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# **Evaluation of methods to assess relative abundance, size/age structure and gear-specific traits in northern pike (*Esox lucius*) sampled in brackish lagoons**

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# Abstract

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Accurate information on fish populations, including abundance, age and size structure, growth rate, and condition, is crucial for fisheries science and management. In order to acquire such information, some type of sampling protocol and gear has to be applied in a random pattern in a given aquatic ecosystem. All gears have a certain type of bias, which is related to factors such as catchability, seasonality, fish behaviour and the habitats sampled. Those biases need to be considered when deriving population-level statements from a sample taken from this population. Northern pike (*Esox lucius*) are a widespread species in the northern hemisphere and is an important species in many fisheries. In the Baltic lagoons around the island of Rügen, Germany, pike stocks have been drastically declining in the recent years and no standardized monitoring approach is in place. The present thesis compared five different sampling gears and methods – e-DNA, angling, gillnetting, ROUV's and longlining – with each other over different seasons and along a gradient of areas with varying fishing pressure and protection. The goal was to derive relative abundance estimates of pike and to compare growth rates, age/size structures and condition revealed by each gear. From all the five gears applied, only three gears – e-DNA, angling and gillnetting – were providing sufficient data.

It was found that angling was the most consistent gear across all the seasons, while e-DNA and gillnetting were mainly applicable in spring, when pike are spawning. However, pike captured with angling showed a very high growth rates in comparison to gillnet pike. That indicates that angling is strongly selective towards highly active fish, while less active and more shy pike are underrepresented due to timidity. This was found to be a large bias in angling based assessments of abundance of pike. The effect of timidity might be as strong as the effect of abundance, when comparing protected and unprotected sites with each other. In order to rule out timidity, a second gear must be applied, that is more resistant to timidity. Gillnets have the potential in doing so, but higher efforts are required than in this thesis. Pike, being a low abundance and low activity species remains to be a challenging species to sample. Given that all tested gears exhibit some form of bias, it is crucial for fisheries management to implement a multi-gear sampling strategy to mitigate these biases and improve the accuracy of population assessments of pike. Angling, despite its biases, should be included due to its consistency across different seasons.

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# 1. Introduction

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Sampling of fishes is the process of inferring insight for the target population from samples taken from this population. The quality of those insights and derived estimates, e.g. catch per unit effort (CPUE), is dependent on the quality of the samples taken and whether those samples are representative units of the population (Murphy & Willis, 1996; Stamatopoulos, 2002). As a general rule to sampling fish, proper sampling effort and sampling design need to be tailored to the objectives of the study and the related research questions, to the habitats and the fish species investigated and to the time of the year, where sampling will take place (Portt et al., 2006).

Very commonly investigated metrics of fish in the field of fish ecology are species richness, abundance, weight-length relationships and size or age structure, which is also an input to infer population-level metrics such as growth rate. Other typical metrics in fish ecology include measurements of individual traits such as body length, age or growth rate (Murphy & Willis, 1996; Nielsen & Johnson, 1983).

In order to gather data on the aforementioned metrics, scientists and fisheries management typically deploy some form of fishing gear and method in a randomized pattern. The choice of sampling gears and methods however is large and is ranging from non-invasive methods such as observations of fish assemblages with genetic and visual methods or echo-sounding (e.g. diving, camera systems, sonar, environmental DNA (e-DNA)) to more invasive and often lethal approaches such as trawling, seining, electrofishing, gillnetting, long-lining, trapping and rod-and-reel fishing (Lacoursière-Roussel et al., 2016a; Mueller et al., 2017)

Typically, those gears are classified into active and passive gear types (Brandt, 1984). In active gear, capturing the fish happens through active movement of the gear (e.g. trawling, electrofishing) towards the fish, while in passive gear (e.g. gillnetting, fyke nets), the capture process depends on fish movements and decisions (Dahm 1980; Kubečka et al., 2012; Mehdi et al., 2021).

All the gears mentioned have their strength and weaknesses, which is why scientists and fisheries management have to apply due diligence to choose the gear and method or multiple gears and methods that meet the study objectives and samples the target population representatively.

One metric that is of particular interest to fisheries scientists and management is abundance of fish. Total abundance of fish has a spatial relation and can be measured as individuals per volume or area of water. For gathering data about abundance in spatial relationship, only few gears exist that can provide such an index such as trawling, echo-sounding, electrofishing and visual observations such as snorkelling for smaller waterbodies and rivers (Merz et al., 2021; Emmrich et al., 2010; Hankin & Reeves, 1988; Hayes et al., 2007). However, even when such (active) gears are used, abundance estimates can be heavily biased. Fish show avoidance and fleeing behaviour, where they are hiding in structures or are evading the gear by swimming, what will result in undetected individuals (Bohlin et al., 1989; Engås & Godø, 1989; Reynolds & Dean, 2020).

Another possibility for total abundance measurements are capture-mark-recapture studies. In such studies, fish are captured, marked, and recaptured to estimate the total population size (Cormack, 1972; Jolly, 1982; Seber, 1982). Those methods are very time consuming and also rely on unbiased samples of fish to be marked, a non-selective recapture gear, population closure and even mixing of marked and unmarked fish and also tag loss is an issue (Agnew et al., 2006; Arnason et al., 1981; Isley & Tomasso 1998).

Hence, given the limitations of total abundance assessments of fish, scientists and fisheries management often rely on relative measures of abundance through catch-per-unit-effort (CPUE). CPUE is assumed proportional to abundance via the classical catch equation  $CPUE = q \times N$ , where  $q$  is the catchability coefficient (defined as the proportion of fish sampled per unit of effort, see Ricker, 1975) and  $N$  is the abundance of fish. In reality, the relationship of CPUE and abundance is often disproportional and affected by a wide range of factors such as gear and method limitations (e.g. to reach all available habitats), the biology of the target species (e.g. seasonal migrations), fisher behaviour, learning of fish (e.g. to avoid angling lures after a capture event) and fisheries induced evolution (Alós et al., 2019; Arlinghaus et al. 2016; Hilborn & Walters, 1992).

Catchability is also a variable parameter and influenced by the type of gear used, seasonality, environmental factors and fish density, possibly leading to hyperstable or hyperdepleted catch rates, where CPUE is not linear to true abundance (Hilborn & Walters, 1992).

