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Ballast water samples as "scientific evidence"

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BALLAST WATER SAMPLES AS "SCIENTIFIC EVIDENCE"

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TABLE OF CONTENTS

Abstract
The Ballast Water Management Convention was established as a response to the global issue of the spread of invasive species. The entry into force of the Convention is supposedly imminent, but many issues remain to be solved to ensure its success in preventing the spread of invasive species. Considering the implications involved, it is of great importance for the success of the Convention that sampling procedures to determine compliance are robust and reliable. Further, sampling results must gain acceptance as scientific evidence and basis for enforcement actions. The IMO has produced guidelines and recommendations that are to be evaluated during a trial period and a uniform protocol for sampling procedures and protocols has yet to be established. Ballast water samples fall in the category of scientific evidence, a well-researched topic, and as such, many lessons learned can be applied in the case of ballast water. The use of ballast water samples as evidence shows many similarities to the use of DNA profiling. DNA profiling was initially highly controversial and much criticized, but has gained general acceptance as evidence in today's courts. Important features behind the success of DNA evidence were firm quantitative estimates of risk for errors close to zero, formal recommendations on how probability calculations should be conducted from official bodies, the establishment of quality control standards to minimize laboratory errors, and administrative protocols. These features resulted in favourable judicial decisions that has materialized general legal acceptance. Although the guidelines produced by the IMO have made significant progress, for ballast water to be used as evidentiary basis for *e.g*. criminal sanctions they need to be a lot more robust. Formal statistical calculations for representativeness is likely to one of the most important features to have in place, as well as protocols for the obtaining and handling of samples. It is further suggested that preparations are made for the use of expert testimonies by courts in ballast water cases.

1. INTRODUCTION

The IMO International Convention for the control and management of ships' ballast water and sediments (the BWM Convention) was adopted in 2004 in response to one of the biggest threats to global ecology and one of the major vectors behind it; invasive species and ballast water. As of 28 of February 2014, 38 states have ratified the Convention and a mere 4,62 % of the world gross tonnage is currently all that is lacking. Several media are reporting the full ratification as imminent and ratification by only one major flag state would meet the requirement.[1,2]

However, ineffective application and enforcement of legal instruments has been shown to be a larger problem in environmental law than in any other area of legal practice and for the success of the Convention it is vital that it is efficiently enforced.[3,4] Unfortunately, several stakeholders have uttered their concern regarding these very aspects of the Convention. The main cause of the concern is the unique D-2 performance standard. The Convention sets discharge-limits based on an allowed maximum number of *viable* organisms of different size groups. The large volumes of ballast water involved in relation to the small number of organisms allowed, coupled with the viability aspect, make determining compliance a complicated procedure. Since non-compliance with the provisions of the Convention may result in criminal charges and heavy fines, and ship-owners are investing millions of dollars in ballast water treatment systems, proper and fair determination of compliance is of great importance.

However, to date, there is no general consensus on how to carry out compliance sampling for the D-2 standard, which is the root of concern with many stakeholders. For example, "*Ballast water experts wrestle with sampling clauses*", was one of the first global headlines on the topic of ballast water in 2014, referring to a recently formed sub-committee with representatives from among others A.P. Moller Maersk, Wilhelmsen Maritime Services and the Managers of the UK P&I Club. During their first meeting in late January, the topic of concern on the agenda was "*legal and contractual implications of sampling to demonstrate compliance with ballast water performance standards*".[5,6] Further, at the "Ballast Water Summit 2014" in Hamburg, Katharina Stanzel, Managing Director at INTERTANKO, spoke about the "*owner's reality*". Ms Stanzel stated that "*owners live in fear of non-compliance and criminalisation – fear of the 'what ifs'*". As examples she mentioned what if port state control does not stick to the agreed trial period for sampling and enforcement, and what if sampling results show violation even though BWMS are operated as designed? [7] The International Chamber of Shipping (ICS) considers the uncertainties regarding the Convention to be so severe that it is calling upon governments around the world, asking them not to ratify the Convention. The main concerns regard the "*lack of robustness of the current IMO type-approval standards*" as well as "*the criteria to be used for sampling ballast water during Port State Control inspections*".[8] It is clear that many issues remain to be solved regarding ballast water sampling and until so has been done, several parties are pushing a principle of "no criminal sanctions solely on the basis of sampling".[9,10]

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[1] Ship and Bunker, 'Fathom Spotlight: Part Two – Active or Non-Active? That is the Ballast Question' (*Ship and bunker,* 2014-02-13) http://shipandbunker.com/news/features/fathom-spotlight/512633-fathom-spotlight-part-two-active-or-non-active-that-is-the-ballast-question (accessed 2014-03-07)

[2] See for example Gavin, Fiona and Hickey, Richard, 'Contractual Pitfalls Relating To The Ballast Water Management Convention' (*Mondaq,* 2014-02-07) http://www.mondaq.com/x/290100/Marine+Shipping/Contractual+pitfalls+relating+to+the+Ballast+Water+Management+Convention (accessed 2014-03-07)

[3] Gunningham, Neil, 'Enforcing Environmental Regulation', *Journal of Environmental Law* 23:2, 2011, p. 169.

