Effect of organic compounds on the acid-base system of the Baltic Sea

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Contents

Abstract

Calculations related to the marine CO_2 system that are based on alkalinity data may be strongly biased if the contributions of organic compounds are ignored. In coastal seas, the concentrations of dissolved organic matter (DOM) are frequently high and the alkalinity from inorganic compounds is low. In this study, measurements of total alkalinity, total CO_2, and pH were used to determine the organic alkalinity, A_{org}, in water from the central Baltic Sea. A_{org} reached a maximum (> 50 μmol/kg-SW) in the surface mixed layer during the spring bloom but it decreased with depth and approached zero below the permanent halocline. This behavior could be attributed to the decreased pH of the deeper water layers. The data were used to calculate the bulk dissociation constant, K_{DOM}, for marine DOM and the fraction f of dissolved organic carbon (DOC) that acts as a carrier for acid-base functional groups. The pK_{DOM} (7.27) agreed well with the value (7.34) previously estimated in a preliminary study of A_{org} in the Baltic Sea (Kuliński et al., 2014). The fraction of carbon atoms carrying acid-base groups was 17 %, which was higher than the previously reported value (12 %).

Spike experiments using artificial seawater and three different humic/fulvic substances were performed to determine whether the acid-base properties of these substances explained the results of the field study. Measurements of A_{org} at different concentrations (DOC) of the added substances indicated that they were more acidic ($\mathrm{p}K_{\mathrm{DOM}} < 6.5$) than the naturally occurring bulk DOC in the Baltic Sea.

Additionally, the contribution of low molecular weight organic acids, specifically of short chain carboxylic acids was evaluated by measuring seasonal and regional variations in the concentrations of formic, acetic, and propionic acids in the surface water of the Baltic Sea. Total mixing of the waters at the near-coast sampling site (Heiligendamm, Germany) with deeper water masses explained the detection of frequently high saline waters during a time series of weekly sampling. The advanced biodegradation of high molecular weight organic acids, resulted in elevated carboxylic acid concentrations. The maximum concentration of acetic acid, as the most abundant carboxylic acid, was > 11 μmol/L. At the estuary of the Oder River (Szczecin Lagoon, Germany/Poland), a correlation between the elevated acetic acid concentrations (2 μmol/L) and an algal bloom was observed in samples taken along the salinity gradient. This suggested a potential saisonality of A_{org} as a consequence of highly variable DOM composition that derived from the intense biological activity, such that acetic acid concentrations are included to bulk DOM parameters.

A theoretical study confirmed that neglecting the DOM contribution in calculations of the pH (from A_{T} and C_{T}) of marine waters would result in a miscalculation, with 25 %, attributable to short chain carboxylic acids, assuming central Baltic Sea conditions at equilibrium ($p\mathrm{CO}_2 = 400$ μatm) and acetic acid concentrations of the same magnitude as measured in the field studies.

The present study has not only stressed the not negligible importance of A_{org}, but also focused on the contribution of distinct substance classes to DOM. It was shown, that humic and fulvic acids alone are not suitable to represent DOM acid-base properties and other compounds like short chain carboxylic acids with their seasonal and regional variability have to be considered as potential contributors to A_{org}.

Zusammenfassung

Berechnet man das marine CO_2 System auf der Grundlage von Alkalinitätskonzentrationen ohne dabei organische Beiträge zu berücksichtigen, können erhebliche Fehler auftreten, denn in Küstengewässern, wie der Ostsee, stehen sehr geringen anorganischen Alkalinitätskonzentrationen hohe Konzentrationen gelösten organischen Materials (DOM) gegenüber. Die vorliegende Studie umfasst daher Messungen von A_T, C_T und pH-Werten, mit deren Hilfe ein organischer Beitrag A_org bestimmt werden kann. Dabei wurden im Oberflächenwasser während einer Algenblüte Maximalkonzentrationen von $A_\mathrm{org} > 50$ μmol/kg-SW beobachtet, die mit zunehmender Wassertiefe bis unterhalb der Halokline auf ungefähr 0 μmol/kg-SW absinken. Damit liegt die Vermutung nahe, dass ein Zusammenhang von A_org mit dem in tieferen Wasserschichten aufgrund des Abbaus organischen Materials sinkenden pH-Wert besteht. Mit Hilfe eines umfassenden Datensatzes konnte sowohl eine Dissoziationskonstante für die Gesamtheit an DOM als auch der Anteil gelösten organischen Kohlenstoffs (DOC), der Protonen aufnehmen oder abgeben kann, bestimmt werden. Der Wert von $\mathrm{p}K_\mathrm{DOM} = 7.27$ ist dabei vergleichbar mit dem Wert (7.34) einer vorangegangen Studie (Kuliński et al., 2014), die denselben methodischen Ansatz verfolgt, A_org jedoch auf Grundlage eines vergleichsweise geringen Datensatzes bestimmt hat. Der protonierbare Anteil des DOC umfasste im Rahmen der vorliegenden Studie 17 % und liegt damit etwas höher als der bereits veröffentlichte Wert von 12 %.

Um die Bedingungen der Feldstudie im Labor nachzustellen, wurde DOC-freies,

künstliches Meerwasser mit Humin- und Fulvinsäuren in unterschiedlichen Konzentrationen versetzt mit dem Ziel, die Säure-Baseeigenschaften des Ostsee-DOM durch nur eine Substanzklasse zu beschreiben. Infolge der Bestimmung von A_{org} ergab sich dabei eine sehr viel größere Dissoziationskonstante (p$K < 6.5$) als ausgehend von der Feldstudie angenommen wurde.

Als eine weitere Substanzklasse, die potentiell einen kausalen Bezug zu A_{org} hat, wurden darüberhinaus kurzkettige Karbonsäuren und ihr saisonal und regional variierendes Verhalten untersucht. Dabei konnten während einer Zeitserie erhöhte Konzentrationen ($c_{\mathrm{max}} > 11$ μmol Essigsäure/L) von Ameisen-, Essig- und Propionsäure insbesondere im Zeitraum zweier Salzwassereinströme aus der Nordsee detektiert werden. Grund dafür ist der verstärkte Abbau hochmolekularer gelöster organischer Substanzen in tiefen salzreichen Wasserschichten, die nach vertikaler Durchmischung aufgrund der küstennahen Probennahmestation (Heiligendamm, Deutschland) auch in das beprobte Oberflächenwasser gelangen. Ebenfalls erhöhte Essigsäurekonzentrationen wurden während einer Algenblüte im Oderästuar (Stettiner Haff, Deutschland/Polen) gemessen (2 μmol/L). Diese Korrelation lässt eine saisonale Varianz von A_{org} vermuten, da sich die Zusammensetzung des DOM infolge biologischer Aktivität ändert und pK_{DOM} als Gesamtdissoziationskonstante kurzkettige Karbonsäuren der Definition nach einschließt.

Der potentielle Beitrag niedermolekularer organischer Substanzen zum Säure-Base System war im Rahmen der Feldstudien zwar nicht signifikant festzustellen, konnte jedoch mit einer theoretischen Studie bestätigt werden. Rechnerische Voraussetzungen waren dabei eine konstante Konzentration von DOC (300 μmol C/L), ein marines System, das im Gleichgewicht mit der Atmosphäre ist ($p\mathrm{CO}_2 = 400$ μatm), und die Verwendung von Essigsäurekonzentrationen, die mit denen der Feldstudien vergleichbar sind. Im Ergebnis konnte der Anteil kurzkettiger Karbonsäuren (exemplarisch Essigsäure) zur Fehlkalkulation von pH Werten (aus A_{T} und C_{T}) aufgrund nicht berücksichtigter organischer Beiträge mit 25 % bestimmt werden.

Die vorliegende Studie hat in ihren einzelnen Teilen nicht nur unterstreichen können, dass A_{org} in alkalinitäts-basierten Berechnungen von marinen CO_2-Systemen nicht zu vernachlässigen ist, sondern überdies die Zusammensetzung von DOM in den Blick genommen. Dabei zeigte sich, dass für A_{org} Humin- und Fulvinäuren allein nicht repräsentativ verwendbar sind, sondern darüberhinaus z.B. niedermolekulare Substanzen wie kurzkettige Karbonsäuren berücksichtigt werden müssen, da sie einen saisonalen und regionalen Einfluss auf A_{org} haben können.

1 Introduction

The ocean is one of the planet's largest sinks for CO_2 of either anthropogenic or geologic origin. Although amounting only to 0.04 % in the atmosphere, CO_2 is, after water vapor, the most effective greenhouse gas. Compared to preindustrial times, the atmospheric CO_2 concentration has increased by 40 % (IPCC, 2007), mainly due to fossil fuel burning, cement production (minor) (Sabine et al., 2004), and deforestation (Houghton and Hackler, 2002). With a CO_2 inventory 50 times larger than that of the atmosphere (Archer, 2005), the ocean can presumably buffer changes in atmospheric CO_2 concentrations with only marginal effects on the marine environment. However, despite this large buffering capacity, the marine system is in fact influenced by the absorption of CO_2, including in many ways that cannot yet be predicted.

Coastal waters are particularly vulnerable to the effects of an elevated atmospheric CO_2 level. Omstedt et al. (2009) showed that the Baltic Sea, a CO_2 source during preindustrial times, now alternates as both source and sink. Moreover, coastal waters are very sensitive to anthropogenically related environmental changes, because: (1) they are strongly influenced by land use in the catchment areas, which are surrounded by numerous CO_2 emission sources and (2) their response time is shorter than that of oceans. For research purposes, however, coastal waters are ideal study sites to investigate the impact of CO_2 because by definition they exhibit a broad range in properties under conditions ranging from oceanic to freshwater.

Despite many experimental and theoretical analyses in coastal and brackish waters, elevated marine CO_2 concentrations have yet to be confirmed, because the large seasonal variation of the marine carbonate system masks the more subtle trends reflecting the effects of increasing CO_2 (Schneider, 2011). Consequently, investigations of ocean acidification conducted in coastal waters remain challenging. Nonetheless, there are many ongoing studies of the marine CO_2 system aimed at understanding the oceanic uptake of anthropogenic CO_2, including ocean acidification phenomena, and at unraveling biogeochemical processes that are linked to the consumption and production of CO_2.

1.1 Theoretical background

The marine acid-base system mainly constitutes inorganic dissolved carbon species and is thus also referred to as the marine CO_2 system. The dissolution of atmospheric CO_2 at the ocean surface (Eq. 1.1) is followed by the formation of carbonic acid (Eq. 1.2), which is immediately dissociated into bicarbonate and carbonate ions (Eq. 1.3, 1.4), releasing a proton.

$$CO_{2\ (g)} \rightleftharpoons CO_{2\ (aq)} \tag{1.1}$$

$$CO_{2\ (aq)} + H_2O_{(l)} \rightleftharpoons H_2CO_{3\ (aq)} \tag{1.2}$$

$$H_2CO_{3\ (aq)} \rightleftharpoons HCO^-_{3\ (aq)} + H^+_{(aq)} \tag{1.3}$$

$$HCO^-_{3\ (aq)} \rightleftharpoons CO^{2-}_{3\ (aq)} + H^+_{(aq)} \tag{1.4}$$

For each of these equilibria, equilibrium constants can be expressed as follows:

$$\frac{[HCO_3^-][H^+]}{[CO_2^*]} = k_1 \tag{1.5}$$

$$\frac{[CO_3^{2-}][H^+]}{[HCO_3^-]} = k_2 \tag{1.6}$$

$$\frac{[CO_2^*]}{pCO_2} = k_0 \tag{1.7}$$

Since the concentration of H_2CO_3 is very low ($\lesssim 0.3$ %) (Zeebe and Wolf-Gladrow, 2001), $[H_2CO_3]$ and $[CO_2]_{(aq)}$, can be added up in the term of $[CO_2^*]$; k_0 is the solubility constant.

Four measurable key variables are used to characterize the status of the marine CO_2 system: total alkalinity (A_T), total CO_2 (C_T), the CO_2 partial pressure (pCO_2), and the pH. In general, any two of these variables are sufficient to determine the other two as well as further properties of the CO_2 system on the basis of the carbonic acid dissociation constants. The constants have to be known with sufficient accuracy and as a function of temperature, pressure, and salinity.

The definition of alkalinity applied in this study was introduced by Dickson (1981) as: "...the number of moles of hydrogen ion equivalent to the excess of proton acceptors (bases formed from weak acids with a dissociation constant $K \leq 10^{-4.5}$ at 25 $°C$ and zero ionic strength) over proton donors (acids with $K > 10^{-4.5}$) in one kilogram sample." The corresponding equation is shown as follows:

$$\begin{aligned} A_T = {} & [HCO_3^-] + 2[CO_3^{2-}] + [B(OH)_4^-] + [OH^-] + [HPO_4^{2-}] \\ & + 2[PO_4^{3-}] + [SiO(OH)_3^-] + [NH_3] + [HS^-] + ... \\ & - [H^+]_F - [HSO_4^{2-}] - [HF] - [H_3PO_4] - ... \end{aligned} \tag{1.8}$$

The concentration of $B(OH)_4^-$ is a relative parameter and is calculated from measured salinity (Culkin, 1965).

Total CO_2 (C_T), or total dissolved inorganic carbon (DIC), is the sum of all DIC species:

$$C_T = [CO_2^*] + [HCO_3^-] + [CO_3^{2-}] \tag{1.9}$$

The $p\mathrm{CO}_2$ is a thermodynamic property of a solution of CO_2 in water. It is equal to the $p\mathrm{CO}_2$ in a gas phase that is at equilibrium with the solution. For simplicity, CO_2 is treated in this work as an ideal gas, to circumvent the expression of fugacity, replacing it with the partial pressure instead (Weiss, 1974). Although partial pressure cannot be measured, it is calculated from the mole fraction measured in the gas phase and can be reformulated from Eq. 1.7:

$$pCO_2 = \frac{[CO_2^*]}{k_0} \tag{1.10}$$

The pH is defined as the negative, decadal logarithm of the hydrogen ion concentration.

$$pH = -log[H^+] \tag{1.11}$$

Depending on the applied pH scale, the hydrogen ion concentration $[\mathrm{H}^+]$ comprises:

- $[H^+]_\mathrm{F}$ – free scale
- $[H^+]_\mathrm{F} + [HSO_4^-]$ – total scale
- $[H^+]_\mathrm{F} + [HSO_4^-] + [HF]$ – seawater scale

The subscript F denotes free protons. In this study, for all experiments and calculations, the total scale was applied and the hydrogen ion concentration was thus defined according to Hansson (1973) as:

$$[H^+] = [H^+]_\mathrm{F}\ (1 + \frac{S_\mathrm{T}}{K_\mathrm{S}}) \tag{1.12}$$

where S_T denotes the total concentration of sulfate ions and K_S is the dissociation constant of HSO_4^-.

1.2 The marine CO_2 system: Ocean vs. Baltic Sea

Ocean

The marine carbon cycle in the oceans can be divided into three pump-based mechanisms: the solubility pump, the organic carbon pump, and the carbonate pump (Hansell and Carlson, 2014). The solubility pump consists of the carbon species $CO_2(g)$, $CO_2(aq)$ and $H_2CO_3(aq)$ and it is driven by the solubility constant expressed by Henry's law (Eq. 1.7, 1.10). Since $CO_2(g)$ is more soluble in cold water, C_T-rich surface water is formed at higher latitudes. At low temperatures, high-density water sinks and thus "pumps" atmospheric carbon down into the deep ocean. Furthermore, the carbonic acid dissociation constants depend on pressure, salinity and temperature and consequently contribute to dissociation equilibria resulting in elevated uptake of CO_2 at low temperatures.

The second conceptual pump, the organic carbon pump, comprises the transformation of DIC to organic carbon; the latter consists of dissolved organic carbon (DOC) and particulate organic carbon (POC). DIC species are consumed, thus promoting primary production, by e.g., photosynthesis. This in turn leads to partial sinking of organic bound carbon to deep water layers. Although slightly degraded and remineralized on its path downwards, up to 6 % of DIC (or C_T) reaches the bottom water (Dunne et al., 2007).

The third (carbonate) pump describes the biogenic precipitation of Ca^{2+} and Mg^{2+} carbonates by coccolithophorids, foraminafera, pteropods, and corals (Zeebe and Wolf-Gladrow, 2001). Carbonates are formed in the upper mixed layer above the lysocline which is the boundary layer, where saturation decreases an thus, the dissolution rate of calcite increases with water depth and pressure. About 50% of these precipitated carbonates redissolve before reaching the deep ocean (Feely et al., 2004), thus releasing CO_3^{2-} and increasing both C_T and especially A_T.

Baltic Sea

In general, the CO_2 carbon cycle of the ocean and Baltic Sea are analogous, but the Baltic Sea has several particularities that must be considered. The Baltic Sea is located between the latitudes 54°N and 66°N and the longitudes 10°E and 28°E. Because it is semi-enclosed, its waters are brackish or coastal. Consequently, both river runoff and the atmosphere have a greater impact than is the case in the North Sea, which is connected to the Baltic Sea by the Kattegat and Skagerrak. The limited inflow of oxygen-rich, high-density saline water from the North Sea gives rise to several phenomena controlling hydrographical characteristics unique to the Baltic Sea. The shallow and narrow topography of the Baltic seafloor, with prominent sills and basins in its southern portion, restrains horizontal mixing of the different water masses entering the sea from different origins (precipitation, rivers, North Sea). The result is a stratification of Baltic Sea waters with respect to salinity (halocline). Additionally, stratification develops as a result of seasonal temperature variations (thermocline). Both phenomena inhibit vertical mixing. Periods of stagnation, characterized by suboxic or anoxic conditions in the deep waters of the central Baltic Sea, develop when inflows from the North Sea are rare. Oxygen depletion in deep water is due to the heterotrophic degradation and respiration processes that oxidize organic material.

From the southwest to the northeast, a surface salinity gradient is the result of the high impact of river runoff. The southwestern Baltic is mainly influenced by the high saline water $S = 35$ from the North Sea whereas in the most northern part, Bothnian Bay, salinity is at a minimum ($S = 2$). The 1978 definition of salinity is based on only physical parameters (Müller, 2007); thus, unlike the ocean, where the ion composition is stable, salinity measurements in the Baltic Sea do not describe potential changes in its ionic composition. Alkalinity, as a key parameter of the marine acid-base system, is closely linked to salinity. Alkalinity is mainly influenced by river runoff into the Baltic Sea's bays and differs depending on the nature of the catchment area and its weathering behavior (Beldowski et al., 2010). In the same study, a distinct linear $A_\mathrm{T} - S$ correlation for each of the four different areas of the Baltic Sea was introduced. The different behaviors in the catchment areas of the Gulf of Bothnia, Gulf of Riga, Gulf of Finland, and the central Baltic Sea, including the transition to the North Sea via the Skagerrak and Kattegat, are a function of geological conditions and are described by the different $A_\mathrm{T} - S$ correlations. Autochthonous processes will also increase or decrease the alkalinity of the Baltic Sea's waters. This was demonstrated in the studies by Brewer and Goldman (1976) and Goldman and Brewer (1980), who showed an increase in alkalinity following $\mathrm{NO_3^-}$ uptake by phytoplankton. In these organisms, charge balance is maintained by the contemporaneous release of $\mathrm{OH^-}$ or the uptake of $\mathrm{H^+}$.

A side effect of intensive agricultural utilization of ground water is that the rivers become enriched in nutrients, such that river runoff also elevates the nutrient supply, which is called eutrophication. Phosphate and nitrate are strong enhancers of primary production; their effects are manifested as extensive algal blooms in coastal waters during spring and summer months. The mineralization of organic material in reactions that consume oxygen cause oxygen depletion in deeper water layers, and is reinforced by the natural stratification (thermocline, halocline), since mixing with oxygen rich waters is inhibited.

Finally, rivers entering the Baltic Sea account for large inputs of dissolved organic matter (DOM, measured as DOC), which influences both carbon and nutrient cycling. For example, in summer, DOC concentrations in the Vistula river reach 783 $\mu\mathrm{mol\,C/L}$ (to facilitate comparability, the units in the original study were converted; Pempkowiak (1985)) whereas the maximum values for the proper Baltic Sea are $\sim$ 330 $\mu\mathrm{mol\,C/L}$. This latter value is still three to five times higher than the maximum concentrations measured in the oceans (60 − 90 μmol C/L, Hansell and Carlson (2014)). Pempkowiak and Kupryszewski (1980) estimated that 50 % of Baltic Sea DOM was of terrestrial origin. The large DOM concentrations are one of the most important differences between coastal waters and open oceans. They are considered in detail in the present study.