For the ability of a certain gear to capture or otherwise sample fish (e.g. observe) in their natural environment, there are additional factors involved besides catchability. Those factors are detectability of the target species and selectivity patterns of the respective gear applied (Ricker, 1975). While catchability is strongly associated with spatial patterns of fish movement and the reactivity of fish to the gear, detectability relates to the abundance of a species, where rare species or low abundance species are much less likely being “detected” with the sampling gear compared to high abundance species (Thompson & Seber, 1994). Selectivity relates to properties of the fish (e.g. body length or shape) in relation to the properties of the sampling gear. Lennox et al. (2017) conceptualized the capture process of being composed of encountering a fish (which is also a function of behavioural decisions and aggregation patterns) and the technical properties of the gear (e.g. mesh sizes in a gill net).

In passive gear types, the behavioural decisions of fish are a key factor to affect selectivity (Lennox et al., 2017). While selectivity in fisheries science is mostly thought of as relating to morphological aspects of body size, other individual traits of fish such as activity levels or boldness, can determine the vulnerability of an individual fish towards a certain gear (Lennox et al., 2017; Monk & Arlinghaus, 2017).

Given that, it seems that there is no gear type that comes without a certain degree of selectivity towards certain individual traits of fish such as body length, growth rate and condition. In littoral electrofishing for pike for example, smaller and slower growing pike are captured compared to gears (e.g. rod-and-reel angling) deployed in deeper areas of the water body (Crane et al., 2015). It is therefore necessary for researchers and management to understand that all gears have a certain bias, which needs to be considered when inferring insights for the entire population from a specific gear and sampling protocol.

Furthermore, gears differ in their ability to capture specific species (catchability) and associated size classes of individual fish. Both, catchability and selectivity, are strongly dependent on the species of interest (in relation to the gear used), habitat structures, environmental conditions (e.g. daytime or season), and is further influenced by species or size-specific behaviour and morphological traits (Portt et al., 2006).

For examples, Mueller et al., (2017) compared nine different sampling gears (electrofishing, seining, snorkelling, multi-mesh-gillnets, lift netting, baited fish traps,

fyke nets, angling and longlines) in their ability to assess fish communities in flood plain habitats. The authors found very strong differences in species richness, CPUE and size structure between the applied gears. They concluded that only two of the used gears (electrofishing and seining) were reasonably unbiased to assess the fish community in the flood plains.

However, this study is also fraught with issues as the authors did not know the true fish community composition. Another study in drainable ponds compared the ability of three gears to reveal the fish community composition prior to draining, what allowed a comparison of the true composition and the composition revealed by the respective gears. In this study, gillnets detected five out of the seven species, but with strong selectivity for larger individuals. These results of the different catchability and detectability of distinct species with different gears can be explained by large differences in behaviours between species and different habitat types within a sampled system (Ravn et al., 2019). Therefore, the usage of a single gear to fully assess an entire fish community or even a single specific species and its full spectrum of individual traits, is often not sufficient (Casselman et al., 1990; Lapointe et al., 2006; Weaver et al., 1993).

From the material discussed so far, it can be concluded that the choice of sampling gear is strongly dependent on the research question and different gears and methods will result in possibly very different samples of the targeted fish species (e.g. different size classes). Portt et al. (2006) developed four main factors that determine the choice of sampling method: First, the research question that wants to be answered. Second, the surrounding habitat where sampling will take place. Third, the fish species that will be investigated and fourth, the time of the year of sampling. The last three points (habitat, fish species and time) mentioned by Portt et al. (2006) are in a very dynamic relationship to each other. Moreover, individuals of a single species might show totally different behaviour and hence catchability even for the same size (Kobler et al., 2009) influenced by consistent individual differences in behaviour (so-called personalities), different life stages (mature or immature) and associated sizes or time of the year, what can also result in different habitat usage and activities (Byström et al., 2003; Werner & Gilliam, 1984; Werner & Hall, 1988).

Therefore, sampling gear must not only be tailored to the species, but also to the life-stage and the season. Even if all these factors are met, individual variation in behaviour



(Conrad et al., 2011) can result in systematic relationships to life-history traits, e.g., bold fish might also be fast growers, which can both influence, which individuals are catchable and who are not (Monk et al., 2021). Especially aging and growth studies are plagued by selective capture of faster growing fish, so that inferences about population level growth taken from a biased sample of fast growing individuals can strongly bias the underlying growth estimates (Ricker, 1969). While studies exist that show that fish captured on one gear might systematically grow more slowly than in another gear (Crane et al., 2015), more methodological research is needed beyond simple size-selectivity of different gear types in the wild.

In lake fish ecology and fisheries science, two gear types are regularly used when the study aims at also sampling biological data from actual fish: electrofishing and gill netting. Electrofishing is suitable to sample littoral fish, but the gear is positively size-selective (i.e., larger fish have a larger chance of being captured, Ruetz, 2007). However, the gear is not suitable to sample fish species that regularly roam outside the littoral zone, and this gear type is not usable when the conductivity of the water is too high (Hill & Willis, 1994). Gill nets, due to the involvement of certain mesh sizes, is also size-selective, typically showing dome-shaped selectivity for specific mesh sizes (Hommik et al., 2020; Kuparinen et al., 2009). The issue can be resolved with multi-mesh gill nets, but catchability strongly depends on active movements of fish and of the fish having a certain shape and condition, and thus shows strong species-selectivity and preferentially samples better conditioned fish (Rudstam et al., 1984). Also, the largest fish of a population are typically undersampled with gill nets. The largest fish in a population can often be best be sampled by rod-and-reel angling (Pope et al., 2005; Wilde et al., 2003).

Especially difficult to sample are species that occur in low abundance (where more effort has to be devoted to capture the fish) and that have a more cryptic lifestyle in terms of movements. The more resident the lifestyle is, the poorer the fish encounters a passive gear. Active gear types such as electrofishing might help, but only for the littoral phase of the fish. All of these conditions apply to freshwater esocids, such as muskellunge or northern pike. These fish species are top predators and occur in low abundances in lakes, rivers and in the case of northern pike also in brackish coastal areas (Cooke et al., 2016; Craig, 1995; Craig, 2008). Esocids are large-bodied, but they show low movement activity and are thus not easily captured with passive gear types

such as gill nets (Arlinghaus et al., 2023; Bregazzi & Kennedy, 1980; Craig, 2008; Dhellemmes et al., 2023; Pierce, 2012).

