Saliña Goto and reduced flamingo abundance since 2010

Ecological and ecotoxicological research

DME Slijkerman[1], P de Vries[1], MJJ Kotterman[1], J Cuperus[1],
CJAF Kwadijk[1], R van Wijngaarden[2]

Report number C211/13

1: IMARES
2: Alterra

IMARES Wageningen UR
Institute for Marine Resources & Ecosystem Studies

Client:	Ministry of Economic Affairs PO Box 20401 2500 EK Den Haag The Netherlands
	BAS code BO-11-011.05-021
Publication date:	19 December 2013

IMARES
WAGENINGEN UR

IMARES is:

- an independent, objective and authoritative institute that provides knowledge necessary for an integrated sustainable protection, exploitation and spatial use of the sea and coastal zones;
- an institute that provides knowledge necessary for an integrated sustainable protection, exploitation and spatial use of the sea and coastal zones;
- a key, proactive player in national and international marine networks (including ICES and EFARO).

This research is part of the BO program Caribbean Netherlands (BO-11-011.05-021) and has been financed by the Ministry of Economic Affairs (EZ) under project number 4308701022.

© Photos titlepage
1rst photo: Damian Davalos
2nd photo: google earth
3rd and 4rd photo: Joel Cuperus

P.O. Box 68
1970 AB IJmuiden
Phone: +31 (0)317 48 09 00
Fax: +31 (0)317 48 73 26
E-Mail: imares@wur.nl
www.imares.wur.nl

P.O. Box 77
4400 AB Yerseke
Phone: +31 (0)317 48 09 00
Fax: +31 (0)317 48 73 59
E-Mail: imares@wur.nl
www.imares.wur.nl

P.O. Box 57
1780 AB Den Helder
Phone: +31 (0)317 48 09 00
Fax: +31 (0)223 63 06 87
E-Mail: imares@wur.nl
www.imares.wur.nl

P.O. Box 167
1790 AD Den Burg Texel
Phone: +31 (0)317 48 09 00
Fax: +31 (0)317 48 73 62
E-Mail: imares@wur.nl
www.imares.wur.nl

IMARES, institute of Stichting DLO
is registered in the Dutch trade
record nr. 09098104,
BTW nr. NL 806511618

A_4_3_2-V13

Contents

Acknowledgements

Frank van Slobbe (OLB) is acknowledged for providing data on flamingo counts
STINAPA Bonaire facilitated during sampling of Benthos and *Artemia*. Ramón de Leon, Fernando Simal and Paulo Bertuol, (all STINAPA Bonaire), thanks for the help and advice given.
CIEE Bonaire facilitated laboratory space and provided the sampling equipment and field assistance.
RIVM is acknowledged by sharing data and efforts on the chemical sampling in 2012, and for including IMARES in their study.
Erik Meesters (IMARES) is acknowledged for contributing largely to the GAM results. Dolfi Debrot for his broad knowledge on local conditions, flamingo populations and contributing to the general discussion of the results. Edwin Foekema (IMARES) is thanked for critically commenting the draft version of the report.
Erika Koelemij (IMARES) is acknowledged for performing the Microtox test. Babeth van der Weijden (IMARES) and Tristan da Graca (student) for assistance in benthic analysis. Maadjieda Tjon Atsoi (IMARES) for assistance during GC-GC analysis.
Paul Hoetjes is acknowledged for his comments on the draft version in name of the ministry of economic affairs.

Abstract

In 2010 a petrochemical fire took place at the BOPEC oil terminals on Bonaire. These facilities are located on the shores of the Goto lake, a legally protected RAMSAR wetland and important flamingo foraging area. Before the fire, daily flamingo counts averaged approximately 400 birds that used the area to feed on Artemia (brine shrimp) and Ephydra (brine fly larvae). Immediately after the fire, flamingo densities plummeted to nearly none and have not recovered. A large amount of fire retardants were used to combat the fire, and were hypothesised to be a potential cause for the flamingo declines. Our analyses of 15 years of baseline flamingo monitoring data show that rainfall does influence flamingo densities but only on the short-term and steering seasonal dynamics of flamingos. Therefore the rainfall event/change in the rainfall regime cannot account for lasting absence of flamingos. Nearby control lakes that were not affected by the fire showed no lasting reduction in flamingo densities, but instead an increase due to the birds no longer feeding in Goto.

In 2012, we measured the concentrations of polycyclic aromatic hydrocarbons (PAHs) and perfluorinated compounds (PFCs, which includes PFOS) in Goto and control-lake waters and conducted additional chemical screening (fingerprinting) of sediments and biota. These measurements showed both lasting elevated levels of PFCs, in water, sediments and biota (fish) and lowered food-species concentrations in Goto as compared to control areas. Based on calculated Risk Quotients combined with the chronic exposure, for the documented PFOS levels, toxicological effects on benthic organisms such as *Artemia* and *Ephydra* are likely. Nevertheless additional impact by other associated retardant toxicant is also probable. Goto was found to be chemically different based on GC*GC chemical fingerprinting indicative of elevated Butylated Hydroxytoluene (BHT) concentrations, a compound used in petrochemical industries as a solvent.

In conclusion, our results demonstrate a close link between the 2010 Bopec fires and the subsequent abandonment of the adjacent Goto lake by foraging flamingos. Compared to nearby control lakes, Goto was found to have elevated (and toxic) concentrations of PFCs and associated low food species concentrations. Therefore, our results suggest that the lasting abandonment of the lake by flamingos after the fire have been due to the drastically low food-species densities as likely caused by toxic ecosystem effects resulting from retardants released into the environment while combatting the fires.

Extended management summary

An ecotoxicological study was conducted concerning Goto lake pollution related to the petrochemical fire at the BOPEC facilities in 2010, and the subsequent absence of flamingos in Goto. Before the fire, approximately 400 flamingos used to feed in Goto, on both *Artemia* (brineshrimp) and *Ephydra* (brine fly larvae). Not only the pollution related to the fires is assumed to have impacted Goto and flamingo abundance, but as well extreme rain events shortly after the fire - that lasted for weeks- are proposed as a reason for the absence of flamingos in Goto.

The current study focuses on the possible causes of the absence of flamingos. For this we explored three aspects of Goto:
Ecological aspects (food availability and abiotic (rain) events)
Chemical aspects situation (presence of substances related to the BOPEC fire))
Toxicological aspects (chemical risk and effects)

Ecology.
In general, flamingo abundance can be influenced by rain events via increased water levels and feed dilution. The question whether heavy rainfall that coincided with the fire explain the decline in flamingo abundance in Goto was studied. This was done using Generalized Additive Models (GAM), taking into account long term monitoring data of flamingos on Bonaire to calculate their normal trends. Local long term climate data on rain events were added to study if this could be of any explanatory value.
To set this research question in a more general perspective , also trends of other salinas were studied, as well a possible recovery of Goto flamingos.

The hypothesis was:
<u>The drop of flamingo abundance in Goto can be addressed to the heavy rains of 2010.</u>

We conclude that this hypothesis can be rejected.
The modelling study revealed that extreme rainfall after the fire is not likely to be an explanatory parameter in the decline of flamingo in Goto. Furthermore, recent flamingo counts in Goto show no signs of recovery (based on data early 2013). For the other analysed locations contrasting trends are observed (some show a decline, some show an increase). However, none of the areas showed such a sharp decline comparable to that found for Goto following the fire. The absent birds of Goto (~400) seemed to have redistributed among the other salinas in Washington Slagbaai National Park in recent years. Besides the sharp decline of flamingos in Goto, this study also revealed a longer term (~15 year) decline of flamingos in Cargill, of ~1000 birds.

Besides rain conditions, the availability of food is known to be a steering factor in flamingo abundance. Therefore, the food availability (benthic status) in Goto and other salinas was studied in terms of historical and current densities (via literature and field monitoring).

The hypothesis was:
<u>Food availability in Goto explains the absence of flamingo.</u>

We conclude that this hypothesis cannot be rejected.
The study on food availability in Goto revealed that Goto shows hardly any signs of Ephyra and *Artemia* presence during the samplings conducted. Although all sampled salinas had, and still have considerable differences in benthic composition between and within salinas, and within a year, Goto seems to have no food year-round. The lack of food is likely to be the most important reason for the absence of flamingos. Yet, the reason for this lack of food is studied via the other mechanisms (chemistry and toxicology).

Chemistry.

Together with RIVM in 2012, the chemical status of water and sediment in Goto and other salinas was studied, focusing on polycyclic aromatic hydrocarbons (PAHs) and perfluorinated compounds (PFCs, which includes PFOS) as suspect compounds as they are key-compounds in petrochemical firefighting and the fires itself. In this study, additional chemical screening (fingerprinting) on sediments and biota was performed.

The hypotheses were:

“Water and sediment quality of Goto is affected by the BOPEC fires” And: Next to PFOS, other substances are found in the Goto ecosystem which are not found in other salinas.

We conclude that these hypotheses cannot be rejected.

The additional chemical study revealed that not only water and sediment in Goto contain elevated levels of PFCs, but biota (fish) too. Goto is chemically different than the other salinas, not only based on PFCs and PAHs, but on other (preliminary identified) compounds as well based on GC*GC chemical fingerprinting. The (preliminary) identified compound Butylated Hydroxytoluene (BHT) is related to petrochemical industries as a solvent.

Toxicology.

The research under this aspect was aimed to answer the question whether the observed concentrations of the analyzed compounds in water, sediment and biota pose a risk for toxicological effects, and furthermore, to what extent water and/or sediment samples are direct toxic to organisms. This was done by using so called Risk Quotient ,RQ, (a value representing environmental risk when the ratio “measured field concentration/Risk limit” > 1), and by application of two bioassays.

The hypothesis was:

“Water and sediment quality are at levels that impact the environment of Goto is such a way that flamingos are affected”

This hypothesis cannot be rejected.

The bioassay results did not indicate acute toxicity of both water and sediment samples. However, based on the calculated RQ of PFOS in both water and sediment of Goto it is likely that chronic exposure to the observed PFOS concentrations have induced toxicological effects on benthic organisms such as *Artemia* and *Ephydra*. Reference salinas are exposed to lower PFOS levels without a serious risk on toxicological effects. The observed internal levels of PFCs in fish are at levels known to induce physiological effects on fish embryos.

Environmental toxicology

In summary, based on this study, toxicity could be held accountable as the steering factor for environmental impact in Goto. Due to lack of causal relationships we however cannot confirm this. Still, the conclusions provide circumstantial evidence that toxicological factors have severely impacted the food availability of flamingos in Goto. Thus, the absence of flamingos is a consequence of the lack of food.

The cause of this is likely to be a result of the BOPEC fires which resulted in PFOS levels affecting the macrobenthic community of Goto.

Besides PFOS, it is likely that not only the analysed compounds, but also other compounds that were not analysed impacted the Goto ecosystem by means of acute (in 2010) and chronic exposure (2010 till present). It is unsure whether the ecosystem Goto can ever recover from this state, and if so, when, and with which additional measures to support this. Ecological recovery from this polluted state is hampered by to compound characteristics of PFOS that is regarded as the main toxicant. It is a very persistent compound and recalcitrant to degrade under natural environmental conditions.

Remediation of PFOS is challenged by the same persistent character. Technologies used to address PFCs in general include groundwater extraction, ex-situ treatment and excavation and disposal of contaminated soil/water. Chemical oxidation is seen as a promising technique but yet has to be tested under field conditions and scale.

Suggestions for upcoming work

- Continue to study the benthic community of Goto on a more structural basis
- Study the planktonic community of Goto
- Study regional dynamics in flamingo feeding sites in terms of both quality and quantity. Synchronise flamingo trend data of Bonaire with regional data such as from Venezuela and Curacao to address the missing ~1000 of Cargill
- Look into observations, or study other birds depending on e,g. Brinefly, like for instance Yellow Warbler, in order to assess additional environmental impact than only flamingo.
- Study the status of metal contamination and (photo-) transformation products of PAHs and evaluate environmental risk of these compounds.
- Study the recovery potential of Goto

Nederlandse samenvatting

In dit rapport wordt een onderzoek beschreven gericht op de oorzaak van de afwezigheid van flamingo's in Goto sinds 2010. Een mogelijke verklaring wordt gevormd door de vervuiling van het Gotomeer als gevolg van de petrochemische brand bij de BOPEC faciliteiten in het najaar van 2010. Voor deze brand gebruikten ongeveer 400 flamingo’s Goto als foerageerplek. Sinds 2010 worden ze hier niet meer waargenomen. Toevalligerwijs volgde kort na de brand een periode van enkele weken met extreme regenval . Ook dit kan mogelijk een reden zijn voor de afwezigheid van de flamingo’s.

Het onderzoek richtte zich op :

- Ecologische aspecten (effect van extreme regenval en voedselaanbod)
- Chemische aspecten (aantoonbare verontreiniging als gevolg van de brand)
- Toxicologische aspecten (mogelijke effecten van aanwezige verontreinigingen)

Ecologie

Kort na de brand was er zware regenval op het eiland die enkele weken duurde. In het algemeen worden aantallen flamingo’s beïnvloed door regen via toenemende waterstanden en/of doordat de dichtheid aan voedsel verdund wordt en foerageer-efficiency afneemt. In deze studie is onderzocht of de zware regenval een verklaring is voor de langdurige afwezigheid van flamingo’s in Goto. Dit werd gedaan met behulp GAM modellering. In deze modellering is de meerjarige trend (> 15 jaar) van flamingo aantallen bepaald, en gecorreleerd met regenval data over dezelfde periode. Een mogelijk herstel van de aantallen flamingo’s in Goto is ook onderzocht. Naast het onderzoek op Goto, zijn ook de aantallen vogels in andere (referentie) salinas gemodelleerd.

De onderzoekshypothese was:
“De afname van flamingo’s in Goto kan worden toegeschreven aan de hevige regenval in 2010”

Wij concluderen dat deze hypothese verworpen kan worden.
De modelstudie laat zien dat de extreme regenval na de brand waarschijnlijk niet de verklarende factor is in de langdurige afname van de flamingo’s in Goto. Ook is er geen sprake van herstel van de aantallen na afloop van de regenperiode (gebaseerd op data tot en met februari 2013). Voor ander locaties worden trends gezien in zowel afname als toename van aantallen vogels, maar geen laat een dergelijk scherpe daling zien zoals bij Goto rond de periode van de brand. De afwezige vogels die eerder in Goto fourageerden (zo’n 400) lijken zich te hebben herverdeeld over de andere salinas van Washington Slagbaai National Park. Naast de afname van flamingo’s in Goto laat de modelering tevens zien dat een afname over een langere periode (~15 jaar) gaande is in Cargill gebied, van ~1000 flamingo’s.

Naast regen is voedselbeschikbaarheid een bekend als belangrijke sturende factor in het voorkomen van flamingos. Daarom is de voedselbeschikbaarheid onderzocht (bentisch) in Goto en referentie salinas gebaseerd op zowel historische, als huidige dichtheden (via literatuur en veld monitoring).

De onderzoekshypothese was:
“Voedselbeschikbaarheid in Goto verklaart de afwezigheid van flamingo’s”

Wij concluderen dat deze hypothese niet verworpen kan worden.
De studie naar voedselbeschikbaarheid in Goto laat zien dat er nauwelijks *Ephydra* en *Artemia* (belangrijke voedselorganismen voor flamingo’s) in Goto aanwezig zijn. De bemonsterde salinas laten allen variatie in dichtheden zien, zowel in de ruimte (binnen een salina en tussen salinas) als in de tijd (seizoen). In het Gotomeer wordt echter op geen enkel moment, en op geen enkele locatie een

substantiële hoeveelheid benthische organismen aangetroffen . De afwezigheid van voedsel is daarmee de voor de hand liggende reden voor de afwezigheid van flamingo's. De reden voor de afwezigheid van voedsel wordt nader onderzocht aan de hand van de andere mechanismen (chemisch en toxicologisch).

Chemie

Samen met het RIVM is in 2012 de chemische status van het water en het sediment bepaald in Goto en referentie Salinas. Hierbij lag de focus op PAKs (polycyclische aromatische koolwaterstoffen) en PFCs (perfluor verbindingen) omdat van deze stoffen bekend is dat zij vrij komen bij petrochemische branden en de bestrijding ervan. In deze aanvullende studie zijn sedimenten en biota (vis) afkomstig van de salinas chemische gescreend op alle mogelijke organische componenten.

De hypotheses waren:
"Water- en sedimentkwaliteit van Goto is beïnvloed door de BOPC branden" En: Naast PFOS, zijn andere stoffen aanwezig in Goto die niet in andere Salinas worden aangetroffen.

Wij concluderen dat deze hypotheses niet verworpen kunnen worden.
Deze aanvullende studie laat zien dat niet alleen water en sedimenten verhoogde niveaus van PFCs bevatten, maar biota (vis) ook. Bovendien is Goto chemisch anders dan de overige salinas, niet alleen gebaseerd op PAKs en PFCs, maar ook wat betreft een stof –BHT- gebutylateerde hydroxytolueen (voorlopige identificatie) die met de GC-GC screening aangetroffen is. Deze stof is gerelateerd aan petrochemische industrie waar het wordt gebruikt als oplosmiddel.

Toxicologie

Het toxicologische onderzoek richtte zich op de vraag wat het risico is dat de concentraties van de geanalyseerde stoffen in water, sediment en biota tot toxicologische effecten kunnen leiden. Daarnaast was een vraag in welke mate water en sediment (nog) direct giftig zijn voor organismen. Het risico op effecten is bepaald met behulp van zgn. Risico Quotiënten- RQ. De RQ waarde geeft de mate van milieurisico aan en wordt bepaald door de gemeten veldconcentraties te delen door een risicolimiet. Als de waarde >1 is, dan is er milieurisico. Daarnaast zijn twee bioassays toegepast (Bacteriën en *Artemia*).

De hypothese was:
"Water en sediment kwaliteit zijn zodanig dat het impact heeft op het ecosysteem van Goto"

Wij concluderen dat deze hypothese niet verworpen kan worden.
Gebaseerd op de berekende RQ van PFOS in zowel water als sediment van Goto gecombineerd met de chronische blootstelingstellingsperiode van inmiddels jaren, is het aannemelijk dat de waargenomen concentraties toxicologische effecten op kreeftachtigen als *Artemia* en insecten zoals *Ephydra* heeft gehad en nog steeds heeft op diens populaties. Referentie salinas bevatten ook PFOS, maar in concentraties dat geen risico op toxicologische effecten inhoudt.
De concentraties PFCs in vis zijn komen overeen met gehaltes in de literatuur waarbij effecten op de overleving en de fysiologie van vis-embryo's worden beschreven.
Acute toxiciteit van zowel water als sediment is in de toegepaste bioassays niet waargenomen.

Milieu toxicologie

Op basis van deze studie wordt verondersteld, dat de toxiciteit van de aanwezige stoffen verantwoordelijk kunnen worden gehouden voor de milieu impact in Goto. Causale relaties ontbreken, zodat dit niet bevestigd kan worden. De conclusies van de afzonderlijke onderdelen bieden echter voldoende indirect bewijs voor de conclusie dat toxicologische effecten bepalend zijn voor de impact op de voedselbeschikbaarheid voor flamingo's in Goto. Kortom, de afwezigheid van flamingo's is een resultaat van gebrek aan voedsel.

De reden voor het gebrek aan voedsel is waarschijnlijk een consequentie van de BOPEC brand die heeft geresulteerd in verhoogde PFOS concentraties in Goto die de macrofauna gemeenschap beïnvloed hebben. In elk geval is duidelijk dat regenval geen verklarende factor is voor de langdurige afwezigheid van flamingo's.

Naast PFOS is het aannemelijk dat ook andere – niet geanalyseerde stoffen zoals metalen of PAKs het milieu van Goto hebben beïnvloed via zowel acute als chronische blootstelling. Het is onzeker of het ecosysteem van Goto ooit zonder hulp kan herstellen, en welke aanvullende maatregelen herstel eventueel kan ondersteunen. Ecologisch herstel wordt belemmerd door stofeigenschappen van PFOS, dat onder natuurlijke omstandigheden niet of nauwelijks afgebroken wordt. Ook saneringsmogelijkheden van PFOS worden bemoeilijkt door diens persistente eigenschappen. Technologieën die worden ingezet om PFOS af te breken betreffen hoogwaardige technologische toepassingen, zoals grondwater extractie, ex-situ behandeling (niet op locatie) en afgraven en elders storten van verontreinigd water en sedimenten. Chemische oxidatie wordt gezien als een veelbelovende techniek bij het afbreken van PFOS, maar is nog niet getest op veldschaal- en condities.

Suggesties voor eventueel vervolg

- Vervolgen bentische survey Goto op meer structurele basis
- Bestuderen van de ontwikkeling van het plankton in Goto
- Bestuderen van regionale dynamiek in flamingo foerageer locaties, zowel in termen van kwaliteit als in kwantiteit. De flamingo trend data van Bonaire synchroniseren met regionale data van bijvoorbeeld Venezuela en Curacao om zicht te krijgen waar de "missende " 1000 vogels zich mogelijk bevinden.
- Bestuderen van ander vogelsoorten bij Goto die ook afhankelijk zijn van bv brinefly, zoals de Yellow Warbler. Mogelijk heeft de impact als gevolg van de BOPEC brand ook andere dieren dan flamingo beïnvloed.
- Bestuderen van de status van metaal verontreiniging en PAK-transformatie producten en evalueer het ecologisch risico.
- Bestuderen van eventueel herstelvermogen van Goto

1 Introduction

1.1 Background to the problem

On 8 and 9 September 2010 there was a big petrochemical fire at the BOPEC facility on Bonaire (Figure 1). The BOnaire PEtroleum Corporation (BOPEC) is a fuel oil storage and transshipment terminal that is fully owned by the Venezuelan oil company Petróleos de Venezuela S.A. (PDVSA). It is supposed that 32 million liters of crude and naphtha went up in flames over a period of 2 days (http://www.beautiful-bonaire.nl/natuur/gotomeer.html).