[4] Demmke, Christoph, 'Towards effective environmental regulation: Innovative approaches in implementing and enforcing European Environmental law and policy'*.* Jean Monnet Working Paper 5/01, New York University School of Law, 2001, p.2.

[5] BIMCO, 'Ballast water experts wrestle with sampling clauses', (*BIMCO*, 2014-01-29) https://www.bimco.org/news/2014/01/29_ballast_water_clauses.aspx (accessed 2014-02-04)

[6] According to Mrs Anna Wollin Ellevsen, Legal and Contractual Affairs Officer at BIMCO, "the focus of the project is to develop voyage and time charter party clauses that allocate responsibility for costs and delays resulting from sampling procedures".
Ibid.

[7] Ship and Bunker, 'Fathom Spotlight: The Ballast Water Summit 2014 – Insights from Day One (*Ship and bunker,* 2014-03-10) http://shipandbunker.com/news/features/fathom-spotlight/185607-fathom-spotlight-the-ballast-water-summit-2014-insights-from-day-one (accessed 2014-03-10)

[8] Kristiansen, Tomas, 'ICS: Hold off on ballast water ratification!', (*Shippingwatch,* 2014-02-12) http://shippingwatch.com/carriers/article6482443.ece (accessed 2014-03-07)

[9] Bierman, S.M., de Vries, P. and Kaag, N.H.B.M., 'The development of a full standard methodology for testing ballast water discharges for gross non-compliance of the IMO's Ballast Water Management Convention' (EMSA/NEG/12/2012).

The use of ballast water samples as basis for enforcement actions, fall within the category of "scientific evidence", a topic that has been the target of extensive research, legal analysis and debate. Since the Convention has yet to enter into force, ballast water samples have yet to be tried as evidence and it remains to be seen what standards they will be expected to meet. It is proposed that the interdisciplinary aspects of ballast water regulations also can be used to its advantage in cases where research done on similar topics in other fields can be applied. This report will use the discourse and research on scientific evidence as basis for trying to determine what standards and what requirements ballast water sampling will have to meet to suffice for evidentiary use in cases of non-compliance.

2. BALLAST WATER SAMPLING

The D2 standard of the BWM Convention

The enforcement of the BWM Convention, especially sampling to determine compliance with the D-2 standard, is evidently the cause of much concern. This is however quite surprising considering the purpose of the values of the D-2 standard. During the development of the standard it was reasoned that the treatment systems, that had yet to be developed, were unlikely to perform perfectly, which is why the standards were established as to allow for low numbers of organisms in the ballast water discharges.[11]

In accordance with the Convention, less than 10 viable organisms greater than or equal to 50 µm in minimum dimension can be discharged per m^3 ballast water. For organisms less than 50 µm and greater than, or equal to 10 µm, in minimum dimension, less than 10 viable organisms per ml can be discharged. Indicator microbe limits are given in specific concentrations. For toxicogenic *Vibrio Cholerae*, less than one colony-forming unit (cfu) per 100 ml or less than 1 cfu per 1 g (wet weight) zooplankton sample is allowed. For *Escherichia coli,* the limit is less than 250 cfu per 100 ml and for Intestinal Enterococci, less than 100 cfu per 100 ml.[12]

Guidelines for ballast water sampling (G2)

The G2 guidelines were adopted in 2008 with the objective of providing Convention parties with practical and technical guidance on ballast water sampling.[13] The guidelines apply to sampling for assessment of compliance with both the D-1 and D-2 standards. And as such they contribute some needed specifications regarding some of the concepts of the Convention, for example, on what is to be considered the "minimum dimension" of an organism.[14] Further, the guidelines contain recommendations on how sampling should be conducted.[15] According to the guidelines, sampling for compliance should follow a protocol that is in line with certain listed principles. It should be conducted so that the sample is *representative* for the whole discharge and possibly accumulated sediment should be taken into account. Quantity and quality of samples should be sufficient to demonstrate compliance with the standard and samples should be concentrated to a manageable size.[16] The number of samples and their volume will be dependent on the objective of the sampling, the analytical method to be used and the statistical significance and certainty required.[17] Samples should be sealed and stored to make sure that they can be used to test for compliance with the Convention and tests should be analysed within a "test method holding time limit" by an accredited laboratory. Finally, samples should be transported, handled and

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[10] IMO Sub-committee on bulk liquids and gases, *Report to the maritime safety Committee and the marine environmental protection committee*, (8 February 2013), BLG 17/18, [4.10].