Electrofishing is effective in capturing the smaller life stages in rivers and lakes nearshore, but the larger pike increasingly loose attachment to the immediate littoral zone and are then not easily sampled with electrofishing (Eklöv, 1997). Moreover, electrofishing does not work in brackish water systems, so that gear type is useless in low salinity environments. An alternative sampling gear could be large fyke nets, which have proved to generate proper indices of spawning stock size in lakes (Pierce, 2012). Given its large body size, especially adult pike are very difficult to sample with the standard EU multi-mesh gill nets commonly used in the monitoring of the EU Water Framework Directive (Olsson et al., 2023).

This becomes problematic given the fact that the overall pike abundance in coastal areas along the Baltic Sea is declining (Olsson et al., 2023) and this calls for improved methods and gears that assess pike populations more precisely especially in coastal areas. Tibblin et al. (2023) argue that a proper index of local abundance could be generated with experimental angling in coastal areas, but this gear might only be sampling adult pikes and underrepresent small pike.

In the past, many different sampling gears and methods have been used to sample pike populations in many different habitats following a wide range of research questions. Besides electrofishing and fyke nets, gill nets with specifically tailored mesh sizes were the most commonly used gear in order to assess pike stocks (Moyle, 1950; Pierce, 2012).

The combination of mesh sizes is an important factor that determines the sizes and amount of the caught fish. It also affects the size selectivity, meaning that specific mesh size only catch a specific size range of fish, what typically results in a bell shaped selectivity curve for single mesh sizes (Hubert et al., 2012; Lobyrev et al., 2018). To counter this strong selectivity, typically a range of different mesh sizes is used in scientific gill net surveys (Hubert et al., 2012; Pierce et al., 1994; Reddin, 1986).

In addition, gill nets tend to catch the faster growing components of each age class, what can result in potential biases regarding age and size structures, overestimated growth rates and conditions (Backiel & Welcome, 1980; Hamley & Regier, 1973).

As gill nets depends on active movement of fish, it can be expected that the gear is not equally suitable in different seasons. In small lakes, Pierce & Tomcko (2003) found summer gill netting to be an appropriate method to track pike CPUE (fish/net) as an index of population size. However, given the low movements of pike in summer, a high “contact rate” of gill nets relative to water area is important, such that the gill nets may not be operational in widespread, large ecosystem as the total number of gill nets needed to cover a water body and achieve a sufficient catchability might quickly become prohibitive. Importantly, vulnerability to capture varies among individuals within the same size class due to behavioural differences and gill nets as a passive gear are likely to catch active pike more likely (e.g. higher swimming distances, Carlson et al. 2009; Edeline et al. 2017; Monk et al. 2021).

Importantly, historically high fishing pressure might systematically remove the more vulnerable phenotypes, leaving behind fish that are less and less catchable, contributing to hyperdepleted CPUE-abundance relationships (Bell et al., 2009, Pierce & Tomcko, 2003). This is especially relevant when sampling natural populations that experienced periods of removal by recreational and/or commercial fisheries, where certain traits such as a more active swimming behaviour have been (partly) removed (Heino et al., 2015, Phillip et al., 2015, Pierce & Tomcko, 2003). Such patterns can also pollute estimates based on recreational rod and reel fishing as a sampling method (Eklöf et al., 2023, Kuparinen et al., 2010).

Another passive sampling gear that has been used to sample pike populations in lakes are fyke nets. Pierce (2012) has shown in long term studies in Minnesota lakes that trap nets prove to be very efficient during the spawning period in spring, where pike accumulate in warmer near shore areas of the lakes in order to spawn, but catch rates significantly dropped after this period. In comparison to gillnetting within the same lakes, trap nets covered a slightly wider size range and fish caught in trap nets had a lower average length than those captured by gill nets (Pierce, 2012). In general, trap nets are considered to be less selective than gillnets as fish do not have to get entangled in the net, but trap nets are limited to near shore placement (Hubert et al., 2012) and may lose catchability quickly throughout the year.

In addition to gill and trap nets, another passive gear that is commonly used in order to catch and sample pike are angling gears, which includes rod and reel fishing (Tibblin et al. 2023) and longlines, where the latter is usually used commercially and both gears

require fish to actively ingest the bait or lure in order to capture them (Hubert et al., 2012; Kuparinen et al., 2010; van Gemert et al., 2022)

Few stock assessment have been conducted using rod and reel angling in pike (Hansen et al., 2005; Lehtonen et al., 2009; Pierce & Tomcko, 2003) and these studies concluded, that there is a high variability in catch rates in rod and reel angling and therefore not always reflect abundance accurately. However, in coastal bays, CPUE assessed by recreational gear was deemed a suitable metric of relative abundance (Tibblin et al. 2023), and Arlinghaus et al. (2016) showed a tight correlation of angling CPUE and pike abundance (measured by mark-recapture) in lakes.

There are several factors that influence the catch rates to angling, such as a wide range of environmental factors (e.g., water temperature, wind strength), fishing pressure, angler skill, movement and foraging activity, daytime and season and hook avoidance due to learning and/or selection and evolution (Arlinghaus et al., 2017; Beukema, 1970; Czapla et al., 2023; Kuparinen et al., 2010; Lennox et al., 2017; Usui-Heikkilä et al., 2008). Especially low temperatures, strong winds and fishing during evening hours had significant positive influence while strong fishing effort before sampling pike with rod and reel significantly reduced catch rates (Kuparinen et al., 2010). Pike catch rates can drop rapidly over time when using artificial lures via learning (Beukema, 1970).

Also, in seasons where pike are not actively foraging, e.g., spawning seasons, catch rates might drop. It is also very likely that the fish that attack a lure are not a random fraction of the fish as research has shown that the largest and the more active pike are more vulnerable to angling gear (Monk et al. 2021). This could create bias in pike stock assessments using rod and reel angling with artificial lures. Furthermore, in comparison to gill and trap nets and electrofishing, angling tends to catch larger fish (Pope et al., 2005) and especially the smallest length captured by angling can be higher than with other gears due to mouth-gape limitations and the fact that angling will operate outside dense refuges (Pope et al., 2005).