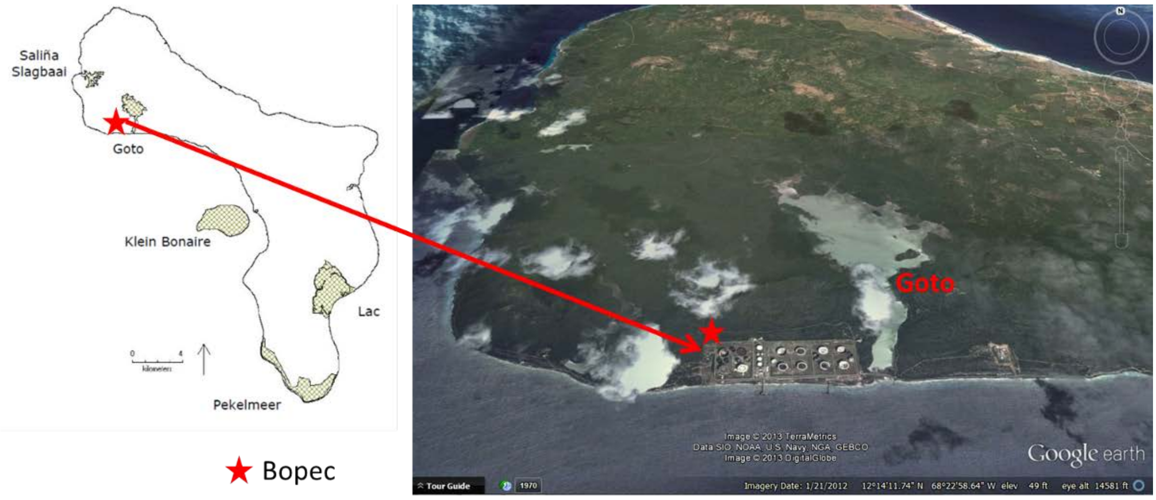


Figure 1 *Overview of Bonaire and its Ramsar Sites, figure Bonaire taken from BNMP mngt plan, close up Bopec and Goto area from Google Earth.*

Goto is a salina (salt lake) and designated as a wetland of international importance under the Ramsar Convention[1] with historically, amongst others, a healthy flamingo population (*Phoenicopterus ruber*). Goto is located in the proximity of the BOPEC facilities, and a channel connecting Goto with the sea lies next to the facility, within a distance of ~30-50 meters. Media and local observers report that flamingos basically disappeared from Goto since that time. It is indicated that the flamingo population displayed a vast reduction of abundance within 4 months after the fire (various personal observations on the island).

The current study focuses on the possible causes of the absence of flamingos. For this we explored three aspects of Goto:

- Ecological aspects (food availability and abiotic (rain) events)
- Chemical aspects situation (presence of substances related to the BOPEC fire))
- Toxicological aspects (chemical risk and effects)

---

[1] The Convention on Wetlands of International Importance, adopted in Ramsar, Iran, in 1971 and known as the Ramsar Convention is an international treaty for the conservation and sustainable utilization of wetlands, and lists so-called Ramsar sites. These sites are considered internationally important and assigned based on fundamental ecological functions and their economic, cultural, scientific, and recreational value

The three aspects are explained in the next sections in terms of working hypotheses and research questions. Furthermore, these three “headers” are used to structure this report.

1.2 Ecology

The absence of flamingos is attributed by many to the BOPEC fires. However, shortly after the fires, heavy rain events occurred and lasted for weeks to months, and since this event was likely to be extreme, it could have impacted flamingo abundance. Data on rain events and long term counting on flamingo numbers for Bonaire are available for this study.

The following hypothesis was studied:

- *Flamingo dynamics on Bonaire relate to rain events; hence the drop of flamingo abundance in Goto can be attributed to the heavy rains of 2010.*

To answer this hypothesis, the following research question was addressed:

1. Could heavy rainfall that coincided with the fire explain the decline in flamingo abundance in Goto?

Additional research questions are drafted in order to set the flamingo abundance in Goto in perspective of flamingo dynamics on Goto and other Salinas of Bonaire:

2. There is a lot of migration of flamingos between the different locations on the island but also with the population in Venezuela and Curaçao (pers. communication A. Debrot (IMARES) and P. Hoetjes (EZ)). If the decline is also observed at other locations on Bonaire, this may indicate a deterioration of the total population that may not be related to the fire. The question to this end is: Is the decrease in flamingo counts also observed at other locations of the island?
3. Do the numbers of flamingos show signs of recovery, now that counting data over a longer period is available? E.g. Where did the “Goto Flamingos” go to?.

Next to extreme rain events, the availability of food is reported as a steering factor in flamingo abundance (e.g. Rooth, 1965).
Therefore, the following hypothesis is studied:

Food availability in Goto explains the absence of flamingos.

The following research questions were addressed:

4. What is the general feeding ecology of flamingos on Bonaire?
5. What is the benthic status of the salinas? In terms of current and historical densities of its main food source in Goto and other salinas?

1.3 Chemistry

Immediately after the fire, the environmental impact resulting from the release of mainly oil, polycyclic aromatic hydrocarbons (PAH) and perfluorinated constituents of firefighting foams was assessed and reported by RIVM (RIVM 2011). RIVM (2011) reported elevated levels of both polycyclic aromatic hydrocarbons (PAHs) and perfluorooctane sulfonate (PFOS) , compounds that are related to petrochemicals fires and firefighting foams respectively.

Two years after the fire, the biological condition of Goto lead to the question whether the observed deterioration of ecological values may be related to delayed ecotoxicological impact caused by the 2010 BOPEC fire and the release of associated firefighting chemicals. On October 15, 2012, the Dutch Ministry of Infrastructure and the Environment (I&M) as requested by the Public Body of Bonaire ordered RIVM to conduct a follow-up study to assess the present chemical condition of Goto and a few other salinas.

In September 2012, IMARES was asked by EZ to prepare a work plan to study the deterioration of Goto. To avoid double effort, and to share knowledge and data, IMARES and RIVM combined efforts in synchronizing each other's work plan and field campaign. The field sampling for sediment and water intended for chemical analysis was conducted by both institutes together at the same time.

IMARES carried out the chemical analysis of PFOS in the RIVM study. The report by RIVM was co-authored by IMARES. During the combined field study IMARES sampled biota and additional water and sediments. Data of both the chemical analysis and the biota are part of this report.

Research questions on chemical status of Goto are answered in the RIVM study, and for completeness are included in this report as well. To study the hypotheses that "Water and sediment quality of Goto is affected by the BOPEC fires" the following research questions were addressed by RIVM (De Zwart et al., 2012) and IMARES (this report):

6. Does water, sediment and biota of Goto contain pollutants that may be related to the BOPEC fire or the firefighting foams used (focusing on PAHs and perfluorated compounds, PFCs)

Besides PFCs and PAHs (the suspect compounds in the study of De Zwart et al., 2012), it could be that other compounds are present in Goto as well.
The hypothesis to *this end is*: Next to PFCs (including PFOS) other substances are found the Goto ecosystem which are not found in other salinas.

In this study the following research questions are added to the RIVM study:

7. Is Goto chemically different than other salinas in terms of chemical fingerprinting by using GC*GC (sediment and biota as the matrix).

1.4 Toxicology

The third aspect is the toxicological aspect in which impact of pollutants is evaluated in general and more specifically to ecological observations related to the flamingos and their food sources .

The hypothesis to this end is: "Water and sediment quality are at levels that impact the environment of Goto is such a way that flamingos are affected"

To study this hypothesis the following research question was addressed by RIVM (De Zwart et al 2012) and IMARES (this report):

8. Are the observed concentrations of the analyzed compounds in water, sediment and biota high enough to induce toxicological effects?
9. To what extent are water and/or sediment samples direct toxic to organisms?

1.5 Environmental toxicology

All three aspects -ecology- chemistry- toxicology- integrated result in an environmental toxicological assessment. Based on the information obtained a qualitative assessment will be described on the environmental toxicological status of Goto.

2 Methods

2.1 Ecology- Flamingo

2.1.1 *Literature review on flamingo ecology*

A literature study on flamingo ecology of Bonaire's population was executed using internet search and SCOPUS and Web of Science database to find scientific records. Background information on flamingos, migration patterns, and their feeding ecology if described. In addition, personal communication with regional experts (Dolfi Debrot (IMARES) , Bart de Boer (regional expert, retired) added to the discussion of some results.

2.1.2 *Flamingo GAM analysis*

The trends in flamingo counts were analysed with Generalized Additive Models (GAM, using a quasipoisson distribution for the errors and a logarithmic link function). In this analysis, two non-linear lines, so called smoothers, are fit to the data. One smoother is used to model the seasonal pattern which is assumed to be similar over the years and one smoother is used to model the trend over the whole period. Possible signs of recovery may be detected as the counts include data until early 2013.

Data of flamingo countings on Bonaire were made available by Frank van Slobbe of Directie R&O Bonaire. These data comprise countings of flamingos over time, starting at June 26h 1981 till February 15th 2013 (most recent date when receiving the file). Data represent countings of total numbers of birds, juvenile, adults and if relevant breeding couples for three regions (North, Lac, Cargill) and total sum of Bonaire. Sub totals are given for individual locations within regions (mainly based on total numbers- no distinction between juvenile, adult, breeding couple).

Only a part of the dataset was taken into account for this study as some factors interfere with a proper analysis. Most important factor to consider was that in time, monitoring locations were added, and counting effort increased. Any trend analysis will be distorted by this fact. Therefore, only the range from 1996 till 2013 was used in this study since counting effort was assumed to be more or less stable in this period. Some locations were discarded due to infrequent monitoring in this period.

2.1.2.1 Trends at different locations

Data for several locations on the island are available for comparison. Because observation effort is not constant for all locations or counts are too low to analyse, a selection of these locations were analysed similar to the analyses of Goto data.

Apart from Goto, the following locations were analysed (for location of the regions see figure 1) :

1. Typical salinas in Washington Slagbaai National park: Saliña Matijs.
2. The total of the counts of the northern locations[2] in Washington Slagbaai National park, excluding Goto
3. Counts from several counting stations at Lac Bay (Lac Bacuna, Lac Cai and Lac West).
4. Total counts from the Cargill/Pekelmeer area.

---

[2] Saliña Matijs, Saliña Bartol east, Saliña Bartol west, Saliña Funchi, Saliña Wayaká, Boca Slagbaai, Saliñas Slagbaai, Saliña Tam, Saliña Frans, Onima, Saliña Lechi and counts at other northern locations

2.1.2.2 Flamingo abundance and rain events

The relation between flamingo abundance and rain fall was studied via literature review. Furthermore flamingo abundance data were analysed together with rainfall statistics.

Required meteorological data was downloaded from www.tutiempo.net on March 12th, 2013. Data is only available for the weather station at Flamingo Airport on Bonaire (789900 (TNCB); latitude: 12.15, longitude: -68.28, altitude: 6). A long time series of precipitation levels (mm/day) are available for this station (Figure 2).

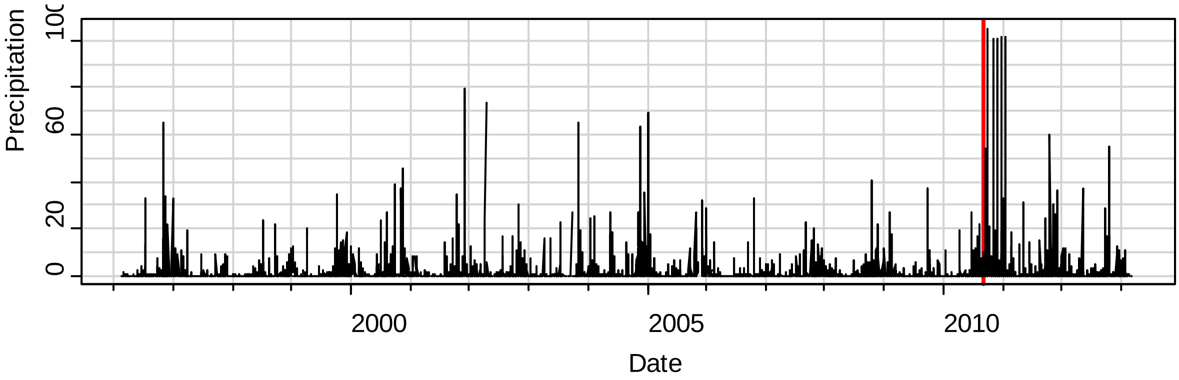


Figure 2 Raw precipitation data (mm/day) at the weather station of Flamingo Airport on Bonaire from 1996 up to early 2013. Vertical red line indicates the date of the fire.

To link the rainfall with the flamingos counts, the average precipitation in the two weeks prior to the date of each counting event was taken (markers in Figure 3).

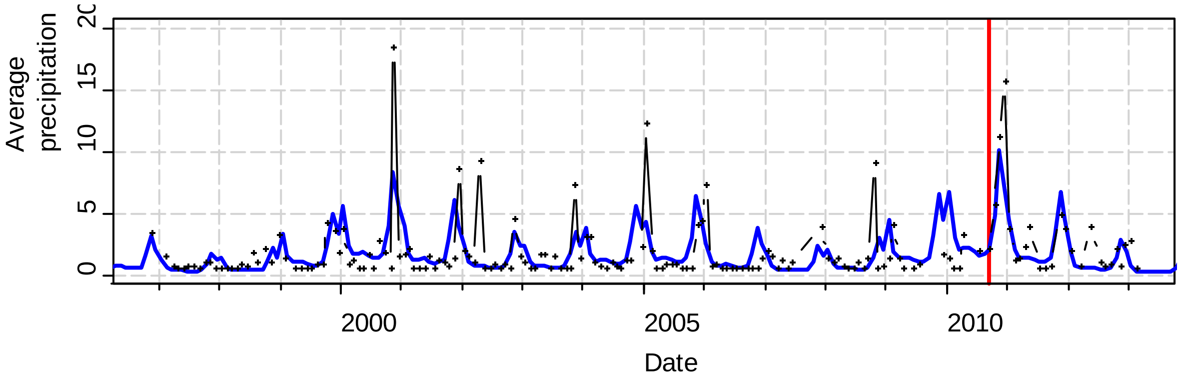


Figure 3 Black markers show the average precipitation (mm/day) in the two weeks prior to the flamingo counting dates (0.5 mm/day was added as a background level). The blue line shows the Generalized Additive Model fitted to average precipitation data. Vertical red line shows the date of the fire.

2.2 Ecology- Benthic composition

2.2.1 Benthic sampling

Benthic samples were collected twice, in October 2012 and in May 2013. In October 2012 a dip net with a width of 30 cm and a mesh size of 2 mm was used. During the sampling on May 30th 2013, a selection of locations was sampled. A macrofauna net was used with a width of 40 cm, and mesh-size of 1 mm. In Table 1 an overview of sampled location is presented.

Table 1 Overview of sampled locations for benthic analysis.

Location	October 2012	May 2013
Matijs1	Yes	No; salina dry
Matijs2	Yes	No; salina dry
Bartol3	Yes	Yes
Slagbaai 5	Yes	Yes
Slagbaai6	Yes	No
Goto7	Yes	Yes
Goto8	Yes	Yes
Goto9	Yes	No; sampling planned , but not succeeded
Goto10	Yes	No

In both sampling moments, the net was dragged 2-3 cm into the sediment for a distance of 5 meters. The collected material was rinsed by dipping the net into the water to remove most sand and clay particles. From the remaining material (biota, shells, gravel, coral and other particles) a subsample was taken, if the sample consisted of more than 1 litre. There after the sample was stored in a polyethylene container or ziplock bag. The sample was preserved with 6-10 % buffered formaldehyde in seawater solution.

After sampling the samples were taken to the lab and rinsed with water over a sieve with a mesh size of 0.5 mm. All the biota was sorted and at least identified on class in the laboratory of IMARES. Of each species type pictures were taken for further identification.

2.2.2 Artemia sampling

On March 18 and 19, 2013 Goto and other saliñas in Washington Slagbaai National Park were sampled for *Artemia* sp. Saliña Matijs was not part of the survey as it was completely dry at the time of sampling. Depending on the size of the saliña, one to three samples were collected that corresponded to the locations at which benthic samples were taken. Sampling was carried out by means of dragging a plankton net (mesh size: 250 µm) for 50 m directly under the water surface. In a few cases the water was too shallow for net dragging. Then, a total of approximately 75L of water, collected from four different spots was filtered through the plankton net. The material collected was conserved in a 70% ethanol solution. Water characterization parameters were collected by means of a YSI 85 multimeter. Measurements were done from shore at a water depth between 10 -15 cm. Additionally, numbers of flamingos was noted.

2.3 Chemistry

2.3.1 Sampling and storage of samples

The sampling of sediment, water and biota took place in conjunction with the RIVM study of 2012, and is described in detail in De Zwart et al., 2012.

In summary, sampling took place in the week from October 29th to November 2nd, 2012. Before the actual sampling took place at the preselected sampling locations, readings on depth, pH, conductivity, dissolved oxygen and temperature were taken in the field. Water and sediment samples were taken in duplicate. First, the water samples were taken by submersing a clean hard plastic beaker[3] to approximately mid depth and filling two glass 1 L bottles. Then, two 250 mL sediment samples were taken by carefully scraping the sediment of the bottom surface layer and transferring the collected sediment to a 250 ml glass container. All samples were labeled and stored in a cool box. After a sampling day, the samples where store in a refrigerator, until transport to the Netherlands.

Figure 4 Field locations in the salinas of Washington Slagbaai, Bonaire.

In the RIVM study, the analytical expenditure was restricted to the two groups of chemicals that are most likely associated to the 2010 BOPEC fire:.

- Polycyclic aromatic hydrocarbons (PAH). These analyses were performed by the laboratory of TNO Earth, Environmental and Life Sciences, Utrecht, the Netherlands.
- Perfluorinated alkanoic acids and perfluorinated alkane sulfonic acids, possibly limited to perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) as the most prominently used constituents of firefighting foams. These analyses were performed by the laboratory of IMARES, IJmuiden, the Netherlands.

Details can be retrieved via De Zwart et al., (2012).

In this study, additional GC-GC analyses was performed on sediment and biota (fish) in order to screen for other suspect compounds in general, and to detect chemical differences between Salinas.

---

[3] Plastic is not the preferred storage material for organic contaminants like PAHs, but due to the short contact time it can be used for quick sampling.

2.3.2 Fingerprinting by GCxGC

GCxGC (GC= Gas chromatography) is a technique that allows for extra discrimination compared to normal GC. When applying this technique in a non-selective way the results of the analysis can be used as a kind of chemical fingerprint. With the help of this technique it is possible to tell if organisms are being exposed to the same environmental conditions (chemical components). A selection of sediments and biota from Matijs, Slagbaai, and Goto (2 sampling locations, number 7 and 9) were analysed.

2.3.2.1 Extraction

Samples are homogenised with a blender after which a subsample is taken and dried using sodium sulphate. The dried sample is then loaded into an Accelerated Solvent Extraction (ASE) cel (Dionex) together with 25 grams of florisil (VWR). Cells are subsequently extracted with a mixture of pentane/dichloromethane (85/15, Promochem) using an ASE300 (Dionex). The extracts were then concentrated to 1 ml using a rotavap (Heidolph) and transferred to a vial for analysis.

2.3.2.2 Analysis by GCxGC

1 µl of sample was injected on a Shimadzu GCMS2010 (GC) coupled to a GCMS-QP2010 Ultra (MS) detector (Shimadzu, ‘s Hertogenbosch, the Netherlands). Analysis was performed in GCxGC mode using a Zoex ZX2 modulator (Shimadzu, ‘s Hertogenbosch, the Netherlands) with a modulation of 6 s. 1st dimension column was a 30m x 0.25 mm i.d. HT8 with a film thickness of 0.25 µm. The second dimension was a 2.3 m x 0.25 mm i.d. BPX-50 column with a film thickness of 0.15 µm. Chromatograms were processed using the GCImage software package (Shimadzu, ‘s Hertogenbosch, the Netherlands).

Chromatograms were compared by making a template from the reference location (Matijs) and then applying this template to the chromatograms of the other locations. This way, peaks not present in the reference location, but present in the other locations were easily spotted. These deviating peaks were identified using the NIST library. The comparison with the NIST library gives a match factor and a probability. The match factor is a score with a maximum of 1000. The higher the score, the higher the similarity with the spectrum from the peak and thus the higher the chance that it is in fact this compound. Probability is the estimated relative likelihood of that the compound multi-spectrum is the correct match for the submitted multi-spectrum.

2.4 Toxicology

Toxicology aspect of Goto is evaluated on two aspects, Environmental Risk Quotients and application of bioassays. An ecotoxicological quick scan with bioassays was conducted with two tests, using crustaceans (ARTOXKIT) and bacteria (MICROTOX).
Artemia and bacteria are both relevant organisms. *Artemia* is found in general in salinas and serves as a food source for flamingos. Bacteria are important organisms in each ecosystem as they reflect the turnover of organic and anorganic compounds (e.g. nutrients).

2.4.1 Risk Quotients

Environmental risk can be evaluated by means of so called Risk Quotients (RQ), which are described by De Zwart et al., 2012. Risk Quotients are defined as the measured concentration divided by the risk limit. The risk limit in this study is the maximum permissible concentration. This limit is compound specific. If the RQ < 1, no environmental risk is expected. Arbitrary classes of risk are redefined to indicate the levels of risk. Risk categories used by De Zwart et al., (2012) are slightly adapted in this report, based on .
RQ <1: insignificant risk (green).
RQ 1-10: moderate environmental risk (orange)
RQ>10: high environmental risk (red)

2.4.2 ARTOXKIT

Toxicity screening was done using ARTOXKIT M ^{TM} , which is an *Artemia* toxicity screening test for estuarine and marine water, to test the acute toxicity of water samples of a selection of Goto sites, and 3 reference sites obtained from Slagbaai and Bartol (Matijs was dry and could not be used as reference). The standard operational procedure was used. The basic principle of the test is a simple, sensitive and rapid screening of acute toxicity for chemicals using brineshrimp *Artemia franciscana* as a test organism. *Artemia* is cultured by hatching cysts in seawater (36 ‰) and after 30 hours the instar II- III larvae can be used for the test.

Figure 5 Cysts and hatching Artemia larvae

The test is performed in a multiwell test plate (Figure 6), in which multiple samples can be tested in replicated series. Each well is filled with 1 ml of sample. The brine shrimp are transferred using a small pipette, in total 10 individual larvae per well. The actual number of exposed larvae deviated a bit from the intended 10/well. The totals are summed in Table 2.

Table 2 Number of Artemia larvae exposed per sample.

sample	Location in Figure 4	Total exposed larvae
Goto7	Loc 7	36
Goto8	Loc 8	38
Goto9	Loc 9	34
Slagbaai5	Loc 5	29
Slagbaai6	Loc 6	32
Bartol3	Loc 3	42

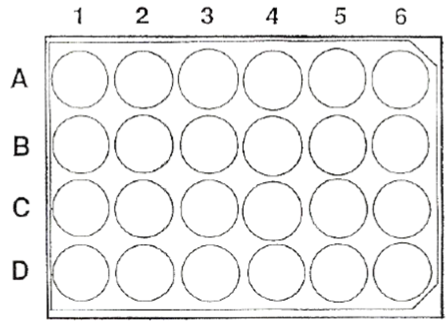


Figure 6 Multiwell test plate composed of 6 x 4 wells. A- D were used as replicate cells, 1-6 were used for the different samples.

This test is normally used to screen chemicals over a range of concentrations in order to calculate the concentration at which 50% of the organisms die (LC_{50}). In this study, the test was applied as 1 first screening of acute toxicity of the water in Goto. Water of other salinas (Bartol and Slagbaai) were used as reference. Water samples of the salinas (500 ml) were taken during the benthic sampling of 30th May 2013. In the laboratory the water samples were filtered over a 100 µm filter to remove any larger zooplankton from the samples and stored in the refridgerator until the toxicity screening the next day. No dilution of the tested water was applied.

The original test lasts for 24 hours after which the surviving larvae are counted. In this case, the test was extended to 48, 72 and 96 hours as well to screen more chronic toxicity. However, the test is not developed as such, and shortage of food is a serious limitation.