[11] International Maritime Organization. (2013). Additional guidelines for implementation of the BWM Convention, Establishing benchmarks in compliance testing by port State control. PPR 1/INF.4, 27 November 2013, p. 3.

[12] International Maritime Organization. (2004). International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004, BWM/CONF/36,16 February 2004, Regulation D-1 and D-2.

[13] International Maritime Organization. (2008). Resolution MEPC.173(58), Guidelines for ballast water sampling (G2), p. 2.

[14] *Ibid.* at 3.1.1.

Minimum dimension is said to mean "the minimum dimension of an organism based upon the dimensions of that organism's body, ignoring e.g., the size of spines, flagellae, or antenna. The minimum dimension between main body surfaces of an individual when looked at from all perspectives. For spherical shaped organisms, the minimum dimension should be the spherical diameter. For colony forming species, the individual should be measured as it is the smallest unit able to reproduce that needs to be tested in viability tests."

[15] *Ibid.* at 5.2 and 5.3.

[16] *Ibid.* at 6.2.

[17] *Ibid.* at Part 3, 1.2.

stored with consideration to the chain of custody.[18] How samples are *handled* after they are taken is, from a legal point of view, just as important as how they are taken and a collection data form and chain of custody record should be kept with each individual sample.[19]

Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention and Guidelines (G2)

Following the adoption of the G2 guidelines, the Subcommittee on Bulk Liquids and Gases was instructed to develop a circular to provide further guidance on sampling and analysis.[20] The result was a state of the art guidance document on the principles of ballast water sampling and methodologies. However, the procedures and methodologies in the circular still have yet to be adequately validated. [21] For most of the approaches mentioned in the document, *e.g.* visual identification, photometry, ATP and flow cytometry, the level of confidence or detection limits remain to be determined.[22] The intention is for states to try out the approaches and "*make sure they are practical and fit for the purpose*" during a trial period of two to three years following the entry into force of the Convention.[23]

Current state of the art sampling methodologies – a brief overview [24]

The guidance document separates indicative, considered as a first step in determining potential non-compliance, from detailed analysis, with the main difference being the level of statistical confidence. [25, 26] The D-2 standard relies on counts of *viable* organisms or colony forming units in the discharges. Methods for this sampling can roughly be divided into those using biological indicators such as nucleic acid, ATP or chlorophyll a, and those using direct counts of living organisms, either manually or automatically.[27]

The presence of ATP (adenosine triphosphate) in a sample can be taken as an indication of life. Sampling using filters and applying ATP analysis could potentially provide information on viability within specific size classes. However, ATP can exist in dead organisms, why this method cannot currently be used to determine organism counts.[28]

Since many of the organisms found in ballast water are likely to be phytoplankton, measuring levels of chlorophyll seems a feasible option. However, chlorophyll a can persist in water outside of a cell, why such a measurement would not provide an organism count. This may be overcome through the use of a Pulse-Amplitude Modulated Fluorometer, which excites the chlorophyll a molecule and register the subsequent fluorescent response signal. Still, this method would only be applicable to phytoplankton and would not provide size distinction.[29] Further, experienced analysts are required and it does not always work on all species or in all salinities.[30]

A flow cytometer automatically counts objects per size class in fluids and can be used for organisms larger than 10 µm. Complex systems can even distinguish sediment and detritus from organisms. Combined with other techniques such as staining, it can potentially distinguish between living and dead phytoplankton.[31] The technique has shown great promise, and allows for both visual and computer processing. However, high particle loads are thought to potentially lower detection limits and presently there are no portable versions of the technology available.[32]

Out of the currently available techniques for ballast water sample analysis, visual inspection with a microscope seems to provide the most robust results. Not to mention the advantage of expertise and equipment being readily available worldwide. Body movements, which can be stimulated, can be used as