Besides the aforementioned traditionally used gears, there are also non-invasive methods available, such as environmental DNA (e-DNA). e-DNA is a molecular approach that utilizes detectable DNA traces of organisms such as mucus, feces, urine, gametes and skin cells in the water column and such DNA can be collected from water

samples and analysed in order to detect the presence of species, so called metabarcoding, or to quantify the amount of DNA, what can potentially be linked to actual biomasses of a particular species (Berry et al., 2019; Cristescu et al., 2018; Kamoroff & Goldberg, 2018; Rourke et al., 2022; Thomsen et al., 2012).

Linking e-DNA concentrations to known abundances of pike in controlled conditions (tank and pond trials) showed a strong positive relationship (Karlsson et al., 2022), but in natural environments, this relationship is frequently less pronounced and appears highly context dependent and variable (Rourke et al., 2022; Yates et al., 2019). Natural ecosystems show strong variability in environmental factors that can influence e-DNA concentrations, degradation and distribution such as temperature in particular, pH, sunlight, salinity, currents and microbial activity (Lacoursière-Roussel et al., 2016b).

Furthermore, animal behaviour patterns such as migration, accumulation and spawning events have strong effects on e-DNA release (Doi et al., 2017; Jane et al., 2015) and especially during spawning, e-DNA concentrations are peaking (Tillotsen et al., 2018; Tsuji & Shibata, 2021).

Also, technical aspects of e-DNA approaches (e.g. filter size, PCR method) strongly influence the results of e-DNA studies and for many fish species, the lack of standardized or best practice protocols make precise abundance/biomass estimates relatively difficult in natural systems (Lacoursière-Roussel et al., 2016a, 2016b; Yates et al., 2019) but not impossible. Brantschen and Altermatt (2024) have shown a significant moderate correlation between e-DNA concentrations and biomasses of fish caught with electrofishing in Switzerland lakes. However, being a non-catch method, e-DNA currently does not allow any estimates of growth, size structure and condition of fish.

Another non-invasive method to assess fish populations or single species are visual or sonar-based observations with camera or echosounder systems or by visual sighting through snorkelling. In marine environments, baited cameras are a frequently used tool to assess fish (Cappo et al., 2006; Priede & Merrett, 1996) but there is no indication that pike would actively swim towards baited cameras. Pike in pelagic areas might be detectable with sonars, but pike associated with refuges and structure, which is often case, will be impossible to be detected from sonar alone (Eklöv, 1997; Holland & Huston, 1984).

The effectiveness of visual methods, e.g., cameras or snorkelling, strongly depend on clear water conditions and therefore “catch rates” can be limited in very turbid waters (Mueller et al., 2006). Turbidity varies with algal blooms seasonally, such that, depending on the ecosystems, visual methods might show strong seasonal variation in detection rates. Besides that, the spatial extend of the habitat itself and the distribution of fish play a major role in terms of the effectiveness of visual, but also all other methods. In small streams for example, visual observations can be a useful tool to estimate fish abundance (Hankin & Reeves, 1988), while in large lakes or vast coastal environments, visual method might not be suitable as the sampling effort needed for good data might be prohibitive (Priede & Merrett, 1996).

While different sampling gears and methods all have their bias and limitations, another issue with sampling gears is their comparability with each other, especially regarding CPUE. Gill nets, for example, have a unit of effort that relates to gill net dimension and deployment time, while angling data have a unit of fish per time and area searched and because of the unit problem, data from different gears are often analysed separately from each other (Gibson-Reinemer et al., 2016; Mueller et al., 2017). Standardizing CPUE to a common unit, usually, relative to some maximum CPUE within a given area, can be a helpful approach to make different gears comparable with each other (Gibson-Reinemer et al., 2016). Standardization best works if multiple gears complement each other, e.g., when there are gear-specific impacts of seasonality on catchability, but methodological research is needed to assess CPUE data from different gears provide a similar or a different picture.

Especially seasonal patterns will influence the spatial location and activity of pike, which should have strong impacts on all passive gear types (gill nets, angling) or alternative methods, e.g., local eDNA concentrations of pike tissues. One way to understand if the CPUE values derived from different gears in the same location align to each other, is to complete methodological research deploying the same gears in the same locations over time and analysing the correlation of CPUE values of the same gear. For gear that lethally samples fish, also metrics of the fishes can be compared to each other, e.g., size structure, age, condition or the growth rate from individuals from different gears sample from different environment. CPUE values of different gears that show similar spatial and temporal trends point to the fact that the CPUE signal is consistent among two gear types. Alternatively, if the correlations of the CPUE values

of different gears are low or non-existent, it either indicates some gear type shows strong seasonal pattern or there are habitat-specific catchabilities that differentially affect the different gear types.

The present thesis completed such assessment for pike populations inhabiting spatially vast lagoon areas in the southern Baltic Sea, where there is no agreed-upon sampling method to reliably index local pike abundance. While recreational rod and reel is suggested for adult pike in some Swedish work in coastal (Tibblin et al. 2023) and lake environments (Arlinghaus et al. 2016), work on gill nets from lakes suggest that this gear might index abundance well in selected seasons (Pierce, 2012). Novel assessment methods such as eDNA do not rely on active capture and might offer a suitable relative abundance index, as shown in laboratory work with low salinity brackish water (Karlsson et al. 2022).

Being passive gear types, more active and faster growing fish should be preferentially captured by gill nets and angling relative to what is available in the population, but it can be assumed that the bias is most pronounced in angling who not only capture more active fish, but possibly also select on aggression, which can involve the fastest growing fish of the population. Angling shall also capture the largest individuals and may capture more hungry, less well conditioned fish relative to gill nets (Lennox et al., 2017).

The objectives of this study was to compare different passive and active sampling gears and methods, specifically experimental gill nets, baited longlines, e-DNA, rod and reel angling with lures and a camera system in their ability in assessing relative abundance (indexed by gear specific CPUE), size and age structures and individual growth rates and condition of pike in the Baltic lagoons around the island of Rügen, Germany (for a review, Arlinghaus et al., 2023). Current monitoring methods of pike in coastal areas vary widely, and many stocks are considered as data-poor (Arlinghaus et al., 2023; Bergström et al., 2022; Olsson, 2019; Olsson et al., 2023; van Gemert et al., 2022).