1.3 Dissolved organic matter

1.3.1 What is dissolved organic matter?

The marine pool of DOM is one of the largest reservoirs of organic matter on Earth (Mopper and Degens, 1979). Its main sources are extracellular phytoplankton, the products of grazing and cell lysis, the solubilization of particles, and prokaryote activity. DOM sinks are generated by biotic consumption (prokaryotes and eukaryotes) as well as by abiotic removal, e.g., phototransformation or sorption onto particles. (Carlson and Hansell, 2014)

But what is DOM? Its definition, the fraction of organic matter that passes through a filter (Hansell and Carlson, 2014), is very vague as neither the pore size nor the filter material is specified. Detailed knowledge of the constitution of DOM as a carrier of functional groups is crucial for understanding its role in marine carbon and nutrient cycling.

Several different methods can be used to more accurately describe DOM. First, it can be separated by size, into high molecular weight (HMW) and low molecular weight (LMW) DOM fractions, most commonly by ultrafiltration. Second, it can be separated by solid-phase extraction (SPE, Dittmar et al. (2008)) into hydrophobic and hydrophilic compounds. A third approach is to classify DOM according to its fate and origin. For example, in coastal waters such as those of the Baltic

Sea, the supply of allochtonous terrestrial DOM from rivers is just as important as autochtonous marine-derived DOM. Alternatively, DOM can be categorized as labile, semi-labile, and refractory when its stability and the determination of mineralization rate constants are of interest (Kuliński et al., 2016).

Nonetheless, a constant challenge in analyzing DOM is that it comprises an enormous variety of compounds with different chemical properties. Thus, depending on its origin, DOM is a diverse mixture of small, simple biochemicals (amino acids, simple sugars, vitamins, fatty acids), complex biopolymers (proteins, polysaccharides, lignins), and complex humic substances and black carbon with still undefined structures (Repeta, 2015).

Current analytical methods allow the molecular characterization of DOM using sophisticated devices, including ^{13}C and ^{1}H NMR, both of which provide information about the different functional groups and the elemental composition of the samples, and chromatographic separation via packed or coated columns. The optimal chromatographic method (gas or liquid chromatography) and column material depend on the target analyte; its retention time will be a product of its interaction with the packed material, allowing both the identification and quantification of the compound. A more sensitive method to identify organic compounds is to subject the chromatographically separated compounds to mass spectrometry. The mass to charge ratio (m/z) and fragmentation behavior provide structural information that enable the precise identification of the compounds eluted from a chromatographic column.

Despite these sophisticated methods, they do not provide a complete description of the whole DOM pool and the choice must often be made about ”knowing a lot about a little” (molecular parameters) or ”knowing a little about a lot” (bulk parameters).

1.3.2 High molecular weight organic acids

DOC consists of at least 50 % of humic substances, which can be subdivided into humins (insoluble), humic acids (soluble $\mathrm{pH} > 2$), and fulvic acids (soluble throughout the whole pH range) (Hessen and Tranvik, 2013). Since the focus of this study is the marine environment, the properties, sources, and fates of dissolved humic and fulvic acids are examined in greater detail.

Humic and fulvic acids are heterogenous macromolecules with molecular masses ranging from 500 to 100 000 Dalton (Hessen and Tranvik, 2013). They result from the microbial degradation of organic matter released by plants or animals and are, if not further degraded, biologically unavailable. Aquatic humic and fulvic substances can be allochthonous or autochthonous, which can be distinguished based on their aromaticity and the proportions of lignins (Tan, 2014). During the degradation of organic matter such as lignins, which are terrestrial humic substance precursor, carboxylic acid functional groups are predominantly formed. Because they are able to release a proton, humic substances are relevant for the acid-base system of aquatic environments.

Fulvic acids account for the majority of humic substances in natural waters with a mean fulvic/ humic acids ratio of 2.6 Tan et al. (1991). Because of the above-described differences in their solubility, humic substances can be easily separated. The procedure for extracting humic substances developed by Thurman and Malcolm (1981) and adopted by Aiken (1985) became standardized procedure of the International Humic Substance Society (IHSS). The procedure roughly comprises first, the filtration of the water sample through a filter with a pore size $> 0.45\ \mu\mathrm{m}$ to remove insoluble components. Afterwards, the sample is acidified to $\mathrm{pH} = 2$ and humic and fulvic acids are extracted by XAD-8 gel column, that was prepared by several conditioning steps in advance. With 0.2 M NaOH solution, the retained sub-

Table 1.1: Summary of basic characteristics of humic and fulvic acids.

	Humic acids	Fulvic acids
Molecular weight	high	low
Color	brown - black	fulvus (lat.) - golden/yellow
Aromaticity	high	low
Acidity	low	high
Proportion of N	high	low

stances can be eluted and humic acids are precipitated by adjusting the brownish solution to pH = 1 using HCl. Humic and fulvic acids can be separated by centrifugation (10 000 rpm, 15 min) (Tan, 2014). The basic characteristics of humic and fulvic acids are summarized in Table 1.1. The abundance of humic substances in seawater is closely related to the level of primary production and is thus highly variable (Rashid, 2012). As their abundance is coupled to DOC, the highest seawater concentrations of humic substances occur in the water layer between 0 and 200 m (Lisitzin and Rodolfo, 1972). Humic substances also accumulate in coastal waters and river plumes (Rashid, 2012), but this elevated proportion is not necessarily attributable to higher terrestrial organic matter loads (Repeta et al., 2002). In their study of coastal salt marshes in the southeastern USA, Moran and Hodson (1994) determined that, after SPE with and XAD-8 gel column, humic substances accounted for 37 % of total DOC. Repeta et al. (2002) similarly determined a 30 % contribution of humic substances to HMWDOM in the surface seawater of Woods Hole (MA, USA).

1.3.3 Low molecular weight organic acids

In addition to HMW organic acids, smaller, organic carbon molecules such as amino acids and carboxylic acids may influence the marine acid-base system. Amino acids and substances with amino groups act as proton acceptors without realeasing a proton and thereby increase the alkalinity of the medium. Since amino acids (usually measured as dissolved free amino acids) in DOM do not exceed concentrations of 1 μmol/L (Mopper and Lindroth, 1982), they were not further evaluated in this study.

As shown in Fig. 1.1, short chain carboxylic acids, such as acetic acid, have a pK between 4 and 5 and thus their anions (in this case acetate) can hardly be considered as proton acceptors (p$K > 4.5$) as introduced by Dickson (1981). Nevertheless, they occur in concentrations higher than those of amino acids, although their impact on the marine acid-base system is unclear.

There is little information in the literature on marine concentrations of LMW carboxylic acids in the water column, since most studies have instead focused on their major role in anaerobic pathways within sediments. Albert et al. (1995) measured maximum surface concentrations in the Black Sea of 2.1 μmol/L for acetate and 8.1 μmol/L for formate. Yang et al. (1993) similarly reported 2.56 μmol/L for acetate as well as 0.26 μmol/L for propionate in Flax Pond (NY, USA) water samples. In the present study, these three acids also had the highest concentrations and were thus chosen for further investigations.

Short chain carboxylic acids (SCCA) derive from microbial degradation of high molecular weight organic acids (HMWOA, (Kieber et al., 1989; Bertilsson and Allard, 1996)), with photooxidation as an additional pathway contributing to the release of LMW organic acids from DOM (Brinkmann et al., 2003; Ho et al., 2002). Both pathways provide bioavailable degradation products that promote the growth of heterotrophic organisms. Alternatively, these products may be mineralized, forming CO_2. Ho et al. (2002) showed that the organic carbon released by primary producers correlates with heterotrophic activity. Thus, the reutilization of organic degradation products by bacteria was expected. Formic and acetic acid may also derive from atmospheric sources, in the form of dry aerosols (Morikami

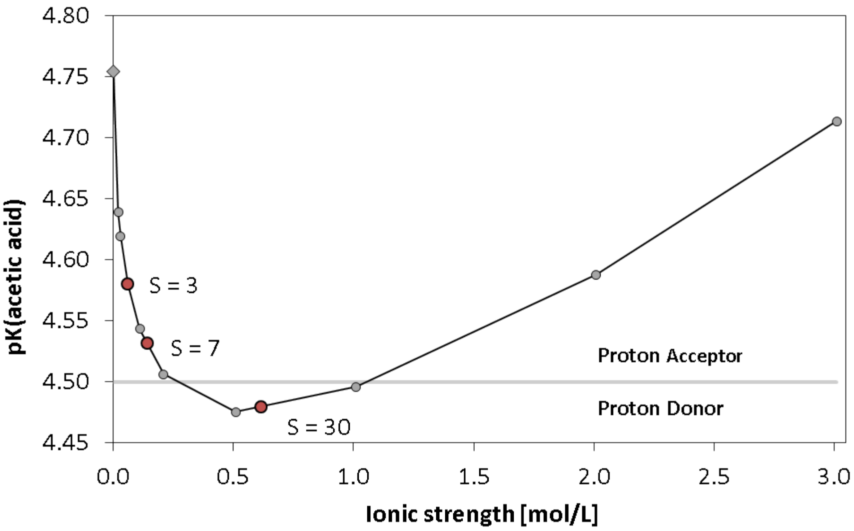


Figure 1.1: Dissociation constants of acetic acid/acetate at 20 °C and different ionic strengths, calculated based on the equation published by Harned and Hickey (1937).

et al., 1993; Khare et al., 1999) and precipitation (Keene et al., 1989). Loads of either one tend to be larger near the shore. However, there is still scant knowledge of LMWOA and their fate in organic carbon cycling are still pending (Ho et al., 2002).

1.4 Contribution of dissolved organic matter to alkalinity

The following section also draws on parts reported in a manuscript entitled "Acid-base properties of Baltic Sea dissolved organic matter", by Karoline Hammer, Bernd Schneider, Karol Kuliński, Detlef E. Schulz-Bull. The manuscript was submitted to the Journal of Marine Systems.

Alkalinity is defined in Eq. 1.8 and differs from the other three key parameters

(C_T, pCO_2, and pH) since it refers to the status of all acid-base components in seawater. In ocean water, except for the phosphate and silicate in deep stagnant waters, borate is the only other relevant contribution besides carbonate to A_T. The contribution by organic proton acceptors to A_T can be neglected because of their very low concentrations with respect to high alkalinity of $\sim$ 2000 μmol/kg-seawater (SW) in ocean water. However, the ellipses in Eq. 1.8 represent the contribution of minor acids and bases to alkalinity, thus, the calculation allows the inclusion of conditions different from those in the ocean, as is the case in estuarine and coastal waters (Fig. 1.2). In both, DOM concentrations are high (Section 1.2) and the alkalinity in some regions of the Baltic Sea may be as low as a few 100 μmol/kg-SW. The potentially large share of organic compounds contributing to the acid-base system is such that the effect on A_T is potentially large. Several studies have described the substantial impact of DOM on A_T (Cai et al., 1998; Hruška et al., 2003; Hongve, 1990; Yang et al., 2015; Hernández-Ayón et al., 1999, 2007; Kim and Lee, 2009). Kuliński et al. (2014) determined the A_T, C_T, pH, and pCO_2 of seawater samples spiked with DOC extracts from river water. They found large differences between the measured pCO_2 and the pCO_2 calculated from A_T and C_T assuming only the presence of inorganic A_T (carbonates and borate) and ignoring the organic proton acceptors in the chemical A_T model they used in their calculations. The $26 - 56$ % lower pCO_2 correlated with the added amount of DOM, which clearly affected the alkalinity. Therefore, the values determined for the CO_2 system in waters with high DOM loads may be questionable if the calculations include measurements of A_T limited to only inorganic components. This consideration is also relevant to biogeochemical models that use the conservative variables A_T and C_T to simulate the marine CO_2 system. However, current knowledge of the complex composition and structure of DOM does not yet allow the inclusion of the acid-base properties of individual organic acids into numerical

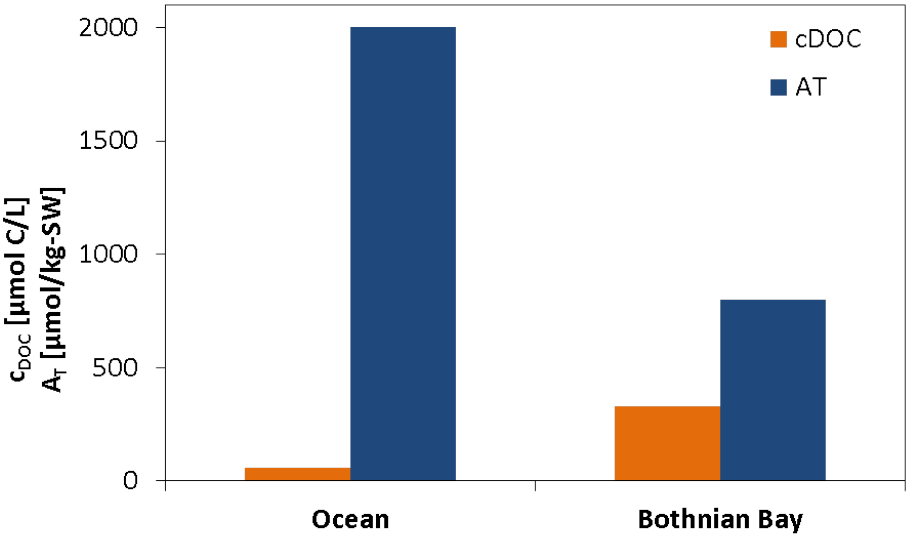


Figure 1.2: Comparison of the dussolved organic carbon concentration (orange) and total alkalinity (blue) in oceanic vs. brackish conditions in the Bothnian Bay.

models. Nevertheless, Ulfsbo et al. (2015) performed a theoretical approach by fitting modeled titration data of fulvic acids under brackish conditions, assuming them to account for the major acid-base effective substance class.

Instead, a more practical approach was suggested by Kuliński et al. (2014) that was applied before by Hernández-Ayón et al. (1999); Kim and Lee (2009); Köhler et al. (2000) and Muller and Bleie (2008). Those authors characterized the effect of organic compounds on A_T by including the term A_org, defined as the difference between the measured A_T and the A_T calculated from measurements of C_T and pH. The latter corresponds to the inorganic alkalinity since organic contribution is ignored:

$$A_\mathrm{org} = A_\mathrm{T(measured)} - A_\mathrm{T(calculated)} \tag{1.13}$$

In case of elevated nutrient concentrations (phosphate and silicate) a contribution has to be considered for the calculated A_T.

In addition, Kuliński et al. (2014) defined a bulk dissociation constant, K_{DOM}, for the DOC fraction f that acts as a carrier of acid-base groups and therefore has the potential to generate A_{org}. On the basis of the principal mass action law,

$$K = \frac{[\mathrm{H^+}]\ [\mathrm{Org^-}]}{[\mathrm{HOrg}]} \tag{1.14}$$

organic anions Org$^-$ can now be illustrated by A_{org}, calculated from three different key parameters. The undissociated (HOrg) species can be represented by the share of DOC that acts as a carrier of functional groups ($f\ c_{\mathrm{DOC}}$) reduced by the proportion that was found deprotonized at a distinct pH. The corresponding apparent dissociation equilibrium including the above introduced terms, may then be expressed as:

$$K_{\mathrm{DOM}} = \frac{[\mathrm{H^+}]A_{\mathrm{org}}}{f\,c_{\mathrm{DOC}} - A_{\mathrm{org}}} \tag{1.15}$$

By measuring A_{T}, C_{T}, and pH of Baltic Sea surface water, Kuliński et al. (2014) determined A_{org} according to Eq. 1.13 and used Eq. 1.15 with the parallel measurements of c_{DOC} to obtain f and K_{DOM}. However, the study was based on a rather limited set of samples ($n = 6$) and must be considered as a preliminary attempt to quantitatively account for the effect of organic compounds on the acid-base system of the Baltic Sea. Based on the results of pilot studies, their study sought to fill in the knowledge gaps regarding the role of dissolved organic compounds in the marine acid-base system of the Baltic Sea. The present work seeks to extend this knowledge through four main objectives, described in the following.

1.5 Objectives

The primary aim of this study was the quantification, evaluation, and parameterization of the contribution of organic compounds to alkalinity and thus to the acid-base system in the Baltic Sea. However, a method for the determination of the precise alkalinity that is also applicable to brackish water, not just to open ocean, needs to be formulated to allow its use in biogeochemical modeling. A_T and C_T are conservative parameters that are the preferred input parameters in model calculations, since, unlike pH measurements, validation using certified reference material is possible.

The neglect of non-inorganic compounds results in a miscalculation that reflects a shift in A_T. Although the absolute value of total A_T itself will not be affected (or at most insignificantly), carbonate alkalinity will be overestimated such that calculations of pH and $p\mathrm{CO}_2$ may be biased. By measuring all four of the above-described key parameters and comparing the measured and calculated results, Kuliński et al. (2014) showed that the pH of Baltic Sea coastal waters was overestimated by 0.4 pH units.

Depending their concentrations and dissociation constants, carboxylic, fulvic, and humic acids, as representatives of LMW- and HMWOAs, influence the acid-base system of coastal waters such as those of the Baltic Sea. The compounds of interest in this work and their relationships are shown schematically in Fig. 1.3. Contrary to Kuliński et al. (2014), I had access to a large amount of data, aiming to extend the recent study. Furthermore, I focused on different substance classes to attribute the magnitude effecting the alkalinity as a result of organic bases formed from weak acids.

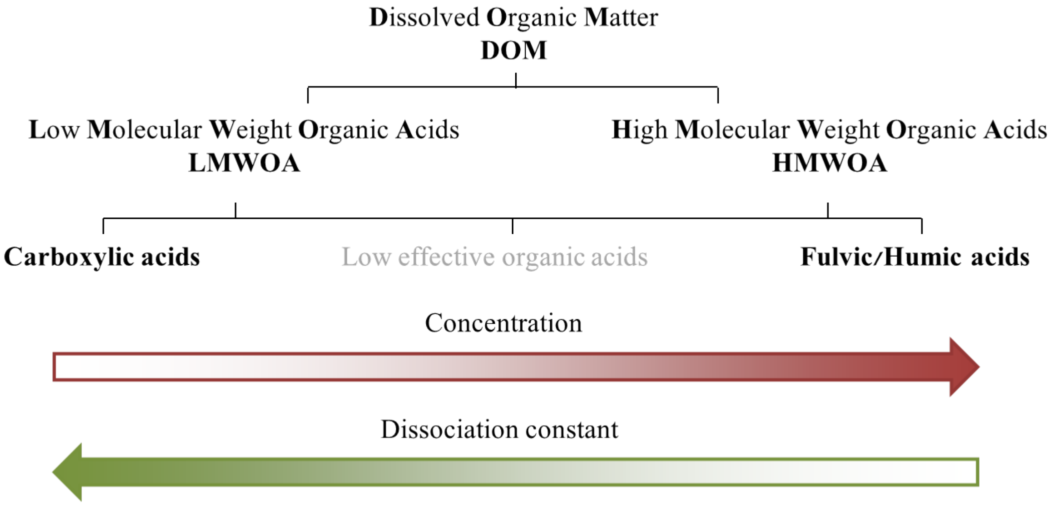


Figure 1.3: Compounds of interest in this study and their relationships.

Two extremes were chosen for investigation:

- Humic and fulvic acids, known for relative low dissociation constants, thus comprising a moderate share of anions at marine pH values, however, assumed to have a distinct influence because of their high concentrations
- Carboxylic acids, occurring mainly in small concentrations, but as a result of high dissociation constants, are mainly present in their deprotonated form and potentially acting as a proton acceptor.

The result of the present study should encourage researchers to consider the properties of organic matter, when dealing with marine CO_2 calculations and models. Four sub-objectives of the study are defined in the following. First the values of the bulk parameter $\mathrm{p}K_{\mathrm{DOM}}$ and of f from natural organic DOM occurring in the central Baltic Sea were determined using data obtained during seven cruises carried out for two years (2014/2015), during which samples were obtained from biogeochemical important stations in the Baltic Sea. The large dataset generated from depth-profile data and collected during this 2-year period covers broad pH and salinity ranges and thus representative conditions in the surface waters of the Baltic Sea during the different seasons. As shown by Kuliński et al. (2014), the dissociation status of organic compounds can be assumed by overestimation of the CO_2 system, by measuring more than two key parameters in combination with c_{DOC}. With the larger dataset available for the present study, I sought to validate the initial but sparse results reported by Kuliński et al. (2014).