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[18] *Ibid.* at 6.2.
[19] *Ibid.* at Part 7, 1.
[20] International Maritime Organization. (2013). Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention and Guidelines (G2), BWM.2/Circ.42, 24 May 2013.
[21] IMO Sub-committee on bulk liquids and gases. (2013). Report to the Maritime Safety Committee and the Marine Environment Protection Committee BLG 17/18, 8 February 2013, Annex 6, page 1.
[22] International Maritime Organization. (2013). BWM.2/Circ.42 Annex I, p. 6-12.
[23] IMO Sub-committee on bulk liquids and gases. (2013). Report to the Maritime Safety Committee and the Marine Environment Protection Committee BLG 17/18, 8 February 2013, Annex 6, p. 1.
[24] Bacteria will not be discussed here since international standards for analysing bacterial presence according to the D-2 standard already exist.
[25] International Maritime Organization. (2013) BWM.2/Circ.42 Annex I, page 1.
[26] *Ibid.,* Annex I, page 4.
[27] *Ibid.,* Annex 2, page 2.
[28] *Ibid.,* Annex 2, page 3.
[29] *Ibid.,* Annex 2, page 3.
[30] *Ibid.,* Annex 2, page 6.
[31] *Ibid.,* Annex 2, page 3-4.
[32] *Ibid.,* Annex 2, page 6.

a parameter for determining viability. Determining minimum size of organisms can be done fairly easily with a microscope, although it should be taken into account that the two dimensional image given in the microscope might not provide a hundred per cent accurate viewing angles, which could result in faulty organism classification.[33]

Several techniques used for analysing ballast water samples may require concentration. In such cases it should be noted that concentration might cause stress in the organisms, which may result in decreased viability. [34] Further, great attention should be paid to make sure that organism mortality is not critically affected by storage conditions and duration before sample analysis.[35]

Representativeness and statistical aspects

Numerical environmental standards should always be robust, recognize scientific assessment and strive towards being statistically verifiable. The European Integrated Pollution Prevention and Control Bureau has stated that "compliance assessments should generally involve a statistical comparison between the measurements, or a summary statistics estimated from the measurements, the uncertainty of the measurements, and the emission limit value or equivalent requirements". In accordance with the G2 Guidelines, ballast water samples should be *representative*. As such, the BWM Convention is the first marine environmental protection regulation to require representative analysis as part of the regulatory process.[36]

The IMO "Guidance on ballast water sampling and analysis for trial use" defines representative sampling as reflecting "*the relative concentrations and composition of the populations (organisms and/or chemicals) in the volume of interest*".[37] The representativeness that should be sought after for ballast water sampling can be separated into biological and statistical representativeness. Biological representativeness refers to the diversity of organisms found in the tanks, while statistical representativeness refers to the number of organisms.[38, 39]

When considering the uncertainties involved in ballast water sampling, it is important to be aware of the fact that uncertainties can add up throughout the whole monitoring process. In dealing with such low concentrations of organisms, as is likely to most often be the case in ballast water sampling, there is a risk that the error that comes from processing the sample and using different assessment methods, could be equal to the difference between a pass and fail of an inspection.[40] Thus, uncertainties need to be estimated and reported to allow for a thorough compliance assessment. [41] Determining the statistical aspects that should apply to any sampling protocol will be a very important step towards establishing sampling procedures and enforcement approaches. Therefore, regulators will have to determine what an acceptable level of certainty is in these cases.[42]

3. SCIENCE IN THE COURTROOM

Legal disputes, finding their way into the courtrooms of today, are growing ever more complex and on this road, science is becoming an indispensable ally. Legal scholars have been said to "*look to science to rescue us from the experience of uncertainty and the discomfort of difficult legal decisions*".[43] But the relationship between law and science is sometimes difficult and require a lot of work to function well.

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[33] *Ibid.,* Annex 2, page 7.
[34] *Ibid.,* Annex 2, page 6.
[35] *Ibid.,* Annex 2, page 7.
[36] Cabezas-Basurko, Oihane and Mesbahi, Ehsan (2011). Statistical representativeness of ballast water sampling. *Proceedings of the Institution of Mechanical Engineers, Part M: Journal of Engineering for the Maritime Environment*, *225*(3), p. 184.
[37] International Maritime Organization. (2013). BWM.2/Circ.42, Annex 1, page 1.
[38] Carney, Katharine J., Cabezas-Basurko, Oihane, Pazouki, Kayvan, Marsham, Sara, Delany, Jane E., Desai, D. V., Anil, A.C. and Mesbahi, Ehsan. (2013). Difficulties in obtaining representative samples for compliance with the Ballast Water Management Convention. *Marine Pollution Bulletin*, *68* (2013), p. 100.
[39] Pazouki, Kayvan, Cabezas-Basurko, Oihane, Mesbahi, Ehsan & Kemp, Jonathan, (2009). The Control of the Spread of Non-Indigenous Species Through Ballast Water – Part B: Ballast water sampling: Methods, Analysis, Representativeness and Legal Issues, p. 52.
[40] Carney, Katharine J., *et al.* (2013), p. 104.
[41] European Commission, European Integrated Pollution Prevention and Control Bureau. (2003). Reference Document on the General Principles of Monitoring, p. ii.
[42] Miller, A. Whitman, Frazier, Melanie, Smith, George E., Perry, Elgin S., Ruiz, Gregory M., and Tamburri, Mario N. (2011). Enumerating Sparse Organisms in Ships' Ballast Water: Why Counting to 10 Is Not So Easy. *Environmental Science & Technology*, *45*(8), p. 3545.
[43] Feldman, Robin. (2009). *The Role of Science in Law*. Oxford University Press. p. 1.