The study was thus aimed at improving our knowledge about the possibilities and limitation of sampling coastal pike for indices of relative local abundance and to infer traits (e.g., size, growth rate, condition) of individual pike. We choose a comparative approach of deploying the same gear types in the same areas over a season. To achieve

variation in local abundance and generate a gradient of local abundance from which to sample, the sampling was completed in areas open to fishing and in areas where recreational fishing was either completely or partially controlled for at least 30 years, and where previous work has shown that local abundances of pike would differ from open (low) to protected areas (higher). Such approach was deemed feasible as lagoon pike population are sedentary (Dhellemmes et al., 2023; Lukyanova et al., 2023) such that we can assume locally closed subpopulation inhabiting different areas and sites.

We tested the following hypotheses:

H1: Rod and reel angling with lures, multi-mesh gill netting and eDNA reliably track variation in local pike abundance, with higher abundances revealed in protected compared to unprotected areas.

H2: CPUE values of a given gear type correlate significantly with each other.

H3: Seasonal effects related to the spawning season or turbidity particularly affect gill nets, eDNA and visual methods and less so lure angling, such that seasonal variation in local CPUE indices are greatest in gill nets, followed by eDNA and least pronounced in lure angling. Therefore, angling CPUE is the most consistent measure of local adult relative pike abundance.

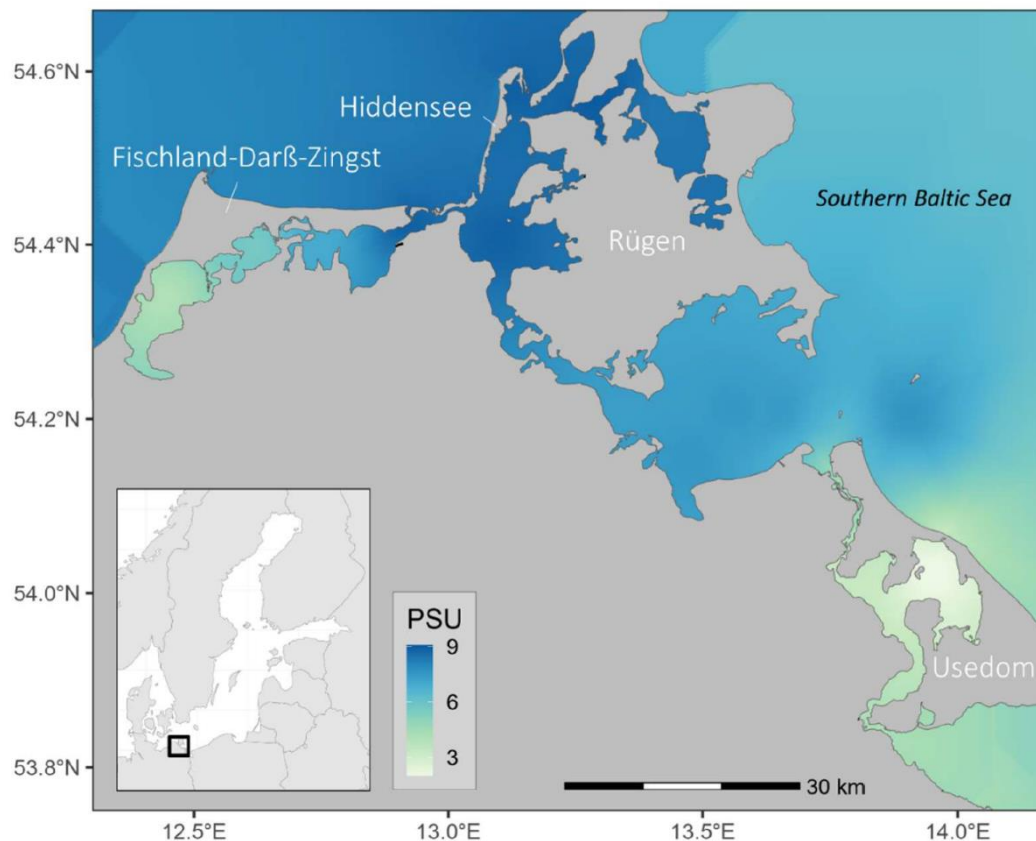
H4: Lure angling captures larger and faster-growing fish than gill netting, while gill netting captures better conditioned fish than angling.



## 2. Methods

### 2.1 Study area

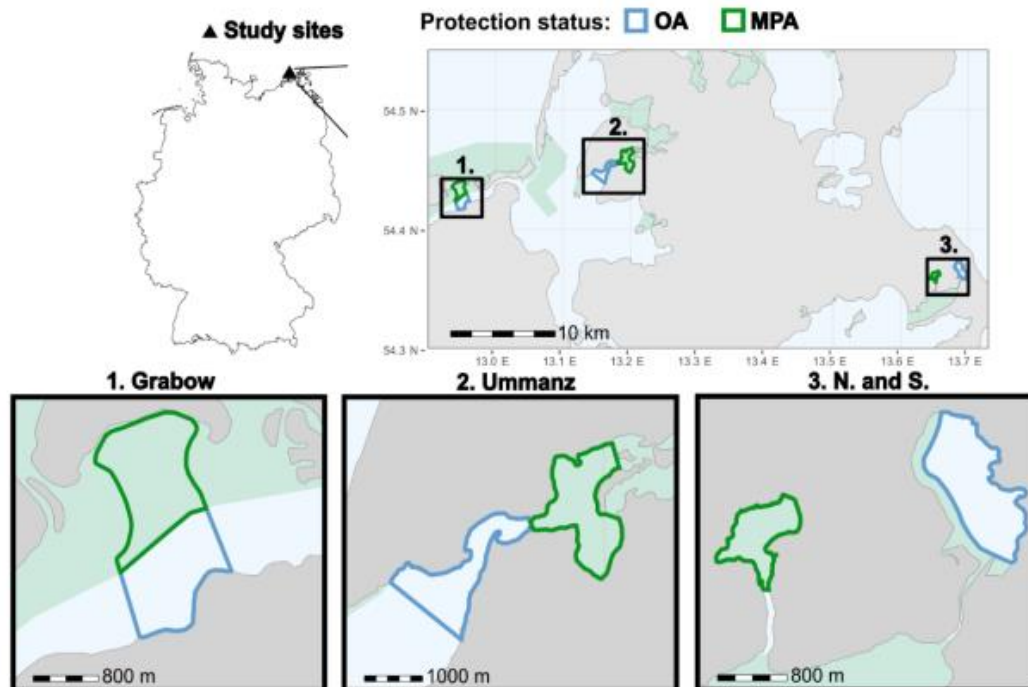
This study took place in an interconnected lagoon system of the southern part of the Baltic Sea around the island of Rügen in Germany (for details: Arlinghaus et al. 2023a, b). This area consists of a large system (around 2.000 km<sup>2</sup>) of shallow lagoons, which are characterized by different salinities ranging from on average 3,2 to 8,7 PSU



**Figure 1: Map of the studied lagoon system with salinity (PSU) gradients.**

(practical salinity units) as a result of freshwater influx from several larger river systems and saltwater influx from the open Baltic Sea (Figure 1). The lagoons vary in nutrient contents (ranging from meso-eutrophic to polytrophic), in visibility (ranging from Secchi depths from 0,4 to 1,9m), in average depth (ranging from 1,8 to 5,8m) and both, freshwater and saltwater fish species, are commonly found (Niessner et al., 2023; Rittweg et al., 2023).