Second, because humic and fulvic acids account for $40-60$ % of DOM (Hessen and Tranvik, 2013), they will have the greatest influence on the acid-base system (Cai et al., 1998) but their contribution to it has been largely neglected. In this study, extracted natural humic and fulvic acids, as model substances, were spiked into DOC-free artificial seawater containing carbonate species and adjusted to brackish seawater conditions. Measurements before and after spiking provided information about the changes in the carbonate system and therefore estimations of the acid-base properties of the model substances. The aim of this experiment based on simulated natural conditions was to investigate the dissociation behavior of DOM representatives.

The third sub-objective of this study was to quantify SCCAs and estimate their impact on the CO_2 system. Despite their low concentrations, carboxylic acids have low pK values and are therefore assumed to contribute to the alkalinity provided by organic compounds. In the Black Sea, acetic acid concentrations between 5 and 80 μmol/L (Mopper and Kieber, 1991) throughout the whole water column have been measured. For the Baltic Sea, as similar data are lacking, seasonal and regional variation in SCCA concentrations were determined in its coastal regions using weekly samples collected from the pier in Heiligendamm (near Rostock, Germany) from winter to autumn. In addition, two river estuaries (Vistula, Oder) were sampled during a research cruise in May 2015. Parallel carbonate, nutrient, and DOC sampling enabled a principal component analysis to support the evaluation of the fate and relationships of carboxylic acids.

Finally, the last chapter of this thesis comprises a theoretical study focusing on the miscalculation of pH that may occur when organic substances are neglected as alkalinity contributors. Therefore, realistic concentrations of organic acids were considered in different regional scenarios describing the inorganic CO_2 system. In addition, A_{org} calculated from measured A_{T}, C_{T}, and pH was compared with A_{org} calculated according to the determined $\mathrm{p}K_{\mathrm{DOM}}$ and f of Baltic Sea organic matter, applying my own results in a preliminary theoretical evaluation of organic substances.

By considering the contribution of dissolved organic compounds to the marine CO_2 system in the Baltic Sea in four different empirical and theoretical approaches, this thesis provides detailed insights that can be extrapolated to other marine coastal systems.

2 Material and Methods

2.1 Inorganic CO_2 parameters

Three (A_T, C_T, and pH) of the four ($p\mathrm{CO}_2$) parameters that characterize the acid-base system can be determined from discrete samples. Prior to sample addition, 250 mL Schott® Duran glass bottles were flushed three times with sample water. The samples were added to the bottles via a silicon tube connected to the CTD Niskin bottle. As the tube reached to the bottom of the sample bottle, extensive gas exchange with the atmosphere was circumvented. The samples where then poisoned with 100 μL saturated mercury(II)-chlorid solution, closed tightly with a glass stopper and clamp, and stored in the dark at 5 °C until analyzed onshore.

2.1.1 A_T, C_T, pH, and pCO_2

Total alkalinity (A_T)

The total alkalinity was measured using the open cell method of Dickson et al. (2007), in which a precisely weighed water sample is titrated with 0.1 M HCl Titrisol® solution, prepared with an ionic strength, which is similar to the ionic strength of the sample. First, acid is added to the sample in excess to the equivalence point followed by titration for reconstructing the equivalence point. Parallel to the addition of acid a high sensitive electrode records the change in electromotive force, which together with the value of consumed acid provides a measure of the alkalinity. The measurement was conducted at 15 °C and gives an accuracy and precision of ±3 μmol/kg-SW and ±2 μmol/kg-SW, respectively. The device performance was assessed each proceeding day by measuring carbon reference material (CRM) obtained from the laboratories of Prof. Dr. Andrew Dickson (University of California, San Diego). The results were corrected when necessary.

Total dissolved inorganic carbon (C_T)

Together with pH, the total dissolved inorganic carbon (DIC or C_T) is sensitive to gas exchange with atmospheric CO_2. Thus, these parameters should be measured first or measured using separate sample bottles to avoid biases from the interaction with the gas phase in the headspace. C_T can be determined coulometrically using the SOMMA-system according to Johnson et al. (1993) and designed by marianda (marine analytics and data, Kiel, Germany). In this method, the CO_2 species are driven out of the sample by the addition of phosphoric acid. The gas is transferred into a cell, where it reacts with ethanolamine to form hydroxyethylcarbamic acid, which is then titrated subsequently. The precision and accuracy of the results were both in the range of ±2 μmol/kg-SW each and were validated using CRMs, as described for the A_T measurements. Measurements were conducted at 20 °C.

CO_2 partial pressure (pCO_2)

Usually $p\mathrm{CO}_2$ is measured with an underway system, where a constant water flow is equlibrated with a invariable volume of air. The CO_2 content from this headspace is then determined by infrared-detection with a LI-7000 system (LI-COR$^®$ Biosciences, Lincoln, NE, USA). In this study, artificial seawater of 10 L reservoirs that were prepared for the spike experiments was tested for its equlibrium status. For this purpose, the CO_2 content from the gas phase, equilibrated with the underlying water sample by an aquarium pump at room temperature was determined. Equilibrium with ambient air ensured no significant loss or gain in C_T of the samples during their preparation, before the addition of humic and fulvic acids.

pH

Because pH is also as sensitive as C_T to gas exchange, it was measured spectrophotometrically as soon as possible the sample bottles were opened onshore. The semi-automated spectrophotometric system uses *m*-cresol purple as the indicator, based on its acid-base properties (Carter et al., 2013). Instead of the traditional potentiometric set up, the pH is measured over the scale of natural ionic strength gradient present in the Baltic Sea (Hammer et al., 2014). The influence of the indicator on sample pH was determined using the MATLAB version of CO_2sys (Van Heuven et al., 2011), in which the properties of the dye are considered with concentration and dissociation constant recommended in Hammer et al. (2014). The results were validated by measurements of CRMs certified for A_T and C_T, which enables comparisons of the measured and calculated pH, although only under oceanic conditions. The performance and accuracy of the system were tested as well by a TRIS (tris(hydroxymethyl)aminomethane) buffer-solution with a defined pH and an adjusted salinity comparable to the ionic strength of the Baltic Proper. The pH values of this measurement method were determined at 25 °C and have an accuracy of ±0.01 to ±0.02 and a precision of ±0.00065 pH units (Hammer et al., 2014).

2.1.2 Reagents

A_T was determined by titration with 0.1 M hydrochloric acid prepared in 0.1 M NaCl (purity $\geq$ 99.5 %) solution to assure an in ionic strength similar to that of the mostly brackish samples. Both chemicals were obtained from Merck® KGaA

(Darmstadt, Germany). To obtain an exact concentration, a Titrisol® ampule was used for the preparation of the HCl solution. Furthermore, a density correction was used in favor of highly accurate results.

Measurement of C_T with the SOMMA-system requires the UIC$_\mathrm{INC.}$ cathode and anode solution. Phosphoric acid (30 %, pro Analysis, Merck®) is used to drive the CO_2 out of the sample. The indicator *m*-cresol purple sodium salt, used unpurified in the spectrophotometric measurement of pH, was from TCI Europe (Zwijndrecht, Belgium).

In general, ultra pure water (Milli-Q water), used in all of the experiments, was generated by a Millipore® system. Artificial seawater used for analytical purposes was prepared by adding NaCl, KCl, $NaSO_4$, $NaHCO_3$, and Na_2CO_3 (Merck®, for analysis) to Milli-Q water as salts. Contrary, $MgCl_2$ and $CaCl_2$, (PanReac AppliChem) were added as solutions with defined concentrations prepared from 1 M standard solutions to artificial seawater. Two of the three investigated humic substances, one humic and one fulvic acid (nos.: 2S101H, 1S102F), were from the International Humic Substance Society (IHSS). The third, a humic acid, that served as a technical standard was from Sigma Aldrich (St. Louis, MO, USA).

2.1.3 CO_2sys, a tool for calculations

CO_2sys, developed by Pelletier et al. (2007), is an Excel-based program for calculating the state of the marine CO_2 system when only two parameters are known. For coastal and estuarine waters, the defined parameters were as follows:

- pH scale: total scale
- K_1, K_2 carbonic acid: Millero et al. (2006)
- $K_{SO_4^{2-}}$: Dickson (1990)

- Approximation: CO_2 as an ideal gas; thus, $p\mathrm{CO}_2$ was used instead of $f\mathrm{CO}_2$

For this study a slightly modified version of the CO_2sys, programmed with MATLAB (Van Heuven et al., 2011), was used. While in principle it is identical with the Excel version, the MATLAB version enables the inclusion of an additional contributor to the acid-base system. This add-on was already used for the determination of the "true" pH to consider the effect of the indicator on the sample pH, as described above in the pH section. With the extended input of both a concentration and a dissociation constant, the organic compounds affecting the marine acid-base system could be considered as a bulk parameter.

2.2 Organic carbon analysis

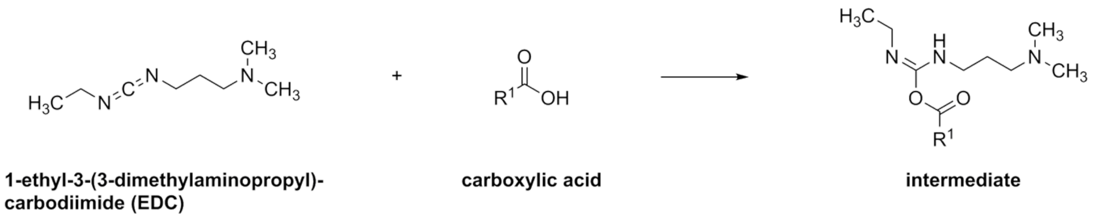
2.2.1 Dissolved organic carbon analysis

The content of DOM measured as DOC was determined with a total organic carbon analyzer from Shimadzu and the high temperature catalytic oxidation (HTCO) method (Sugimura and Suzuki, 1988). To validate the data, consensus reference material from the laboratories of Prof. Dr. Dennis Hansell (University of Miami, RSMAS) was measured. The maximum standard deviation was 3 μmol C/L.

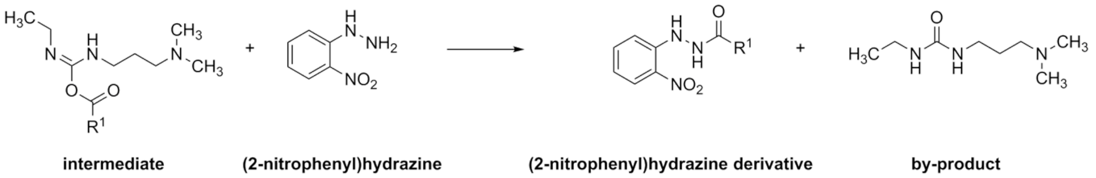
2.2.2 Carboxylic acid analysis

Derivatization

The extraction of highly hydrophilic, short chain, aliphatic, low molecular weight organic acids (LMWOA) from seawater has a poor efficiency. Thus, the derivatization of these compounds, in which they are reacted already within the aqueous phase followed by their extraction into the organic phase, is the preferred isolation method. Miwa et al. (1985) introduced a derivatization method in which carboxylic acids form hydrazides that react with 2-nitrophenylhydrazine (2-NPH), supported by the coupling agent 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as follows:



(2.1)



(2.2)

Instead of liquid chromatography, Vairavamurthy and Mopper (1990) suggested separation by GC to achieve a better resolution and blanks with lower values. In the present study, the procedure described in Vairavamurthy and Mopper (1990) was followed, with slight modifications:

- Extraction with 3 x 1000 μL
- Centrifugation after first extraction step: 1500 rpm, 7 min, 20 °C
- Evaporation with clean air instead of a N_2 stream (no difference in blank samples)
- Resuspend in 50 μL of ethyl acetate for subsequent GC-MS analysis

GC-MS analysis

Carboxylic acid derivatives were separated and detected by GC-MS using an Agilent gaschromatograph (model 6890) equipped with a DB-5 MS capillary column. The dimensions of the column were as follows: length 60 m, I.D. 0.25 mm, and film 0.25 μm (model number: J&W 122-5532). The 2 μL samples were injected by splitless mode. Helium was used as the carrier gas, with a constant flow rate of 1.4 mL/min. The oven temperature was programmed starting with an initial temperature of 120 °C, held for 2 min. The first ramp of 15 °C/min until 210 °C was maintained for 2 min. The second phase consisted of a 4 °C/min ramp until a maximum of 265 °C, held for 6 min. The transfer line was heated to 300 °C. Carboxylic acids were detected based on the following masses: $m/z = 181$ (formic acid), $m/z = 195$ (acetic acid), $m/z = 198$ (d_4-acetic acid), $m/z = 209$ (propionic acid). For all substances the $m/z = 153$ representing the fragment of 2-NPH was also detected in SIM mode.

Reagents

The derivatization reagents were of highest purity grade available. 2-NPH ($> 98\%$) was obtained from TCI Europe and EDC (99 %) was obtained from Merck®. Ethyl acetate (99.8 %, SupraSolv®) was used as an extracting agent and in the GC analysis. The acidification steps were carried out using 1 M and 0.2 M HCl, both prepared from 32 % hydrochloric acid (Merck®). The certified standard material was Supelco TraceCERT® (Sigma Aldrich®), used as a 10 mM volatile free acid mix (Lot: LC02966, XA17116V) of ten different LMW carboxylic acids. Acetic acid-d_4 (99.5 %; Sigma Aldrich) was added to each sample as an internal standard, which shifted the mass by $\sim$ 3 g/mol, since one deuterium is eliminated by the cleavage of water in the first step of Eq. 2.1.

2.3 Specified sampling and experimental setup

The following section also draws on the material and methods reported in a manuscript entitled "Acid-base properties of Baltic Sea dissolved organic matter", by Karoline Hammer, Bernd Schneider, Karol Kuliński, Detlef E. Schulz-Bull. The manuscript was submitted to the Journal of Marine Systems.

2.3.1 Empirical data collection from vertical profiles

In 2014 and 2015, seven vertical profiles were investigated in February, March, May, and November. Samples taken from a CTD rosette at station BY15, located in the Gotland Basin in the central Baltic Sea (Fig. 2.1), were analyzed for A_{T}, C_{T}, and pH. Depending on the constitution of the deep-water masses, samples were collected down to 110 m (2014) or 240 m (2015). Under anoxic conditions, HS^- would bias the alkalinity and thereby cause an error in related calculations. Thus, measurements of alkalinity down to the seafloor in which the data will be used in calculations of the CO_2 system are only recommended after ventilation of the central Baltic basins by inflows from the North Sea. In this study, samples were taken at 1, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, and, 110 and in some cases, 240 m water depth.

Each sample was poisoned with 500 μL of saturated mercury(II)-chloride solution, not only to stop biological activity but also to ensure the complete precipitation of HS^- that, if indeed present in the sample, would alter the C_{T} measurements (Sörgel, 2001). All three parameters were measured using one bottle, with C_{T} and pH measured first to prevent changes in the CO_2 content by gas exchange with the headspace. Samples for DOC measurements were also taken from the CTD rosette and then filtered onboard through GF/F Whatman® filters.

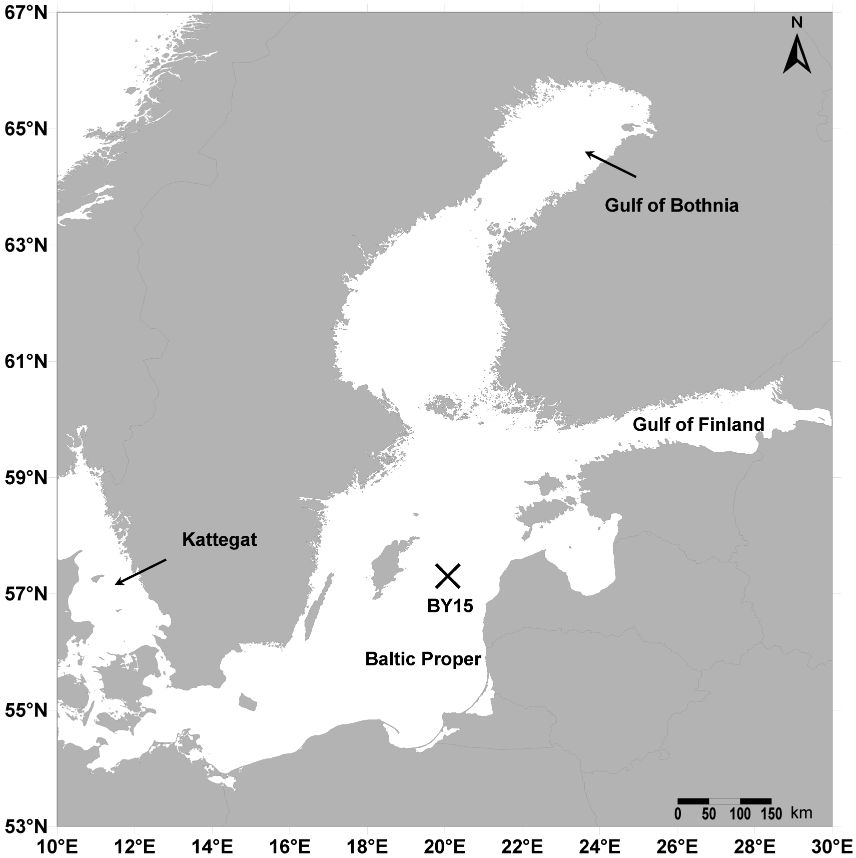


Figure 2.1: Map of the Baltic Sea Sampling station BY15, located in the Gotland Deep, is indicated (X).

All measurements of organic and inorganic parameters were performed as described above.

2.3.2 Laboratory setup for spike experiments

Artificial seawater was spiked with humic or with fulvic substances, as the major components of DOC in the Baltic Sea (Ulfsbo et al., 2015). A salinity of 7, typical for the central Baltic Sea, was obtained using the recipe for artificial seawater presented in DelValls and Dickson (1998), with slight modifications. $NaHCO_3$ and Na_2CO_3 were added to obtain an alkalinity of about $\sim$ 1650 μmol/kg-SW, corresponding to a salinity of 7 according to the $A_\mathrm{T} - S$ relationship described by Beldowski et al. (2010) for the Baltic Proper.

In the first step, the artificial seawater was equilibrated with atmospheric $\mathrm{CO_2}$ by bubbling ambient air through the 10 L sample reservoir. By this, alterations of the $\mathrm{CO_2}$ system by gas exchange during subsequent preparation of the spiked samples could be avoided. The equilibrium $p\mathrm{CO_2}$ of $\sim$ 410 μatm was measured in the headspace via infrared detection with a Li-7000 system. The artificial seawater was then transferred from the reservoir to 250 mL Schott® Duran sample bottles without any headspace.

Three stock solutions of humic/fulvic substances in artificial seawater were then prepared. The two standards, referred to herein as HA IHSS (humic acid) and FA IHSS (fulvic acid), were obtained from the IHSS and consisted of extracts from a section of the Suwannee River located in southern Georgia (USA). The third stock solution contained a technical humic acid, purchased from Sigma Aldrich and referred to herein as HA SA. This humic acid material, extracted from brown coal, was only roughly characterized by the vendor. To dissolve as much as possible of the humic acids (HA IHSS, HA SA) in artificial seawater, the two stock solutions were treated for 4 h in an ultra sonic bath and then filtered through GF/F Whatman® glass microfiber filters and then through 0.2 μm polyethersulfone filters (PESU, Sartorius) to ensure the absence of particles and colloids. Despite ultra-sonic treatment, only $4-5$ % of the HA SA and 50 % of the HA IHSS could be redissolved, whereas the FA IHSS was completely dissolved. A summary of the specific characteristics of the standard material provided by the vendors is shown in Table 2.1.