2.4.3 Microtox

Microtox® is a standardised aquatic toxicity test system which uses the bioluminescent marine bacterium (*Vibrio fischeri*) as the test organism (Azur Environmental, 1998). In general the bacteria are exposed to a range of concentrations of the material being tested. The reduction in intensity of light emitted from the bacteria is measured. The change in light output and concentration of the toxicant produce a dose / response relationship, after which the EC_{50} (concentration producing a 50% reduction in light) is calculated.

In this study, Microtox is used as a screening method for toxicity of sediment samples (porewater) and water samples of Goto (Goto7, Goto8, Goto9) and 4 reference locations (Bartol3, Slagbaai5, Slagbaai6, Matijs1)[4]. If relevant, a dose response relationship was established. The test was conducted according to the ISO protocol (ISO, 2007). All samples (water and porewater) had high salinity values, and had to be diluted to be in compliance with the Microtox test criteria. This means that the original concentration of toxic compounds (if present) is diluted as well, and is thus higher in the actual situation. pH had to be adjusted in some samples (by adding HCL) as was the oxygen level (by aeration of te sample).

Each sample was screened for bioluminescence after 5, 15 and 30 minutes exposure. Results are presented as % change in luminescence.
Control samples were applied with NaCl. Control toxicity substance was not applied due to the fact that this was only a first screening test.

---

[4] The samples are the same samples on which te chemical analyses was performed, sampled in October 2012.

3 Results- Ecology

3.1 Flamingo ecology

3.1.1 General description Caribbean flamingo

Picture 1. Caribbean Flamingo in Washington Slagbaai, Bonaire. *Picture and © Damian Davalos.*

Flamingos (family Phoenicopteridae) are long-legged, pink waterbirds living in the wild in Africa, Asia, Europe, North America and South America. The Caribbean flamingo shares the genus Phoenicopterus with the Greater and Chilean flamingo, and some sources consider the Caribbean flamingo a sub-species of *Phoenicopterus ruber*. When considered a sub-species the scientific name of the Caribbean flamingo is *Phoenicopterus ruber ruber (*Picture 1*)*.

In general, the bird is tall (120-145 cm high). The wading bird has webbed feet, with long legs and a long neck, with a deep pink to red/orange, with black primary and secondary feathers. The pink colour comes from carotenoids, which are ingested via its food.

3.1.2 Distribution and migration

The Caribbean flamingo has four populations (Galapagos Islands - Southern Caribbean (including Bonaire) - Yucatan and Northern Caribbean), and only four main breeding sites of which Bonaire is one, next to the Bahamas, Cuba, and Mexico. In Figure 7 the distribution area is presented.

Figure 7 Distribution range of the Caribbean flamingo. Taken from: BirdLife (Caribbean Flamingo Species Factsheet)

Caribbean flamingos are fairly nomadic throughout the year, following resources that have shifting patterns (e.g. Boylan, 2000, Del Hoyo et al., 1992, Espino-Barros & Baldassarre 1989a, Espino-Barros & Baldassarre, 1989b). Although they are non-migratory birds, they can move to different patches within sites, but also between sites up to hundreds of km apart. Those that do migrate, migrate between summer breeding sites and winter non-breeding areas. In Bonaire, migration does not occur all at once, but in successive waves (Birdlife, 2008). Juvenile birds tend to follow their parents, and copy their routes and feeding places (pers comm De Boer).

Typical habitat where flamingos are found includes saline lagoons, muddy flats, shallow lakes -coastal or inland. Flamingos prefer saline to fresh water. However, the Caribbean flamingo can tolerate twice the salinity of sea water (~75 ppt). Disturbances in the habitat, such as severe weather, affect resources which may cause flamingos to move around during the year, as they adapt to shifting food availability and available breeding sites (Baldassarre & Arengo (2000), Del Hoyo et al., (1992), Elphick, et al., (2001), Sprunt, (1975). The breeding season of flamingos lasts from January to July.

Population size is estimated to be 34,000 in Venezuela and Bonaire (1996 estimate; Espinoza et al. 2000). More recent regional number are not found. The Caribbean flamingo is listed on various international conservation lists (Birdlife, 2008) (Table 3), which states that the Caribbean flamingo is not severely threatened but that care is needed in international perspective.

Table 3 Protection status according to Birdlife, 2008

list	**status**	**Meaning of status**
IUCN Red List	"Least concern"	evaluated to have a low risk of extinction
CMS	"appendix 2"	Migratory species that have an unfavourable conservation status or would benefit significantly from international co-operation
CITES	"appendix 2"	Appendix II lists species that are not necessarily now threatened with extinction but that may become so unless trade is closely controlled.
SPAW	Annex 3	Annex 3 lists species that require regulation and management of use to ensure protection and recovery of their populations

3.1.3 Flamingos on Bonaire in general

Bonaire is of global importance for waterbird populations including Caribbean flamingo. Wells and Debrot (2008) stated that over the last 10 years the numbers have fluctuated between c.1,500 and 7,000 breeding individuals (though most normally averaging c.5,000). Based on data provided by DROB the previous reported numbers by Wells and Debrot seem a bit optimistic. Based on the flamingo abundance data, the breeding population of the Sanctuary- the main breeding site on Bonaire-, shows numbers ~500 in recent years. Around the year 2000 higher numbers were counted up to 2500, but lowered (not significantly) in the last 10 years (Figure 31).

The flamingos fly to mainland Venezuela to feed in lagoons along the coast of the state of Falcón where hundreds are regularly seen but are not known to breed. The movements of the flamingos within the island and to-and-from mainland Venezuela are however poorly known (Wells and Debrot, 2008).

On Bonaire, multiple Important Bird Areas (IBA) are located, in which the flamingo feeds, or nests. Based on Wells and Debrot (2008), the following estimated are provided:
Washington-Slagbaai National Park encompasses ~25% of Bonaire at the northern end of the island. A regionally important concentration of 500 Caribbean flamingo occurs in this IBA. This IBA is a state-owned protected area that includes two Ramsar sites—Goto and Boca Slagbaai (Figure 1 for Ramsar locations).
At *Lac Bay,* numbers of Caribbean flamingo occasionally exceed 200.
Pekelmeer (saltworks) is globally significant for Caribbean flamingos. A maximum of 1,300 pairs nesting in 1996 was recorded (Wells& Debrot, 2008). Much or most of this IBA is government owned but is leased to the commercial salt works company. However, a 55-ha area (including an island) has been set-aside since 1969 as a Flamingo Breeding Reserve, which is where most of the birds nest. Pekelmeer (and the flamingo reserve) are designated as a Ramsar site (Wells& Debrot, 2008).

3.1.4 Data: Trend and recovery Goto

The trend on flamingo abundance is analysed for the various regions and locations where flamingos are counted. In figure 1 the different regions (Ramsar sites) are presented. Not all data are shown, only the most representative figures are included in this report.

The observed flamingo counts in Goto and the resulting statistical model are shown in Figure 8. The number of flamingos start low each year and increase over the months and decline again at the end of each year (Figure 8 and Figure 9, right). In the years following 1996, the number of flamingos are stable at Goto (Figure 8 and Figure 9, left). However around the year 2010, a clear decrease is observed (Figure 8 and Figure 9, left). The exact start (in time) of the decline is difficult to pinpoint with this model due to the behaviour of the smoothing function that fits available data. Flamingo count- dates do not match exactly with the date of the fire, combined with very large differences, and consequently the smoothing function integrates data points before and after the fire, resulting in a declining behaviour of the model, just before the fire.

The counting data of the most recent years(2010-2013) does not show any sign of recovery. A negative trend is still observed for the most recent years (Figure 8 and Figure 9, left).

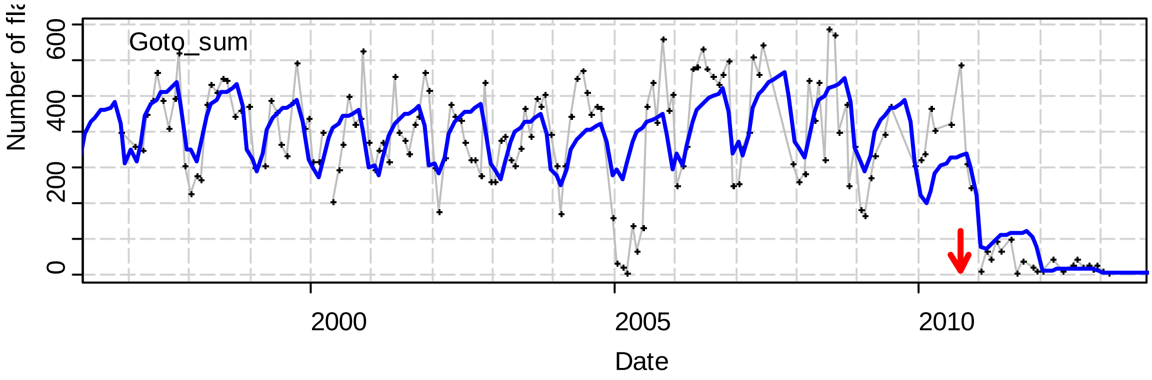


Figure 8 *Observed flamingo counts (black markers) in the Goto area (both entrance and saliña) and fitted model (blue). Red arrow indicates the date of the fire.*

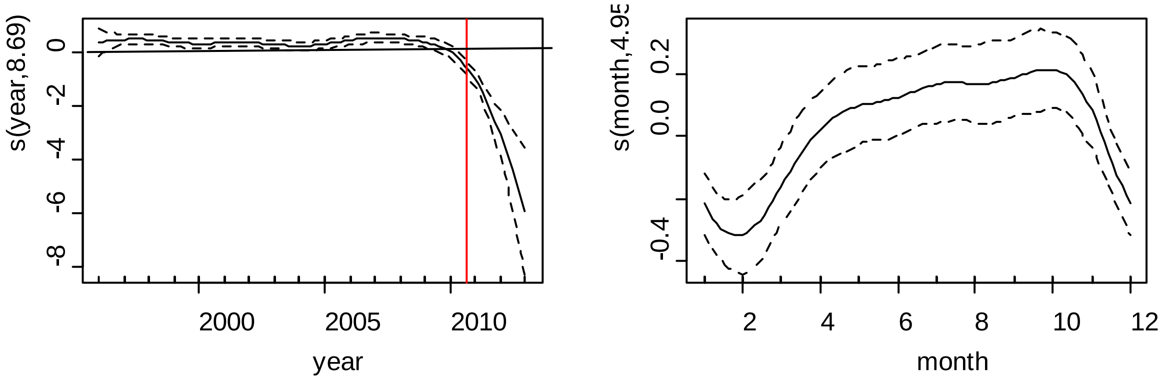


Figure 9 *Smoothed trends over the years (left) and within each year (right) in the Goto area (both entrance and saliña). Red line indicates moment of fire.*

3.1.5 Trends at Washington Slagbaai locations (north Bonaire)

3.1.5.1 <u>All northern locations excluding Goto</u>

For the combined northern locations (excluding Goto) the seasonal patterns are less apparent (Figure 10 and Figure 11) than in Matijs (Figure 13). The trend over the years fluctuates with approximately five year cycles around a count of around 250 flamingos (Figure 10 and Figure 11). However, after the fire in 2010, the numbers in all other northern salinas increase strongly, which can be accounted to numbers usually found at Goto before the fire.

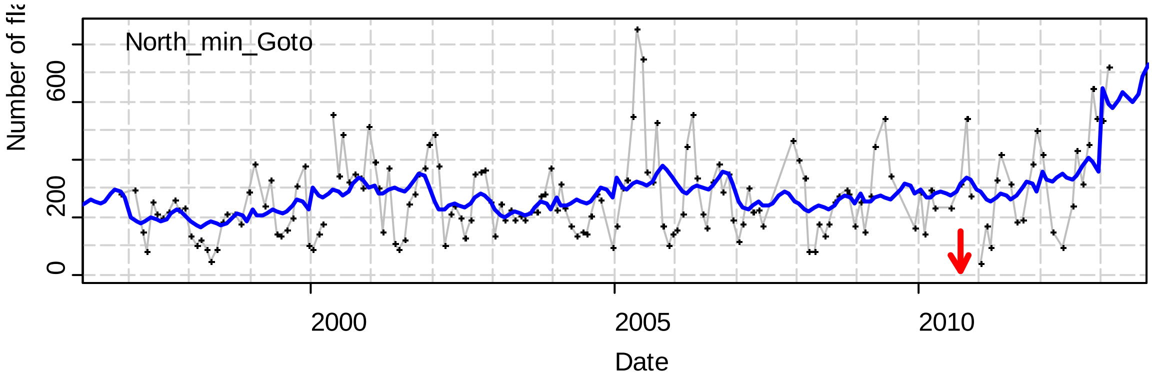


Figure 10 *Observed flamingo counts (black markers) at the combined northern locations (excluding Goto) and fitted model (blue). Red arrow indicates the date of the fire.*

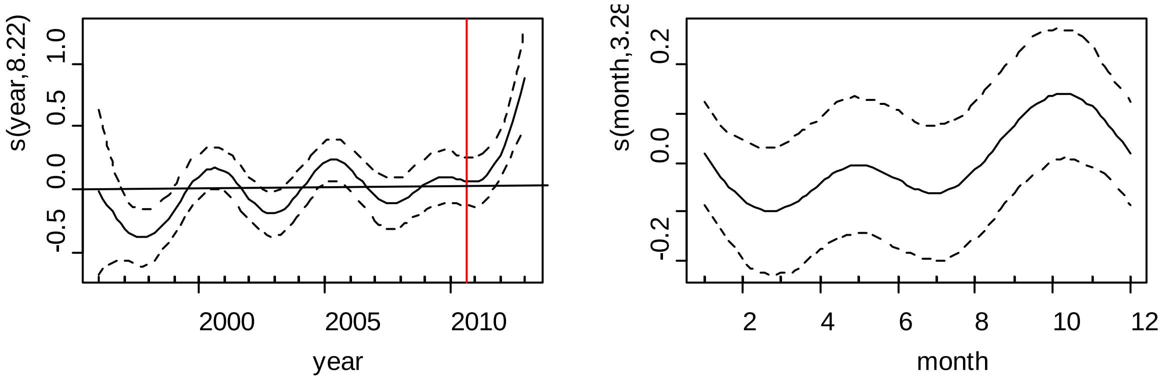


Figure 11 *Smoothed trends over the years (left) and within each year (right) at the combined northern locations (excluding Goto). Red line indicates moment of fire.*

3.1.5.2 Matijs

At Saliña Matijs, the overall number of flamingos has increased, with some fluctuations, in the last decades (Figure 12 and Figure 13 left). Striking at this location is that there are two peaks in flamingo counts for most years (in May and November, Figure 13 right). It seems that in years after much rain, Matijs does not dry in, and fullfills a fouraging place for Flamingo in May.

In Matijs, there is no considerable drop in numbers in recent years, comparable to that observed at Goto, but rather an increase since 2005.

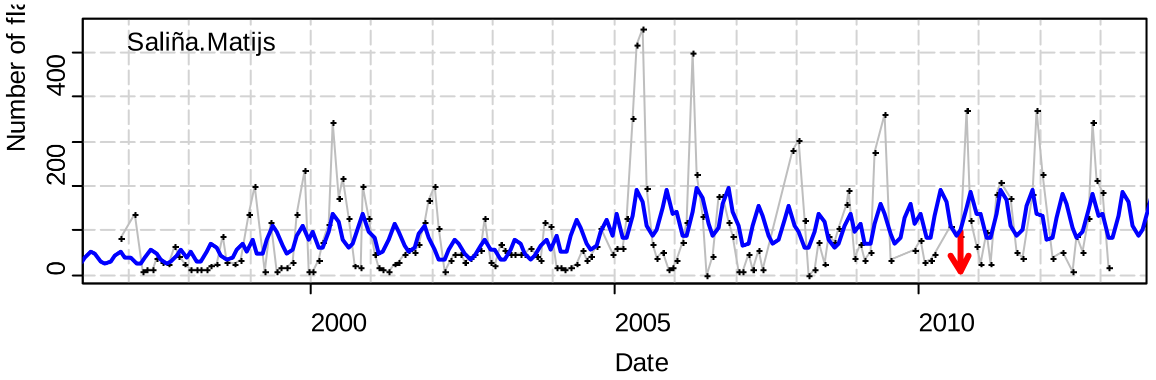


Figure 12 *Observed flamingo counts (black markers) in Saliña Matijs and fitted model (blue). Red arrow indicates the date of the fire.*

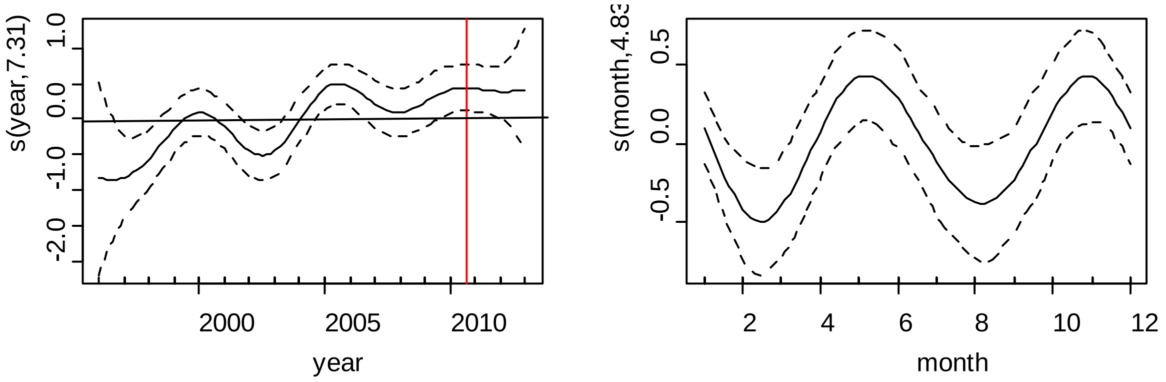


Figure 13 *Smoothed trends over the years (left) and within each year (right) in Saliña Matijs. Red line indicates moment of fire.*

3.1.6 Diet & feeding ecology

Flamingos are known to prefer habitats with high food abundance (Allen, 1956). The diet of Caribbean flamingo consists of small crustaceans (such as amphipods), mollusks, insects (such as midges and brine flies), polychaete worms, fish (rarely), widgeongrass seeds, muskgrass tubercles, and algae ((Arengo & Baldassarre, (2002), del Hoyo et al., (1992), Ehrlich, et al., (1988), Zweers et al., (1995)). A flamingo needs about 270 g of food/ day (10% of body mass), or about 32,000 brine fly pupae, 50,000 brine fly larvae, or 135,000 brine shrimp daily (Rooth 1965).

Rooth (1965) correlated the number of *Ephydra* to the number of eating flamingos in three salinas of Bonaire. He concluded that the 100 present flamingos in Slagbaai eat about 3200000 *Ephydra* a day, but this did not affect the numbers of Epydra. In Pekelmeer flamingos consumed up to 80% of the present *Ephydra*. *Ephydra* recovered to original numbers after the feeding period.
In Goto, 700 Flamingos were present, and ate 22400000 *Ephydra* a day. In Goto, *Ephydra* showed a decrease in numbers when 700 flamingos were eating, indicating that Goto food balance was not enough, and that flamingos needed other food locations to complete their diet.

Since 1965, fouraging sites on the island were diminished by more than 50% (Rooth, 1975, 1976; De Boer, 1979). As a result, a daily migration that often exceeded 1000 birds was observed between Bonaire and Venezuela (De Boer, 1979). Clearly, the aim of this migration was to obtain food in Venezuela. So, in spite of the fact that the breeding colony on Bonaire is well protected, the foraging habitats are under increasing ecological stress (Kristensen, 1976).

3.2 Flamingo dynamics and rainfall

3.2.1 Literature

Spatial-temporal distribution of non-breeding and breeding flamingos seems to be dependent on food density and climatic variation (various authors; Vargas et al. (2008), Arengo & Baldassarre, (1995) Baldassarre & Arengo (2000), Tuite (2000)).

Water depth is an important predictor of the abundance of flamingos (Bucher et al., 2000; Espinoza et al., 2000; Pirela, 2000). Vargas et al., (2008) found significant correlations between rainfall, lagoon water level (LWL), lagoon water temperature (LWT) and flamingo abundance. Extreme rainfall resulted in an increase in LWL and a record decline in flamingo numbers. Vargas et al., (2008) evaluated that reductions in the abundance of flamingos in the rainy season and significant temporary declines during El Niño events are explained by movements between lagoons within the Galapagos Archipelago rather than by mortality of the birds.

Although rainfall and lagoon water levels are highly correlated, the lagoon water temperature was not always a significant predictor of flamingo numbers within the Galapagos Archipelago. The cause can be that increase of LWT, leads to algal growth, and increase of other prey (Vargas et al., 2008). That increased temperature leads to more algae is questioned, it could be rather decrease of salinity which favors algal growth.

Decreased abundance of flamingos at lagoons with higher water levels can be explained by the following:

1. Limited length of legs and neck, therefore being incapable of wading. The impact of increased LWL would become more apparent in deeper lagoons where water levels would reach unfavorable water levels sooner than at shallower lagoons.
2. Changes in prey availability. Density and distribution in the water column of prey might be changed. Prey availability varies more in space and time in low salinity than in high salinity logoons (from Vargas et al., (2008); Arengo & Baldasarre, (1998)).

Both aspects could be relevant for Goto. Hence the first aspect might be more relevant to Goto then to other salinas in WSNP as Goto receives more rainwater due to run off from the surrounding hills. Data on LWL are not available, and the only proxy for this study that can be used is the actual rainfall.

Vargas et al., (2008) showed that no long term impact was observed after severe climate events. The events can however alter flamingo movement patterns and dispersion. After the climatic disturbance (both rainy events, and El Niño) in the Galapagos archapello, the flamingo abundance returned to its average situation. Delayed return to the average situation was observed and was suggested to be a result of slow fall of water levels after the rainy season. Absence of Flamingo's after a severe climate event could take as long as 3-19 months, depending on the lagoon (Vargas et al., 2008).

Strong climatological patterns are observed in Bonaire as well, and seems to occur at a 5 year cycle. In recent decade, heavy rainfalls occurred at Bonaire. The year 2010 was extremely wet year and it is suggested that this rain event rainfall could have caused the drop in flamingo numbers. We tested this hypothesis in next paragraph.

3.2.2 GAM analysis flamingo trends and rainfall on Bonaire

The trend of flamingo counts over years and the seasonal pattern has already been determined (see section 3.1). This analysis already showed climatic and seasonal patterns in flamingo counts. It is therefore not particularly useful to include rainfall data directly as an explanatory variable in analyses. Instead, extreme (high or low) precipitation levels deviating from climatic and seasonal patterns should be used. For this purpose, the trend in average precipitation is determined using a GAM (Gaussian family, with a logarithmic link function, blue line in Figure 3), distinguishing a trend over the years and seasonal trends (Figure 14). Before this analysis 0.5 mm/day, representing a minimal precipitation level, was added to the data. This was done because the logarithmic link function can't process zeros in the data.