This study was embedded in a broader inter- and transdisciplinary research project (BODDENHECHT, [www.boddenhecht-forschung.de](http://www.boddenhecht-forschung.de)). This project researched the biology and ecology of pike inhabiting this lagoon system with additional focus on the usage of the stock by recreational angling and commercial fisheries in the past and currently, the population development of the pike stocks over the last decades and the protection of pike. Pike in the lagoon systems play a major role in local economy, especially for recreational angling nowadays, while the commercial usage of pike has consistently declined over the past decades. The Baltic lagoons around the island of Rügen are famous among anglers for its high abundance of pike in general and associated high catch rates since the reunification of East and West Germany. Also, the high frequency of large individual pike attracted many anglers to fish this lagoon systems (Arlinghaus et al., 2023a). But since around 10 years, the pikes stocks are drastically declining (van Gemert et al., 2022), what caused sever concerns from many local fishing guides and anglers and lead to the realisation of the Boddenhecht project to identify the reasons for this decline and other general research question regarding the biology and ecology of the pike in the Baltic lagoon system.



**Figure 2: Map of the three comparison areas (Grabow, Ummanz and N. and S. with respective sites (MPA=green, OA=blue).**

As a part of this project, areas within this lagoons system with a certain degree of protection against fishing effort (MPA) were systematically assessed and compared with areas without any degree of protection against fishing pressure (OA) to derive insights on the magnitude of impact of recreational and commercial fishing on pike abundance. Choosing suitable comparison areas was a challenge as the respective protected and unprotected sites of each comparison area should not be of totally different characteristics such as size, depth, macrophyte coverage and other environmental factors as this could drive pike abundance. Furthermore, good accessibility regarding logistics and permissions and allowances for sampling of the areas was required.

The here presented thesis and associated research questions regarding suitable and appropriate pike sampling gears and methods was embedded in such comparison, which was conducted in the year 2022 across three comparison areas, where each area consisted of a protected site (MPA) and an open site (OA). All three areas were assessed in spring, summer and autumn, following a standardized sampling protocol.

**Table 1: Overview of the spatial extend, mean depth and SD and maximum depth of the three areas and respective site.**

| Area      | Site | Surface area<br>(km <sup>2</sup> ) | Mean depth in<br>m | Max. depth<br>in m |
|-----------|------|------------------------------------|--------------------|--------------------|
| Grabow    | OA   | 1.1                                | 0.9 ± 0.7          | 3.9                |
|           | MPA  | 1.4                                | 0.9 ± 0.4          | 3.7                |
| Ummanz    | OA   | 1.5                                | 0.9 ± 0.7          | 5.9                |
|           | MPA  | 1.8                                | 0.6 ± 0.4          | 3.9                |
| N. and S. | OA   | 1                                  | 1.3 ± 0.4          | 2.6                |
|           | MPA  | 0.4                                | 1.0 ± 0.7          | 4.2                |

The chosen areas, that were found to be suitable for a comparison are shown in figure 2. Comparison area 1 (“Grabow”) is embedded in the “Darß-Zingster Bodden Ost” (DZB-O)”, which is in western direction of the main island of Rügen. The DZB-O area has a spatial coverage of around 77 km<sup>2</sup> and large parts of this area are under the administration of the national park “Vorpommersche Boddenlandschaft”. The MPA site of the comparison area Grabow lies in the core zone of the national park and had a spatial extent of 1.4 km<sup>2</sup>, an average depth of 0.9 m and a maximum depth of 3.7 m

(Table 1). The core zone does not allow any access by boats and limits angling only to few selected spots along the shoreline. In the Grabow MPA site, no such accessible spots were present and therefore, no recreational angling was present in this site. However, due to special allowances, commercial fishing is allowed in this site and commercial gear has been observed in this MPA in the same timeframe as this study was conducted, but not specially for pike (Braun et al., 2023). The adjacent OA site of the area Grabow is open for recreational angling from boat and shore and for commercial fishing as it lies outside the core zone, but in the developmental zone (“Pflege- und Entwicklungszone”). Angling effort was observed in this OA site during this thesis, but that does not hold true for pike specific commercial gear (Braun et al. 2023). The size, average depth and maximum depth of the OA site of Grabow were comparable similar to the MPA site (Table 1).

The second comparison area Ummanz lies in the wider area called “Westrügener Bodden (WRB)”, south of the sub-island “Ummanz” of the main island of Rügen. The whole comparison area lies within the development zone of the aforementioned national park. However, the MPA site falls within a no access zone and no shore-angling zones fall within this area, therefore totally excluding recreational angling. For this site, allowance for one commercial fisherman exists, who is actively fishing this area, what includes fishing for pike. Commercial activities in the area are not allowed during the month April and May as this area is a spawning protection zone (“Laichschonbezirk”). Given the activity of the commercial fishermen, gillnets have been observed in the Ummanz MPA site (Braun et al. 2023). This MPA had a spatial extend of 1.8 km<sup>2</sup>, an average depth of 0.6 m and a maximum depth of 3.9 m.

The adjacent OA site of the comparison area Ummanz lies also within the development zone of the national park but is not limited in access regulations regarding angling and commercial fisheries, and both, commercial and recreational fishing activities have been observed (Braun et al. 2023), including pike specific gears. The OA site of Ummanz was slightly smaller than the MPA site (1.5 km<sup>2</sup>) and also slightly deeper on average (0.9 m) and also regarding maximum depth (5.9 m).