The DOC concentrations of the HA IHSS, FA IHSS and HA SA stock solutions were analyzed as described in the Section 2.2.1. Defined volumes of each one were added to 250 mL artificial seawater samples to obtain DOC concentrations of $\sim$100, 250, and 500 μmol C/kg-SW (Fig. 2.2). The C_T and pH measurements

Table 2.1: Summary of the vendors' substance specifications for three standards. The pK_1 and pK_2 values were determined from the titration data fitted using the modified Henderson-Hasselbalch equation (Katchalsky and Spitnik, 1947) for the Carboxyl- and Phenolic binding sites.

	elemental composition				acidic functional group	
Substance	C [%]	H [%]	O [%]	N [%]	pK_1	pK_2
HA SA	42.75	3.61	-	0.99	-	-
HA IHSS	52.63	4.28	42.04	1.17	4.35	10.44
FA IHSS	52.44	4.31	42.2	0.72	3.8	9.52

Figure 2.2: Artificial seawater samples spiked with humic acid (from left to right: 0, 100, 250 and 500 μmol C/L).

of the individual spiked samples were measured separately in two freshly opened bottles, thus avoiding CO_2 gas exchange with the headspace after measurement of the first variable (either pH or C_T). Alkalinity is not affected by CO_2 gas exchange and was measured after the pH determination. Each combination of spike substance and DOC concentration was measured in triplicate. Since pH and C_T were determined in separate bottles, six samples for the measurement of A_T and C_T were prepared for each DOC concentration of one spiked substance. One set of six unspiked bottles was used for reference and blank measurements. A_T, C_T, and pH were also measured as described in Section 2.1. In addition, experiments aimed at examining the influence of humic and fulvic acids on the absorption spectra of the spectrophotometric pH measurements were performed by measuring the pH of the TRIS buffer solutions spiked with humic acids were measured. No significant perturbations were detected for concentrations up to 500 μmol C/kg-SW.

2.3.3 Sampling and sample preparation for short chain carboxylic acid analysis

Samples collected weekly taken routinely at the pier of Heiligendamm (MV, Germany) were analyzed for several parameters at the Leibniz-Institute of Baltic Sea Research in Warnemünde (IOW). The following parameters were typically determined: NO_3^{2-}, NO_2^-, PO_4^{3-}, $Si0_4^-$, chlorophyll a (Chl a), salinity (S), and temperature (T). The latter two were measured with a portable conductivity measurement instrument (WTW, model Cond 1970i). Chl a was measured using a fluorometer (Turner Designs, TD - 10 AU005CE).

Acid-base status was determined in two additional samples. For the determination of inorganic parameters (A_T, C_T, and pH) one sample was collected in a 250 mL Schott® Duran bottle, stored and analyzed as described in Section 2.1.1. For the Baltic Sea surface water sampling the CTD rosette was replaced by a plastic bin on a rope. The second sample was taken for SCCA analysis. A plastic syringe (non-sterile, 20 mL, Braun Injekt®) was flushed twice with sample water before it was filled with the sample, which was then filtered through an attached luer-lock (non-sterile, Phenomenex®, RC membrane, 0.45μm) into a 20 mL screw cap glass vial. In preparing the vials, special care was taken to avoid agglomeration of the analyte onto the glass wall. This was ensured by flushing the vials with 1M HCl (1x) and Milli-Q (2x) and then heating them for 14 h at 400 °C. The caps were air dried. The samples for LMWOA were stored at -20 °C until analysis.

In May 2015, a research cruise was conducted whose aim was an investigation of the acid-base status along a salinity gradients. Several polish river estuaries entering the Baltic Sea were sampled for various parameters. The Oder estuary was assessed directly from the research vessel, as it entered the Szczecin Lagoon through the Swine, a branch of the Oder River. The smaller Vistula River was sampled from a rubber boat, since the research vessel was not allowed to enter. The samples were taken at intervals of roughly 1 PSU, verified contemporaneously by an onboard a thermosalinograph. The following parameters were analyzed in the study of acid-base status: A_T, C_T, pH, S, T, Chl a, c_DOC, $\mathrm{NO_3^{2-}}$, $\mathrm{NO_2^-}$, $\mathrm{PO_4^{3-}}$, $\mathrm{Si0_2}$, and LMWOA, more specific, SCCA. Chl a was measured by spectrophotometry. Carboxylic acids were collected from the Vessel's surface seawater pumping system and proceeded as described above. Due to rough transport conditions, some of the glass vials broke and the samples had to be discarded.

The designation of analyzed carboxylic acids might be misleading, because at seawater pH (~ 8) the acids are present in their dissociated forms (anions). As they were analyzed in their protonated constitution, however, hereinafter they are called acids.

3 Determination of dissolved organic matter acid-base bulk parameters

The following section also draws on the results and figures reported in a manuscript entitled "Acid-base properties of Baltic Sea dissolved organic matter", by Karoline Hammer, Bernd Schneider, Karol Kuliński, Detlef E. Schulz-Bull. The manuscript was submitted to the Journal of Marine Systems.

This section examines the acid-base properties of DOM in the Baltic Sea as previously reported by Kuliński et al. (2014). However, in the present study the number of analyzed samples was increased considerably and, more importantly, because sampling was performed at different depths and during different seasons, the samples cover a much broader pH range. Thus, uncertainties in the calculations of the bulk parameter f and K_{DOM} could be reduced substantially.

The A_{T}, C_{T}, and pH data from the monitoring cruises were collected during a 2-year period (2014/2015) and enabled the determination of A_{org}, which together with the dissolved organic carbon concentration (c_{DOC}) provided insights into the dissociation processes of organic matter occurring in the central Baltic Sea.

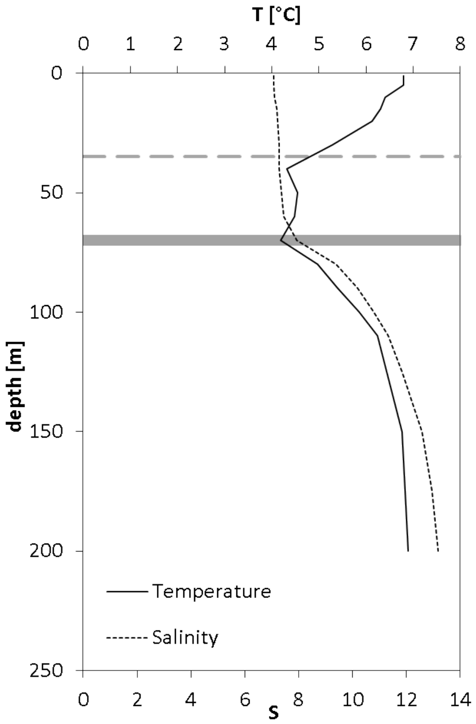


Figure 3.1: Monitoring data of temperature and salinity at station BY15 (Gotland Deep) collected in March 2015. The thermocline and halocline are indicated as dashed and bold lines, respectively.

3.1 Measurements of A_T, C_T, pH, and c_{DOC}

Station BY15 is characterized by a salinity and temperature stratification that is typical for the entire central Baltic Sea. A permanent halocline (Fig. 3.1, grey line) is maintained throughout the year at a depth of $60 - 70$ m. The water below the halocline is regularly subjected to long periods of stagnation that favor the development of anoxic conditions. The water column above the halocline is fully mixed during winter but a thermocline (Fig. 3.1, dashed line) forms at a depth of $30 - 40$ m during spring that rises to ~ 20 m during mid-summer.

This stratification is also reflected in the vertical distribution of biogeochemically reactive elements and thus of the variables that control the marine CO_2 system.

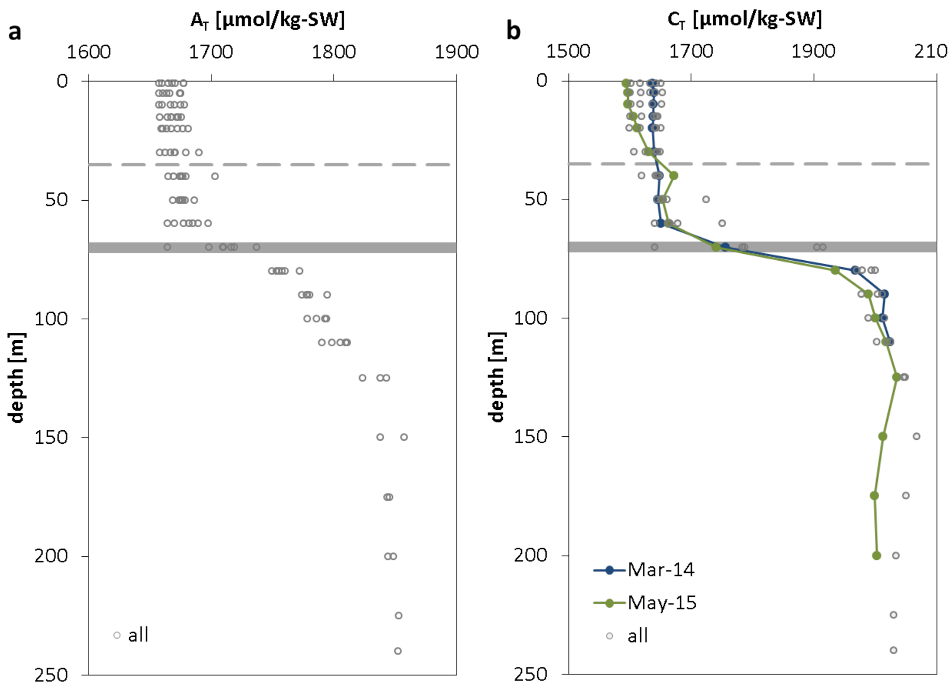


Figure 3.2: Monitoring data of 2014/2015: Vertical profiles of alkalinity (a) and total inorganic carbon (b) at station BY15 (Gotland Deep). The C_T profiles before (blue) and after (green) a spring bloom are shown.

Because alkalinity is closely linked to salinity, it increases sharply below the halocline (Fig. 3.2a). The effect of nitrate consumption during the spring bloom on alkalinity is minor such that a seasonal pattern is not detectable. By contrast, the variability occurring above the halocline can be attributed to inhomogeneous salinity distributions. In the surface mixed layer, total CO_2 is depleted during plankton growth and thus shows a distinct seasonality in the surface mixed layer. These findings are illustrated in Fig. 3.2b, which presents the C_T profiles during the pre- and post-spring bloom periods of March 2014 and May 2015, respectively. The sharp increase of C_T below the permanent halocline is mainly due to the mineralization of organic matter in stagnant deep water layers.

The vertical distribution of C_T was also reflected in the pH profiles measured con-

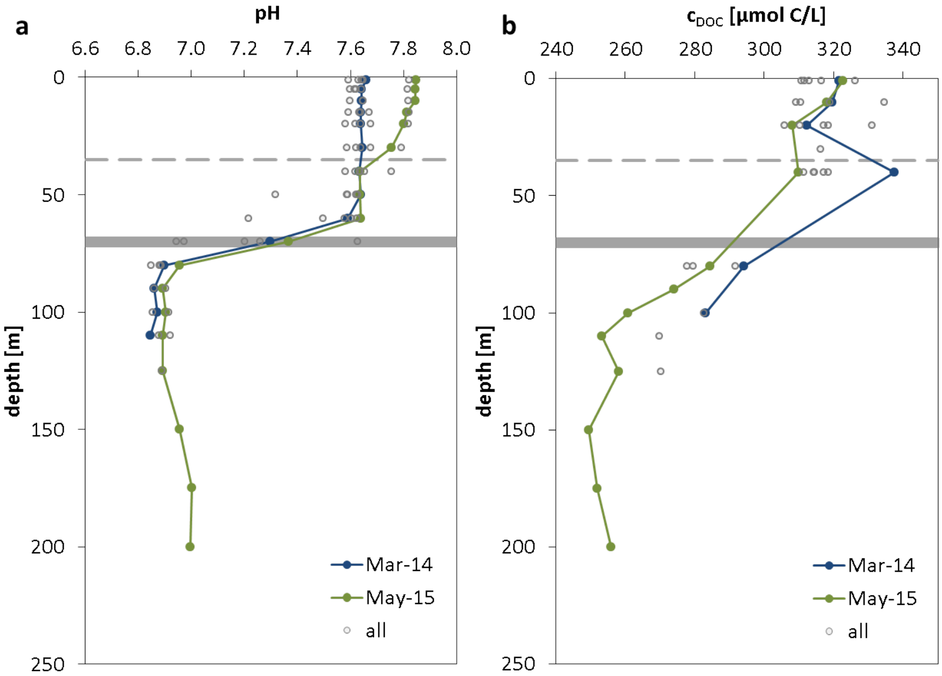


Figure 3.3: Monitoring data of 2014/2015: pH profiles (a) and dissolved organic carbon concentration (b) at station BY15 (Gotland Deep). Two profiles are highlighted, before (blue) and after (green) a spring bloom.

sistently at 25 °C. In parallel with the development of the spring bloom, the pH was low in March and high in May (Fig. 3.3a). The large pH decrease (∼ 1 pH unit) below the permanent halocline could be attributed to the intense mineralization of organic matter.

Finally, the DOC concentrations are relevant to the interpretation of A_{org}. The absence of a seasonality related to spring bloom production was indicated by the c_{DOC} profiles for March (2014) and May (2015) (Fig. 3.3b). However, below the permanent halocline c_{DOC} decreased significantly, by roughly 50 μmol C/L.

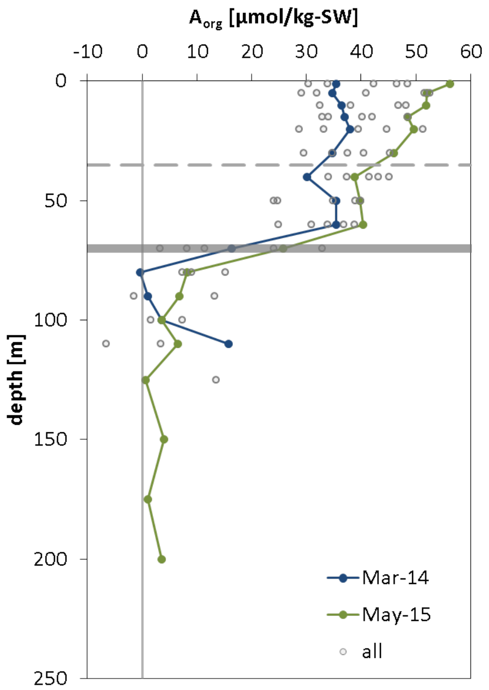


Figure 3.4: Profiles of organic alkalinity A_{org} as the difference between the measured alkalinity and the inorganic alkalinity calculated from C_{T} and pH. Two profiles are highlighted, before (blue) and after (green) a spring bloom.

3.2 Characterization of organic alkalinity

3.2.1 pH dependency

The organic alkalinity A_{org}, defined as the difference between the measured and the inorganic alkalinity calculated on the basis of the C_{T} and pH measurements, is presented as a function of depth in Fig. 3.4. Similar to the distribution of C_{T} and pH, A_{org} showed a seasonality in the surface mixed layer above the halocline, with the higher values related to the spring bloom in May. At the halocline, there was a sharp decrease close to zero in the vertical A_{org} distribution whereas the negative values below the halocline reflected uncertainties in the underlying measurements. The low A_{org} was due to the differences between the two relatively large values of measured and calculated alkalinity. Considering measurement uncertainties in A_{T}, C_{T} (from CRMs), and pH (from TRIS-buffer solution, (Hammer et al., 2014)) the A_{org} standard deviation of $\sigma = \pm 5$ μmol/kg-SW, which is in the same range as predicted by Yang et al. (2015) ($\sigma = \pm 4$ μmol/kg-SW). This would give a reasonable uncertainty of $3\sigma = \pm 15$ μmol/kg-SW.

Following the approach of Kuliński et al. (2014), in which both the a fraction (f) of DOC that has acid-base properties and a bulk dissociation constant of organic acids were considered, K_{DOM} was defined as the practical dissociation equilibrium describing the dependency of A_{org} on pH and the c_{DOC}. This is shown by rearrangement of the in Section 1.4 presented relationship as follows:

$$K_{\mathrm{DOM}} = \frac{[\mathrm{H}^{+}]A_{\mathrm{org}}}{f\,c_{\mathrm{DOC}} - A_{\mathrm{org}}} \tag{3.1}$$

$$\frac{K_{\mathrm{DOM}} f\,c_{\mathrm{DOC}}}{A_{\mathrm{org}}} - K_{\mathrm{DOM}} = [\mathrm{H}^{+}] \tag{3.2}$$

$$A_{org} = \frac{f\ c_{DOC}\ K_{DOM}}{[\mathrm{H}^{+}] + K_{DOM}} \tag{3.3}$$

Since the variability in the c_{DOC} (Fig. 3.3b) was relatively small (300 ± 40 μmol C/L), the variability of A_{org} could be ascribed mainly to the pH. This is illustrated in Fig. 3.5, which shows A_{org} as a function of pH. The pH ranged from ~ 6.8 to 7.8 and thus covered an H^{+} concentration range of one order of magnitude. Although the quadratic regression line in Fig. 3.5 has no physicochemical meaning, it clearly indicates a strong decrease of A_{org} at lower pH. The highest A_{org} (~ 56 μmol/kg-SW) occurred at the highest pH (7.85 at 25 °C), measured in the water at the immediate surface. In deeper water, where the pH decreased due to organic matter mineralization, the degree of protonation of organic substances increased, resulting

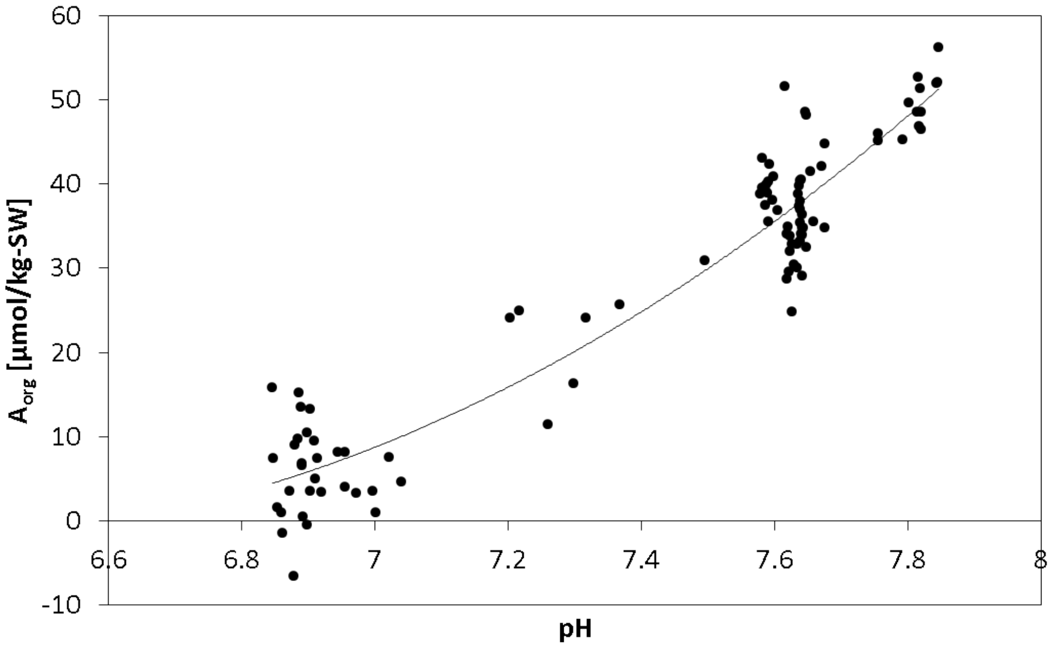


Figure 3.5: Relationship of A_{org} to pH at BY15, based on monitoring data from 2014/2015 ($n = 97$).

in an A_{org} close to the halocline of $10 - 0$ μmol/kg-SW at pH $= 6.85$. At this low pH, weak organic bases are almost completely converted to their corresponding acids, such that the majority of organic substances no longer contribute to the A_{T}. A concurrent change in the composition of the DOM due to the increasing contribution of North Sea water could not be excluded but it would not fully explain the fact that A_{org} approached zero.