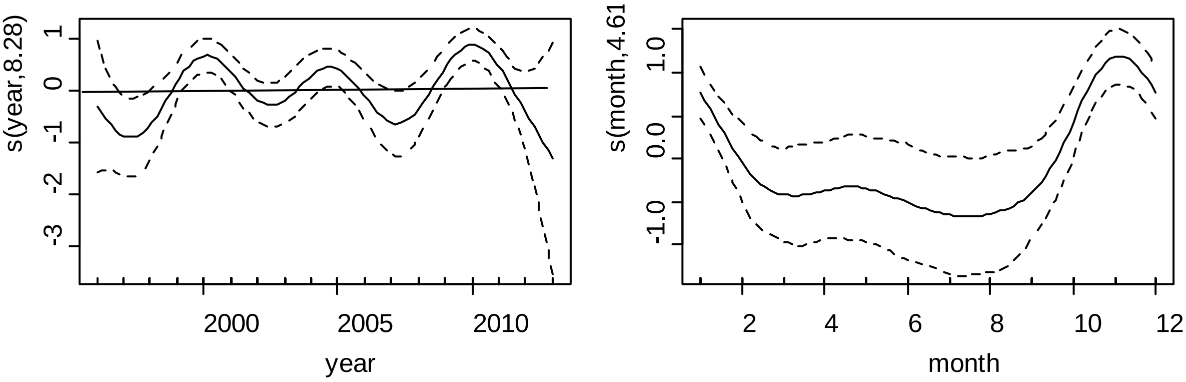


Figure 14 Smoothed trend of the average precipitation data (Figure 3) over the years (left) and within a year (right).

Next the residuals, the differences between the statistical model and the observed average precipitation (vertical distance between black markers and blue line in Figure 3), are included in the analysis of the flamingo count data. It is determined whether the rainfall extremes explain a significant part of the observed variation in flamingo count data and whether the extreme rainfall can explain the drop in flamingo counts at Goto.

For comparison the same analysis, with the rainfall extremes, is also performed for the combined northern location on the island and the Cargill area (presented in annexes).

The trend of rainfall over the years appears to be fluctuating (Figure 14, left) with approximately a five year period. This may be the result of el Niño events or some other factor that is not included in the present study. Similar 5 year cycles were found for flamingo counts at several locations (e.g., Figure 13 left for Matijs) and may therefore be related to weather patterns. There is also a seasonal pattern (Figure 14, right), with a peak of precipitation at the end of each year. Note that after the fire more rain fell than is expected from the fitted model.

When rainfall extremes are included in the statistical model to explain observed flamingo counts at Goto, it does not explain a significant part of variation in flamingo counts (Figure 16). Nor does the extreme rainfall (residual precipitation in Figure 16 right) explain the sharp drop in numbers after the fire, in fact, the model appears to assign a positive effect of extreme rainfall on flamingo counts (Figure 16 right).

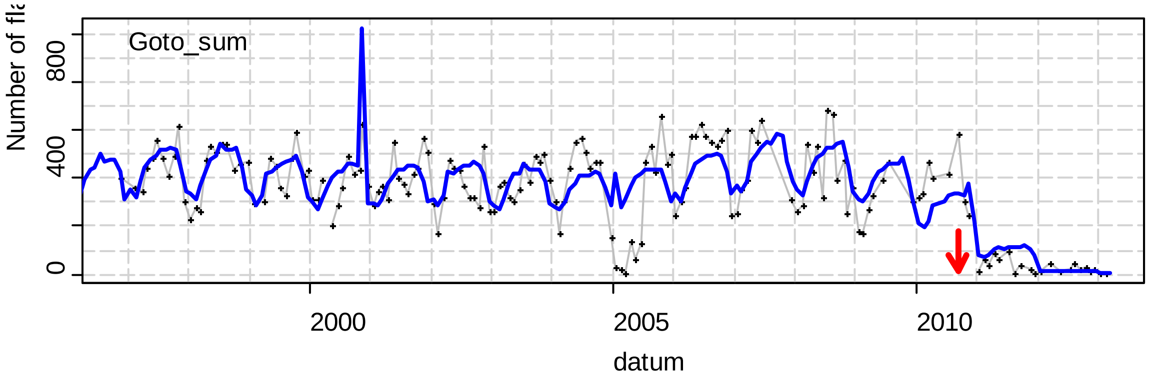


Figure 15 *Observed flamingo counts (black markers) in the Goto area (both entrance and saliña). The blue line shows the model fit to the count data, including extreme precipitation as explanatory variable. Red arrow indicates the date of the fire.*

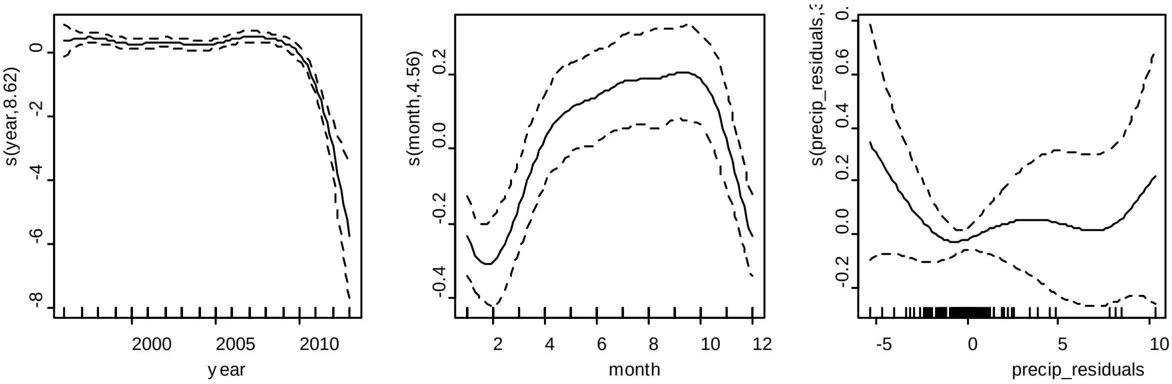


Figure 16 *Smoothed trends over the years (left) within each year (middle) and residual precipitation (right, difference between observed and modelled precipitation in mm/day; Figure 14) in the Goto area (both entrance and saliña).*

The same analysis is also performed with flamingo counts for the combined northern locations on the island (excluding Goto, Figure 17 and Figure 18). In this case the model could not properly include extreme rainfall as an explaining variable (note that the smoother describing the contribution of extreme rainfall and its confidence bands converge in the origin of the plot, Figure 18 right).

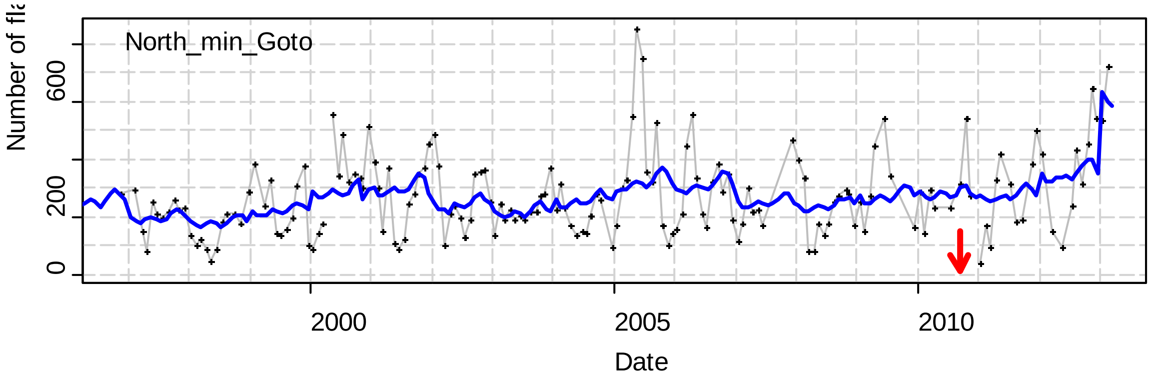


Figure 17 *Observed flamingo counts (black markers) in the northern part of the island (excluding Goto). The blue line shows the model fit to the count data, including extreme precipitation as explanatory variable. Red arrow indicates the date of the fire.*

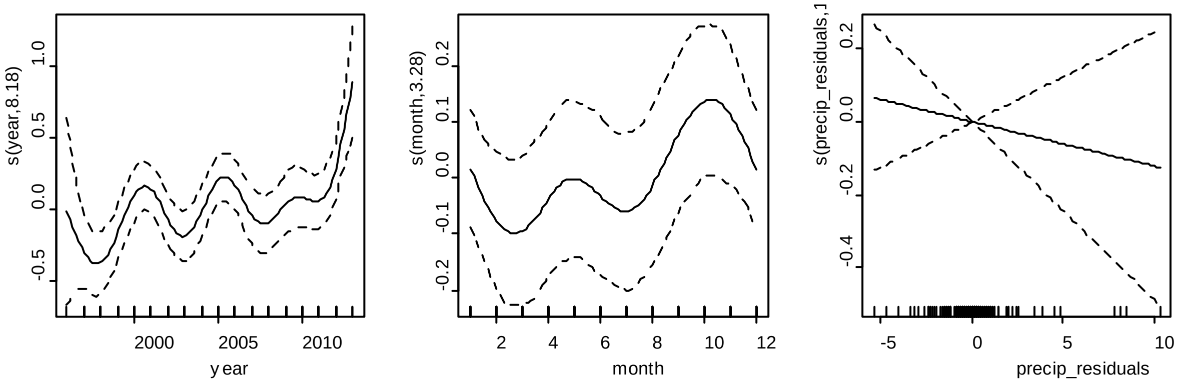


Figure 18 *Smoothed trends over the years (left) within each year (middle) and residual precipitation (right, difference between observed and modelled precipitation in mm/day; Figure 14) in the Northern part of the island (excluding Goto).*

3.3 Ecology: Benthos and *Artemia* abundance

3.3.1 General salina benthic composition

Salinity of salinas steers the benthic fauna composition. Salinity levels of saliñas are known to fluctuate throughout the year due to differences in precipitation levels in the dry and wet season. Due to this fluctuation, the fauna community is expected to change during the year. In waters with salinity above 130 ‰, only two fauna species, the brine shrimp *(Artemia salina)*, and larvae of the brine fly *(Ephydra cinerea)* are able to survive (Kristensen & Hulscher-Emeis, 1972). These euryhaline species use this extreme habitat in order to escape from the effects of competition with other macrozoobenthos and predation by fish. In the absence of fish predation and with only intraspecific competition at these extreme conditions, these species are able to reach very high densities.

At salinities lower than 130 ‰, euryhaline fish *(Cyprinodon dearborni* and *Poecilia vandepolli)* are also able to survive, often diminishing *Artemia* and *Ephydra* populations (Kristensen & Hulscher-Emeis, 1972). Below salinity levels of 60 ‰, a wide variety of fauna species, including several species of Crustacea, Gastropoda, other Pisces, Corixidae, Coleoptera*,* and Protozoa can be found (Kristensen & Hulscher-Emeis, 1972).

3.3.2 Benthos composition Goto before the fire

Recent baseline data on the benthos community before the BOPEC fires are not available. The only known study describing benthos ecology of Bonaires salinas is from Rooth (1965). Rooth (1965) sampled the salinas in the early 1960’s and made rough estimates on the abundance of selected species such as *Ephydra* and *Artemia*, as a food source of flamingo. He sampled *Ephydra* puppea by examining rocks. *Ephydra* densities fluctuated over sampling moments.
In Slagbaai the presence of *Ephydra* varied between 1000-1600 per 25 rocks sampled. In Goto, *Ephydra* varied between 1200-2000 per 25 rocks sampled, and lower numbers were found in March, April and May compared to other months.
In general Rooth found that *Ephydra* was found most in Pekelmeer (20 times more than Goto), and that Goto has 1.5 more *Ephydra* than Slagbaai.
Artemia was found in all salinas sampled (Slagbaai, Goto,), but abundance in Goto (mean 60 ± 34 in 10L) was lower than in Slagbaai (mean 547 ± 977 in 10 L). Rooth (1965) concluded that smaller *Artemia* were less frequently found in high salinity areas, and Goto has higher salinity than Slagbaai. Numbers of *Artemia* did not correlate with numbers of flamingos.
If one should convert this abundance information to biomass estimates, it very well corresponds with the observation of Rooth that in Goto flamingo feed mainly on *Ephydra* (section 3.1.6).

3.3.3 Benthos composition shortly after the fire in 2010

In October 2010, a month after the BOPEC fires, Jorcin and Caglierani Casanova (2011) sampled macrobenthos community across the salinas.

For Goto they observed primarily *Ephydra* in various stages of development. The absolute abundance differed among the locations sampled from 0-2525 ind/m^2 (Table 4). At all locations high densities of algae were found (*Botryococcus* Kuetzing), deposited on the sediment in a high state of decomposition. We have not looked into the state of decomposing algae in 2012 and 2013, but in general our study correspond with findings of Jorcin and Caglierani Casanova (2011). GM6 corresponds with our location Goto9, at which some *Ephydra* was found as well. At locations corresponding with our Goto7 and Goto10 no macrobenthos was found in 2012 and 2013 (next section)- but traces were found in 2010 (Table 4). This observations show that macrobenthos density and occurrence in Goto differs among the locations, and that *Ephydra* is hardly found anymore; only 2 individuals were found in May 2013 (next section).

Table 4 Absolute abundance (ind. m^2) of taxa found in the sampling stations of Goto observed in October 2010 (Jorcin and Caglierani Casanova (2011). Their original sampling names are replaced by ours in the table. GM4= Goto7; GM5= Goto10; GM6= Goto9; GM7= Goto8. Taken from Jorcin and Caglierani Casanova (2011).

Taxon	GM1	GM2	GM3	Goto 7	Goto 8	Goto 9	Goto 10
Insecta							
Diptera: *Ephydra* sp	-	-	2323	-	152	101	101
Coleoptera: Hydrophilidae Latreille	-	-	202	-	-	-	-
Mollusca							
Hydrobiidae Simpsom	-	-	-	-	-	51	-
Total organisms	0	0	2525	0	152	152	101

Slagbaai samples of 2010 were dominated by Ostracoda (Crustacea) varying from 222-3838 ind/m2. Only at one station (S4) the presence of Collembola was recorded. The other species present was a crustacean (ostrocoda). These species were not present in the samples of 2012 and 2013 as the method differed. The species of 2010 are 1-0.5mm in size and are not captured. In 2012 *Ephydra* larvae were the only species present, whereas in 2013 *Artemia* and *Ephydra* dominated both (next section).

Table 5 Absolute abundance (ind. m^2) of taxa found in the sampling stations of the salina Slagbaai (Jorcin and Caglierani Casanova (2011). S5= Slagbaai6 S1= Slagbaai 5. Taken from Jorcin and Caglierani Casanova (2011).

Taxon	Slagbaai5	S2	S3	S4	Slagbaai6
Insecta					
Collembola: Entomobryidae	-	-	-	51	-
Crustacea					
Cytherideidae morphotype 1	1010	222	505	3838	556
Total organisms	1010	222	505	3889	556

The macrobenthic fauna of salina Bartol was represented only by Ostracoda (same as in Slagbaai) and some protozoa. Numbers of Ostracoda were lower then found in Slagbaai, varying from 556-1364. In 2012 mainly oligocheates were observed and a few unidentified larvae. In Bartol 3 no benthic organisms were found. In 2013, Bartol3 had some low numbers of Larvae type B (next section). Bartol3 does not correspond with the Bartol locations of Jorcin and Caglierani Casanova (2011). Bartol4 of our study corresponds with W1 of Jorcin and Caglierani Casanova (2011).

Table 6 Absolute abundance (ind. m^2) of taxa found in the sampling stations of the salina Bartol. Taken from Jorcin and Caglierani Casanova (2011).

Taxon	W1 (Bartol4)	W2	W3
Crustacea			
Cytherideidae morphotype 1	556	657	1364
Protozoa			
Foraminiferida Eichwald, 1830	51	-	-
Total organisms	607	657	1364

Salina Matijs has a different macrobenthic fauna compared to the other salinas. In 2010 the macrobenthos of this salina was represented by crustaceans (Copepoda Harpacticoida and Ostracoda, Cytherideidae), Mollusca (Gastropoda) Foraminifera and Insecta (Diptera: *Ephydra* and Culicoides). Although the total abundance of organisms was similar between the two sampled stations, the richness of zoobenthos and the abundance for each taxon was different between them. In our study Mathijs showed the most species and abundance as well in the 2012 sampling, including *Ephydra*.

Table 7 Absolute abundance (ind. m^2) of the taxa founded in the sampling stations of the salina Matijs. Taken from Jorcin and Caglierani Casanova (2011).

Taxon	**M1**	**M2**
Crustacea		
Copepoda Harpacticoida	48384	-
Cytherideidae morphotype 1	53232	206313
Cytherideidae morphotype 2	6867	15202
Insecta		
Diptera: *Ephydra*	1717	-
Diptera, Ceratopogonidae: Culicoides Latreille, 1809	3435	-
Mollusca		
Gastropoda: Hydrobiidae Simpsom, 1865	68687	-
Protozoa		
Foraminiferida Eichwald, 1830	39495	62980
Total organisms	221817	254495

3.3.4 Benthic composition 2012 and 2013

In 2012 10 locations were sampled for benthic composition and analysed. In 2013 a selection of locations was sampled again. Matijs was planned, but could not be sampled in 2013 as the salina was dry. Goto 9 was sampled, but did not result in a sample due to difficulties with the net. A choice was made to sample only 1 out of 2 Bartol locations, and to discard Goto10 as similar results for 7, 8 and 10 were retrieved in 2012.

In Figure 19 and Figure 20 the results are shown as bar plots. It shows that in October more species types were found compared to May 2013. Up to 14 different species were found in 2012, and only 6 in 2013. 7 species could not be identified, but were all larvae of a kind of invertebrate or analid. In Figure 21 an overview is presented of these species.

In 2012, a large variance in species count and composition is observed between and within salinas. Most individuals were found in Matijs, mostly decapode lavae (crab larvae). A large bloom of juvenile crabs as observed during sampling, and this is reflected in the data as well. Besides crab larvae, Type B larvae were found, followed by decapod juveniles (crabs). In the other reference locations Bartol and Slagbaai, the variance within the salina was large, no species at Bartol 3, compared to Bartol4 with in which mainly olichaetes were found. At Slagbaai6, *Ephydra* (brinefly) larvae were found. At Slagbaai5 only some poppae of *Ephydra* and its larvae. At Goto7, 8 and 10 only 1 water bug was found, but these samples are characterised in general as empty. Only at Goto9, the location near the sea and BOPEC benthic species and fish (*Cyprinodon*) were found. It should be mentioned that on Goto9, some variance due to subsampling is introduced. These countings have to be corrected for variance introduced due to subsampling.

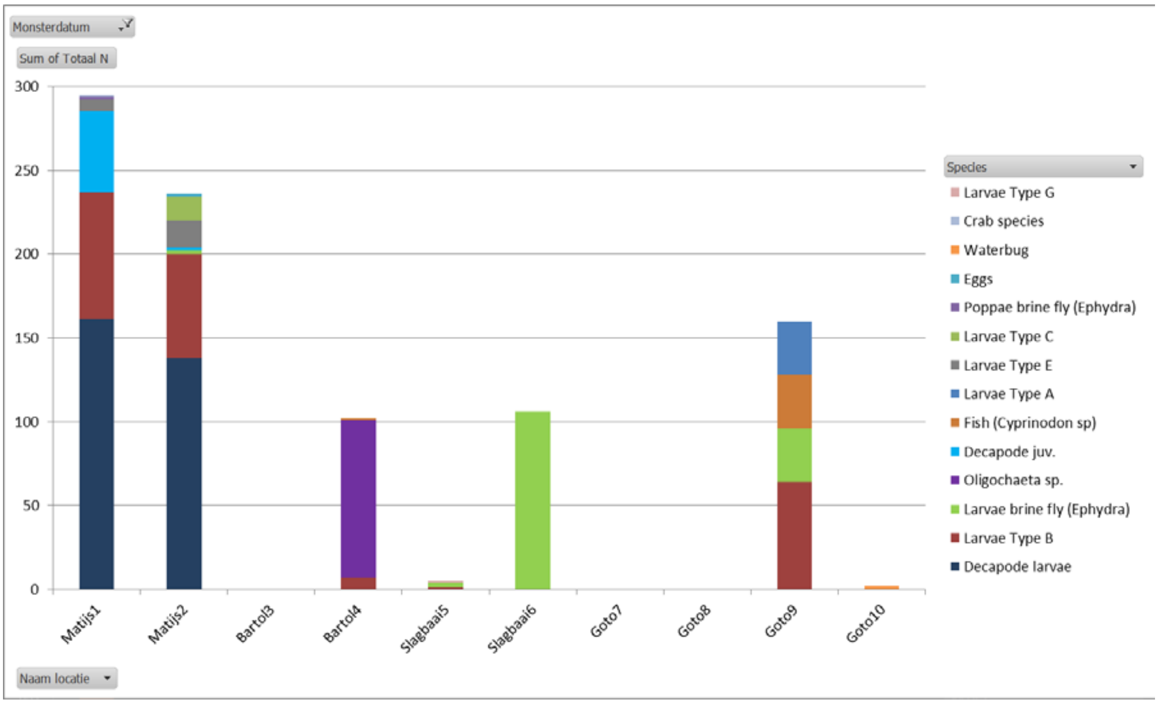


Figure 19 Benthic composition at 10 locations sampled in October 2012.

In 2013, large differences were observed between and within salinas as well. Large numbers of *Artemia* and *Ephydra* were found in both Slagbaai locations, with numbers up to almost 1600/sample in Slagbaai6. During sampling, many Flamingos were fouraging near the sampling locations of Slagbaai. Some individual benthic species at low numbers were found, but could not be identified. At Goto7 some *Ephydra* larvae were found. At Goto8 no species were found. Bartol 3 showed low numbers as well, mainly larvae of type B.

Between 2012-2013 results large differences occur. The abundance of *Artemia* in 2013 and the absence in 2012 can be explained by seasonal variation. Rooth (1965) found large seasonal differences in *Artemia* abundance, varying from total absence to very abundant. In 2012 no visual sightings of *Artemia* were recorded, whereas in 2013 *Artemia* were observed (swarming around) by eye during sampling.

Based on these data, we can say that within salina specifications differ largely. Matijs and Slagbaai have the highest abundance of benthic species, but also with inner-salina variance. Bartol has variable abudance as well, but is lower in 2013 than in Slagbaai. In general, Goto shows no signs of life, expect for Goto9 which in 2012 shows different species.