Professor David Goodstein has described the presentation of scientific evidence in court as "*a kind of shotgun marriage between the two disciplines*".[44] He goes on to say that both "*are obliged to some extent to yield to the central imperatives of the other's way of doing business, and it is likely that neither will be shown in its best light*". Professor of Law, Robin Feldman, goes so far as to say that "*we are constantly seduced into believing that some new science will provide answers to law's dilemmas, and we are constantly disappointed*", she further states that law "*maintains a pure and idealized vision of science, one that is far removed from any contemporary understanding within the scientific field itself. Thus, the problem is not only that science cannot do for law what we think it can, the problem is also that science is not even what we think it is*".[45]

Our efforts are not for nothing though, we can use science to great advantages within our legal system, if we only learn how to best manage the role that we let science play.[46] In doing so, the most important aspects to take into consideration are the evidence itself and, the frequently occurring, expert testimonies.

Scientific evidence

Evidence can be defined as "the available body of facts or information indicating whether a belief or proposition is true or valid" or "information drawn from personal testimony, a document, or a material object, used to establish facts in a legal investigation or admissible as testimony in a law of court".[47] The scientific investigation of a crime is best described like a pyramid; at the bottom you have the facts and physical evidence, on top of that base is the analysis and at the very top are the conclusions. *I.e.* one draws few conclusions based on a much larger amount of facts and evidence. Conclusions should only be made based on the facts and analysis and not on assumptions or hypotheses.[48] Further, it must be ensured that evidence "*has been obtained, transported, handled, analyzed, and preserved in accordance with practices accepted by the court*".[49] However, "*evidence to determine if a company procedure is deficient may be gathered to less stringent standards than evidence to convict a person of murder*". This is to be expected. When the consequences of an investigation can result in the loss of fortune, dishonour, incarceration, or capital punishment, high standards of care are appropriate."[50]

Expert testimony

Regarding complex scientific matters, where it is completely unreasonable to expect a judge to study the necessary background information needed to make a judgement, the use of expert witnesses is inevitable and yet there are many question marks regarding this practice. How should the expert be chosen or hired? How can it be avoided that the expert becomes the judge? How can it be determined who is qualified as an expert? How can the neutrality of the expert be determined?

In the United States, the topics of judicial gatekeeping and expert testimonies have been extensively debated and researched due to some high profile cases. The selection of experts has been facilitated by the development of a program to identify independent experts, providing handbooks for both judges and experts.[51, 52] Courts have also been appointed as "gatekeepers" of scientific evidence. In practice, this role has taken different shapes, such as using pre-trial conferences or pre-trial hearings of experts for example.[53, 54]

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[44] Federal Judicial Center, National Research Council of the National Academics. (2011). *Reference Manual on Scientific Evidence* (3rd ed.). Washington, D.C. p. 52.
[45] *Ibid.,* p. 95.
[46] *Ibid.,* p. 11.
[47] Oxford dictionaries, http://www.oxforddictionaries.com/definition/english/evidence?q=evidence (accessed 2014-02-20)
[48] Noon, Randall K. (2009). Scientific method: Applications in failure investigation and forensic science. p. 6
[49] *Ibid.,* p. 15
[50] *Ibid.,* p. 15
[51] Federal Judicial Center, National Research Council of the National Academics. (2011). p. 8.
[52]American Association for the Advancement of Science, 'CASE: Recruitment and Screening Panel', http://www.aaas.org/page/case-recruitment-and-screening-panel (accessed 2014-02-18)
[53] Federal Judicial Center, National Research Council of the National Academics. (2011). p. 6.
[54] In the case of silicon gel breast implants, one solution that has gotten a lot attention as being very comprehensive in its attempt to bring science into the courtroom in the most accurately possible way is a case in Alabama, where the judge appointed a "a 'neutral science panel' of four scientists from different disciplines to prepare a report and testimony on the scientific basis of claims in silicone gel breast implant product liability cases consolidated as part of a multi district litigation process". The testimony was filmed so that it could be used and referred to later on and thereby save time and costs of litigation. For more information see Federal Judicial Center, National Research Council of the National Academics. (2011). p. 7.