3.2.2 Determination of pK_{DOM} and f

To determine K_{DOM} and f, Eq. 3.1 was rearranged such that $c_{\mathrm{DOC}}/A_{\mathrm{org}}$ was a linear function of [H$^+$]:

$$K_{\mathrm{DOM}} = \frac{[\mathrm{H}^+]A_{\mathrm{org}}}{f\,c_{\mathrm{DOC}} - A_{\mathrm{org}}} \tag{3.4}$$

$$K_{\mathrm{DOM}}\,f\,c_{\mathrm{DOC}} - K_{\mathrm{DOM}}\,A_{\mathrm{org}} = [\mathrm{H}^+]\,A_{\mathrm{org}} \tag{3.5}$$

$$\frac{f\,c_{\mathrm{DOC}}}{A_{\mathrm{org}}} = \frac{[\mathrm{H}^+]}{K_{\mathrm{DOM}}} + 1 \tag{3.6}$$

$$\frac{c_{\mathrm{DOC}}}{A_{\mathrm{org}}} = \frac{1}{f\,K_{\mathrm{DOM}}}[\mathrm{H}^+] + \frac{1}{f} \tag{3.7}$$

Figure 3.6 shows a plot of $c_{\mathrm{DOC}}/A_{\mathrm{org}}$ vs. [H$^+$] and the corresponding linear regression line. Only A_{org} values > 10 μmol/L and thus less affected by uncertainties in the A_{org} determination ($\pm$ 5 μmol/kg) were included; two outliers were excluded. In the absence of c_{DOC} measurements, the averaged data of the c_{DOC} ($\sim$ 300 μmol C/L) at a similar depth were used to calculate $c_{\mathrm{DOC}}/A_{\mathrm{org}}$, and the maximum and minimum observed concentrations to calculate the potential error. The slope and intercept of the regression line allowed the calculation of pK_{DOM} and f, yielding values of 7.27 ± 0.11 and 0.17 ± 0.02, respectively, at a confidence

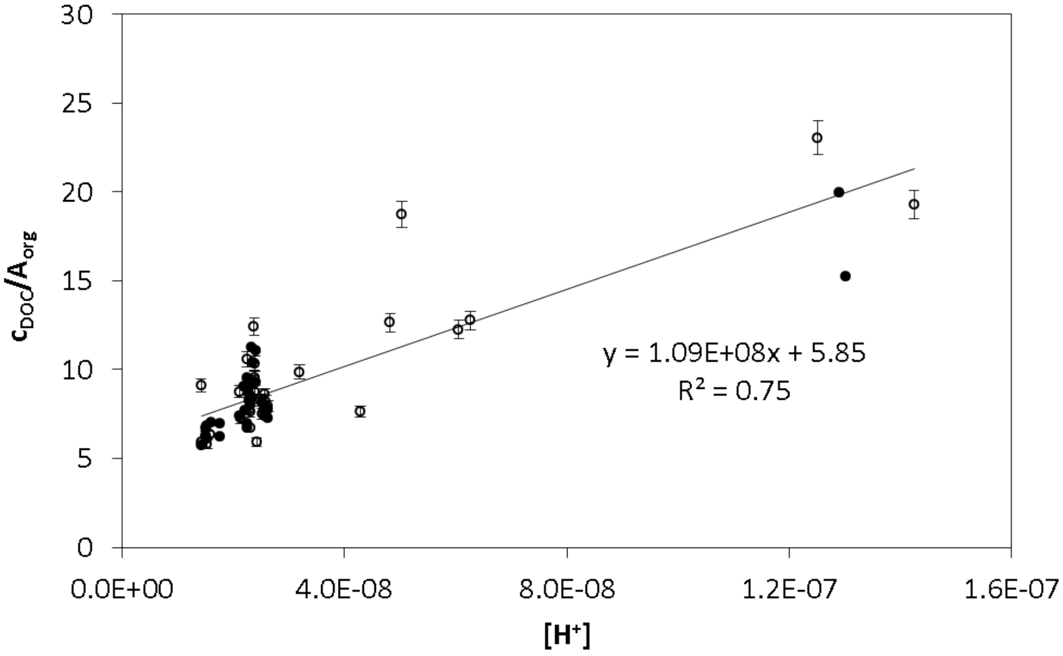


Figure 3.6: Linear correlation of $c_{\mathrm{DOC}}/A_{\mathrm{org}}$ and $[\mathrm{H}^+]$ as determined from monitoring data collected in 2014/2015 ($n = 70$). Measured (filled circles) and in the absence of c_{DOC} measurements, averaged (open circles with vertical error bars) data are shown. A_{org} values ≤ 10 μmol/kg-SW were not considered.

level of $P = 0.95$. Based on a similar approach, but analyzing a limited number of samples ($n = 6$), Kuliński et al. (2014) reported corresponding values 7.34 and 0.12 (revised values, reported in Ulfsbo et al. (2015)). The two sets of values are in reasonable agreement given the natural variability in organic matter composition and the simplification involved in a theoretical approach.

Ulfsbo et al. (2015) used a chemical model of humic substances [Windermere Humic Aqueous Model, WHAM VII, Tipping et al. (2011)] in combination with the Pitzer model to calculate the total concentration of ionizable sites (n) as a function of pH (dn/dpH) for fulvic acids and to estimate the concentration of proton acceptors protonized during an alkalinity titration of a sample with a pH of ~ 8. Using this method they were able to reproduce the experimental A_{org} determinations of Kuliński et al. (2014) and thereby demonstrate that fulvic acids play a major role in generating organic alkalinity in the Baltic Sea.

4 Dissociation of humic and fulvic acids in artificial seawater

The following section also draws on the results and figures reported in a manuscript entitled "Acid-base properties of Baltic Sea dissolved organic matter", by Karoline Hammer, Bernd Schneider, Karol Kuliński, Detlef E. Schulz-Bull. The manuscript was submitted to the Journal of Marine Systems.

The results from a field study formed the basis of the second part of this study. By reproducing the addition of DOM representatives into brackish artifical seawater, I sought to support the findings from field studies in laboratory experiments.

4.1 Thermodynamic consistency

I asked whether the field results could be reproduced in spike experiments using humic/fulvic substance solutions and artificial seawater. However, it was first necessary to examine the thermodynamic consistency of the A_T, C_T, and pH measurements of organic free artificial seawater adjusted to approximately natural conditions by the addition of HCO_3^- and CO_3^{2-}. Thermodynamic consistency, i.e., agreement between the measured and calculated A_T, C_T, and pH, is mandatory to ensure that any measured difference in alkalinity is truly a result of the added organic matter and not an artifact. Therefore, each of the three variables (A_T,

C_T, and pH) was calculated from the remaining two measured variables. Comparisons between the calculated and measured values identified maximum deviations of ±3 μmol/kg-SW for A_T and C_T and 0.017 for pH ($n = 3$). Thus, there was no systematic shift between the calculated and measured values; rather the differences were considered within the range of analytical uncertainties and are acceptable for the further investigations.

4.2 A_T, C_T, and pH

The A_T, C_T, and pH of the spiked samples showed large deviations from the corresponding values of the untreated artificial seawater (Table 4.1). Both the pH and the A_T of the spiked HA IHSS and FA IHSS solutions were lower than the corresponding values of the unspiked artificial seawater, by a maximum of $\sim$ 0.45 units and 35 μmol/kg-SW, respectively. The decrease in the pH was attributed to the addition of the purchased humic substances, which were provided in their protonated (acidic) form by the vendor and included weak [relative to the definition of alkalinity (Dickson, 1981)] as well as strong acidic groups. The latter were responsible for the decrease in alkalinity. In addition, the observed decrease in total CO_2 could be explained by the enhanced pCO$_2$ of the spike solution, due to the acidic character of the dissolved humic acids. This led to a loss of CO_2 by gas exchange during preparation of the spike solution (filtration, ultrasonic treatment). Hence, the C_T of the samples were diluted by the addition of the CO_2-depleted spike solution. However, these modifications of the acid-base system of the original artificial seawater did not in any way affect the determination of A_{org} or the estimation of f and K_{DOM}.

Table 4.1: Spiked samples: Values for c_{DOC}, A_{org}, pH, A_{T}, and C_{T} for artificial seawater ($c_{\mathrm{DOC}} = 0$) and three different humic and fulvic acids, at three different DOC concentrations; $A_{\mathrm{org}} = A_{\mathrm{T(measured)}} - A_{\mathrm{T(calculated)}}$.

Substance	c_{DOC} [μmol C/kg-SW]	A_{org} [μmol/kg-SW]	pH	A_{T} [μmol/kg-SW]	C_{T} [μmol/kg-SW]
HA SA	0	3	7.93	1655	1618
	78	13	7.90	1657	1616
	235	31	7.85	1660	1608
	469	59	7.77	1662	1595
HA IHSS	0	-3	7.98	1654	1615
	100	9	7.90	1649	1612
	249	25	7.76	1638	1607
	498	48	7.53	1619	1599
FA IHSS	0	3	7.99	1658	1611
	100	8	7.92	1650	1609
	200	24	7.79	1642	1606
	500	43	7.57	1623	1602

4.3 A_{org} correlation with c_{DOC}

The A_{org} values of the spiked samples calculated according to Eq. 1.13 were plotted against the c_{DOC} values representing the concentrations of the added humic substances (Fig. 4.1).

The c_{DOC} value measured in the blank of the artificial seawater was $< 20\ \mu$mol C/L and was ignored in the data evaluation. The linear correlations for all three organic acids were nearly perfect ($r^2 \geq 0.99$), with intercepts close to zero. The slope of the regression lines representing the A_{org} contribution per carbon unit of the added humic acid varied between 0.12 and 0.08. The highest value was that of HA SA. This result agrees well with the assumed highest acidity related to high oxygen content inferred from the low carbon content as presented in Table 2.1. Whereas the lowest contribution to A_{T} was, other than expected from higher acidity and lower aromaticity, that of FA IHSS. The highest A_{org} values at the maximum c_{DOC}

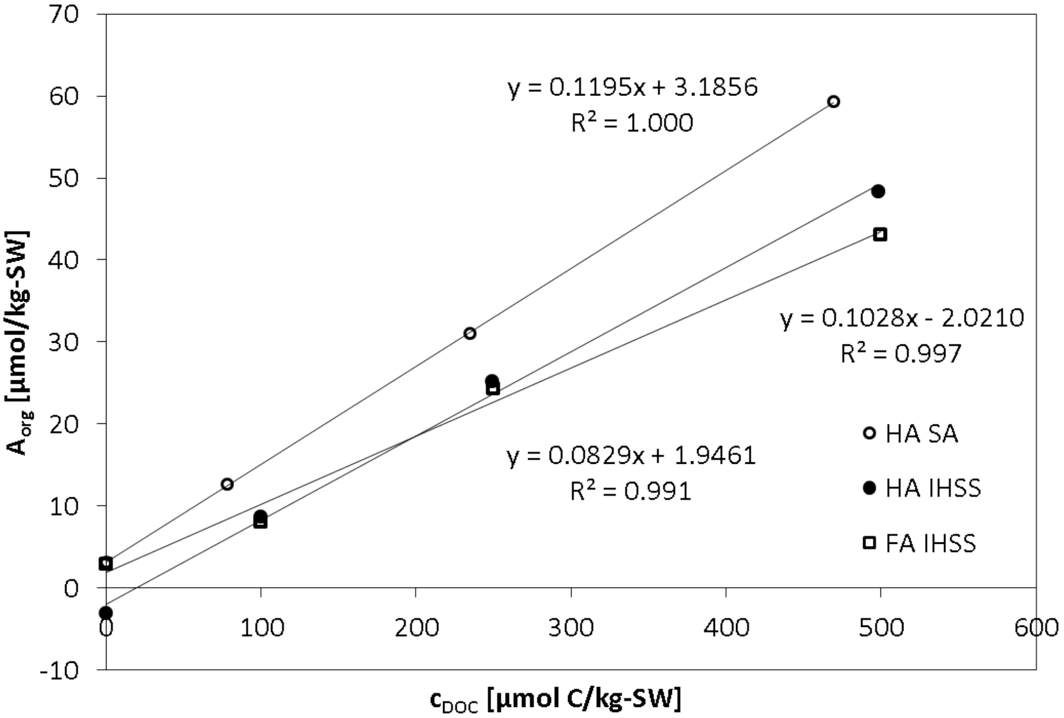


Figure 4.1: Linear correlation of A_{org} determined during laboratory study with the c_{DOC} of the technical humic acid (HA SA, open circles), the IHSS humic acid standard (HA IHSS, filled circles), and IHSS fulvic acid (FA IHSS, open squares).

of 500 μmol C/kg-SW were between 43 and 60 μmol/kg-SW and thus within the order of magnitude of the highest A_{org} determined during the field study in Section 3.2.

While the strict linear relationships between the DOC concentration and A_{org} seemed plausible, A_{org} is also a function of pH [see Fig. 3.5 and Eq. 3.3]. Since the pH dropped with successive humic/fulvic acid additions and f is assumed to be constant (bulk parameter), a decrease in the $A_{\mathrm{org}}/c_{\mathrm{DOC}}$ ratio would be expected according to Eq. 4.1 that was derived from Eq. 1.15:

$$\frac{A_{\mathrm{org}}}{c_{\mathrm{DOC}}} = f \frac{K_{\mathrm{DOM}}}{K_{\mathrm{DOM}} + [\mathrm{H}^+]} \tag{4.1}$$

However, the strict linear character and the near zero intercept of the A_{org} vs. c_{DOC} relationship suggested that $A_{\mathrm{org}}/c_{\mathrm{DOC}}$ was constant within the accuracy limits of my measurements. Thus, according to Eq. 4.1, this implies that K_{DOM} was much larger than the hydrogen ion concentrations (p$K_{\mathrm{DOM}} \ll$ pH). In other words, at pH values as low as 7.5, the lowest pH measured in this experiment, the majority of the acid-base functional groups existed as anions. This was not the case in the natural Baltic Sea DOM, in which a distinct dependency of A_{org} on $pH > 7.5$ was determined and accordingly yielded a K_{DOM} ($5.4 \cdot 10^{-8}$ mol/kg) that was close to the concentration of the H$^+$ ions at which the lab experiments were performed ($3.2 \cdot 10^{-8}$ mol/kg).

4.4 Theoretical pK of humic and fulvic acids

For $K_{\mathrm{DOM}} \gg [\mathrm{H}^+]$, the relationship given in Eq. 4.1 can be approximated by $A_{\mathrm{org}} = f\, c_{\mathrm{DOC}}$ with $K_{\mathrm{DOM}}/(K_{\mathrm{DOM}} + [\mathrm{H}^+]) \sim 1$ and thus the slopes in Fig. 4.1 become equal to f, which ranges between 0.08 and 0.12. While the slope of 0.12 for HA SA is in agreement with the experimentally derived f (0.12) of Kuliński et al. (2014) my field study yielded a considerably higher value ($f = 0.17$). However, it should be stressed that the correspondence of slopes and f-values is due to complete deprotonation of organic acids ignoring the dissociation behavior at lower pH. Furthermore, a comparison with the acid-base properties of naturally occurring DOM is limited by the fact that marine DOM comprises a variety of organic compounds whose acid-base properties differ significantly from those of humic/fulvic substances. To conclude, the pK_{DOM} of humic/fulvic substances in the artificial seawater could not be determined because the dissociation constants were too high (pK_{DOM} too low) to analytically detect changes in $A_{\mathrm{org}}/f\ c_{\mathrm{DOC}}$ (Eq. 4.1) within a pH range of $7.5 - 8.0$.

I therefore calculated the $A_{\mathrm{org}}/f\ c_{\mathrm{DOC}}$ as a function of pH within a pH range of $8.0 - 7.5$ for different hypothetical pK_{DOM} values (6.0, 6.5, 7.0, 7.5) to identify the pK_{DOM} at which my analytical capability would have allowed the detection of a decrease in $A_{\mathrm{org}}/f\ c_{\mathrm{DOC}}$ during a drop in pH from 8.0 to 7.5 (Fig. 4.2).

Setting $A_{\mathrm{org}}/f\ c_{\mathrm{DOC}}$ to 100 %, thus complete deprotonation at pH = 8, for p$K_{\mathrm{DOM}} = 6.5$, the ratio $A_{\mathrm{org}}/f\ c_{\mathrm{DOC}}$ changed by ~ 6 % and decreases further for lower pK_{DOM} values. This change was of the same order of magnitude as the relative uncertainty in A_{org} of ± 3 μmol/kg-SW calculated from averaged uncertainties in A_{T}, C_{T} and pH measurements. Thus, p$K_{\mathrm{DOM}} > 6.5$ would have resulted in a detectable decrease in $A_{\mathrm{org}}/f\ c_{\mathrm{DOC}}$. Hence, 6.5 can be considered as the upper limit for the bulk dissociation constant of humic/fulvic acids (Fig. 4.2, highlighted shade) which were thus considerably more acidic than the bulk DOM in surface water of the Baltic Sea. This statement is consistent with modeled data of Ulfsbo et al. (2015) for fulvic acids assuming a pK of ~ 6.

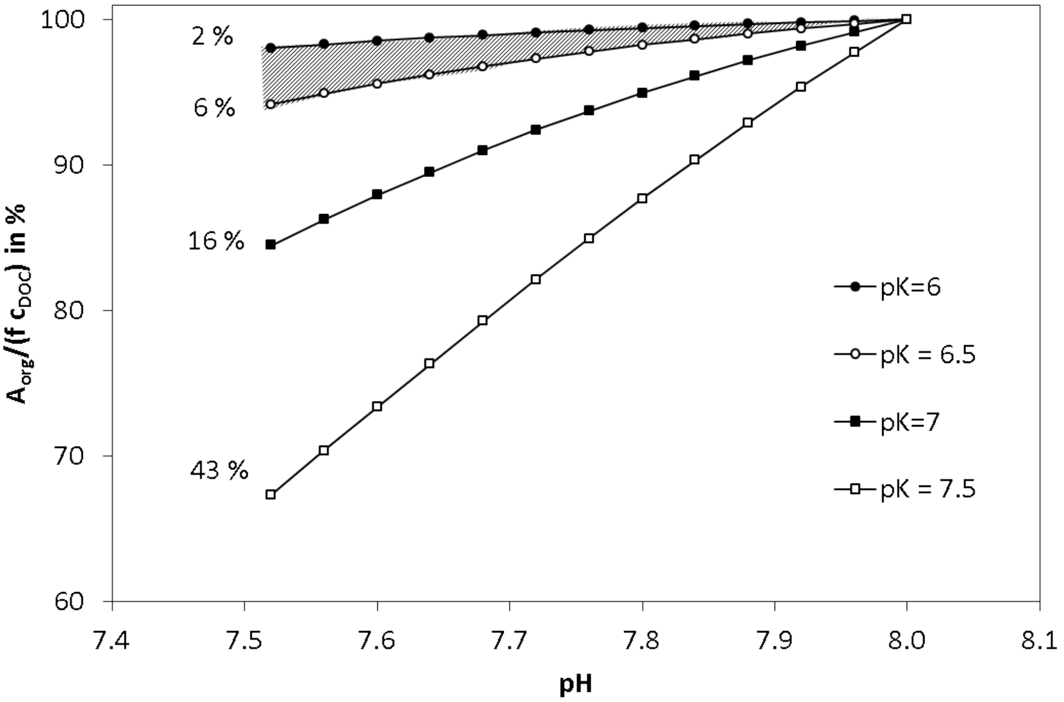


Figure 4.2: Theoretical study: Percentage reduction of $A_{\mathrm{org}}/f\,c_{\mathrm{DOC}}$ with decreasing pH; Assumption: $A_{\mathrm{org}} = 100$ % at pH = 8 calculated for pK = 6, 6.5, 7, 7.5.

5 Seasonal and regional variation of short chain carboxylic acids in the Baltic Sea

Despite the abundance of LMWOA, and specifically of short chain carboxylic acids (SCCA), as metabolites and biodegradation products in the marine environment, their role within the acid-base system has not been adequately addressed. SCCAs have dissociation constants close to that of carbonic acid [$\mathrm{p}K_1^* = 6.082$ at 20 °C and $S = 7.122$, (Millero et al., 2006)], but their behavior during titrations in alkalinity measurements is poorly understood. This chapter is a first attempt to determine the concentrations of SCCAs during a seasonal cycle and along salinity gradients in two Polish river estuaries, by measuring both inorganic and organic parameters. This knowledge will allow estimations of the potential effect of SCCAs on the alkalinity of these waters and on the marine acid-base system.