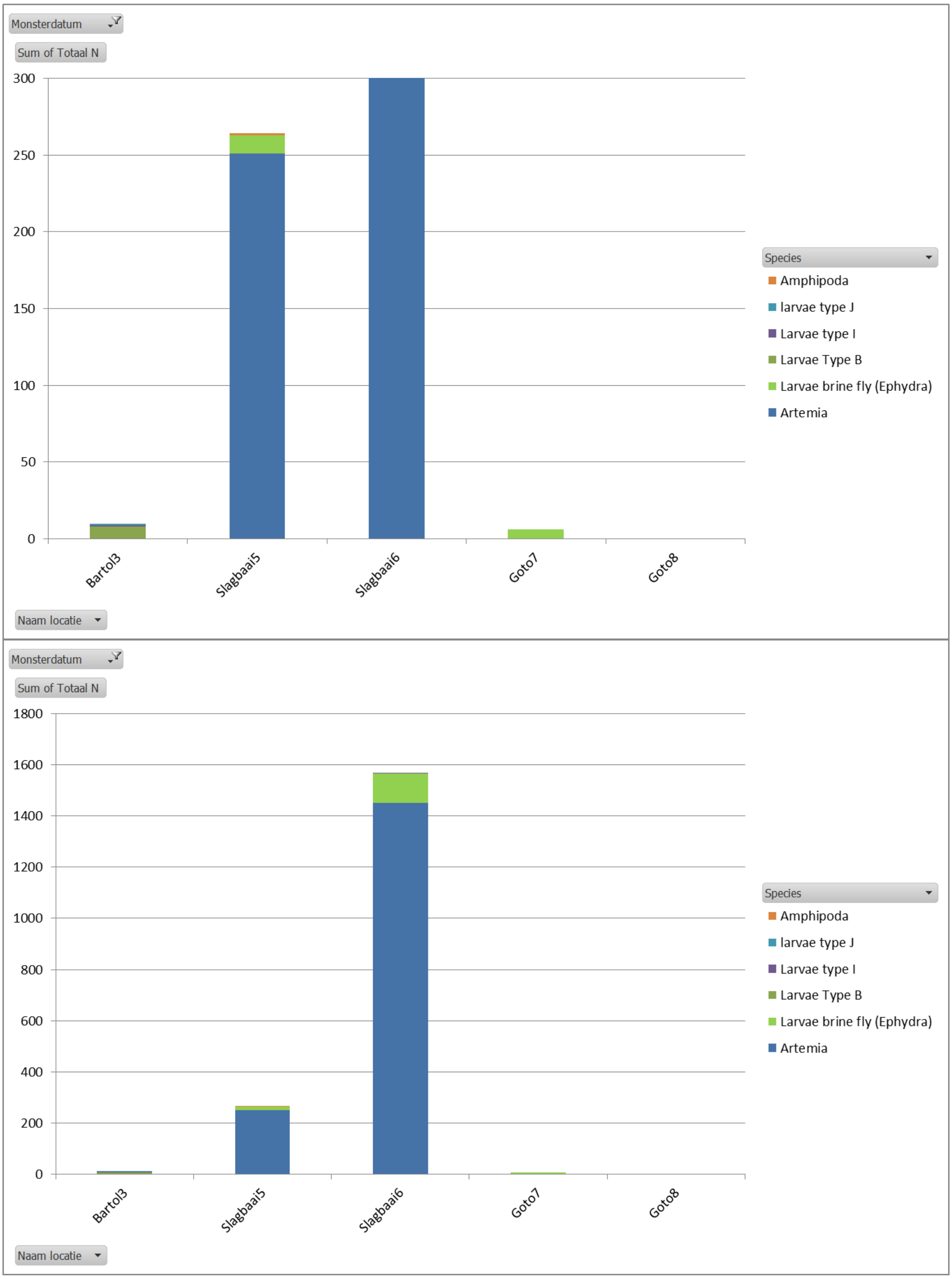


Figure 20 Benthic composition at 6 locations sampled in May 2013. Sampling of Goto 9 was not succesfull. Upper graph shows numbers in the lower range, the lower graph shows the total range.

Larvae types which were not identified are displayed in Figure 21.

Figure 21 overview of un-identified species

3.3.5 Artemia abundance 2013

Water was hypersaline and specifications are presented in Annex 2. Compared to the other salinas, oxygen levels were relatively low in Goto. In Bartol1, and Slagbaai oxygen levels were over-saturated (Annex 2).

Artemia sp. was very abundant in Slagbaai, but almost absent in the other salinas (Table 8). Higher numbers of flamingos were found where *Artemia* sp. was abundant (Table 8).

Table 8 Plankton samples from the saliñas in March 2013

Saliña	**Species**	**Numbers**	**Sampling method**	**Flamingos**
Bartol 3a*	No plankton	0	bucket	17
Bartol 4	No plankton Poecilia sp.	0 2	dragging	
Slagbaai5	*Artemia* sp.	19975	dragging	110
Slagbaai5a**	*Artemia* sp.	12725	dragging	see Slgb 5
Goto10	*Artemia* sp.	5	dragging	0
	Insect larva	2		
Goto8a***	No plankton	0	dragging	2
Goto9	*Artemia* sp.	1	dragging	10

*Bartol 3a, corresponds more or less with Bartol3 in the benthic survey, but is more located in the middle of the salina instead of Bartol3, which is more to the “entrance” of the salina .

** Slagbaai 5a is located near Slagbaai 5, but is more towards the middle of the salina.

***Goto 8a is more or less similar to Goto 8, but ~50 meters more east.

3.3.6 By-catch of fish during sampling 2013

During sampling for water and sediments, and macrofauna in 2012, additionally fish were caught as well. It was not the purpose and the technique was not aimed to catch fish, and thus the results should be taken with care and are only indicative. In Table 9 the number of fish and their specifications on length and wet weight are given.

It is obvious that in Slagbaai and Goto7 up to four times the number of fish were found compared to other locations. It is known by (Kristensen and Hulscher-Emeis, 1972) that these species of fish (inhabit habitats with high salinities (up to 130 ‰). Above salinities of 130 ‰ these fish cannot survive. During sampling, salinities varies, and could be below 130 ‰ somewhere in the water column (e.g. upper halve). At Slagbaai 6, salinities were above this value, and no fish were caught. These fish are predatory, and feed on *Artemia*. It is known that when these fish are present, they can control the foodweb of salinas (Kristensen and Hulscher-Emeis, 1972). Annual variance occurs due to variance in salinities (due to evaporation and precipitation) and when the fish die due to high salinity, *Artemia* will flourish again.

During the benthic sampling of 2013, no fish were caught, but unfortunately, the conductivity meter was broken and no data on salinity are present to relate this observation to high salinity values. It is obvious, that *Artemia* were flourishing at Slagbaai5 and no fish were present. However in Bartol and Goto this relation is not observed- there were no fish, and no *Artemia* either. During the sampling of *Artemia* in March 2013, some fish were caught at two locations, but during this sampling no relation with *Artemia* abundance and occurrence is observed. During studies of Rooth (1965) Goto salinity was stable around 80 g Chlorine/L (~80 ‰), and he reported both *Artemia* abundance, and fish. Unknown is if fish and *Artemia* occurred together.

Table 9 Number of fish found during sampling in 2012, including length and weight specifications. Goto9 (2) refers to sampling in 2013. LIMSnumber is the internal tracking nummer of samples.

	LIMSnumber	**Average Length (mm)**	**Average Wet weight (g)**	**number**
Matijs1	2012/1174	19.0 (11.3)	0.332 (0.5)	2
Bartol4	2012/1176	26.3 (3.5)	0.428 (0.2)	4
Slagbaai5	2012/1177	26.5 (6)	0.432 (0.2)	8
Goto7	2012/1179	29.8 (1.1)	0.594 (0.1)	9
Goto9	2012/1181	16.0 (7.1)	0.163 (0.2)	2
Goto9 (2)	Not available	24.7 (0.6)	0.283 (0.0)	3

4 Results: Chemistry

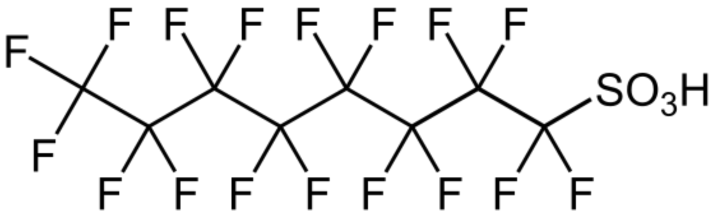
The study by RIVM in 2012 indicated that only the concentrations of fire fighting foam constituents, such as perfluoridated compounds (PFC), mainly represented by perfluorooctane sulfonate (PFOS)- a toxic and the most bioconcentrating compound of PFCs tested-, are still elevated in the water and sediments of Goto. These substances are not present in the two reference salt lakes evaluated in comparison (De Zwart et al., 2012). In the two upcoming paragraphs, more details are provided.

4.1 PAH in water and sediment (summary from De Zwart et al., 2012)

PAHs (Polycyclic aromatic hydrocarbons, analyzed as a set of 16 compounds) were analyzed in water and sediment. In the samples of 2012 very low concentrations are observed (De Zwart et al., 2012). The concentrations were far below the maximum permissible concentrations. PFOS in water and sediment (summary from De Zwart et al., 2012 plus additions by IMARES)

4.2 PFCs including PFOS (summary from De Zwart et al. 2012, with additions)

Picture 2 PFOS chemical structure



12 different PFCs (Perfluor compounds), including PFOS (perfluorooctane sulfonate) were analyzed in water and sediment. PFOS was found in 2010 directly after the fires and is still present in the water and sediments of Goto (De Zwart et al., 2012). PFOS and PFCs are not present in the two reference salinas evaluated in comparison.

At multiple Goto locations, the concentrations in the water phase for the PF-carboxylates is relatively high (above 100 ng/L) for PFHxA and around 10 ng/L for PFOA (Annex 5). In the sediments, the concentrations of these substances are below the detection limit. This can only be explained by a relatively low hydrophobicity (low Koc,) and high polarity, resulting in a low adsorption to soil particulates and a high solubility. This holds for all the PF-carboxylates as compared to the PF-sulfonates.

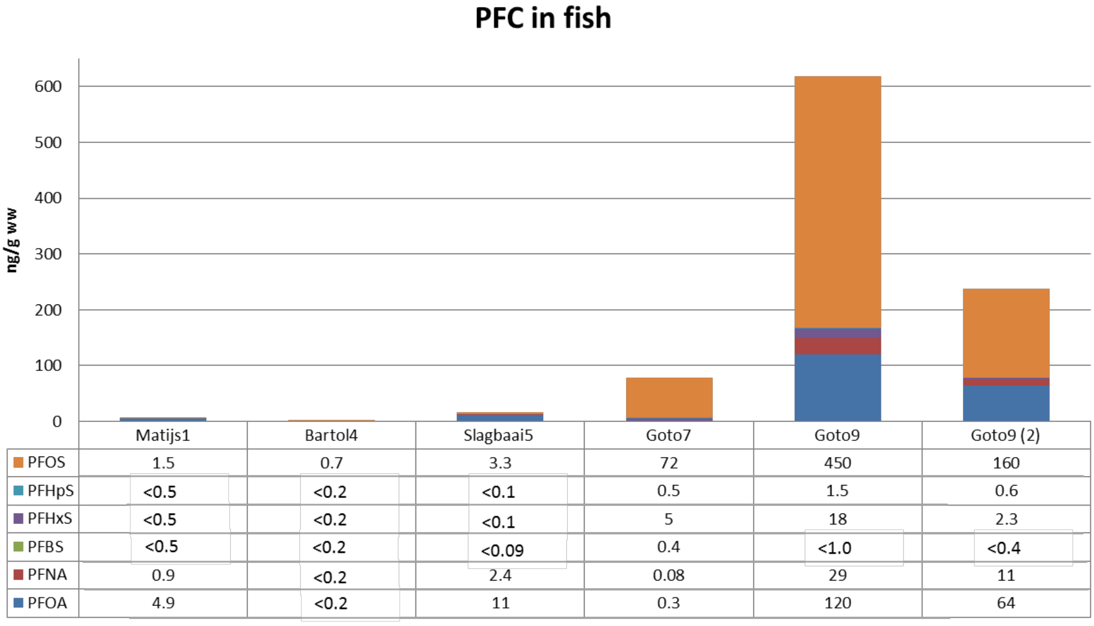
For the PF-sulfonates the situation is different. At Goto locations, high (>100 ng/L) water concentrations for PFBS, PFHxS and PFOS were observed, while the concentrations for PFHpS are detectable, but below 10 ng/L. In the sediments, only the concentrations for PFHxS and PFOS are above the detection limit, but generally below a concentration of 10 µg/kg WW. Overview of concentrations in both water and sediment is provided in Annex 5

4.3 PFCs in biota

In Figure 22 an overview is presented of different PFC concentrations found in fish caught during sampling of 2012 and 2013. The zero values indicate that the compound is below detection limits.

Fish at locations in Goto contain higher levels of PFCs than fish in the reference Salinas. Fish at Goto9, near BOPEC, contain highest levels of PFC's mostly related to PFOS and PFOA. Goto9 is the location closest to BOPEC, and these findings relate to the concentrations found in water and sediment which were highest as well at this location.

PFOA was detected in fish samples in Matijs and Slagbaai as well, which seems strange since this compound was not detected in water and sediment. Fish however serve as “active samplers” of these compounds, and even if the concentrations in water are below the detection limit, accumulation takes place, resulting in detectable concentrations in fish.



	Matijs1	Bartol4	Slagbaai5	Goto7	Goto9	Goto9 (2)
■ PFOS	1.5	0.7	3.3	72	450	160
■ PFHpS	<0.5	<0.2	<0.1	0.5	1.5	0.6
■ PFHxS	<0.5	<0.2	<0.1	5	18	2.3
■ PFBS	<0.5	<0.2	<0.09	0.4	<1.0	<0.4
■ PFNA	0.9	<0.2	2.4	0.08	29	11
■ PFOA	4.9	<0.2	11	0.3	120	64

Figure 22 *Concentration of PFC’s in ng/g ww found in Cyprinodon fish in 6 different salinas during sampling in 2012. Goto9 (2) refers to accidental caught fish in June 2013. Corresponding LIMS numbers are presented in table 9.*

4.4 Chemical fingerprinting: GC*GC results

4.4.1 Sediments

When closely comparing the chromatograms of the samples from the different locations, many small differences were observed. These peaks in the chromatogram were then checked by the mass-spectra of those peaks. In all but one the mass-spectra could not show that these peaks were different in either location. For one peak found in Goto it was demonstrated clearly that it was present in Goto, but not at any of the other locations. The compound corresponding with the peak was identified as Butylated Hydroxytoluene (BHT) by the NIST library (library of mass-spectra) with a match factor of 815 (out of a possible 1000) and probability of 50.2 %. This means that there is a high chance it can be this compound. Additional confirmatory work should be performed to verify the identity of this compound.

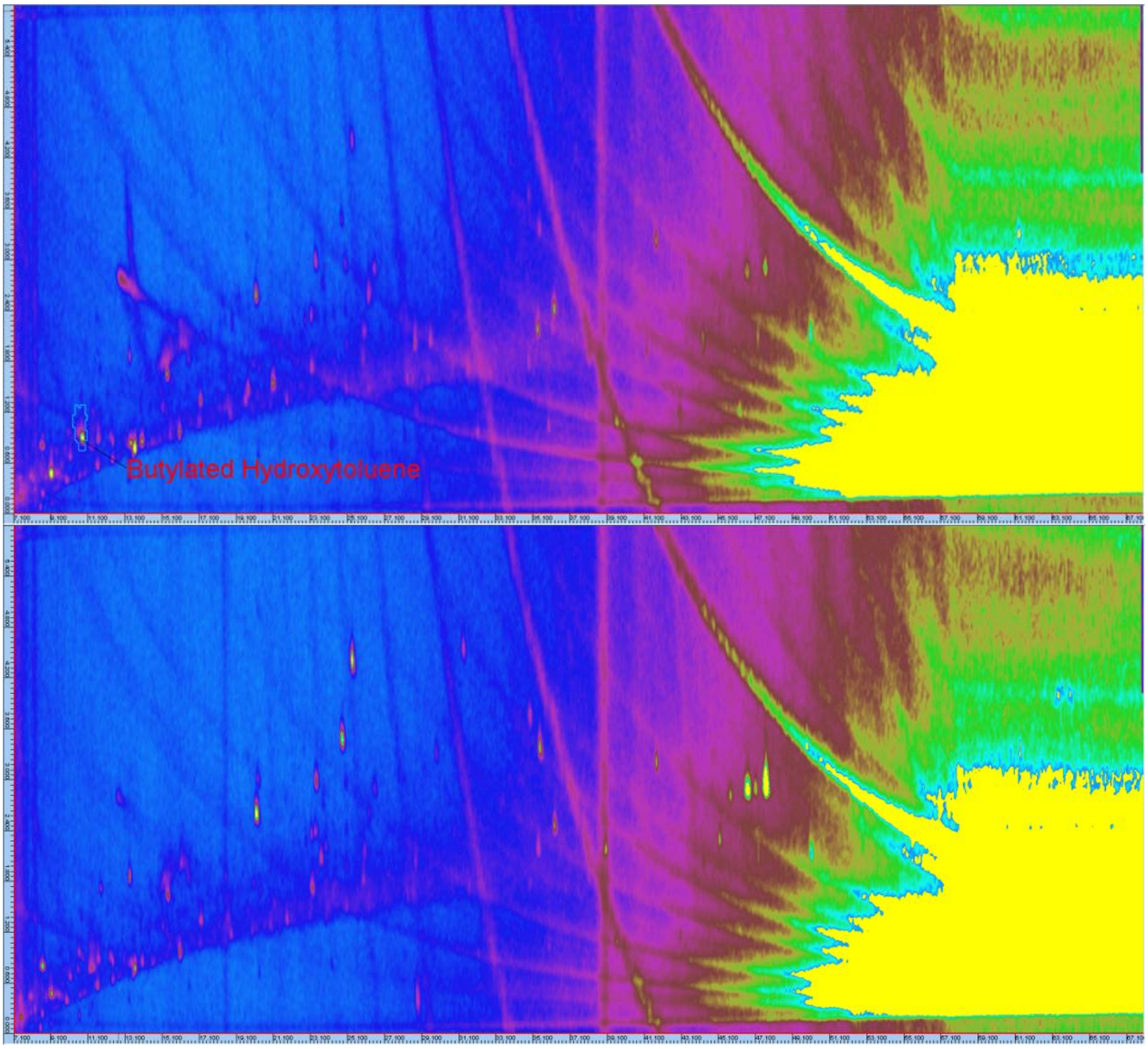


Figure 23 GCxGC chromatogram of location Goto7 (upper graph) in which the deviating peak of butylated hydroxyl toluene is marked. GCxGC chromatogram of location Matijs1 (lower graph) in which the deviating peak is not present.

Butylated hydroxytoluene (BHT) is widely used in industry as an anti-oxidant in fluids (e.g. cosmetics, fuel, oil). In the petroleum industry, BHT is known as the fuel additive AO-29. As such, it's presence in Goto can be explained by the BOPEC fires. The compound is rated as slightly toxic to fish based on 6 studies with freshwater fish(www.pesticideinfo.org[6]).

Based on its chemical characteristic, the compound is defined as unstable in the environment under ambient light and consequently, will decompose relatively quickly. That the compound is still present in Goto can be explained that it is measured in the sediment compartment in which it is not exposed to light, or that the initial concentrations (directly after the fire) were very high. No attempts have been made to confirm the identity of the compound nor to measure the concentration in the sediment.

---

[5] http://www.pesticideinfo.org/Detail_Chemical.jsp?Rec_Id=PC33712

[6] http://www.pesticideinfo.org/Detail_Chemical.jsp?Rec_Id=PC33712

The physico-chemical characteristics of any compound can be used to estimate the fate of this compound in the environment. The relatively high log kow (lipophilic) (Table 10) indicates the potential of this compound to bioaccumulate in biota. Log Koc indicates the potential for accumulation in the soil.

Table 10 Physico-chemical properties of BHT (#: http://www.chemspider.com/Chemical-Structure.13835296.html) ^: http://www.inchem.org/documents/sids/sids/128370.pdf

parameter	**Value BHT**
Log Kow#	5.03-5.1
Log Koc#	4.362
Water solubility^	0.6-1.1. mg/l

4.4.2 Biota

For biota, at Goto and Slagbaai a peak was found in the beginning of the chromatogram that was not present at reference location Mathijs. According to the NIST library this was 5H-1-Pyridine (match factor 922, probability 29.1 %). In general Pyridines are related to benzene, and used as a solvent, and as such, it dissolves easily in water.

Physico-chemical properties and environmental effect information on this specific pyridine cannot be found.

The compound preliminary identified as BHT was not observed in fish. As anti-oxidant, BHT is not likely to be very stable and degradation by the fish can occur. This would explain why this compound is not observed in fish.

5 Results: Toxicology

5.1 Risk Quotients

Risk Quotient categories used by De Zwart et al., (2012) are slightly adapted in this report, based on .
RQ <1: insignificant risk (green).
RQ 1-10: moderate environmental risk (orange)
RQ>10: high environmental risk (red)

5.1.1 PAH

For all sampling locations in both sediments and water, the sum of the risk quotients (ΣRQ) for the different PAHs is considerably below 1, indicating a complete absence of risk in terms of ecosystem impact caused by exposure to the measured PAHs (De Zwart et al., 2012) (see Annex 4). PAH concentrations (this set of 16) were decreased significantly between 2010 and 2012.

5.1.2 PFC (including PFOS)

Proper risk limits for most PFCs analyzed are not available, because these compounds have never been subject to formal toxicity testing. However, PFOS is assumed to be a most toxic and bioconcentrative constituent of the firefighting foam formulations formerly known and used to extinguish the BOPEC fires. Due to the fact that there are only few toxicity data available for PFOS, the risk limits for PFOS are all characterized by the application of rather large assessment factors (Table 11). For the determination of the risk limit for direct ecotoxicity upon (sub)chronic exposure, (Moermond et al., 2011) only found eleven NOEC/EC10 values for the combined freshwater and marine datasets.

Table 11 Risk limits defined for PFOS as the Maximum Permissible Concentration (MPC) based on assessment factors (AF) for direct ecotoxicological effects (eco) and for secondary poisoning (sp) (Bodar et al., 2011, Moermond et al., 2011). For sediments, the risk limits are standardized to dry sediments with 10% organic matter (OM). (Derived from De Zwart et al., 2012).

Abbreviation	**Name**	**MPCeco, water**	**MPCsp, water**	**MPCeco, sediment**	**MPCsp, sediment**
PFOS	Perfluorooctanesulfonic acid	23 ng/L	2.6 ng/L	10 µg/kg DW	3.2 µg/kg DW
AF	Assessment factors applied (AF)	100	30	100	30

The evaluation of ecological risk associated with the observed exposure to PFOS is given as risk quotients (RQ) in Table 12.
All of the observed PFOS concentrations in water and to a lesser extent in the sediment of Goto are clearly indicative for risk on secondary food chain intoxication and chronic effects, varying from moderate risk to high risk. Highest risk is expected at all Goto locations, based on RQ related to water and secondary poisoning. At Goto9, the risk is highest, and as well found in sediment. The characteristics of this location are different then from the other Goto locations, and although the levels are corrected for local aspects as water content and organic matter, PFOS levels at this location are consequently somewhat different than at the other locations.

The exposure period in (sub)chronic toxicity tests, on which the risk limits are based, have a maximum exposure duration of 28 days Potentially, the exposure time to the substances originating from the

BOPEC fire in 2010 is now (end of 2013) more than 36 months. As already mentioned by De Zwart et al., 2012, it is virtually impossible to account for the actual risk and potential effects that may be caused by this prolonged exposure period. The actual environmental risk due to the extreme chronic exposure is likely to be under estimated based on these RQs.