A series of cases known as "the Daubert Trilogy" has provided guidance for courts in the way they deal with expert witnesses for "scientific matters". In the trilogy, some general "themes" can be discerned. Firstly, the trial judge has the obligation to act as a gatekeeper and must therefore screen expert testimonies. In doing so, it must be ensured that the testimony is both relevant and reliable.[55] In order to determine reliability, the judge must decide if the testimony is grounded in "methods and procedures of science", *i.e.* scientific method.[56]
To determine scientific validity, the following general observations are recommended;[57]

- Can the technique/theory be tested and has it been? (Scientific method)
- Has the technique/theory been subjected to peer-review and publication?
- What is the known or potential rate of error?
- Are there standards for the control of the operation of the technique?
- Is there a general acceptance of the technique?

Although specific to the United States, the procedure of relevant considerations laid out by Daubert is highly relevant to the use of scientific evidence in jurisdictions in general. The way that the BWM Convention is currently formulated, it is potentially vulnerable to legal challenge and the use of expert testimonies is a practice likely to be seen frequently as responses to enforcement actions.

DNA-profiling

DNA fingerprinting, or what today is usually referred to as DNA profiling, may not appear to have much in common with ballast water sampling. However, this technique, its use as scientific evidence and its evolution to a generally accepted type of evidence, is the poster child for scientific evidence and valuable lessons can be learned from it. DNA profiling is today often treated as the benchmark against which all other scientific evidence is measured and ballast water sampling is today beginning on a journey that DNA evidence has already taken.[58] Using DNA evidence in court today will not raise many eyebrows, but this was not always the case.

The technique behind DNA profiling was developed for research purposes in the early 1980s.[59] Simply put, the technique is based on the presence of repeat sequences, called mini-satellites, in the DNA. Through the use of probes, restriction enzymes (which cuts the DNA into smaller pieces) and electric current, causing negatively charged DNA-fragments to separate according to size, repeat sequences can be used to obtain a series of "bands", which is referred to as a DNA fingerprint.[60] Through a comparison of banding patterns, one can try to determine if two samples come from the same person (with the exception of identical twins which also have identical DNA).[61] The application of population genetics allows scientists to calculate probabilities of a match. The statistics behind DNA fingerprinting and the application of population genetics are aspects of the technique that has been called into question.[62] Other objections have been aimed at the fact that molecules of crime scene DNA might have been degraded to some extent due to exposure to environmental conditions.[63] Further, inaccurate results can be the case if samples have been contaminated with foreign DNA. There is also of course the risk of two bands ending up on the same place randomly simply because they are of the same size. With the improvement of the technique, ways to minimize these risks have also been established.[64]

So how did DNA profiling go from scepticism and critique to general acceptance? It has been said that *"No single technical solution, legal decision, or other event was responsible for ending controversy in the courts and science press about forensic DNA profiling. Instead, a confluence of technical, legal, and administrative 'fixes' worked to close debate about the reliability of the technique".*[65] From a technical

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[55] *Daubert v. Merrell Dow Pharmaceuticals, Inc.* (1993), 509 US 579 (1993) at 584-587.
[56] *Ibid.* at 590.
[57] *Ibid.* at 593-594.
[58] Lynch, Michael. (2013). Science, truth, and forensic cultures: The exceptional legal status of DNA evidence. *Studies in History and Philosophy of Biol & Biomed Sci*, *44*(1), p. 60.
[59] Clayborn, Charity Lynn. (1989). Evidence -Criminal Law - Evidence of DNA Fingerprinting Admitted for Identification Purposes in Rape Trial, 12 *UALR Law Journal*, p. 544.
[60] *Ibid.,* p. 545-546.
[61] *Ibid.,* p. 546.
[62] "The frequency of the occurrence of the entire pattern is calculated by multiplying the probability of each band's occurrence by the probabilities of the other match bands. This approach assumes that the probability of one band occurring does not affect the probability of any other band occurring. This assumption is valid only if the entire population being studied mates at random and thus satisfies a condition known as Hardy-Weinberg equilibrium"
Ibid., p. 547.
[63] *Ibid.*, p. 547.
[64] *Ibid.*, p. 548.
[65] Lynch, Michael. (2013). p. 65.