5.1 Accuracy of carboxylic acid measurements

The concentrations of carboxylic acids in Baltic Sea surface water were very low and remained near the limits of detection (LOD); however, I chose the three most abundant carboxylic acids for further analysis. For all three, calibrations using regression graphs resulted in r^2 values > 0.99. In a first measurement period in 2015, the LOD for formic, acetic, and propionic acid were 0.37, 0.55, and 0.63 μmol/L, respectively. Because the GC-MS device was not in use for a prolonged period of time, its performance in the second measurement period (2016) suffered to a small extent and resulted in a moderately higher LOD for propionic acid (1.1 μmol/L). For formic and acetic acid, t-tests showed the similarity of calibration graphs obtained for the two measurement periods. All calibrations were performed with $n > 10$ data items. As a reference, a deuterated acetic acid standard was added to each sample, such that absorbance ratios to determine the carboxylic acid concentrations in standard solution prepared in Milli-Q water were obtained. With this approach, both incomplete extraction and derivatization were taken into account. The calibration graphs were evaluated according to DIN 32645.

5.2 Time series of short chain carboxylic acid samples from Heiligendamm pier

A prerequisite to the evaluation of carboxylic acid concentration in a context relevant to the CO_2 system is an analysis of the status of the inorganic acid-base system over a seasonal cycle from winter to autumn.

Figure 5.1 shows the causality between A_T, C_T, and pH. Their inverse agreement (pH decreases with increasing A_T and C_T and vice versa) throughout the seasonal cycle validated the sampling strategy and the data obtained from those samples. By measuring A_T, C_T, and pH in discrete samples, each of the key CO_2 parameters can be calculated from the measured ones and allows to determine A_{org}.

The sampling period began in February, immediately before the anitcipated start of the biological activity. At the end of February, the appearance of moderate spring bloom was reflected by an increase in the pH of > 0.2 pH until mid-March. In parallel, C_T concentration decreased by ~ 50 μmol/kg-SW, due to CO_2 consumption by primary producers. On March 17th, there was an abrupt increase in the salin-

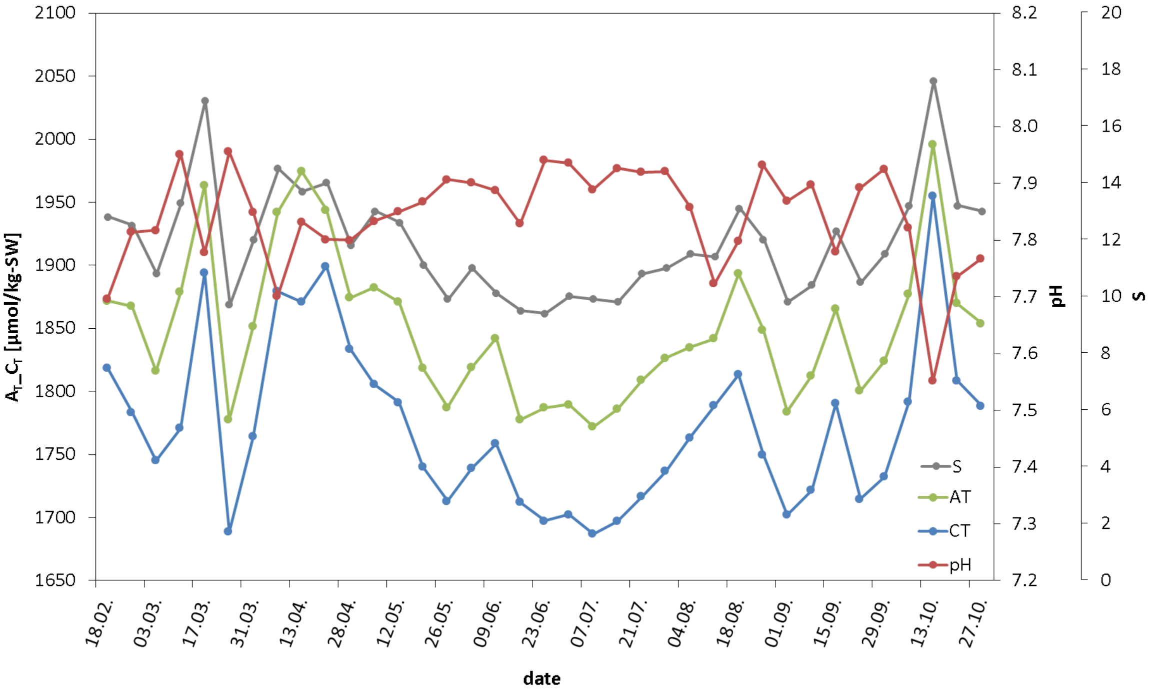


Figure 5.1: Seasonal cycle of A_{T}, C_{T}, pH, and salinity in water samples from Heiligendamm pier collected in 2015.

ity caused by a weak inflow of highly saline water from the North Sea, confirmed by the observation platform MARNET in the Arkona basin (Nausch et al., 2016). The increased salinity was accompanied by an increase in alkalinity and a decrease in pH. The increased salinity of the surface water was attributed to total mixing, since the water samples were collected close to the coast, where stratification of the shallow water does not occur.

During the summer months, A_{T} and C_{T} were relatively low and stable between the end of May and mid-July, interrupted only at the beginning of June by a second increase in salinity and thus alkalinity. Greater variability in inorganic CO_2 parameters began at the end of summer, when the increased wind stress began.

This resulted in the detection of different water masses with slightly different inorganic acid-base statuses at Heiligendamm pier. In mid-October, another weak inflow from the North Sea was detected based on the increase in the salinity > 17 (Fig. 5.2). The inflow was again detected by the monitoring platform in the Arkona basin, on October 18th (Nausch et al., 2016). The changing in water masses were also reflected in a maximum level of alkalinity (1996 μmol/kg-SW) and a minimum pH of 7.55 at 25 °C. The parallel occurrence of a moderate spring bloom, indicated by the elevated Chl *a* concentrations ($c_{\mathrm{max}} = 10.6$ mg/m^3) shown in Fig. 5.2, and an increased salinity suggested the development of an algal bloom in the highly saline westward waters of the Baltic Sea, coinciding with currents and/or strong wind events and water masses that were driven eastwards. The nitrate concentration was at a maximum of 5.7 μmol/L at the beginning of the measurement series in February but was depleted thereafter until March 10th, indicating primary production. Only during subsequent elevations in the salinity, did nitrite and nitrate, summed in the parameter NO_{2_3}, increase moderately, up to 2 μmol/L. Formic acid was detected only at very low concentrations throughout the sampling period, except for a single maximum (3 μmol/L) on March 17th that coincide with the above-described increase in salinity due to a minor inflow of water masses from the North Sea. As the smallest organic acid, formic acid is likely a favorite substrate in heterotrophic assimilation by microorganisms. In addition to the photooxidation of formic acid throughout the euphotic zone, this may explain its constantly low concentrations, near or below LOD (0.37 μmol/L), during the summer and autumn.

Acetic acid is one of the most favored SCCA substrates and it was the most frequently measured carboxylic acid in recent studies investigating the marine seawater content of these compounds [e.g. Albert et al. (1995); Yang et al. (1993)]. As a common metabolite, higher concentrations of acetic acid than of formic or

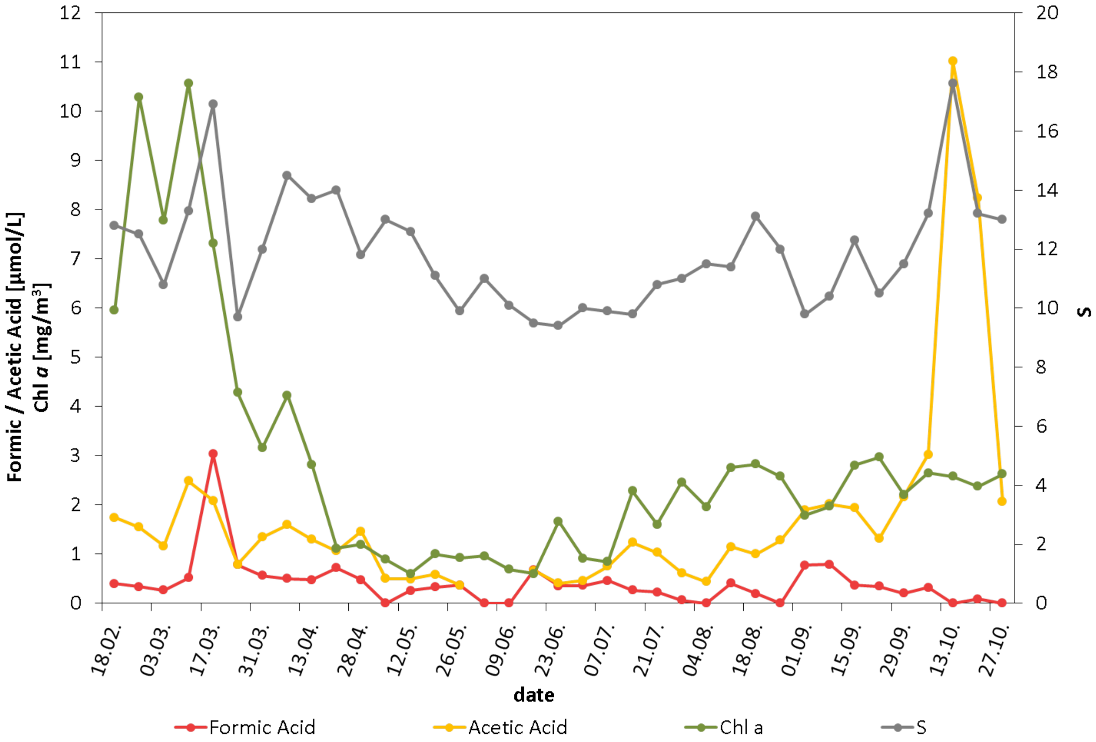


Figure 5.2: Seasonal cycle of formic acid, acetic acid, salinity, and chlorophyll *a* in samples collected in 2015 from Heiligendamm pier.

propionic acid were expected. As shown in Fig. 5.2, during and after the increase in Chl *a* concentrations, elevated concentrations of acetic acid (2.5 μmol/L and 1.6 μmol/L, respectively) were measured. However, because of the observed parallel peak in salinity, the high acetic acid concentrations could not be assigned to on-site developed biological activity. Instead, another source for this SCCAs can be proposed. According to Albert et al. (1995), as a result of the advanced degradation of HMWOA, deeper water layers are enriched in carboxylic acids, whose emergence in the surface layer may be promoted by vertical mixing processes.

Throughout the summer months, low but still detectable concentrations of acetic acid ($1.0 - 1.5$ μmol/L) persisted, most likely due to the combination photooxidation and assimilation processes, both of which proceed rapidly in the surface water layer. Only at the end of August did the concentration again exceed 2 μmol/L, which, as already observed during springtime, coincided with elevated salinity but only slightly elevated Chl *a* concentrations. Beginning on the 6th of October, the acetic acid concentration strongly increased (> 11 μmol/L) until the 13th October, while the salinity was > 17. Immediately after this moderate inflow, acetic acid concentrations rapidly dropped, with a concentration of 3.8 μmol/L recorded on October 27th, when the sampling period ended because further variation corresponding to biological activity was not expected. This discrete peak in the acetic acid concentration, presumably attributable, like the others, to an inflow event, was unlikely to have been generated on-site.

Concentrations of the third investigated short chain organic acid, propionic acid, remained below or near LOD throughout the seasonal cycle. The maximum concentration occurred in autumn, together with elevated acetic acid concentrations. Thus, between September 2nd and 23rd and between October 7th and 21st, concentrations of 0.66 μmol/L and 0.71 μmol/L (LOD = 0.63 μmol/L), respectively, were measured. The coincidence with the weak inflow of highly saline water provided further evidence of the mixing of different water masses, including those enriched in OAs.

Since, to the best of my knowledge, this is the first study in which SCCAs were measured in the water column of the Baltic Sea, comparisons with literature data were not possible. Nevertheless, surface concentrations were similar to those in the marine surface waters studied by Albert et al. (1995) and Yang et al. (1993): 2.1 μmol/L and 2.56 μmol/L, respectively. The evaluation of A_{org} showed that a systematic correlation with one of the parallel measured parameters could not be found. The mean A_{org} was characterized by a high variability 21 ± 10.5 μmol/kg-SW and the values did not coincide with seasonal events: neither elevated organic matter (SCCA and Chl *a*) levels in spring nor different conditions of inorganic CO_2 parameters (spring, autumn). However, A_{org} decreased during the first weak inflow in spring, from 26 to nearly 0 μmol/kg-SW. This likely pH-dependent response was observed only once and was therefore not as significant as it was for the vertical profiles analysis described in Section 3.2.

Principal component analysis of the Heiligendamm dataset

A PCA was performed to identify subtle correlations between the measured parameters. To adress inhomogeneities in their magnitudes, the data were first fourth-root-transformed. PC1, with a variation of 33.6 %, showed strong correlations between formic acid, acetic acid, Chl *a*, and nutrients, with Eigenvectors of $0.3 - 0.5$. This result further supported the conclusion that the measured SCCA were not the result of organic matter formation and degradation, rather, they likely derived from other, introduced sources as described above. If the former were the case, then a negative correlation of Chl *a* with nutrients and SCCA concentrations during the moderate spring bloom period would be expected. PC2 (20 % variation) showed a high negative correlation between formic acid and temperature. Higher irradiation likely gives rise to higher temperatures and thus an increase in photooxidation, resulting in increased OA formation. The correlation between nitrate and Chl *a* suggested that biogeochemical signals are masked by the changing composition of water masses in response to frequent winds and currents.

5.3 Regional variability of organic and inorganic parameters for two Polish river estuaries

5.3.1 Investigation of the Oder estuary

While unexpected, the concentrations of carboxylic acids in the investigated rivers were comparable to those measured near-shore at Heiligendamm pier. The Oder River was sampled eight times, the Swine River three times, and Szczecin Lagoon four times (Fig.5.3).

An assessment of the potential effect of even small carboxylic acid concentrations also relies on an assessment of the inorganic acid-base system. As presented in Fig. 5.4a, both alkalinity and total CO_2 increased at lower salinities, which are representative of a more riverine environment with freshwater conditions. A steep increase in pH to a maximum of 8.9 at 25 °C was detected in the waters leaving the Swine River and entering the Szczecin Lagoon.

The suspected algal bloom was confirmed by the Chl *a* concentrations, which reached a maximum of 41 $\mathrm{mg/m^3}$ (Fig. 5.5). Across the lagoon, elevated Chl *a* concentrations coincided, although with opposing intensities (Fig. 5.5), such that A_{org} reached maxima of 68 and 40 μmol/kg-SW. The maximum in acetic acid concentration was 2 μmol/L. Neither the Chl *a* nor the acetic acid maximum coincide with the maximum of DOC (c = 632 μmol C/L), reflecting the independence of

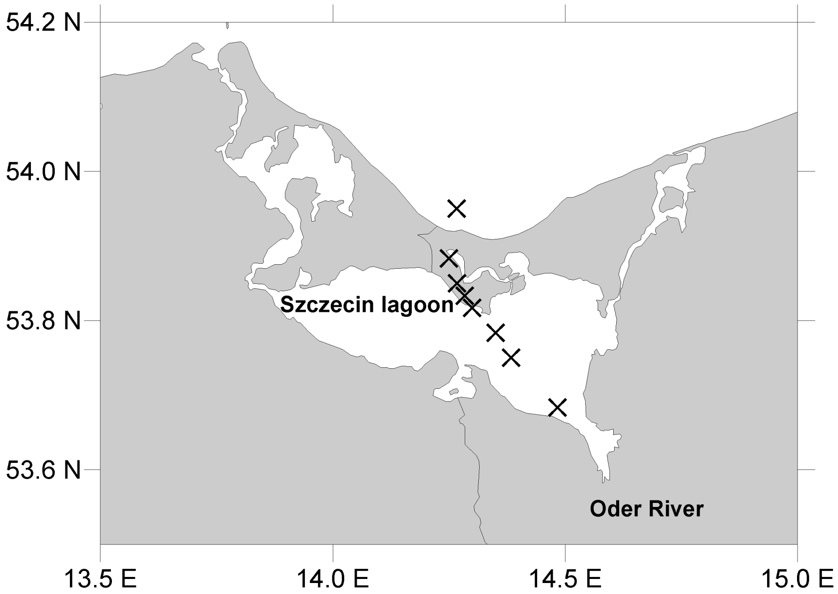


Figure 5.3: Map of the sampling stations in the Oder estuary in May 2015.

A_{org} from total c_{DOC}. However, the changing A_{org} indicated a dependence of its constitution on DOC and the latter's content of proton-exchangeable sites. Despite a change of a 1 pH unit the A_{org} term showed no significant correlation with the hydrogen ion concentration. Thus, a relationship to the dissociation of DOM could not be determined.

An evaluation of the nutrient data showed patterns of nitrate and silicate that were typical of a phytoplankton bloom, with minimum concentrations < 0.6 μmol/L and < 1.7 μmol/L, respectively. At the lowest salinity and near the Oder River proper into the lagoon, the pH is dropped to a moderate value of 8.2, accompanied by a steep increase in total CO_2. Towards the river outflow, nutrient concentrations increased slightly such that the algal bloom developed mainly in the central area of Szczecin Lagoon, where the fluctuations in its water masses are the lowest and algae can grow free of disturbances.

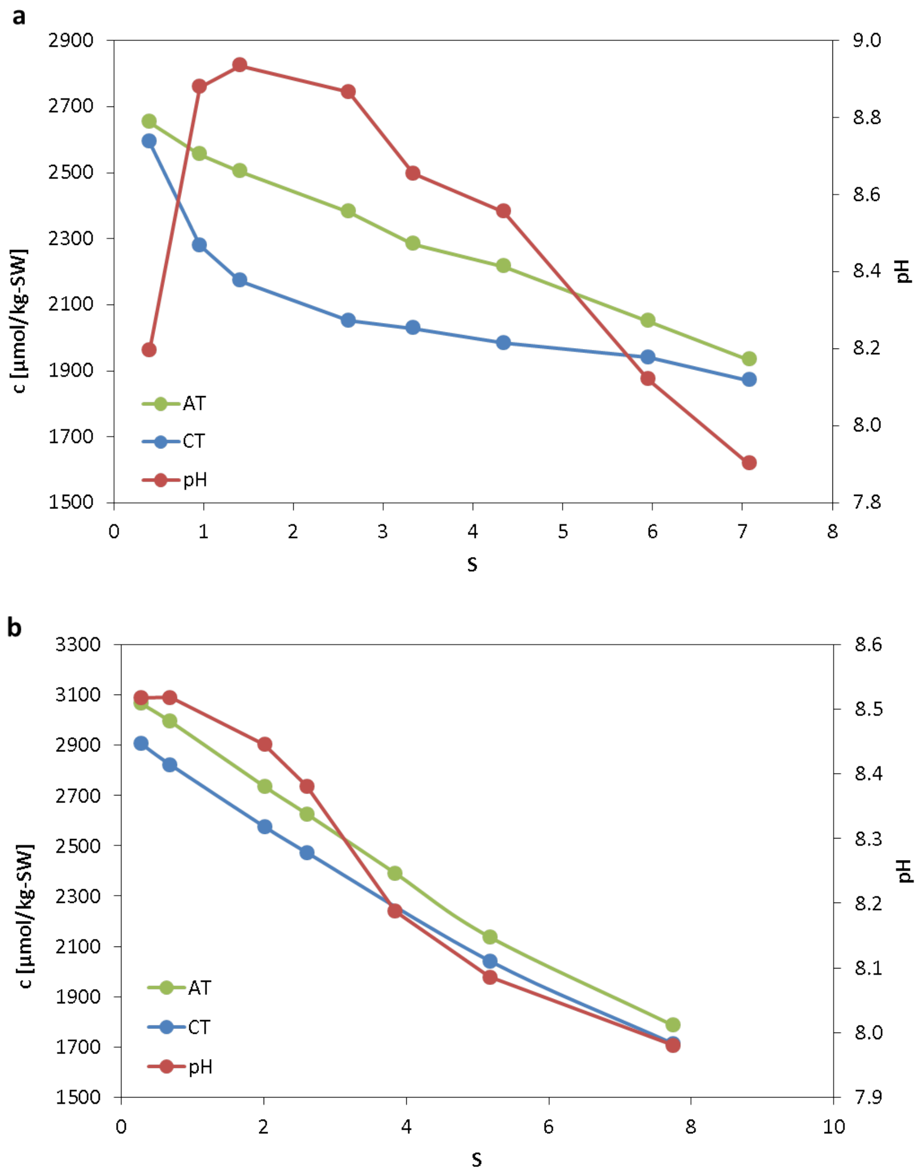


Figure 5.4: Alkalinity (A_T), total carbon (C_T), and pH with increasing salinity in the estuaries of the Oder River (a) and Vistula River (b) in May 2015.