The last comparison area (Neuensiener and Selliner See, N. and S.) is part of a wider area named “Greifswalder Bodden (GB)”, a large lagoon eastwards of the island of Rügen. The area N. and S. lies under the jurisdiction of the biosphere reserve (Biosphärenreservat Südost-Rügen). The two sites in this area are lake-like extension

of larger lagoons and are not in close proximity to each other. Both sites are spawning protection zones (April and May) and hence limit commercial activity in this time. However, commercial fishing exists in both sites, while the recreational access is not fully excluded in the MPA site. Here, around 50 annual licences are issued for the MPA site, while the OA area is fully accessible for recreational angling. For both sites, larger commercial efforts have been observed (Braun et al. 2023), while angling efforts were stronger in the OA site. The OA (1 km<sup>2</sup>) site was around twice the size of the MPA site and slightly deeper on average (Table 1.)

Given the aforementioned description of the areas, especially regarding the access for commercial and recreational efforts, the comparison present in this thesis was conducted along a gradient of protection against fishing pressure, mainly against recreational efforts. None of the areas were free of fishing efforts, but in the area Grabow, pike specific efforts are most likely zero, but that remains unsure. This gradient of protection should correspond well with pike abundances, given the assumption that a certain limitation of fishing effort will increase the number of fish.

In regard to water temperatures, salinities and visibilities of the respective sites (MPA and OA) in the three comparison areas in different season, those parameters were found to be very similar. Also, the coverage of macrophyte coverage was found to be very similar, except in autumn in the area N. and S., where macrophyte coverage was much higher in the OA compared to the MPA site (Table 2). The similarity between the respective sites in the given areas make the areas very comparable with each other and allows conclusions about pike abundance that are not related to environmental factors, but to the presence or absence of a certain degree of fishing pressure.

**Table 2: Overview of environmental variables (macrophyte coverage, water temperature, salinity and visibility) across the different areas, seasons and sites.**

| Area      | Season | Site | Macrophyte coverage (%) | Water temperature (°C ± SD) | Salinity (PSU ± SD) | Visibility (m ± SD) |
|-----------|--------|------|-------------------------|-----------------------------|---------------------|---------------------|
| Grabow    | Spring | OA   | 34.6                    | 15.9 ± 3.3                  | 8.7 ± 0.2           | 0.6 ± 0.2           |
|           |        | MPA  | 20.4                    | 15.7 ± 3.5                  | 8.9 ± 0.3           | 0.6 ± 0.2           |
|           | Summer | OA   | 62.2                    | 18.5 ± 3.8                  | 9.6 ± 0.5           | 2.8 ± 1.5           |
|           |        | MPA  | 65.3                    | 18.3 ± 4.0                  | 9.6 ± 0.6           | 2.8 ± 1.5           |
|           | Autumn | OA   | 45.9                    | 7.4 ± 4.7                   | 8.7 ± 0.2           | 3.7 ± 0.7           |
|           |        | MPA  | 47.6                    | 6.6 ± 3.9                   | 8.8 ± 0.8           | 3.1 ± 1.2           |
| Ummanz    | Spring | OA   | 22.5                    | 14.3 ± 3.5                  | 8.9 ± 0.2           | 1.3 ± 0.6           |
|           |        | MPA  | 24.5                    | 14.2 ± 3.5                  | 9.0 ± 0.1           | 1.3 ± 0.6           |
|           | Summer | OA   | 29.7                    | 19.5 ± 3.2                  | 9.6 ± 0.7           | 1.4 ± 0.6           |
|           |        | MPA  | 24.9                    | 19.4 ± 3.0                  | 9.7 ± 0.7           | 1.3 ± 0.6           |
|           | Autumn | OA   | 54.0                    | 9.4 ± 3.8                   | 8.7 ± 0.5           | 3.9 ± 0.2           |
|           |        | MPA  | 66.9                    | 9.4 ± 3.9                   | 8.7 ± 0.5           | 3.9 ± 0.2           |
| N. and S. | Spring | OA   | 14.5                    | 11.1 ± 7.5                  | 7.5 ± 0.1           | 1.5 ± 0.8           |
|           |        | MPA  | 12.3                    | 11.0 ± 7.0                  | 7.3 ± 0.2           | 1.3 ± 0.6           |
|           | Summer | OA   | 39.6                    | 19.7 ± 3.9                  | 8.0 ± 0.5           | 1.4 ± 0.7           |
|           |        | MPA  | 44.6                    | 19.5 ± 4.1                  | 7.9 ± 0.6           | 1.1 ± 0.1           |
|           | Autumn | OA   | 44.6                    | 7.6 ± 5.1                   | 7.5 ± 0.5           | 2.5 ± 0.4           |
|           |        | MPA  | 6.38                    | 7.5 ± 5.1                   | 7.4 ± 0.5           | 2.4 ± 1.4           |

## 2.2 Sampling protocol

All three areas with corresponding site pairs (MPA and OA) were sampled over the course of an entire year (2022), covering the seasons of spring (April), summer (July) and autumn (October). Note that the methodological research completed for this thesis was embedded in standard MPA assessments as part of the wider Boddenhecht project, this thesis advantaged of sampling protocols with lure and longline angling and gill netting that were completed anyways to fulfil project goals. In addition, other non-lethal methods (eDNA) and underwater visual assessment methods were added to complement the ongoing sampling. In every season, a standardized sampling protocol was applied to sample pike, which included two rod and reel angling days, pike multi-mesh gillnetting, e-DNA sampling, macrophyte and environmental sampling, baitfish-longlining with multiple hooks and visual observations with camera systems. There were three additional angling days conducted after the sampling protocol during main sampling periods to increase effort of angling and to cover daily variance. All sampling gear was deployed in randomized fashion in each site, and also the days of sampling were randomly chosen.