The reference salinas Matijs, Bartol and Slagbaai are exposed to PFOS to a green level, indicating the complete absence of risk, except for Slagbaai5 with a RQ of 1.2 for water samples based on the risk for secondary poisoning, but this value lays within the orange uncertain zone.

Table 12 *RQ risk analysis as performed for the measured PFOS in the sampled water bodies on Bonaire. The RQ values are calculated by dividing the measured concentrations (Appendix 2) by the compound and medium specific risk limit (RL as specified in Table 11), for water and sediment and for direct effects of intoxication and the occurrence of secondary poisoning in the food chain. Concentrations below the limit of detection (DL) are presented as “bd”. For the different RLs the applied assessment factor is used as an uncertainty margin (UM) for the orange RQ range. For sediments the risk limits are corrected for the organic matter content of the local sediments. This table is adapted from De Zwart at al., 2012 by adding reference locations “Slagbaai” and Bartol 4, and discarding Tam. Risk categories are adapted.RQ <1: insignificant risk, green. RQ 1-10: moderate risk. RQ> 10 high risk.*

		Risk Quotient									
Compartment	Risk limit	Matijs1	Bartol3	Bartol4	Slagbaai5	Slagbaai6	Goto7	Goto8	Goto9	Goto10	Goto11
Water	MPC, eco	bd	0.03	0.0	0.1	0.1	3.7	4.3	5.7	5.2	5.2
Water	MPC, sp	bd	0.27	0.3	1.2	0.6	32.7	38.5	50	46.2	46.2
sediment	MPC, eco	bd	bd	bd	bd	bd	2.3	2.8	16.8	2.5	1
sediment	MPC, sp	bd	bd	bd	bd	bd	7.1	8.7	52.4	7.8	3

5.1.3 PFCs in biota

Reported in section 4.3, fish at location Goto9 near BOPEC, contain highest levels of PFCs, mostly related to PFOS and PFOA. Goto9 is a location at which the risk quotients for secondary poisoning due to PFOS was estimated (De Zwart et al., 2012 and Table 12). The is observed for Goto7.

The elevated internal levels of PFCs in fish is in line with PFC’s tendency to cumulate in biota. The ecological risk of the levels found is however not clear. PFC relates to various effects in organism. Depending on the concentration and exposure, effects are described by various authors. A brief overview of effects, not extensive, is given in Table 13. Internal concentrations in fish larvae in the study of Huang et al., (2010) ranged from 15-66 ng/g ww after 5 days of exposure (depending on the level of exposure). These internal concentrations are much lower that the concentrations found in Goto fish. From these data, it is likely that effects of PFOS on fish cannot be excluded. Post hatched larval stage is more sensitive than the embryo or adult stage (Shi et al., 2008), and sensitivity could increase with increased exposure. The long term developmental and reproductive effects are expected to be higher, but not studied yet. The concentrations studied by Du et al., 2009 indicate maternally transferred effects on first generation offspring. In case females are expose to 250 ug/l FPOF, all offspring die. In the case of Goto, it is unknown how much of the fish its life cycle time is spend in Goto, and to what extent impact on its specific life history will affect a population.

Table 13 Examples of effects of PFOS on fish at various concentrations.

Effect	**Concentration + duration**	**Reference**
EC50- Growth reduction embryo zebra fish	3-5 mg/l (132 hpf)	Shi et al., (2008)
Embryo malformation zebra fish	1 mg/l (4 hpf)	Shi et al., (2008)
Spinal curvature zebra fish embryos	0.5 mg/l (96 hpf)	Shi et al., (2008)
100% Mortaility zebra fish embryo	1 mg/L (132hpf)	Shi et al., (2008)
LC50 zebra fish embryo	2.2 mg/l (120 HPF)	Huang et al., (2010)
Swimming behavior increases	0.24-4 mg/l (132hpf)	Huang et al., (2010)
Malformation EC50	1.12 mg/L (120 hpf)	Huang et al., (2010)
Increased hart rate	0.05 mg/L (48 hpf)	Huang et al., (2010)
Growth inhibition of gonade in female fish	50 and 250 ug/l 70 days	Du et al., (2009)
Offspring (F1) malformation and mortality after maternal exposure and transfer	50 and 250 ug/l exposed female (effects on F1 96 hours after hatching)	Du et al., (2009)
Increase of ALT serum	< 107 ng/g ww	Hoff et al., (2003)

5.2 Bioassays

5.2.1 ARTOKIT

In Table 14 and Figure 24 the survival of *Artemia* exposed to different water samples is presented. *Artemia* survival after 24 hours for all 3 Goto samples was 95% or higher. The reference salinas Slagbaai 5 and 6 showed lower survival, respectively 88% and 100%, with large standard error. Bartol3 showed 100% survival after 24 hours of exposure.

Goto samples showed relatively high survival in the extended exposure period, until 96 hours (up to 56% survived), whereas the reference salinas Slagbaai 1 and 2 showed continues decreased survival until 72 hours resulting in almost 100% mortality. After 96 hours exposure, Bartol3 sample showed decreased survival as well, being 15%. Due to high variance, no significant differences in survival was detected on the 24 and 48 hour exposure (ANOVA, $p > 0.05$). For 72 and 96 hour exposure, differences between samples were significant ($p < 0.05$), Goto samples had significant higher survival then Slagbaai 1 and 2 (72 hours).

Results indicate that no acute toxicity is present for *Artemia* to water of Goto, and that a prolonged exposure did not result in increased mortality relative to Bartol as a reference. The mortality at the reference samples- Slagbaai-, especially after 24 hours, can be attributed to food shortage. Goto samples were murky and silty, indicating that organic material could serve as a food source during the prolonged exposure. Slagbaai samples were clear, most probably due to observed grazing *Artemia* in the salina. This probably has led to food shortage for the larvae.

Table 14 Average survival (% of exposed individuals), including standard deviation between brackets.

	Goto7	**Goto8**	**Goto9**	**Slagbaai5**	**Slagbaai6**	**Bartol3**
24	95% (6%)	100% (0%)	100% (0%)	88% (16%)	100% (0%)	100% (0%)
48	95% (6%)	100% (0%)	100% (0%)	64% (47%)	98% (5%)	100% (0%)
72	92% (6%)	98% (5%)	96% (8%)	0% (0%)	3% (6%)	98% (5%)
96	56% (36%)	57% (34%)	83% (20%)	0% (0%)	3% (6%)	15% (6%)

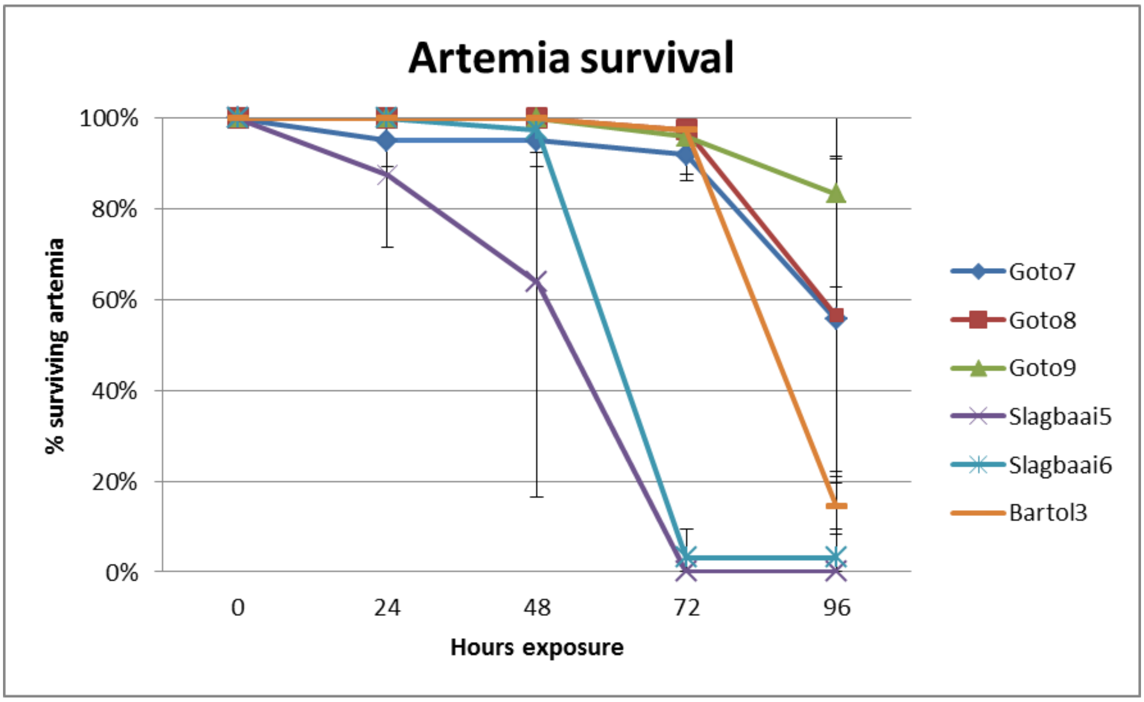


Figure 24 *Artemia survival for 6 samples (3 Goto samples) and 3 reference Salinas (Bartol3, Slagbaai1 and 2)*

5.2.2 Microtox

Samples were diluted by a factor 8 due to their high salinity ranges (factor 4) and microtox test conditions (factor 2). Results of the screening showed no clear difference in effects in this bioassay between reference locations and Goto locations.

All water samples showed an increase of luminescence, as did pore water samples of Slagbaai6, Goto7 and Goto8. Increase of luminescence is known to occur occasionally in case of testing marine samples. In this study, all samples are saline, but so are the samples that did not show an increase
Bartol3, Sagbaai5 and Goto9 pore water samples showed a slight inhibition of luminescence, but not enough to continue screening for EC_{50} calculation. In Annex 6 data of the test are presented.

6 Conclusions

6.1 Ecology

6.1.1 Flamingo abundance

The hypothesis on flamingo abundance and rainfall events was:
<u>Flamingo dynamics on Bonaire relate to rain events; hence the drop of flamingo abundance in Goto can be addressed to the heavy rains of 2010.</u>

We conclude that this hypothesis can be rejected, based on the answers given per research question:

Could heavy rainfall that coincided with the fire explain the decline in flamingo abundance in Goto?
The extreme rainfall after the fire is not likely an explanatory parameter in the decline of flamingo counts at Goto.

Do the number of flamingos show signs of recovery?
Recent flamingo counts at Goto show no signs of recovery (data early 2013)

Is the decrease in flamingo counts also observed at other locations of the island?
No, contrasting trends are observed for the analysed locations (some show a decline, some show an increase). However, none of the areas showed a sharp decline in recent years comparable to that found for Goto around the fire. Slagbaai and Matijs are not affected.

6.1.2 Food availability: benthic status

The hypothesis on flamingo absence and food availability was:
<u>Food availability in Goto explains the absence of flamingo.</u>

We conclude that this hypothesis cannot be rejected, based on the answers given per research question:

What is the general feeding ecology of Flamingos on Bonaire?
Bonaire flamingos mainly feed on Ephydra and Artemia in northern salinas such as Goto

What is, in terms of current and historical densities and composition, the benthic status of the salinas, related to the main organisms that serve as food for flamingos?.

- *In Goto Ephyra and Artemia were consistently absent or present in very low numbers during the sampling events in 2012 and 2013 . Other salinas showed periodically low numbers of macrofauna as well, but these are probably related to seasonality.*
- *Goto lake historically (1965) differed from Slagbaai in food for flamingo (10% Artemia, 150% Ephydra), which did not result in lower number of flamingos. Ephydra seemed to be the most important food in Goto. Temporary decreased numbers of macrofauna due to foraging flamingos showed quick recovery.*
- *A month after the BOPEC fire in 2010, benthic community in Goto was dominated by Ephydra. In Slagbaai no Ephydra or other food for flamingos was found.*
- *The composition of the benthic community shows considerable differences between salinas and varies within a year.*
- *Matijs and Slagbaai currently have the highest densities of food organisms (depending on the season) and variance in benthic species composition is high in Matijs.*

- *Fish are found at all locations. Assuming that fish could be a reason for decreased Artemia abundance, annual variance in fish numbers should result in periods with elevated Artemia and thus in temporary elevated flamingo numbers. No recovery of flamingo in Goto is observed. Other Salinas inhabited by fish as well, and no correlation with Artemia was observed based on the limited data. We conclude that fish are not the main reason for long term flamingo absence in Goto.*

6.2 Chemistry

The hypotheses on chemical status were:
<u>"Water and sediment quality of Goto is affected by the BOPEC fires" And: Next to PFOS, other substances are found in the Goto ecosystem that are not found in other salinas.</u>

We conclude that these hypotheses cannot be rejected, based on the answers given per research question:

Do water, sediment, and biota of Goto contain pollutants that may be related to the BOPEC fire or the firefighting foams used (focusing on PAHs and PFCs)

> *Yes, all three environmental matrices of Goto contain substances that can be related to the BOPEC fires. More specifically PFCs, (of which PFOS is the compound with highest concentration)- are present. These substances are found in other salinas as well, but often below limits of detection, or in concentrations much lower than in Goto.*

Is Goto chemically different than other salinas in terms of chemical fingerprinting by using GC*GC (sediment and biota as the matrix).

> *Yes, Goto is chemically different than the other Salinas based on GC*GC fingerprinting of its sediments. For biota this is less apparent.*

6.3 Toxicology

The hypothesis on chemical status was:
<u>"Water and sediment quality are at levels that impact the environment of Goto"</u>

This hypothesis cannot be rejected, based on the answers given per research questions:

Are the observed concentrations of the analyzed compounds in water, sediment and biota high enough to induce toxicological effects in Goto?

> *Yes, the risk quotients of PFOS in water and sediment of Goto, combined with the chronic exposure of years is likely to have induced toxicological effects on benthic organisms such as Artemia and Ephydra. Reference salinas Matijs, Slagbaai and Bartol are exposed to PFOS levels without any serious risk on toxicological effects.*

To what extent are water and/or sediment samples from Goto directly toxic to organisms?

> *Acute toxicity of both water and sediment samples is not indicated in the two toxicity tests. Internal levels of PFCs in fish were observed, at levels known to induce physiological effects on fish embryos. Acute impact on adult fish populations are not expected, but effects on fish embryos cannot be excluded. Depending on the life history mechanisms of these fish population effects might occur. Full exposure of all fish in the Goto is unlikely. Due to niches (rainwater layers) population effects might be limited.*

7 Discussion on environmental toxicology of Goto

Based on this study, toxicity could be held accountable as the steering factor for environmental impact in Goto. Due to lack of causal relationships however, we cannot confirm this. Still, the conclusions in chapter 6 provide circumstantial evidence that toxicological factors have severely impacted the food availability of flamingos in Goto. Consequently, the absence of flamingos is a result of a lack of food. The cause of this absence is likely to be a result of the BOPEC fires which resulted in PFOS levels affecting the macrobenthic community and *Artemia* numbers of Goto.

Ecological recovery from this polluted state is hampered- and unlikely to happen- due to the compound held accountable, PFOS. Besides PFOS, it is likely that not only the analysed compounds, but also other compounds that were not analysed impacted the Goto ecosystem by means of acute (in 2010) and chronic exposure (2010 till present). It is unsure whether the ecosystem of Goto can ever recover from this state, and if so, when, and with which additional measures to support this.
If PFOS is the only compound accountable, remediation of PFOS is challenged by the fact that it is a very persistent compound and recalcitrant to degrade under natural environmental conditions (Hawley et al., 2012). Technologies used to address PFCs in general include groundwater extraction, ex-situ treatment and excavation and disposal of contaminated soil/water (Hawley et al., 2012). Chemical oxidation is seen as a promising technique (Hawley et al., 2012), but has yet to be tested under field conditions and scale.

More specifically, the three aspects of environmental toxicology- ecology, chemistry and toxicology- are discussed in next sections.

7.1 Ecology

7.1.1 Flamingo dynamics

The variance in counting effort of flamingos between locations and over the decades was not corrected by means of statistical procedures. We selected the most stable counting period instead, resulting in a shorter period to analyse flamingo dynamics. More analysis can be done on the complete dataset when corrections for counting effort are included.

Besides the flamingo dynamics presented in chapter 3 of this report, additional results were obtained on the flamingo dataset for other regions in Bonaire. Flamingo numbers in the Cargill region decreased (Annex 1) with approximately ~1000 birds in the last decennium. The breeding colony (data not shown) is however stable and we assume that only the numbers of birds feeding at Cargill decrease. On Bonaire, some salinas increase in numbers, this increase does not account for ~1000 birds, but much less (~100). Regional shifts to feeding places in Curacao and Venezuela might explain the decrease in Cargill. A steering factor might be a combination with a decreasing carrying capacity of Cargill to feed the historic numbers of flamingos, together with increased activity on the islands. Regional data could help interpret these observations.

7.1.2 Benthos /Food availability

The benthic surveys of 2012 and 2013 are only quick scan observations, and although long term personal observations by many people (e.g. de Leon, Slijkerman) subscribe the benthic data, statistical coincidence cannot be excluded. It is recommended to continue to study the status of the benthic community on a more structural basis.

Although not presented as a result, many people have reported the colour change of Goto since a few years. From a blue coloured salina, Goto has turned into a brown murky water mass. This subscribes the fact that Goto has turned into another ecosystem. *Artemia* is a non-specific feeder on algae and bacteria. Plankton ecology of the salinas and a potential relation with toxicology was not included in this study, but might be part of the explanation why Goto has turned into another colour.

Although speculative, it could be that since the BOPEC fires Goto turned into an alternative stable state (Scheffer et al., 1993; Scheffer, 1998) with another planktonic food web based on algae that are not suitable to be consumed by *Artemia*. This does not yet explain the absence of *Ephydra* though, and this hypothesis is additional to the absence of *Artemia* only. Planktonic studies, together with in situ or laboratory studies aimed to study the recovery potential of *Artemia* in Goto are needed to pinpoint this speculation.

7.2 Chemistry

A very important aspect to realize when evaluating current levels of chemical compounds is that the reported levels of 2012 represent the levels present two years after the fire. In the meantime, environmental levels might have decreased due to degradation , dilution and redistribution over matrices, including biota.

The chemical study focused on compounds related to the firefighting activity, and the fire itself. PAHs and PFCs were the main suspect groups to focus on. PAHs are studied as a representative set of standard 16 individual compounds. As a result of photo-transformation some PAH can be transformed into other compounds, that can have higher toxicity. Besides this aspect, many other PAHs can be present, combined with e.g. nitro or methylated groups. We have not studied these other PAHs.

Two years after the fires, PFOS is still present in Goto, and based on its persistent characteristics, it is not likely that without additional measures the concentration of this compound will rapidly decrease.

The finger printing with double gas chromatography, GC*GC, revealed another preliminary identified organic compound, Butylated hydroxytoluene (BHT), to be present in Goto and not in other salinas.

In this study the focus was on PAH, PFC, and an organic chemical finger printing of both water and sediments. Anorganic compounds such as metals were not studied. Zinc, copper, lead, iron and manganese are examples of heavy metals associated with oil products, and it is likely that after the BOPEC fire, Goto contains increased levels of these metals as well due to run off firewater containing oil products. Each type of metal will partition in the ecosystem over water, sediment, and biota, depending on its partitioning characteristics and fluxes between matrices. Partitioning in general is affected by pH, redox state, (dissolved) organic content, salinity, temperature and other environmental factors which in turn result in the bio availability of the metal. Depending on bio availability, metals pose a risk for the environment of Goto.

7.3 Toxicology

As mentioned already in the previous chapter, it is important to realize that the reported concentrations of potentially toxic substances in 2012 represent the levels present two years after the fire, and that initial concentrations must have been higher.
The toxicity test were not expected to show effects based on PFOS data, but accounted for the fact that not all present compounds are known. The *Artemia* exposed were starved to death after 96 hours, and it

could be interesting to study the impact on *Artemia* to the same samples with elevated food and long term exposure- focusing on endpoints related to reproduction.

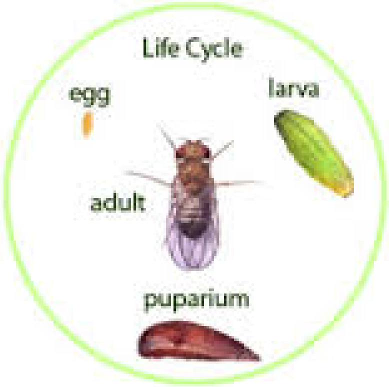
De Zwart et al., (2012) evaluated the ecological risk associated with the observed exposure to PFOS, in which specific aspects were not yet incorporated in the derivation of the risk quotients, and are slightly adapted in this discussion:

1) multi-stress due to co-exposure of organisms to compounds other than PFOS and PFCs,
2) multi-stress due to brine circumstances
3) the very long exposure time in the situation of Goto
4) the Risk limit is not based on a NOEC divided by an assessment factor, but on a LOEC (i.e. concentration level at which effects will occur).

Related to 1): multistress due to other compounds is a very relevant aspect since we have detected another discriminative compound (likely to be) BHT. Metals are not determined but are also likely to be present in Goto after the fire. Besides the unknown status of metals, the environmental fate of PAH is variable since these compounds are suspect to degradation/transformation by light, microorganisms, and other chemical reactions. Light-transformation of these compounds can lead to either detoxification or to more toxic species by transformation into oxidation products that can be better photosensitizers (quinones) or direct acting mutagens (amino to nitro-PAHs) (Yu, 2002). The mechanism of light transformation is likely to occur in Bonaire where plenty of sunlight is present. The risk of photo-transformed PAH cannot be excluded, but was not studied.

The brine circumstances (2) can cause additional stress to the organisms which can result in increased sensitivity to toxicants. Variation in environmental aspects that could interfere in such a way are taken into account in the derivation of risk limits (by additional safety factor). In the salinas however, these brine aspects are out of the “ordinary” so to speak, and it can be discussed whether the normal factors compensate for this situation. Extensive literature exists on multi stress, but often these relate to fresh water organisms. Brine environments are not extensively studied with ecotoxicity aspects, and specific examples cannot be provided in this study. Increasing salinity can both affect the bio availability of the pollutant and the sensitivity of the organism that has to cope with the salt stress.

Acute toxic effects are currently not likely to expect as the toxicity screening by Artox and Microtox did not indicate any effects. Low (bio available) concentrations explain the lack of direct toxicity (in the tests), but long term exposure (3), is a relevant aspect for all persistent compounds present in Goto. Since PFOS persists in the environment, the exposure is very long, as not to say, indefinite when not diluted or drained in any way. Toxicity studies focus normally on acute (24 hour exposure) or chronic (days to weeks of exposure) effects. Studies covering longer exposure times are not available or scarce. At Longer exposure time lower concentrations will cause stronger effects, for instance on the life cycle of *Artemia* and brine fly larvae (the main food of flamingos on Bonaire). It is known that PFOS can affect insects via e.g. the moulting cycle, reproduction or survival (e.g. Mommaerts et al., 2011). Bots et al., (2010) found sub-lethal effects to the life cycle of damselflies after exposure to levels (10 µg/l) of PFOS. Metamorphosis was indicated to be the most sensitive endpoint in the study. Bots et al., (2010) assume that much lower concentrations of PFOS (then 10 µg/l) might be toxic for organisms when they are exposed for longer periods. This assumption is strengthened by the PFOS research of Van Gossum et al., (2009) who found that behavioral performance of damselfly larvae was ten times lower exposed for four months, than those exposed for only one month.