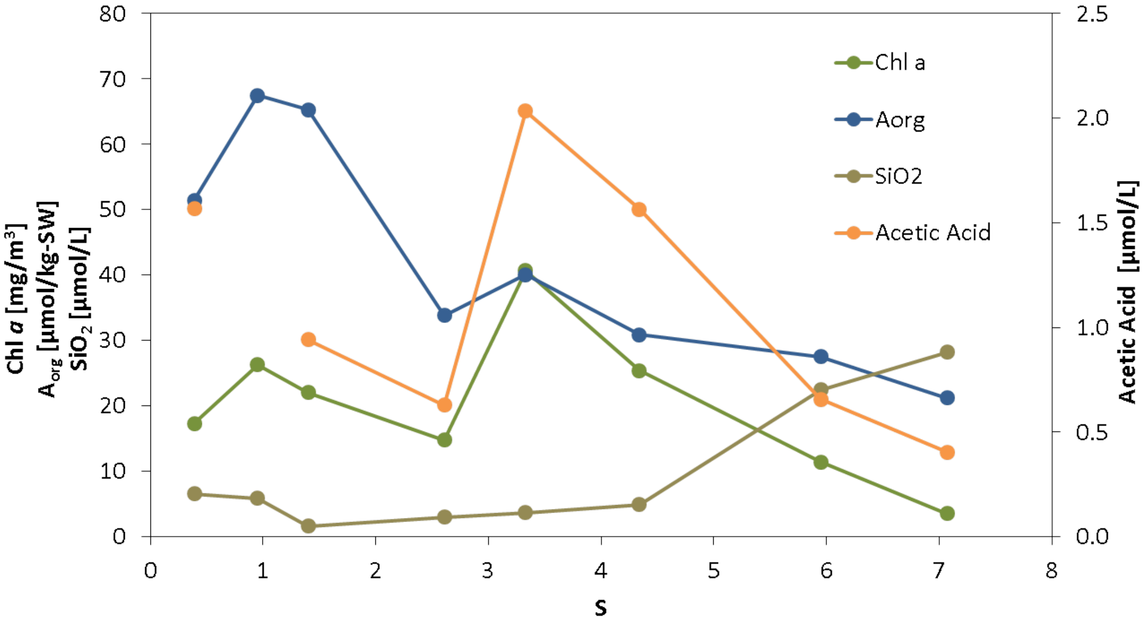


Figure 5.5: Chl a, A_{org} (calculated from measured A_{T}, C_{T} and pH), SiO$_2$ and acetic acid concentrations with increasing salinity the Oder River estuary in May 2015.

Principal component analysis of the Oder estuary dataset

A PCA was conducted after a fourth-root transformation of the data. PC1, with 51.7 % variation, was mainly controlled by the negative correlation between alkalinity and salinity and their inverse behavior with respect to marine conditions; thus, alkalinity decreased with increasing salinity and pointed to rivers as the alkalinity source. PC2 (23.2 % variation) reflected the negative correlation (Eigenvectors: 0.56 and -0.41) of acetic acid, as the major carboxylic acid, with nitrate. This result indicated that carboxylic acid formation was a consequence of HMWOA degradation rather than consumption promoting primary production. PC3 (17.8 % variation), in which there was a significant negative correlation of silicate with Chl a, suggested diatom growth.

5.3.2 Investigation of the Vistula estuary

For the Vistula River, one C_T data point was neglected in the evaluation, because its very low value was likely due to an incorrect measurement. Based on analysis of remaining six data points and in contrast to the Oder River, both A_T and C_T increased stably (Fig: 5.4b). At S $<$ 4, the pH increased with a slightly steeper slope than at higher S values. As there were no Chl a data, particulate organic matter could not be evaluated. Interestingly, the DOC concentration increased at the same sampling station, to $\sim$ 70 μmol C/L and both nitrate and acetic acid showed discrete peaks at $S = 2.6$ (Fig. 5.6), suggesting the invasion of a slightly different water stream, such as a nutrient-enriched plume. The absence of a C_T signal suggested that there was no biological activity.

Overall, carboxylic acid concentrations remained mostly near their LODs as they did not exceed 2.3 μmol/L (acetic acid). In contrast to the Oder River, formic acid was detected across the entire salinity gradient at concentrations of $1.2-2$ μmol/L.

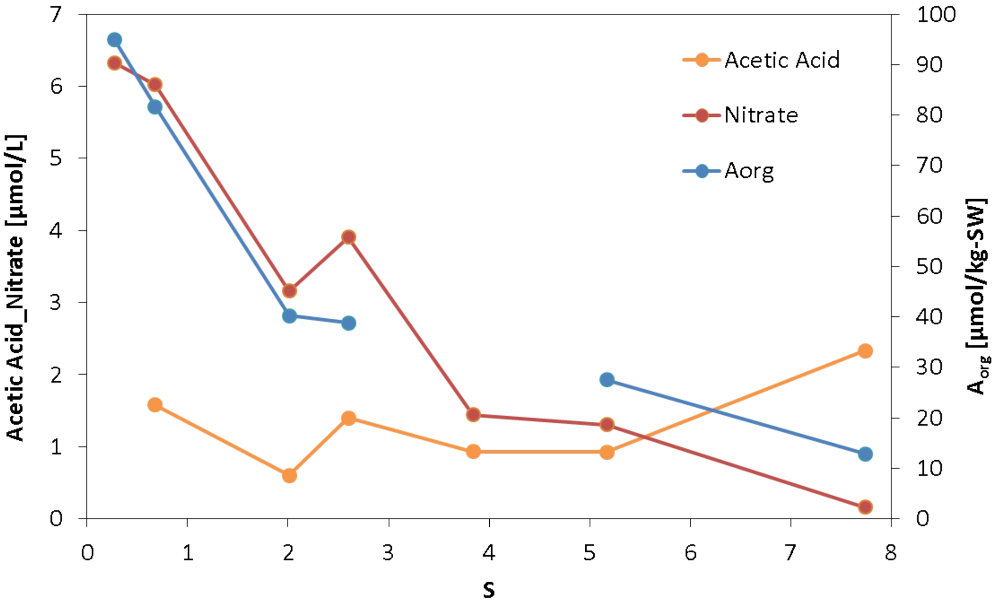


Figure 5.6: Concentrations of acetic acid, nitrate, and A_{org} (calculated from measured A_T, C_T and pH values) as a function of increasing salinity in the Vistula River estuary in May 2015.

This strongly suggested that formic acid was biologically consumed during the algal bloom in the Oder estuary, since the concentrations at this location exceeded the LOD only twice. The patterns of inorganic and organic compounds were consistent with the predominance of physical mixing effects of river and seawater, without signs of primary production.

As unlike the Oder River and despite the small changes in pH (< 0.54 pH units), in the Vistula River A_{org} correlated ($r^2 = 0.71$) with the hydrogen ion concentration. This result suggested that for the Oder River, despite of mixing processes, the pH-dependence of A_{org} was perturbed, presumably due to a phytoplankton bloom that generated high variability in the composition of DOC ($f \neq$ constant).

Principal component analysis of the Vistula estuary dataset

After a forth-root-transformation of the dataset, PC1, with a variation as high as 67.9 %, showed the significant negative correlation of salinity (Eigenvector = 0.23) with alkalinity (Eigenvector = −0.2), as expected for river water. The PCA also revealed the river as a source of nitrate and A_{org}, based on the negative correlation with salinity. The significance of A_{org} was high, evidenced by an Eigenvector of −0.82. A correlation of formic and acetic acid with A_{org} by similar Eigenvectors (0.3 − 0.5) was shown by PC2 (20.7 % of the variation), suggesting the river as source for organic alkalinity contribution in marine systems. However, it should be noted that because the PCA was based on only few data points, in the case of both the Oder River and the Vistula River, the results should be interpreted with caution.

Because a significant contribution of SCCA to the marine acid-base system could only be suspected from evaluation of the sparse Oder River dataset, a study to estimate their theoretical influence using results from conducted field and laboratory investigations was performed.

6 Theoretical study

6.1 Miscalculation of pH for three different alkalinity regimes

The results obtained in my study were used to calculate the potential overestimation of pH as a CO_2 key parameter and the potential effect of OA on the marine acid-base system. The different concentrations of OAs were assumed to reflect DOM of differing composition. Since carboxylic acids were measured within the range of < 1 to 11 μmol/L, concentrations of 5, 10, and 15 μmol/L were used to calculate the pH (from A_T and C_T) in respect to the unpertubed organic free status with $c_\mathrm{OA} = 0$ μmol/L. In addition, the same calculation was conducted using $\mathrm{p}K_\mathrm{DOM} = 7.27$ determined in Section 3.2 for three different f values (0.1, 0.15, 0.2), thus allowing calculation of the ΔpH (error in the pH) when the influence of OAs was neglected. A constant DOC concentration of 300 μmol C/L and a CO_2 partial pressure of 400 μatm (equilibrium with atmosphere) were assumed.

By incorporating realistic concentrations of carboxylic acids and different levels of proton-exchangeable functional groups of DOM into the error that arise when pH is calculated from A_T and C_T, their influence on the CO_2 system in different areas of the Baltic Sea could be modeled. Figure 6.1 shows the error in ΔpH for ocean water (Kattegat, $S = 30$), brackish water (proper Baltic Sea, $S = 7$) and

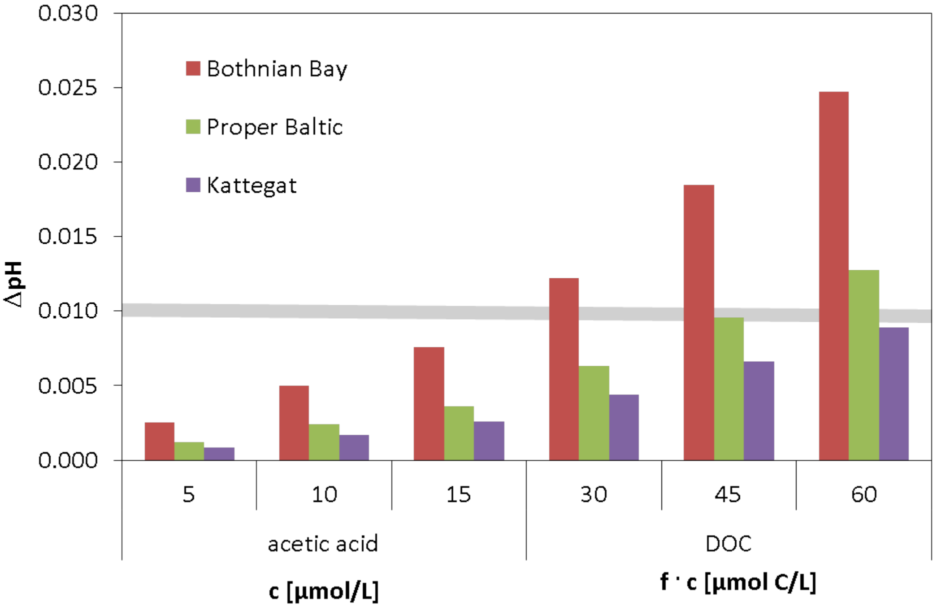


Figure 6.1: Theoretical miscalculation of the pH (ΔpH) at three different concentrations of acetic acid (left) and three different f values (0.10, 0.15, 0.20) representing the acid-base effect of DOM (right).

nearly fresh water (Bothnian Bay, $S = 3$). The left side of Fig. 6.1 illustrates the ΔpH resulting from carboxylic acids for at $S = 30$, 7, and 3. Since acetic acid was the most abundant carboxylic acid, it was used in this calculations based on the dissociation constants reported by Harned and Hickey (1937), as presented in Fig. 1.1 of Section 1.3.3. The determined error in the pH ranged from 0.001 for the Kattegat at 5 μmol acetic acid/L to a maximum error of 0.008 for the Bothnian Bay at an acetic acid concentration of 15 μmol/L. At first glance, this level of error seemed very low and thus negligible, assuming an overall uncertainty in the pH measurement between of $0.01 - 0.02$ (Section 2.1.1). However, as shown on the right side of the figure, in which the total influence of DOM was applied to ΔpH, the content of carboxylic acids affecting the overall miscalculation is higher than would be expected based on their concentrations alone. Assuming a stable DOM concentration but three different contributions (f) of acid-base effective functional groups depending on region and season, ΔpH ranged from a minimum of 0.004 pH-units for $f = 0.1$ in the Kattegat to a maximum of 0.025 pH-units in the Bothnian Bay at $f = 0.2$. The contribution of the carboxylic-acid-related error in pH to the error arising from the total DOM spectrum was 25%, assuming 10 μmol acetic acid/L in the Baltic Proper and a 15% contribution of acid-base functional groups to c_{DOC}. This is a realistic scenario describing the organic contribution in the central Baltic Sea. A much serious, yet not unrealistic scenario is a 60 % contribution of SCCAs to the miscalculation in pH arising from total DOM containing $c = 15$ μmol acetic acid/L and an overall acid-base functional proportion of $f = 0.1$.

6.2 Measured A_{org} vs. theoretical A_{org} during a seasonal cycle

In a second theoretical study, the previously determined bulk dissociation constant pK_{DOM} and the share of DOM (f) comprising acid-base functional groups as presented in Section 3.2 were applied to predict A_{org}. For this purpose, the time series from Heiligendamm, which contained a sufficient number of data ($n = 37$) was used.

Two calculated organic alkalinities were compared. The first A_{org} was calculated as applied before throughout the whole study, determining the difference between the measured alkalinity, in which the organic contribution is considered, and the calculated alkalinity, representing carbonate alkalinity, from the measured C_{T} and pH values. The second A_{org} (A_{org} I) was calculated from the measured C_{T} and pH, but with the addition of K_{DOM} and f as input parameters, thereby considering the organic contribution. Thus, the measured alkalinity was substituted with the

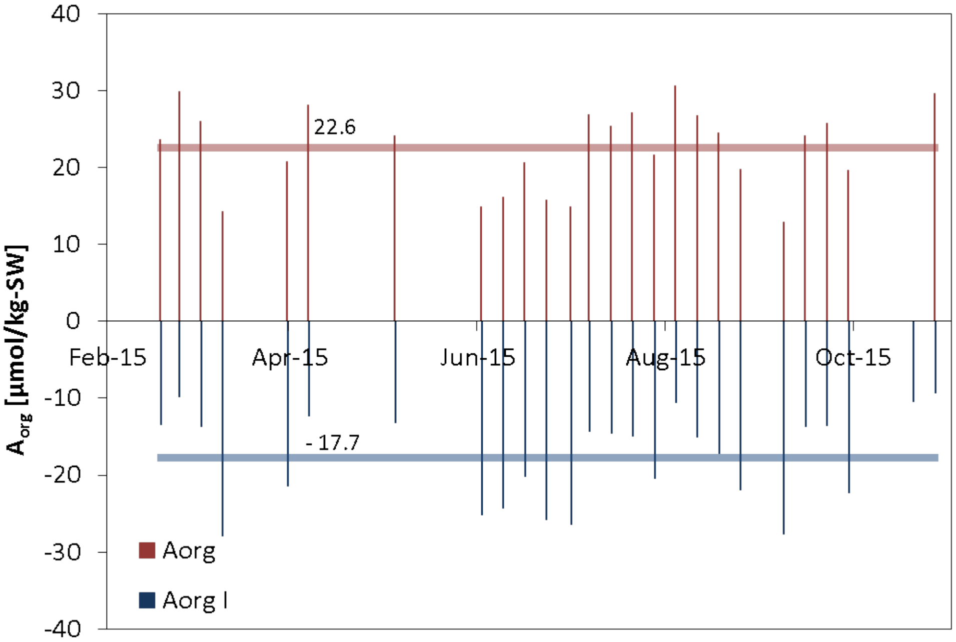


Figure 6.2: Difference of two A_{org} for a time series in Heiligendamm (2015), one calculated from measured A_{T}, C_{T}, and pH (red vertical lines) and one determined using C_{T}, pH, $f = 0.17$ and p$K_{\mathrm{DOM}} = 7.27$ (A_{org} I, blue vertical lines).

carbonate alkalinity, bulk pK, and the acid-base effective DOC concentration as the input parameters in the CO$_2$sys program. The results are shown in Fig. 6.2.

The data were purged by eliminating the upper most and lowest 10 % of both the determined A_{org} and the A_{org} I values. The averages are shown in the figure as translucent lines. The calculation with the traditional input gave a mean deviation of the measured and calculated alkalinity of $A_{\mathrm{org}} = 22.6$ μmol/kg-SW (red). With the new approach, the deviation was reversed and A_{org} I was negative; the mean was -17.7 μmol/kg-SW (blue translucent line).

This improvement, albeit small, was important as it demonstrated that: (1) The DOM in Heiligendamm differs significantly from that in the central Baltic Sea (investigated in Section 3.2). Whether contribute to the dissociation behavior (pK)

or to the number of protonated sites of DOM (f) remains to be investigated. (2) With the application of pK_{DOM} and f in theoretical calculations, the deviation between the measured and calculated alkalinity could be reduced by ~ 5 μmol/kg-SW.

7 Conclusions and future perspectives

The following section also draws on the conclusions reported in a manuscript entitled "Acid-base properties of Baltic Sea dissolved organic matter", by Karoline Hammer, Bernd Schneider, Karol Kuliński, Detlef E. Schulz-Bull. The manuscript was submitted to the Journal of Marine Systems.

The results of the present study expand current knowledge of the acid-base properties of the marine DOM present in coastal waters and estuaries. The decreasing salinity that characterizes the transition from oceanic conditions to these waters is accompanied by decreasing alkalinity. By focusing on distinct classes of substances that significantly affect the marine acid-base system in the Baltic Sea, my study extends previous examinations of bulk DOM as a contributor to the alkalinity in coastal waters.

The first chapter followed up on a previous study of organic alkalinity in the Baltic Sea (Kuliński et al., 2014). Based on a more comprehensive dataset for A_{org} that took into account the diversity of coastal water conditions (large pH and salinity gradients), I characterized the acid-base properties of Baltic Sea DOM by determining its bulk dissociation coefficient and estimating the fraction of its carbon atoms carrying acid-base groups. After showing a distinct correlation between A_{org} and pH, the terms of the principal mass action law were substituted with measurable and calculable parameters and then linearized. This allowed pK and f to be determined by plotting the resulting terms against each other. The pK_{DOM} of 7.27 agreed well with the value (7.34) obtained by Kuliński et al. (2014) and supported the validity of their findings, despite the small dataset used in that study. This concurrence suggests that, although the samples for these two studies were obtained during different seasons and from different depths and regions of the Baltic Sea, the chemical composition and acid-base properties of its DOM seems to be rather stable, at least for the major constituents. The f value determined in my investigations (0.17) was somewhat larger than that reported by Kuliński et al. (2014) (0.12). This discrepancy is acceptable given the voidable dynamic treatment of the acid-base equilibrium as a bulk acid-base system and not as distinct acid-base pairs.

However, I was unable to attribute the bulk properties of Baltic Sea DOM to a single class of compounds, such as humic or fulvic acids, which are major contributors to Baltic Sea DOM. The dissociation behavior of humic and fulvic acids was investigated by adding both compounds to artificial seawater adjusted to Baltic Sea conditions and then measuring three acid-base parameters (A_{T}, C_{T}, pH) before and after spiking. In contrast to the results of the field study, in the lab experiments the acid-base properties of neither humic nor fulvic substances alone explain the behavior of natural DOM. The theoretically estimated upper limit of 6.5 for the pK of humic and fulvic substances clearly indicated that these compounds are much more acidic than the bulk DOM in the Baltic Sea. However, this conclusion is limited to the fraction of the two humic substances soluble in seawater. The possible irreversible transfer of an originally soluble fraction to the particulate phase during the isolation of humic substances from their natural matrix cannot be ruled out. Thus, while acid-base properties of the natural DOM in the Baltic Sea cannot be entirely attributed to humic substances, they may contribute to the bulk pK_{DOM} in the Baltic Sea.