point of view, courts required quantitative estimates of random match probabilities and as those odds eventually decreased to one in millions, and are today approaching zero, the acceptance by courts came to grow. However, discussions on how to calculate the statistical probabilities has continued but formal recommendations from organisations like the U.S. National Research Council has been of great benefit. Other key features in DNA profiling gaining general acceptance as scientific evidence has been the establishment of quality control standards to avoid laboratory error as well as administrative recommendations on collection of, handling, and analysis of samples. So for DNA profiling, low probabilities for random matches combined with administrative protocols and firm control standards turned out to be the recipe for success ensuring judicial decisions in its favour and in turn legal acceptance.[66]

4. BALLAST WATER SAMPLES AS SCIENTIFIC EVIDENCE

Using DNA profiling as the benchmark for ballast water samples as scientific evidence, what are then the aspects that need to be considered and focused on? A critical aspect of ballast water sampling is that the "pollution" to be dealt with is living organisms and the provision that the amount of *viable* organisms needs to be measured. With the aspiration of simplifying sampling, organisms where divided into size categories, but the result may not have been the desired. The standards for different categories were set considering a general distribution of organisms between the groups. However, in certain areas for example, the species composition may to a large degree consist of phytoplankton that are smaller than 10 µm and thereby fall outside of the plankton spectrum targeted by the D-2 standard. Algal blooms may result in heavy densities of organisms in the larger than 50 µm category, which would pose serious difficulties in reaching the needed mortality rates considering only 10 per m^3 ballast water are allowed. [67]

Ballast water can contain a very wide variety of organisms, something that makes determining viability in a simple manner very difficult. For zooplankton belonging to the larger size category, determining viability based visual inspections of mobility is a possible approach, but even so, the concept of viability might be too vague to provide certainty. For example, should organisms where the reproductive potential has been compromised be considered viable? And if so, how could this be determined in a feasible manner and within a reasonable amount of time?[68] For organisms belonging to the smaller category, which usually is comprised of "simpler" non-motile organisms, determining viability becomes even more of a challenge.[69] Especially considering that any such methodology would have to be affordable, available world wide, not too time-consuming and applicable to a large spectrum of organisms.[70, 71] Extensive efforts have gone into accurately identifying and counting microplankton, but no single method has been proven to determine viability in a reliable manner.[72]

The aspect of sample timing is also an important aspect to consider when determining at what point during a discharge samples should be obtained. Not all parts of a ballast water tank are within reach and organisms, especially zooplankton, tend to be unevenly distributed inside the tanks.[73] The distribution of organisms in the ballast tanks can be affected by the duration in the tanks and it is likely that organisms to a certain extent will settle at the bottom of the tank or attach to the walls.[74] Small-scale tests have shown high variability in organism distribution over time and high uncertainties that are likely to be much larger in reality.[75] Further, organism counts may not be constant and death or re-growth may

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[66] *Ibid.*
[67] International Maritime Organization (2013), PPR 1/INF.4, p. 5.
[68] International Maritime Organization (2013), PPR 1/INF.4, p. 7.
[69] Steinberg, Mia K., Lemieux, Edward J., and Drake, Lisa A., (2011). Determining the viability of marine protists using a combination of vital, fluorescent stains. *Marine Biology*, 158 (2011), p. 1432.
[70] Steinberg, Mia K. *et al.,* (2011) p. 1432.
[71] *"In sum, the combination of FDA and CMFDA stains proved a useful tool for enumerating viable protists in Florida and Maryland but was less successful in Washington and Maine, owing largely to false positives from heterotrophic and mixotrophic dinoflagellates. Using a carefully selected threshold for separating the fluorescence of live and dead organisms, the amount of error when using this method would decrease. Despite these few limitaitons, fluorescent vital stains are useful tools for evaluating the efficacy of ballast water managemetn systems against a discharge standard and for any areas of research that examine viabilty in diverse plankton assemblages", "To date, we know of no other viability and enumeration method that is as robust as this procedure for analyzing diverse plankton assemblages (including heterotrophs) at low densities. Considering the variation in false positive errors between sample sites, stains must be validated at each location before use".*
Steinberg, Mia K. *et al.,* (2011) p. 1436.
[72] Steinberg, Mia K. *et al.,* (2011) p.1431.
[73] Cabezas-Basurko, Oihane and Mesbahi, Ehsan (2011). p. 183.
[74] Carney, Katharine J., *et al.* (2013), p. 100.
[75] Carney, Katharine J., *et al.* (2013), p, 104.