Picture 3 life cycle of flies, e.g. brine fly (Ephydra)

Some *Ephydra* in Goto7 are found in 2013. The presence of some *Ephydra* larvae does however not indicate that recovery takes place. Given the local PFOS levels it is possible that these larvae do not emerge to adult flies.

Furthermore, it is to be discussed if the effects on macrofauna are only direct (via long term expose, and effects on emergence/life history) or that PFOS affects the food structure of macrofauna e.g. via effects on algae and bacteria as well. In that case, surviving macrofauna are starved instead of affected directly.

In general, risk limits are derived from NOEC values, which is the estimated concentration at which no effects are observed. In the case of the risk limit for PFOS, a NOEC value was not available, and a LOEC value was used instead. LOEC is a less conservative value as it represents the estimated lowest concentration at which effects are observed.

The above mentioned aspects could indicate a chance that that the risk assessment as it was presented by De Zwart et al., (2012) underestimated the actual impact on the ecosystem in Goto.

7.4 Suggestions for upcoming work

In this study, not all aspects of interest could be covered, or could be studied extensively. Furthermore, based on knowledge gained during this study, new questions are raised which are relevant to understand the Goto system better, and to explore options for recovery. The most relevant suggestions for additional studies are listed below:

- Although long term personal observations by many people (e.g. DeLeon, Slijkerman, Van Wijngaarden) subscribe the survey data, statistical coincidence (these two data points) cannot be excluded. It is recommended to continue study the benthic status on a more structural basis as this study was only a quick scan.
- Study the planktonic status of Goto
- Study regional dynamics in flamingo feeding sites in terms of both quality and quantity of the food source. Synchronise flamingo trend data of Bonaire with regional data such as from Venezuela and Curacao to identify the “missing” ~1000 birds.
- Other bird species depending on Goto benthic fauna are likely to be impacted as well. Look into observations, or study other birds depending on e,g. brinefly, like Yellow Warbler, in order to assess additional environmental impact than only flamingo.
- Study the status of metal contamination and photo transformation products of PAH and evaluate environmental risk of these compounds.
- Study the recovery potential of Goto:

- Enclosure studies in laboratory or in situ with stocked *Artemia* and brinefly. Covering full life cycles .
- Long term effects to be studied to indicate causes (e.g. using Toxicity Identification and Evaluation techniques) and search for measures
- Model mass balance Goto lake combined with PFOS concentration in water. Model measures such as drainage, and open channel[7] to the sea during rainy season.

---

[7] Opening Goto might result in higher abundance of predatory fish, that could result in depressed food availability and depressed flamingo numbers

References

Arengo, F. and Baldassarre, G. A. (1995). Effects of food density on the behavior and distribution of nonbreeding American Flamingos in Yucatán, Mexico. The Condor 97: 325-334.

Arengo, F., and Baldassarre, G. A. (1998). Potential food availability and flamingo use of commercial salt impoundments in the Ria Lagartos Biosphere Reserve, Mexico. Colonial Waterbirds 21 (2): 211-221

Arengo, F., and G.A. Baldassarre (2002). Patch choice and foraging behavior of nonbreeding American flamingos in Yucatan, Mexico. The Condor, 104(2):452-457.

AZUR Environmental (1998): Microtox Basic test procedure. Templateid3.pdf

Baldassarre, G.A., and F. Arengo. (2000). A review of the ecology and conservation of Caribbean flamingos in Yucatan, Mexico. Waterbirds, 23, Special Publication 1: 70-79

BirdLife. 2008. Caribbean Flamingo Species Factsheet.

Bodar, C.W.M., et al. 2011. Advies risicogrenzen grond en grondwater voor PFOS. RIVM Briefrapport 601050002/2011, RIVM, Bilthoven, the Netherlands

Bots, J., De Bruyn, L., Snijkers, T., Van den Branden, B., and Van Gossum, H. (2010). Exposure to perfluorooctane sulfonic acid (PFOS) adversely affects the life cycle of the damselfly Enallagma cyathigerum. Environmental Pollution 158:901-905

Boylan, J. 2000. Sonographic analysis of the vocalizations of Chilean and Carebbean flamingos. Waterbirds, 23, Special Publication 1:179-184.

Bucher, E. H.; Chani, J. M.; Echevarria, A. L. (2000). Andean Flamigos breeding at Laguna Brava, la Rioja, Argentina. Waterbirds 23(Special publication): 119-120.

De Boer, B.A. (1979). Flamingos on Bonaire and in Venezuela. STINAPA Doc. 3

De Zwart D, de Groot A, Kotterman M, van der Aa M, Aalbers T, Slijkerman D, Bodar C (2012) Follow-up study on the chemical status of Lake Goto, Bonaire. Measurements and risk assessment. RIVM Letter report 609224001/2012

Del Hoyo, J., A. Elliott, and J. Sargatal, eds. 1992. Handbook of Birds of the World, Vol 1. Barcelona: Lynx Edicions

Du, Yongbing, et al. "Chronic effects of water-borne PFOS exposure on growth, survival and hepatotoxicity in zebrafish: a partial life-cycle test." Chemosphere 74.5 (2009): 723-729.

Ehrlich, P.R., D.S. Dobkin, and D. Wheye. (1988). The Birder's Handbook. A Field Guide to the Natural History of North American Birds. New York: Simon & Schuster Inc.

Elphick, C., J.B. Dunning, Jr., and D.A. Sibley. (2001). National Audubon Society: The Sibley Guide to Bird Life and Behavior, pp. 187-189. New York: Alfred A. Knopf. 587 pp.

Espino-Barros, R., and G.A. Baldassarre. (1989a). Activity and habitat-use patterns of breeding Caribbean flamingos in Yucatan, Mexico. The Condor, 91:585-591.

Espino-Barros, R., and G.A. Baldassarre. (1989b). Numbers, migration chronology, and activity patterns, of nonbreeding Caribbean flamingos in Yucatan, Mexico. The Condor, 91: 592-597

Espinoza, F., et al. (2000). Numbers and distribution of the Caribbean flamingo in Venezuela. Waterbirds, 23, Special Publication 1: 80-86.

Furdui, V.I., Stock, N., Whittle, D.M., Crozier, P., Reiner, E., Muir, D.C.G. and Mabury, S.A. (2005). Perfluoroalkyl contaminants in lake trout from the Great Lakes. ENV024 Furdui. "Fluoros" 9th

International Symposium on Fluorinated Alkyl Organics in the Environment, August 2005, Toronto, Canada.

Hoff, P. T., K. V. Vijver, W. V. Dongen, E. L. Esmans, R. Blust and W. M. De Coen (2003): Perfluorooctane sulfonic acid in bib (Trisopterus luscus) and plaice (Pleuronectes platessa) from the Western Scheldt and the Belgian North Sea: distribution and biochemical effects. Environ. Toxicol. Chem., 22, 608–614.

Huang H, Huang C, Wang L, Ye X, Bai C, Simonich MT, Tanguay RL, Dong Q. (2010) Toxicity, uptake kinetics and behavior assessment in zebrafish embryos following exposure to perfluorooctanesulphonicacid (PFOS). Aquat Toxicol. 2010 Jun 10;98(2):139-47.

ISO (2007) Protocol11348-3:2007 Water quality -- Determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (Luminescent bacteria test) -- Part 3: Method using freeze-dried bacteria.

Jorcin and Caglierani Casanova (2011) Zoobentonic community of the salinas of the slagbaai washington national park - isle of bonaire - netherlands antilles. Report HidroLógica Asesoría y Consultoría Ambiental Ltda. Botucatu, São Paulo, Brasil.

Kristensen, I. (1976). Discussion on flamingo problems. STINAPA 11: 42-41

Kristensen, I. and Hulscher-Emeis, T. M. (1972). Factors influencing *Artemia* populations in Antillean salines. Studies Fauna Curaçao 135: 87-111

Moermond, C. T. A., Verbruggen, E. M. J., and Smit, C. E. (2011). Environmental risk limits for PFOS - A proposal for water quality standards in accordance with the Water Framework Directive. RIVM Report 601714013/2010, RIVM, Bilthoven, the Netherlands.

Mommaerts V, Hagenaars A, Meyer J, De Coen W, Swevers L, Mosallanejad H, Smagghe G. (2011). Impact of a perfluorinated organic compound PFOS on the terrestrial pollinator Bombus terrestris (Insecta, Hymenoptera). Ecotoxicology. 2011 Mar;20(2):447-56.

RIVM. (2011). Compound depositions from the BOPEC fires on Bonaire - Measurements and risk assessment. RIVM Letter report 609022067/2011, RIVM, Bilthoven, the Netherlands.

Rooth, J. (1965) The flamingos on Bonaire: habitat, diet and reproduction of Pheonicopterus ruber ruber. The Natural Science Study Group of Suriname and the Dutch Antilles, Publication 41.

Rooth, J. (1975). Caribbean Flamingos in a man-made habitat. edit: Kear, J. and Duplaix-Hall, N. Flamingos: 75-79

Rooth, J. (1976). Ecological aspects of the Flamingos on Bonaire. STINAPA 11: 16-32. 151pp +appendices.

Shi, X., Du, Y., Lam, P.K.S., Wu, R.S.S., Zhou, B., (2008) Developmental toxicity and alteration of gene expression in zebrafish embryos exposed to PFOS. Toxicol. Appl. Pharmacol. 230, 23–32.

Sprunt, A. (1975). The Caribbean. In: J. Kear, and N. Duplaix-Hall eds. Flamingos. Berkhamsted, England: T. & A. D Poyser.

Vargas, F. H., S. Barlow, T. Hart et al., (2008). Effects of climate variation on the abundance and distribution of flamingos in the Galápagos Islands. Journal of Zoology, 276 (3), 252–265.

Wells, J.V., and A. Debrot. (2008). Important Bird Areas of Bonaire. Pp 95-102 in Important Bird Areas in the Caribbean: key sites for conservation. Cambridge, UK: BirdLife International. (BirdLife Conservation Series No. 15).

Yu, H (2002) Environmental carcinogenic polycyclic aromatic hydrocarbons: photochemistry and phototoxicity. J Environ Sci Health C Environ Carcinog Ecotoxicol Volume 20, Issue 2

Zweers, G., F. de Jong, and H. Berkhoudt. (1995). Filter feeding in flamingos (Pheonicopterus ruber). The Condor. 97(2): 297-324.

Quality Assurance

IMARES utilises an ISO 9001:2008 certified quality management system (certificate number: 124296-2012-AQ-NLD-RvA). This certificate is valid until 15 December 2015. The organisation has been certified since 27 February 2001. The certification was issued by DNV Certification B.V. Furthermore, the chemical laboratory of the Fish Division has NEN-EN-ISO/IEC 17025:2005 accreditation for test laboratories with number L097. This accreditation is valid until 27 March 2017 and was first issued on 27 March 1997. Accreditation was granted by the Council for Accreditation.

Justification

Report C211/13
Project Number: 430.87010.22

The lab coordinator has checked the analyses results and approved for publishing:

Approved: Mw. M. Hoek
Lab coordinator

Signature: M Hoek

Datum: December 19th 2013

The scientific quality of this report has been peer reviewed by a colleague scientist and the head of the department of IMARES.

Approved: Dr. Edwin M. Foekema
Sr researcher

Signature:

Date: December 17th 2013

Approved: drs. Floris L. Groenendijk
Head of department

Signature:

Date: December 17th 2013

Annex 1. Flamingo dynamics in other locations than Northern Bonaire

Lac area

For the Lac area, the numbers of Lac Bacuna and total Lac region are shown. Data of Lac Bacuna shows that flamingo counts in these areas fluctuate with peaks approximately every 5 years (Figure 27 and Figure 28 left). There is clearly no considerable drop in numbers in recent years, comparable to that observed at Goto.

For Lac total, no 5 year cycle is observed, nor an increase or decrease over time. No drop as seen in Goto is observed in Lac.

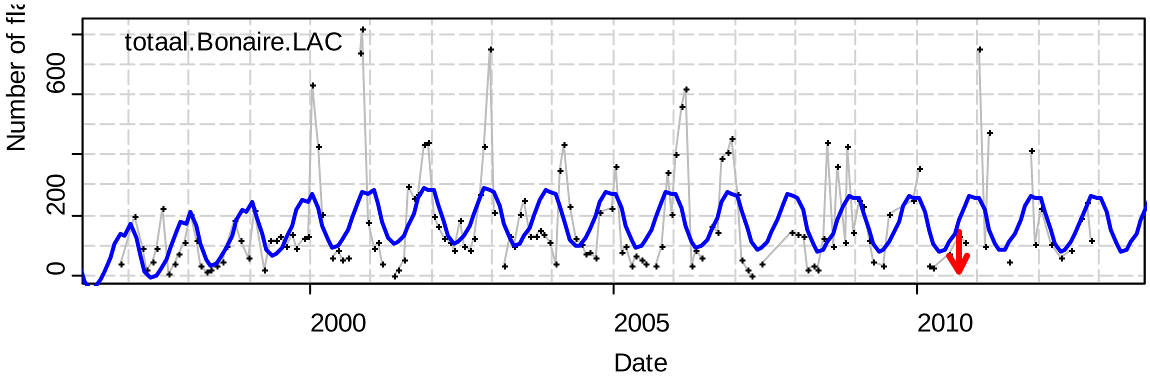


Figure 25 Observed flamingo counts (black markers) at Lac region and fitted model (blue). Red arrow indicates the date of the fire.

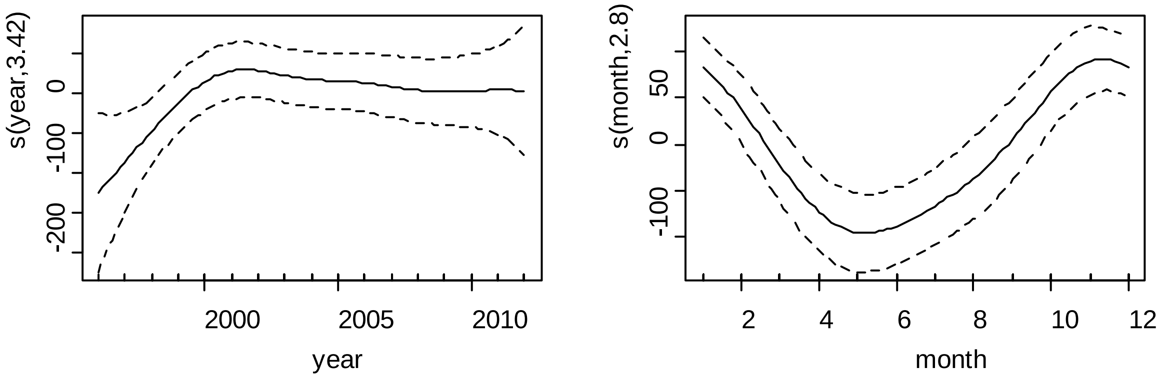


Figure 26 Smoothed trends over the years (left) and within each year (right) at Lac region.

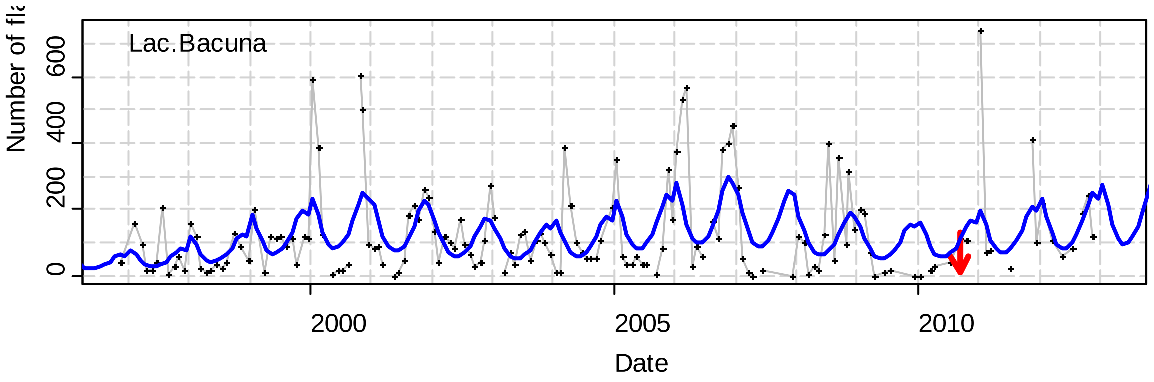


Figure 27 *Observed flamingo counts (black markers) at Lac Bacuna and fitted model (blue). Red arrow indicates the date of the fire.*

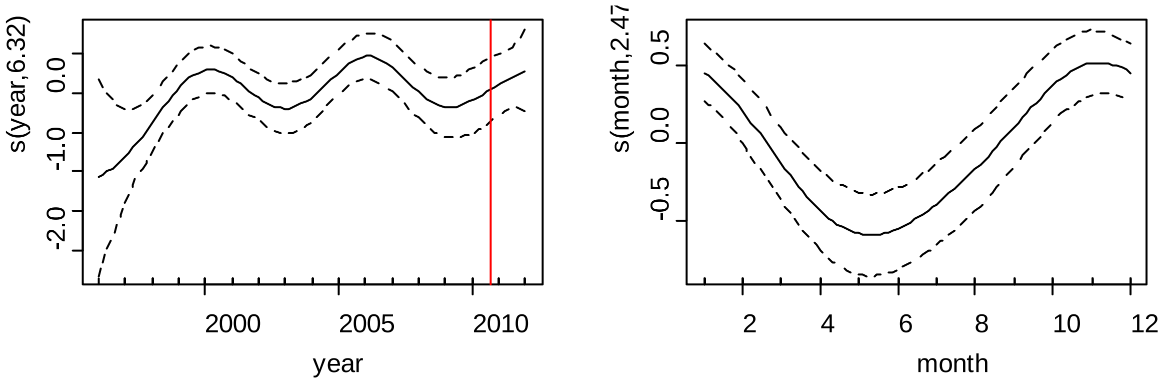


Figure 28 *Smoothed trends over the years (left) and within each year (right) at Lac Bacuna. Red line indicates moment of fire.*

Cargill / Pekelmeer

Flamingo counts are relatively high in the Cargill area (Figure 29), in fact this area harbours the largest part of Bonaire's flamingo population. Flamingo counts at this location peak early in the year (around March and April) (Figure 29 and Figure 30 right), whereas this peak is observed at the end of the year (in October and November) for most other locations. The Cargill area shows an overall declining trend in flamingo counts in the past decades (Figure 29 and Figure 30 left). This decline may have accelerated in recent years although this acceleration is a bit uncertain (Figure 30 left). The decline is not as steep as that observed at Goto.

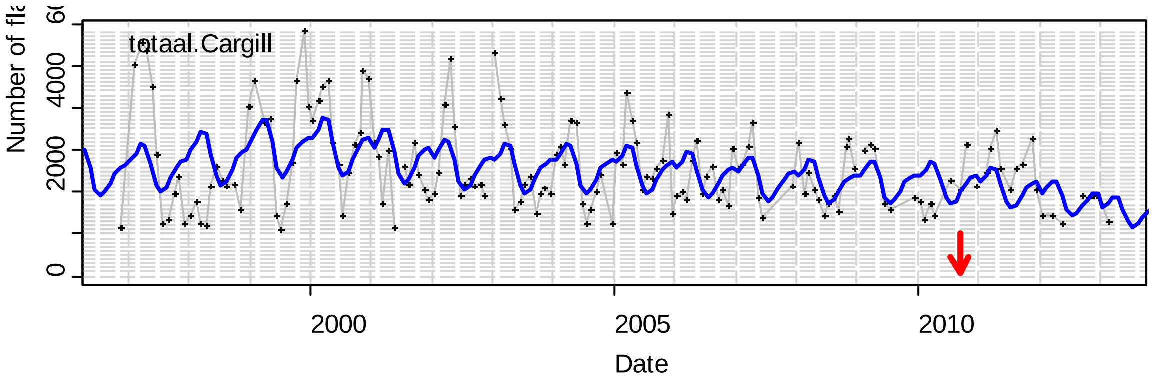


Figure 29 *Observed flamingo counts (black markers) at Cargill and fitted model (blue). Red arrow indicates the date of the fire.*

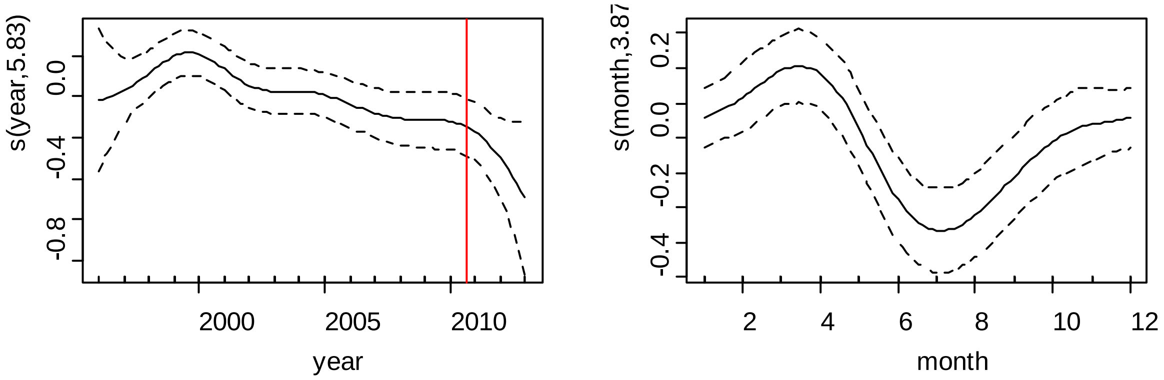


Figure 30 *Smoothed trends over the years (left) and within each year (right) at Cargill. Red line indicates moment of fire.*

Based on the Flamingo abundance data, the overall flamingo numbers (total and breeding) a steady decrease over the last 20 years is observed, represented by the area inhabiting the most flamingos on Bonaire, Cargill (Figure 32 and Figure 33). In Cargill area (includes Pekelmeer), the highest numbers of around 6000 individuals were recorded in the years 2000, but show maximum numbers up to 3500 now a days.