The time series from the Heiligendamm pier samples showed the large variability of inorganic CO_2 parameters, especially in spring and autumn. The high salinities measured in parallel indicated the presence of water masses from a different local origin. The elevated nitrate and SCCA concentrations ($c_{\mathrm{max}} = 3, 11, 0.7$ μmol/L for formic, acetic, and propionic acid, respectively) coincided with inflows of highly saline water and were therefore unlikely to have resulted from on-site biologic degradation or consumption; rather, they appeared to derive from the deep-water masses enriched in biodegradation products. This conclusion was supported by the lack of a correlation of A_{org} with the measured parameters, neither for the intensified biological activity during spring (indicated by Chl a) nor for the inflow events in autumn. Thus, a distinct pH dependence of A_{org} on the variation in biological activity could not be detected by a seasonal time series and the reason for the large variability in A_{org} observed in this study remains unclear. The likely long-term diversity in the composition of DOM ($f \neq$ constant) may instead account for the disturbed relationship between pH and A_{org}.

Two different Polish rivers, representing two different biological states, were investigated in this study. In the Oder estuary, a pH of 8.9 and a Chl a concentration of 41 mg/m^3 against a background of a nearly depleted nutrients were consistend with a phytoplankton bloom, particularly in Szczecin Lagoon. The abundance of OAs likely derived from the degradation of POC to DOC, hence releasing A_{org} contributors, as indicated by a parallel peak in Chl a, A_{org}, and the acetic acid concentration. However, despite a wide pH range, the correlation between A_{org} and pH was not significant. The data from the Oder estuary thus showed a distinct correlation between biological activity and both SCCA and A_{org}, but a pH dependence of organic alkalinity could not be demonstrated, in contrast to my findings for the vertical profiles in the central Baltic Sea, also assessed in this study. Thus, high levels of primary production may mask the sensitive relationship of A_{org} with pH.

In the Vistula estuary, however, the inorganic CO_2 parameters showed a pattern indicative of the conservative mixing of river water with seawater. Despite the narrow pH range (< 0.54) a pH dependence of A_{org} could be detected in the Vistula estuary. Since no detectable biological activity occurred, a correlation between A_{org} and SCCAs could not be established.

Nonetheless, this study was a first a attempt to consider other contributions besides that of HMWOA to A_{org}. In a theoretical study using the CO_2sys program and the SCCA concentrations measured during a seasonal cycle and in two river estuaries, I was able to show the distinct effect of these compounds on the acid-base system. Indeed, under the conditions of the central Baltic Sea at equilibrium ($p\mathrm{CO}_2 = 400\ \mu\mathrm{atm}$), the failure to account for the contribution of SCCA can result in a miscalculation of the pH by 25 %. For example, during elevated biological activity and in deep water layers, both of which are characterized by intense biodegradation, small organic acids, normally present in low concentrations, will reach high abundances and thus contribute to determining the pH of the respective waters. As a result they thus cause a saisonality in A_{org}.

My work clearly had several limitations. First, in the absence of full molecular characterization of DOM, bulk parameters were applied that ignored the highly heterogeneous nature of DOM. Second, measurement uncertainties resulted in a large variability of A_{org}. Third, the DOM reference material was only of terrestrial origin. Fourth, since the pK of SCCA is close to that of carbonic acid, the behavior of these compounds during a titration for alkalinity determination remains unclear. These and other issues support the need for further investigations of DOM and its influence on the acid-base system in marine waters. For example, the temperature dependency of $\mathrm{p}K_{\mathrm{DOM}}$ should be investigated to account for the high variability of seawater temperatures. Furthermore, the results from the Oder estuary remain to be validated in a comprehensive investigation of A_{org}, which is responsive to seasonal signals, especially those from algal blooms. Further important issues are the proportion of humic substances in bulk DOM and the nature of the contribution of organic acids; the latter can be determined by calculations of their dissociation constant and concentrations. By expanding the capability of programs such as CO_2sys it will be possible to include these values to account for more than one class of organic substances and thus to obtain more detailed and differentiated insights into contribution of organics to A_{org}.

There is a growing awareness of the importance of A_{org} in characterizations of the marine CO_2 system and considerably progress has been made in its quantitative descriptions. Nevertheless, an exact thermodynamic treatment of A_{org} is not yet possible, as it requires knowledge of the seawater concentrations and dissociation constants of the major individual organic compounds with acid-base properties. In the meantime, studies such as the present one provide useful tools for approximating the potential error in calculations of the properties of the marine CO_2 system on the basis of alkalinity data. This results are relevant not only to experimental investigations, but also to models of the CO_2 system in which alkalinity and total CO_2 are included as conservative variables.

References

Aiken, G. R., 1985. Isolation and concentration techniques for aquatic humic substances.

Albert, D. B., Taylor, C., Martens, C. S., 1995. Sulfate reduction rates and low molecular weight fatty acid concentrations in the water column and surficial sediments of the black sea. Deep Sea Research Part I: Oceanographic Research Papers 42 (7), 1239–1260.

Archer, D., 2005. Fate of fossil fuel co2 in geologic time. Journal of Geophysical Research: Oceans 110 (C9).

Beldowski, J., Löffler, A., Schneider, B., Joensuu, L., 2010. Distribution and biogeochemical control of total co2 and total alkalinity in the baltic sea. Journal of Marine Systems 81 (3), 252–259.

Bertilsson, S., Allard, B., 1996. Sequential photochemical and microbial degradation of refractory dissolved organic matter in a humic freshwater system. Ergebnisse der Limnologie 48, 133–141.

Brewer, P. G., Goldman, J. C., 1976. Alkalinity changes generated by phytoplankton growth. Limnol. Oceanogr 21 (1), 108–117.

Brinkmann, T., Hörsch, P., Sartorius, D., Frimmel, F. H., 2003. Photoformation of low-molecular-weight organic acids from brown water dissolved organic matter. Environmental science & technology 37 (18), 4190–4198.

Cai, W.-J., Wang, Y., Hodson, R. E., 1998. Acid-base properties of dissolved organic matter in the estuarine waters of georgia, usa. Geochimica et cosmochimica acta 62 (3), 473–483.

Carlson, C. A., Hansell, D. A., 2014. Dom sources, sinks, reactivity, and budgets. In: Elsevier Inc.

Carter, B., Radich, J., Doyle, H., Dickson, A., 2013. An automated system for spectrophotometric seawater ph measurements. Limnol. Oceanogr.: Methods 11, 16–27.

Culkin, F., 1965. The major constituents of sea water. Chemical oceanography 1, 121–161.

DelValls, T. A., Dickson, A. G., 1998. The ph of buffers based on 2-amino-2-hydroxymethyl-1,3-propanediol ('tris') in synthetic sea water. Deep Sea Research Part I: Oceanographic Research Papers 45 (9), 1541–1554.

Dickson, A., 1981. An exact definition of total alkalinity and a procedure for the estimation of alkalinity and total inorganic carbon from titration data. Deep Sea Research Part A. Oceanographic Research Papers 28 (6), 609–623.

Dickson, A. G., 1990. Standard potential of the reaction: Agcl (s)+ 12h2 (g)= ag (s)+ hcl (aq), and and the standard acidity constant of the ion hso$_4^-$ in synthetic sea water from 273.15 to 318.15 k. The Journal of Chemical Thermodynamics 22 (2), 113–127.

Dickson, A. G., Sabine, C. L., Christian, J. R., 2007. Guide to best practices for ocean co2 measurements.

Dittmar, T., Koch, B., Hertkorn, N., Kattner, G., 2008. A simple and efficient method for the solid-phase extraction of dissolved organic matter (spe-dom) from seawater. Limnol. Oceanogr. Methods 6, 230–235.

Dunne, J. P., Sarmiento, J. L., Gnanadesikan, A., 2007. A synthesis of global particle export from the surface ocean and cycling through the ocean interior and on the seafloor. Global Biogeochemical Cycles 21 (4).

Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J., Millero, F. J., 2004. Impact of anthropogenic co2 on the caco3 system in the oceans. Science 305 (5682), 362–366.

Goldman, J. C., Brewer, P. G., 1980. Effect of nitrogen source and growth rate on phytoplankton-mediated changes in alkalinity. Limnology and Oceanography 25 (2), 352–357.

Hammer, K., Schneider, B., Kuliński, K., Schulz-Bull, D. E., 2014. Precision and accuracy of spectrophotometric ph measurements at environmental conditions in the baltic sea. Estuarine, Coastal and Shelf Science.

Hansell, D. A., Carlson, C. A., 2014. Biogeochemistry of marine dissolved organic matter. Academic Press.

Hansson, I., 1973. A new set of acidity constants for carbonic acid and boric acid in sea water. Deep Sea Research and Oceanographic Abstracts 20 (5), 461–478.

Harned, H. S., Hickey, F., 1937. The ionization of acetic acid in aqueous sodium chloride solutions from 0 to 40°. Journal of the American Chemical Society 59 (7), 1284–1288.

Hernández-Ayón, J., Zirino, A., Dickson, A., Camiro-Vargas, T., Valenzuela, E., 2007. Estimating the contribution of organic bases from microalgae to the titration alkalinity in coastal seawaters. Limnology and Oceanography: Methods 5, 225–232.

Hernández-Ayón, J. M., Belli, S. L., Zirino, A., 1999. ph, alkalinity and total co_2 in coastal seawater by potentiometric titration with a difference derivative readout. Analytica chimica acta 394 (1), 101–108.

Hessen, D., Tranvik, L., 2013. Aquatic humic substances: ecology and biogeochemistry. Vol. 133. Springer Science & Business Media.

Ho, T.-Y., Scranton, M. I., Taylor, G. T., Varela, R., Thunell, R. C., Muller-Karger, F., 2002. Acetate cycling in the water column of the cariaco basin: Seasonal and vertical variability and implication for carbon cycling. Limnology and oceanography 47 (4), 1119–1128.

Hongve, D., 1990. Shortcomings of gran titration procedures for determination of alkalinity and weak acids in humic water. Water Research 24 (10), 1305–1308.

Houghton, R., Hackler, J., 2002. Carbon flux to the atmosphere from land-use changes. in trends: A compendium of data on global change. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tenn., USA.

Hruška, J., Köhler, S., Laudon, H., Bishop, K., 2003. Is a universal model of organic acidity possible: Comparison of the acid/base properties of dissolved organic carbon in the boreal and temperate zones. Environmental science & technology 37 (9), 1726–1730.

IPCC, 2007. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental

Panel on Climate Change (IPCC). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Johnson, K. M., Wills, K. D., Butler, D. B., Johnson, W. K., Wong, C. S., 1993. Coulometric total carbon dioxide analysis for marine studies: maximizing the performance of an automated gas extraction system and coulometric detector. Marine Chemistry 44 (2-4), 167–187.

Katchalsky, A., Spitnik, P., 1947. Potentiometric titrations of polymethacrylic acid. Journal of Polymer Science 2 (4), 432–446.

Keene, W. C., Talbot, R. W., Andreae, M. O., Beecher, K., Berresheim, H., Castro, M., Farmer, J. C., Galloway, J. N., Hoffmann, M. R., Li, S.-M., 1989. An intercomparison of measurement systems for vapor and particulate phase concentrations of formic and acetic acids. Journal of Geophysical Research D 94 (D5), 6457.

Khare, P., Kumar, N., Kumari, K., Srivastava, S., 1999. Atmospheric formic and acetic acids: An overview. Reviews of geophysics 37 (2), 227–248.

Kieber, D. J., McDaniel, J., Mopper, K., 1989. Photochemical source of biological substrates in sea water: Implications for carbon cycling. Nature 341 (6243), 637–639.

Kim, H., Lee, K., 2009. Significant contribution of dissolved organic matter to seawater alkalinity. Geophysical Research Letters 36 (20).

Köhler, S., Laudon, H., Wilander, A., Bishop, K., 2000. Estimating organic acid dissociation in natural surface waters using total alkalinity and toc. Water Research 34 (5), 1425–1434.

Kuliński, K., Hammer, K., Schneider, B., Schulz-Bull, D., 2016. Remineralization of terrestrial dissolved organic carbon in the baltic sea. Marine Chemistry 181, 10–17.

Kuliński, K., Schneider, B., Hammer, K., Machulik, U., Schulz-Bull, D., 2014. The influence of dissolved organic matter on the acid-base system of the baltic sea. Journal of Marine Systems 132 (0), 106–115.

Lisitzin, A. P., Rodolfo, K. S., 1972. Sedimentation in the world ocean with emphasis on the nature, distribution and behavior of marine suspensions. Society of Economic Paleontologists and Mineralogists.

Millero, F. J., Graham, T. B., Huang, F., Bustos-Serrano, H., Pierrot, D., 2006. Dissociation constants of carbonic acid in seawater as a function of salinity and temperature. Marine Chemistry 100 (1-2), 80–94.

Miwa, H., Hiyama, C., Yamamoto, M., 1985. High-performance liquid chromatography of short-and long-chain fatty acids as 2-nitrophenylhydrazides. Journal of Chromatography A 321, 165–174.

Mopper, K., Degens, E., 1979. Organic carbon in the ocean: nature and cycling. The global carbon cycle, 293–316.

Mopper, K., Kieber, D. J., 1991. Distribution and biological turnover of dissolved organic compounds in the water column of the black sea. Deep Sea Research Part A. Oceanographic Research Papers 38, Supplement 2 (0), S1021–S1047.

Mopper, K., Lindroth, P., 1982. Diel and depth variations in dissolved free amino acids and ammonium in the baltic sea determined by shipboard hplc analysis. Limnology and Oceanography, 336–347.

Moran, M. A., Hodson, R. E., 1994. Dissolved humic substances of vascular plant origin in a coastal marine environment. Limnology and Oceanography 39 (4), 762–771.

Morikami, T., Tanaka, S., Hashimoto, Y., 1993. Formic and acetic acids in the marine atmosphere. Nippon Kagaku Kaishi, 98–98.

Muller, F. L., Bleie, B., 2008. Estimating the organic acid contribution to coastal seawater alkalinity by potentiometric titrations in a closed cell. Analytica chimica acta 619 (2), 183–191.

Müller, T. J., 2007. Determination of salinity. Wiley-VCH Verlag GmbH, pp. 41–73.

Nausch, G., Naumann, M., Umlauf, L., Mohrholz, V., Siegel, H., Schulz-Bull, D. E., 2016. Hydrographic-hydrochemical assessment of the baltic sea 2015. Report.

Omstedt, A., Gustafsson, E., Wesslander, K., 2009. Modelling the uptake and release of carbon dioxide in the baltic sea surface water. Continental Shelf Research 29 (7), 870–885.

Pelletier, G., Lewis, E., Wallace, D., 2007. Co2sys. xls: A calculator for the co 2 system in seawater for microsoft excel/vba. Washington State Department of Ecology/Brookhaven National Laboratory, Olympia, WA/Upton, NY, USA.

Pempkowiak, J., 1985. The input of biochemically labile and resistant organic matter to the baltic sea from the vistula river. Mitt. Geol.-Paläont. Inst. Univ. Hamburg 58, 345–350.

Pempkowiak, J., Kupryszewski, G., 1980. The input of organic matter to the baltic from the vistula river. Oceanologia 12 (7).

Rashid, M. A., 2012. Geochemistry of marine humic compounds. Springer Science & Business Media.

Repeta, D. J., 2015. Chemical characterization and cycling of dissolved organic matter. Biogeochemistry of Marine Dissolved Organic Matter, 21–63.

Repeta, D. J., Quan, T. M., Aluwihare, L. I., Accardi, A., 2002. Chemical characterization of high molecular weight dissolved organic matter in fresh and marine waters. Geochimica et Cosmochimica Acta 66 (6), 955–962.

Sabine, C. L., Feely, R. A., Gruber, N., Key, R. M., Lee, K., Bullister, J. L., Wanninkhof, R., Wong, C., Wallace, D. W., Tilbrook, B., 2004. The oceanic sink for anthropogenic co2. science 305 (5682), 367–371.

Schneider, B., 2011. The CO2 system of the Baltic Sea: Biogeochemical control and impact of anthropogenic CO2. Springer, pp. 33–49.

Sörgel, K., 2001. Sequenzielle coulometrische bestimmung von schwefelwaaserstoff und kohlendioxid im meerwasser.

Sugimura, Y., Suzuki, Y., 1988. A high-temperature catalytic oxidation method for the determination of non-volatile dissolved organic carbon in seawater by direct injection of a liquid sample. Marine Chemistry 24 (2), 105–131.

Tan, K., Lobartini, J., Himmelsbach, D., Asmussen, L., 1991. Composition of humic acids extracted under air and nitrogen atmosphere 1. Communications in Soil Science & Plant Analysis 22 (9-10), 861–877.

Tan, K. H., 2014. Humic matter in soil and the environment: principles and controversies. CRC Press.

Thurman, E. M., Malcolm, R. L., 1981. Preparative isolation of aquatic humic substances. Environmental Science & Technology 15 (4), 463–466.

Tipping, E., Lofts, S., Sonke, J., 2011. Humic ion-binding model vii: a revised parameterisation of cation-binding by humic substances. Environmental Chemistry 8 (3), 225–235.

Ulfsbo, A., Kuliński, K., Anderson, L. G., Turner, D. R., 2015. Modelling organic alkalinity in the baltic sea using a humic-pitzer approach. Marine Chemistry 168, 18–26.

Vairavamurthy, A., Mopper, K., 1990. Determination of low-molecular-weight carboxylic acids in aqueous samples by gas chromatography and nitrogen-selective detection of 2-nitrophenylhydrazides. Analytica Chimica Acta 237, 215–221.

Van Heuven, S., Pierrot, D., Rae, J., Lewis, E., Wallace, D., 2011. Matlab program developed for co2 system calculations http://cdiac. ornl. gov/ftp/co2sys. CO2SYS_calc_ MATLAB_v1 1.

Weiss, R., 1974. Carbon dioxide in water and seawater: the solubility of a non-ideal gas. Marine chemistry 2 (3), 203–215.

Yang, B., Byrne, R. H., Lindemuth, M., 2015. Contributions of organic alkalinity to total alkalinity in coastal waters: A spectrophotometric approach. Marine Chemistry 176, 199–207.

Yang, X. H., Lee, C., Scranton, M. I., 1993. Determination of nanomolar concentrations of individual dissolved low molecular weight amines and organic acids in seawater. Analytical Chemistry 65 (5), 572–576.

Zeebe, R. E., Wolf-Gladrow, D. A., 2001. CO2 in seawater: equilibrium, kinetics, isotopes. Gulf Professional Publishing.

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List of Abbreviations

2-NPH	2-nitrophenylhydrazine
CA	carboxylic acis
Chl *a*	chlorophyll *a*
CRM	carbon reference material
CTD	conductivity temperature depth
DFAA	dissolved free amino acids
DIC	dissolved inorganic carbon
DIN	Deutsches Institut für Normung
DOC	dissolved organic carbon
DOM	dissolved organic matter
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
FA	fulvic acid
GC	gas chromatography
GF/F	borosilicate glass microfibre filters, 0.7μm particle retention
HA	humic acid
HMW	high molecular weight
HMWOA	high molecular weight organic acids
HTCO	high temperature catalytic oxidation
I.D.	inside diameter
IHSS	International Humic Substance Society
IOPAN	Institute of Oceanology Polish Academy of Science

IOW	Institut für Ostseeforschung Warnemünde
IPCC	Intergovernmental Panel on Climate Change
IR	infrared
LMW	low molecular weight
LMWOA	low molecular weight organic acids
LOD	limit of detection
MA	Massachusetts
marianda	marine analytics and data
MARNET	Marines Umweltmessnetz in Nord- und Ostsee des Bundesamts für Seeschifffahrt und Hydrographie
MS	mass spectrometry
MV	Mecklenburg-Vorpommern
NE	Nebraska
NMR	nuclear magnetic resonance spectroscopy
NY	New York
OA	organic acids
PCA	principal component analysis
PESU	polyethersulfone
POC	particulate organic carbon
PSU	practical salinity units
rpm	revolutions per minute
SA	Sigma Aldrich
SCCA	short chain carboxylic acid
SIM	single ion monitoring
SOMMA	single operator multiparameter metabolic analyzer
SPE	solid phase extraction
SW	seawater

TOC	total organic carbon
TRIS	tris(hydroxymethyl)aminomethane

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Eidesstattliche Erklärung

Ich versichere hiermit an Eides statt, dass ich die vorliegende Arbeit selbstständig angefertigt und ohne fremde Hilfe verfasst habe, keine außer den von mir angegebenen Hilfsmitteln und Quellen dazu verwendet habe und die den benutzten Werken inhaltlich und wörtlich entnommenen Stellen als solche kenntlich gemacht habe.

Karoline Hammer

Rostock, den 24. März 2017