occur inside the ballast tanks. Studies have shown that organism counts might be negatively impacted by long sampling times and long holding times have been found to decrease cell density.[76, 77]

As previously mentioned, representativeness is a central feature in ballast water sampling and consequently, the volume needing to be sampled to obtain this needs to be determined. Research conducted on this question shows that it is difficult to find a consensus. And even if statistical representativeness is achieved, how can one be sure that a sample also is biologically representative?[78] One study indicates that 7 m^3 time-integrated samples could provide "reasonable balance of statistical power and logistic achievability when applied to zooplankton discharge". Although it was noted that other sources of error would have to be added to identify proper sample volumes. [79] Another study found that representative samples for 10,000 and 50,000 m^3 of ballast water required samples of 4899 and 8000 m^3 respectively.[80] (These results do not take into account organism viability) Volumes that could not easily be sampled in a practical manner, especially considering the time needed to obtain them, the space needed to process them and the costs for analysing them. Also, the aspect of undue delay must be considered.[81]

5. CONCLUSIONS

The stakes are high for ship operators where they would be found to be non-compliant with the provisions of the BWM Convention, not only could they be facing heavy fines but the could also be risking criminal prosecution. Consequently, adequate and reliable inspections and monitoring programs will inevitably be an important feature of the Convention. In order for ballast water samples to be used as evidence and basis for enforcement actions they need to be statistically representative and live up to a high level of confidence.[82] In this quest, many issues need to be taken into consideration, issues that are likely to give rise to difficult legal obstacles once the Convention enters into force.

The G2 guidelines and G2 guidance circular for trial use provide a great basis for the continuing work. However, without validated methodologies, evidentiary use will be very difficult and open to legal challenge. Considering the experiences from DNA profiling and the conclusions regarding the features of successful use of scientific evidence, a successful sampling regime for ballast water would need to be standardized and harmonized between Port State Controls around the world. Although several methods for analysing ballast water samples are showing potential, as long as they are not applicable across the board, on all species and in all salinities etc., establishing a protocol for general application in many jurisdiction could prove to be very difficult. And as has been seen in the case of DNA profiling, the establishment of administrative protocols and formal recommendations, both for the actual analysis of samples and for their handling upon collection, transport to laboratories, storage etc. are important factors of success in this context. This is also supported by experiences from drinking water regulations, where sampling results have been known to vary due to sampling and analytical recovery errors. Standardized sampling and analytical protocols have allowed for the establishment of comprehensive models and quantifications of all potential sources of error.[83] The statistics underlying DNA profiling was one if its most criticized aspects and only the development of well-established quantitative estimates of random match probabilities and results as low as approaching zero could silence the critique. For ballast water sampling, is has yet to be determined what levels of uncertainty can be reached and what statistical power should be required, something that undeniably would be an important step towards gaining acceptance.

Currently, the most robust methodology is visual inspection, and it has several advantages such as being easily verifiable through, for instance, photography of samples in microscopes. However, it is not the universal solution that will work across the board, neither is any of the other available methods and it is quite evident that a "one-fits-all" solution, at least at the moment, is eluding us. Ballast water sampling has a long way to go before reaching the success and acceptance that DNA profiling has seen and to further the full ratification and subsequent entry into force of the convention and perhaps it is time to consider alternate solutions until more "workable" techniques are readily available. Surrogate parameters could perhaps be considered or current requirements could be revised, for example, it has been suggested that a single size-class determination of viability and extrapolation from the most sensitive to

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[76] David, Matej, and Gollasch, Stephan. (2011) Representative ballast water sampling for ballast water management compliance monitoring. Proceedings of 14 ICTS, Portoroz, Slovenia. p. 3.
[77] Carney, Katharine J., *et al.* (2013), p. 104.
[78] Carney, Katharine J., *et al.* (2013), p. 100.
[79] Miller, A. Whitman, *et al.* (2011), p. 3544.
[80] Carney, Katharine J., *et al.* (2013), p. 104.
[81] Carney, Katharine J., *et al.* (2013), p. 104.
[82] Cabezas-Basurko, Oihane and Mesbahi, Ehsan (2011), p. 184.
[83] Miller, A. Whitman, *et al.* (2011), p. 3540.

other groups could be a possible option.[84] It could also be beneficial to prepare for the highly likely employment of expert testimonies of courts in cases regarding ballast water offences. It is for example suggested that the establishment of an independent expert witness directory would be beneficial, as well as the drafting of handbooks containing guidelines and recommendations, aimed at legal professionals, for determining the scientific validity of sampling procedures and results.

[84] International Maritime Organization (2013), PPR 1/INF.4, p. 7.

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