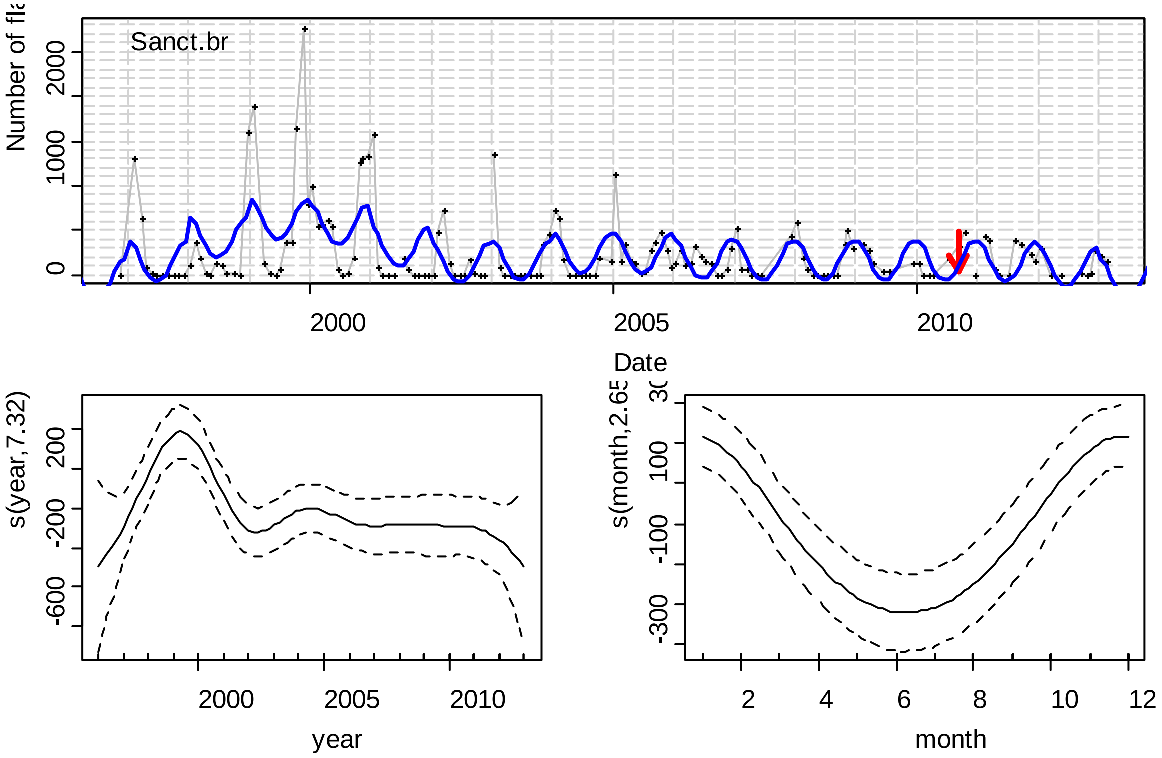


Figure 31 *Breeding Flamingo numbers in Pekelmeer sanctuary, Bonaire. Black dots are counted numbers, blue line represents the fitted model. Red arrow is Fire event at BOPEC. Below: Smoothed trends over the years (left) and within each year (right).*

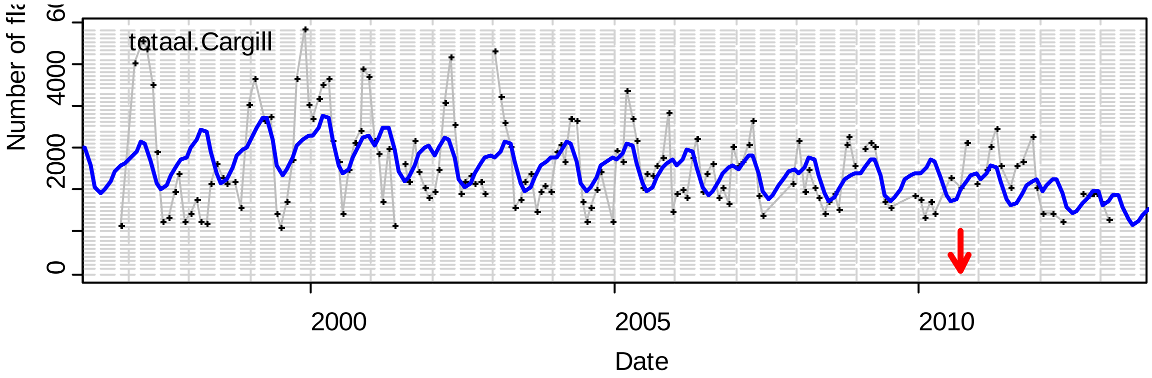


Figure 32 *Observed flamingo counts (black markers) at Cargill and fitted model (blue). Red arrow indicates the date of the fire.*

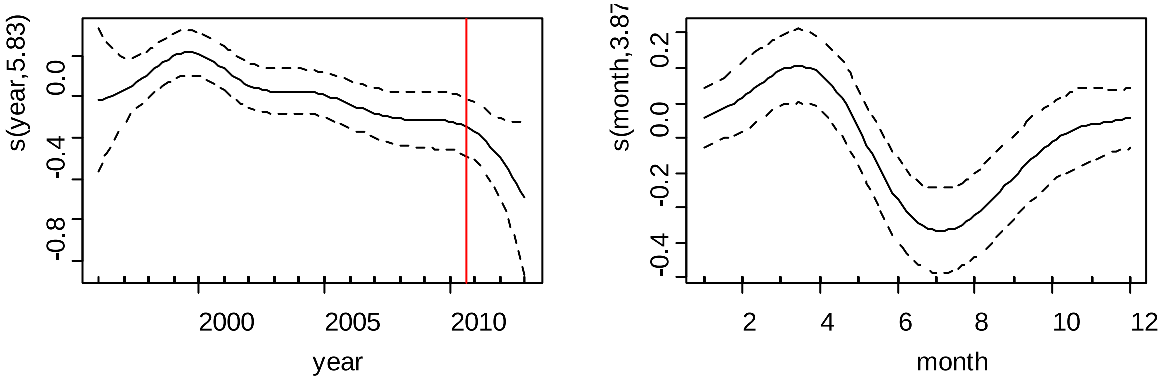


Figure 33 *Smoothed trends over the years (left) and within each year (right) at Cargill. Red line indicates moment of fire.*

Rainfall and Cargill area

The analysis to integrate rain fall extremes with Flamingo countings was also performed for the Cargill area where flamingo counts are highest (Figure 34 and Figure 35). For this location extreme rainfall does explain a significant part of the variation in flamingo counts. However, the relation with residual precipitation (right in Figure 35) is not very coherent.

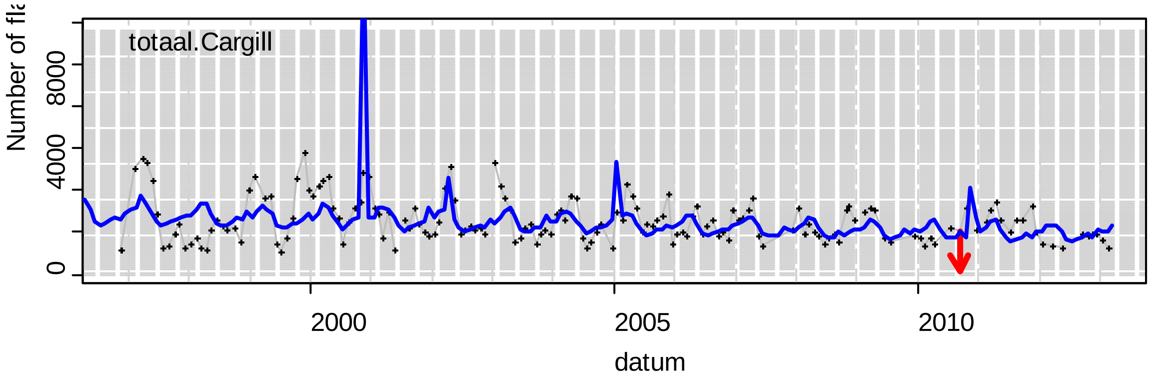


Figure 34 *Observed flamingo counts (black markers) in the Cargill area. The blue line shows the model fit to the count data, including extreme precipitation as explanatory variable. Red arrow indicates the date of the fire.*

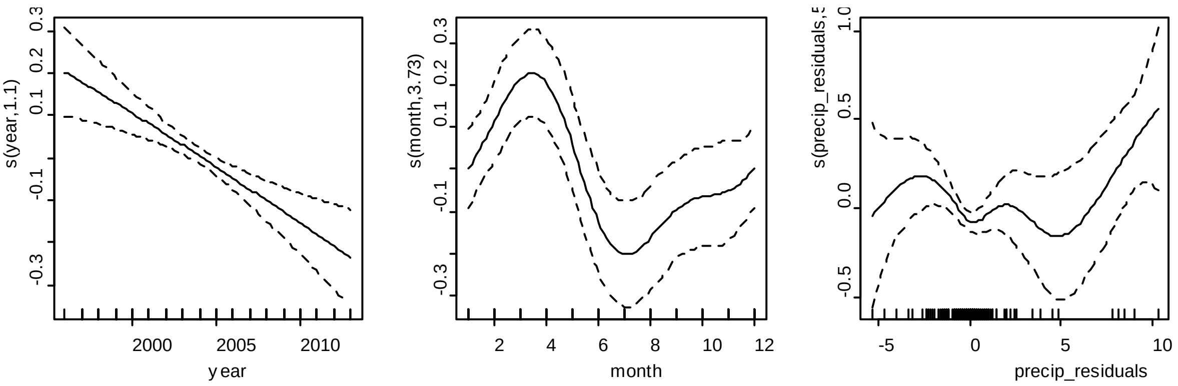


Figure 35 *Smoothed trends over the years (left) within each year (middle) and residual precipitation (right, difference between observed and modelled precipitation in mm/day; Figure 14) in Cargill area.*

Annex 2. Water parameters during Artemia smapling

Water characterization parameters for the saliñas. OoR: Out of Range

Saliña	**Water Temp °C**	**O_2 %**	**EC mS/cm**	**Salinity ppt**
Bartol 3a*	28	130	OoR	--
Bartol 4	29	105	OoR	--
Slagbaai	30	140	70-OoR	60-65
Goto 10	29	94	142	OoR
Goto 8a***	28	62	138	OoR
Goto 9	30	75	OoR	OoR

Annex 3. Description of the locations

Description of samples taken in 2012, together with field measured site characteristics. (Based on De Zwart et al., 2012 plus additions)

Date dd/mm/yyyy	**Sampling location**	**Sample code**	**Northing** degree	**Easting** degree	**Sample taken**	**Water Temp.** ºC	**Conductivity** ms/cm	**Barometric pressure** hPa	**DO** mg/L	**pH** unit	**Sample depth** cm	**Avifauna observations**
29/10/2012	Salina Matijs	Loc. 1	12.27497	68.35562	Sed 2x250 ml	34.91	49.9	1025.0	9.8	9.21	10-15	ca 400 flamingo's
					Water 2x1 L	34.87	49.7	102.4	9.84	9.40	5-7.5	
29/10/2012	Salina Bartol east	Loc. 3	12.29925	68.38962	Sed 2x250 ml	32.44	93.2	1023.6	3.86	8.34	15-20	3 flamingo's + 1 pelican
					Water 2x1 L	32.4	104.2	1023.5	5.17	8.35	7.5=10	
10/29/2012	Bartol west	Loc.4			Sed 2x250 ml							
					Water 2x1 L							
10/30/2012	Slagbaai West	Loc. 5	12. 15785	68.24806	Sed 2x250 ml	35.58	147.6	1026.8	3.68	7.79	30-40	3 adult Flamingo, 2 juveniles. 2 silver herons.
					Water 2x1 L	29.83	59.11	1026.8	13.4	8.34		
10/30/2012	Slagbaai Dam noord	Loc. 6	12 15.296	068 24.502	Sed 2x250 ml	33.68	154.4	1027.3	2.64	7.76	30-40	No Flamingos
					Water 2x1 L	31.46	139.4	1027.4	3.89	7.79		
30/10/2012	Lake Goto in the park	Loc. 7	12.24280	68.37103	Sed 2x250 ml	32.34	123.1	1026.9	4.25	7.96	10	4 flamingo's + 1 silver heron + 2 grey herons
					Water 2x1 L	32.34	123.1	1026.9	4.25	7.96	5	
30/10/2012	Lake Goto polluted spot according to STINAPA ranger	Loc. 8	12.23507	68.36933	Sed 2x250 ml	31.63	123.7	1026.9	3.79	7.97	30-40	no flamingo's present
					Water 2x1 L	31.68	124.8	102.9	5.1	7.99	15-20	
30/10/2012	Lake Goto near BOPEC	Loc. 9	12.22213	68.37563	Sed 2x250 ml	34.14	125.8	1026.4	6.22	7.99	30-40	no flamingo's present
					Water	35.19	122.7	1026.4	6.73	7.97	15-20	

Date dd/mm/yyyy	**Sampling location**	**Sample code**	**Northing** degree	**Easting** degree	**Sample taken**	**Water Temp.** ºC	**Conductivity** ms/cm	**Barometric pressure** hPa	**DO** mg/L	**pH** unit	**Sample depth** cm	**Avifauna observations**
					2x1 L							
30/10/2012	Lake Goto	Loc. 10	12.23342	68.37175	Sed 2x250 ml	33.01	122.4	1023.7	5.16	7.99	30-40	no flamingo's present
					Water 2x1 L	33.29	114.6	1023.7	5.06	7.97	15-20	

Annex 4. Risk Quotients of PAHs

Table 15 *Sigma RQ risk analysis as performed for the measured PAH compounds in the sampled water bodies on Bonaire. The RQ values are calculated by dividing the measured concentrations by the compound and medium specific risk limit (RL as specified in de Zwart et al., 2012) Concentrations below the limit of detection (DL) are omitted. For sediments the risk limits are corrected for the organic carbon content of the local sediments. The green color being < 0, indicates complete absence of the risk for ecosystem damage based on QR of these compounds.*

PAH compound	**Medium**	**RL**	**Unit RL**	**Loc.1**	**Loc. 3**	**Loc. 7**	**Loc. 8**	**Loc. 9**	**Loc. 10**	**Unit**
Naphthalene	Water	5400	ng/L				0.000			RQ
Acenaphthylene	Water	4000	ng/L	0.003	0.001	0.000	0.000	0.000	0.000	RQ
Acenaphthene	Water	1700	ng/L							RQ
Fluorene	Water	1100	ng/L	0.001	0.002	0.001	0.001	0.001	0.001	RQ
Phenanthrene	Water	580	ng/L	0.001	0.004	0.001			0.001	RQ
Anthracene	Water	410	ng/L							RQ
Pyrene	Water	270	ng/L			0.003	0.002			RQ
Fluoranthene	Water	180	ng/L			0.004				RQ
Chrysene	Water	74	ng/L							RQ
Benz[a]anthracene	Water	64	ng/L							RQ
Benzo[k]fluoranthene	Water	54	ng/L							RQ
Benzo[b]fluoranthene	Water	53	ng/L							RQ
Benzo[a]pyrene	Water	53	ng/L							RQ
Benzo[ghi]perylene	Water	52	ng/L							RQ
Dibenz[a,h]anthracene	Water	36	ng/L							RQ
Indeno[1,2,3-cd]pyrene	Water	35	ng/L							RQ
			ΣRQ	**0.005**	**0.006**	**0.009**	**0.004**	**0.001**	**0.003**	RQ
Organic matter content	**Medium**	**RL 10% OM**	**Unit RL**	7.2%	10.0%	8.2%	7.4%	29.8%	5.6%	OM%
Naphthalene	Sediment	430	µg/kg DW		0.017					RQ
Acenaphthylene	Sediment	510	µg/kg DW	0.004		0.002		0.000	0.002	RQ
Acenaphthene	Sediment	530	µg/kg DW	0.042		0.009			0.002	RQ
Fluorene	Sediment	580	µg/kg DW	0.004						RQ
Phenanthrene	Sediment	670	µg/kg DW				0.000			RQ
Anthracene	Sediment	710	µg/kg DW				0.001		0.001	RQ
Pyrene	Sediment	890	µg/kg DW							RQ
Fluoranthene	Sediment	990	µg/kg DW					0.001		RQ
Chrysene	Sediment	1700	µg/kg DW		0.000	0.000	0.001	0.001	0.001	RQ

PAH compound	Medium	RL	Unit RL	Loc.1	Loc. 3	Loc. 7	Loc. 8	Loc. 9	Loc. 10	Unit
Benz[a]anthracene	Sediment	1900	µg/kg DW				0.000	0.000		RQ
Benzo[k]fluoranthene	Sediment	2500	µg/kg DW				0.000	0.000	0.000	RQ
Benzo[b]fluoranthene	Sediment	2600	µg/kg DW		0.000	0.000	0.000	0.000	0.000	RQ
Benzo[a]pyrene	Sediment	2600	µg/kg DW					0.000		RQ
Benzo[ghi]perylene	Sediment	3100	µg/kg DW				0.000	0.000	0.001	RQ
Dibenz[a,h]anthracene	Sediment	4700	µg/kg DW						0.000	RQ
Indeno[1,2,3-cd]pyrene	Sediment	4900	µg/kg DW		0.000	0.000	0.000	0.000	0.000	RQ
			ΣRQ	0.051	0.017	0.012	0.002	0.003	0.006	RQ

Annex 5. PFC in water (µg/l) and sediment (µg/kg ds corrected for salt content)

Analytical results of the PFC measurements as performed by IMARES for water samples and wet sediment samples. The sediment concentrations calculated on a dry weight basis (lower block of data) are calculated from wet weight by a correction for sample dry weight, excluding the salt content, mainly attributable to the water phase of the sample. Grey highlighted cells are above the limit of detection (LD). The dark grey highlight indicates a sediment sample with a relatively high water content. These data are previously reported by De Zwart et al., 2012. In this annex additions are made on loc 2, loc 5, and loc6.

Table 16 PFC in water (µg/l)

Location	Sample number	PFPA	PFHxA	PFHpA	PFOA	PFNA	PFDcA	PFUnA	PFDoA	PFTrA	PFBS	PFHxS	PFHpS	PFOS	PFDS
Loc 1 (Matijs)	2012/1158	<3.1	<3.1	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<3.1	<0.3	<0.3	<0.3	<0.3	<0.3
Loc 2 (Matijs)	2012/1159	<3.2	<3.2	<0.3	3.5	<0.3	<0.3	<0.3	<0.3	<3.2	<0.3	<0.3	<0.3	<0.3	<0.4
Loc 3 (Bartol oost)	2012/1160	<3.0	<3.0	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<3.0	<0.3	<0.3	<0.3	0.7	<0.3
Loc 4 (Bartol west)	2012/1161	<3.0	<3.0	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<3.0	<0.3	<0.3	<0.3	0.7	<0.3
Loc 5 (Slagbaai west)	2012/1163	<3.0	<3.0	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<3.0	<0.3	<0.3	<0.3	3.2	<0.3
Loc 6 (Slagbaai dam)	2012/1164	<2.9	<2.9	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<2.9	<0.3	0.2	<0.3	1.5	<0.3
Loc 7 (Goto park)	2012/1165	89	100	15	8.7	<0.3	<0.3	<0.3	<0.3	<2.9	160	170	3.4	85	<0.3
Loc 8 (Goto eiland)	2012/1166	120	140	20	14	<0.3	<0.3	<0.3	<0.3	<2.9	190	210	6.1	100	<0.3
Loc 9 (Goto Bopec)	2012/1167	99	130	32	11	<0.3	<0.3	<0.3	<0.3	<2.9	170	210	8.3	130	<0.3
Loc 10 (Goto tov eiland)	2012/1168	82	180	22	3.5	<0.3	<0.3	<0.3	<0.3	<2.9	150	210	5.7	120	<0.3

Table 17 PFC in sediment (µg/kg ds -corrected for salt content)

Location	Sample number	PFPA	PFHxA	PFHpA	PFOA	PFNA	PFDcA	PFUnA	PFDoA	PFTrA	PFBS	PFHxS	PFHpS	PFOS	PFDS
Loc 1 (Matijs)	2012/1144	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.2	<0.2	<0.2	<0.2	<0.2
Loc 2 (Matijs)	2012/1145	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.2	<0.2	<0.2	<0.2	<0.2
Loc 3 (Bartol oost)	2012/1146	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3	<0.3	<0.3
Loc 4 (Bartol west)	2012/1147	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.3	<0.3	<0.3	<0.3	<0.3
Loc 5 (Slagbaai west)	2012/1148	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.2	<0.2	<0.2	<0.2	<0.2
Loc 6 (Slagbaai dam)	2012/1149	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.2	<0.2	<0.2	<0.2	<0.2
Loc 7 (Goto park)	2012/1150	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2	3	<0.2	18.5	<0.2
Loc 8 (Goto eiland)	2012/1151	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.3	0.3	<0.3	20.6	<0.3
Loc 9 (Goto Bopec)	2012/1152	<1.9	<1.9	<1.9	<1.9	<1.9	<1.9	<1.9	<1.9	<1.9	<1.3	7.7	<1.3	499.6	<1.3
Loc 10 (Goto tov eiland)	2012/1153	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4	<0.2	0.4	<0.2	14	<0.2

Annex 6. Microtox data

		after 5 minutes		after 15 minutes		after 30 minutes	
Sample no (2012-)	Field location	% effect	Ave. effect	% effect	Ave. effect	% effect	Ave. effect
1158	Matijs1	-38.17%		-60.66%		-54.62%	
1158	Matijs1	-36.49%	-37.33%	-60.23%	-60.44%	-55.34%	-54.98%
1160	Bartol3	-25.78%		-36.18%		-34.45%	
1160	Bartol3	-24.70%	-25.24%	-33.44%	-34.81%	-36.49%	-35.47%
1163	Slagbaai5	-18.51%		-27.02%		-23.75%	
1163	Slagbaai5	-5.85%	-12.18%	-14.74%	-20.88%	-10.35%	-17.05%
1164	Slagbaai6	-41.08%		-52.76%		-39.34%	
1164	Slagbaai6	-34.03%	-37.55%	-44.49%	-48.62%	-32.56%	-35.95%
1165	Goto7	-42.65%		-50.80%		-45.76%	
1165	Goto7	-19.50%	-31.08%	-34.25%	-42.52%	-35.51%	-40.64%
1166	Goto8	-36.99%		-53.34%		-47.49%	
1166	Goto8	-23.04%	-30.02%	-39.66%	-46.50%	-37.62%	-42.56%
1167	Goto9	-50.57%		-69.94%		-65.81%	
1167	Goto9	-26.56%	-38.56%	-44.11%	-57.02%	-42.72%	-54.26%
1144	Matijs1	nd	nd	nd	nd	nd	nd
1144	Matijs1	nd	nd	nd	nd	nd	nd
1146	Bartol3	35.63%		26.31%		24.18%	
1146	Bartol3	40.01%	37.82%	32.03%	29.17%	29.94%	27.06%
1148	Slagbaai5	17.81%		9.76%		13.56%	
1148	Slagbaai5	17.65%	17.73%	10.58%	10.17%	11.67%	12.61%
1149	Slagbaai6	-30.83%		-46.08%		-42.60%	
1149	Slagbaai6	-36.06%	-33.45%	-52.20%	-49.14%	-46.07%	-44.34%
1150	Goto7	-46.52%		-61.02%		-58.66%	
1150	Goto7	-26.96%	-36.74%	-37.49%	-49.26%	-38.77%	-48.72%
1151	Goto8	-38.25%		-41.97%		-39.34%	
1151	Goto8	-29.44%	-33.84%	-41.31%	-41.64%	-38.46%	-38.90%
1152	Goto9	6.80%		2.06%		1.49%	
1152	Goto9	5.26%	6.03%	-0.71%	0.67%	-0.53%	0.48%