### 2.2.1 Gillnetting

Benthic multimesh gillnets for pike had the dimensions of 100 x 1.5 m with five different mesh sizes (50, 60, 75, 95 and 110 mm, knot-to-knot). Panel length for each mesh size was 20m. Gillnets were set once per season in each area (Grabow, Ummanz, N. and. S) and for each site (MPA and OA). In each site, six nets were randomly deployed during sunset and lifted shortly after sunrise on the next day (overnight gillnetting, Figure 3, right). Gillnet points were chosen randomly within each site, and gillnet were deployed on average for  $15.5 \pm 2.5$  hours. All fish caught were measured (total length, TL) to nearest millimetre (mm), externally sexed (Casselman, 1975), sampled for scales near the dorsal fin, and weighted to the closest gram (g). Pike were assigned to the mesh size in which they were entangled in and if alive, pike were marked with external floy-tags and released afterwards near the capture location. Net locations were recorded with GPS and set depth was measured at the start, middle and end of each net. Environmental data such as oxygen, salinity, water temperature, wind strength and direction and visibility were collected for each site (MPA and OA). Also, the bycatch (mainly common bream, *Abramis brama*) was accessed for each net.

### 2.2.2 Angling

During the standardized sampling protocol, two boat-angling days were conducted during each season in each area, while three additional angling days were conducted shortly after the main sampling period within the same areas. Each site (MPA and OA) was fished with equal effort on each angling day by alternating between sites (MPA and OA) within a fixed time period (session). Starting site and fishing locations were randomly chosen. Average fishing duration for each site was  $1.32 \pm 0.24$  hours per session. All angling days (N=40, 13 days in Grabow and N. and S., 14 days in Ummanz) were performed with rod and reel angling from a drifting boat using artificial pike lures that are commonly used by anglers in the region. For each angler on the boat, specific information about the lure (lure length, colour and type), the fish interaction (number of followers seen by the angler, bites, and fish captured) and effort (fishing hours) were recorded. Total fishing effort of all anglers involved for all areas was 964,4 hours. It was mandatory to fish each lure with the same effort in each site and no lure change was allowed within a session. As with gillnetting, all pike captured were measured, weighted, sampled for scales and genetics, externally sexed, tagged with floy-tags and released afterwards near the capture location. Environmental data such as oxygen, salinity, water temperature, wind strength and direction and visibility were collected for each site (MPA and OA).

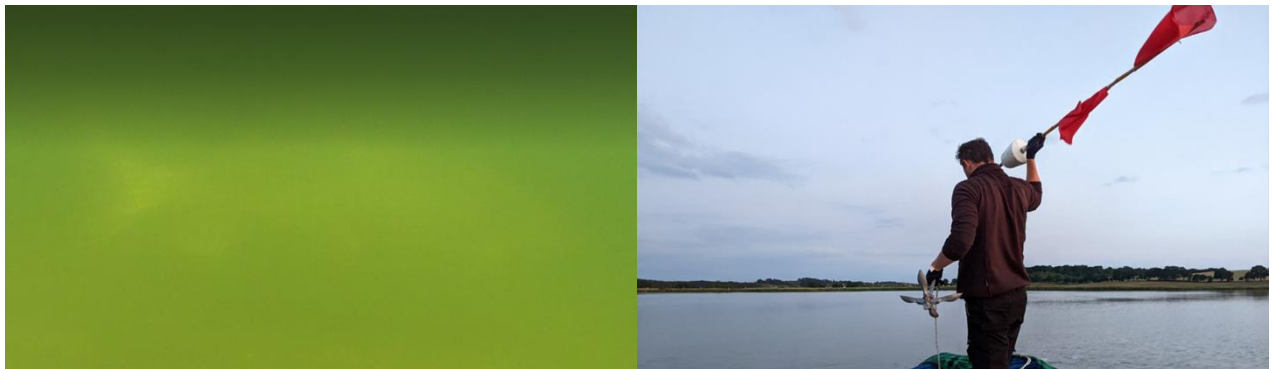
### 2.2.3 Longlining

Similar to gillnetting, longlines were set overnight at random locations and were set once per season in each area. In each site (MPA and OA), three longlines of 30 meter length with 5 hooks on 50cm steel leader, baited with baitfish (size range: 100 to 250mm), were deployed. Environmental data such as oxygen, salinity, water temperature, wind strength and direction and visibility were collected for each site (MPA and OA) and set depth and locations were recorded. There were no pike captured at all with longlines, and therefore, longlines are not further statistically analyzed, but discussed within this study.



#### 2.2.4 Camera system

Visual sampling of pike was conducted with a “remote operated underwater vehicle” (ROUV) of type ‘Videoray Pro III’, which was equipped with two additional underwater cameras of type ‘Sony HDR-AS50’. The two additional cameras were mounted facing forward in a 60° angle to each other, covering a 180° frontal view into driving direction. The ROUV was driven in a total of 25 random 50 meter transects per site (total distance per site = 1250m; 2500m per area). Transect length was limited to 50 meter as the ROUV cable was 50 meters long. The ROUV was only used during the spring season due to technical issues and no further visual observations methods were applied in the summer and autumn sampling phase. Recorded videos were later screened for pike detections. Only two confirmed (Figure 3, left) and one unsafe pike observation were detected and therefore, further statistical analysis of the ROUV visual sampling was not done, but is discussed.



**Figure 3: A pike observed with the ROUV in Ummanz (left) and setting of pike gillnets during evening hours (right)**

#### 2.2.5 e-DNA

For e-DNA sampling, ten samples were taken per area (5 per site) in each season. Each sample consisted of five subsamples of 500 ml surface water (Figure 4, left), which were taken in around 50 meter distance to each other and mixed after in a 3 litre container. Three out of the five samples for each site were taken in close distance to the random gillnet points prior to the deployment of the gillnets to cover potential relationships between gillnet catches and e-DNA concentrations. The remaining two samples were taken at nearshore transects at the leeward and windward shores of each site to cover possible wind driven accumulations of e-DNA. Pooled subsamples were then filtered following Sinsoma instructions using standardized manual filter kits (Sinsoma GmbH, Austria) until filters clogged (Figure 4, right). The amount of filtered

water ranged from 356 to 1000 ml and the mean amount was  $813 \pm 193$  ml. e-DNA within the filters was fixed with a buffer solution (Longmire's solution, see (Williams et al., 2016) and send within 24 hours to Sinsoma GmbH for analysis. To determine concentrations, ddPCR (digital droplet polymerase chain reaction) at 57°C was used utilizing a pike specific assay (Olsen, 2015; 2016) with the following primer and probe combination:

F-Primer: 5'-CCTTCCCCCGCATAAATAATATAA-3'

R-Primer: 5'-GTACCAGCACCAGCTTCAACAC-3'

Probe: 5'-FAM-CTTCTGACTTCTCCCC-BHQ-1-3'

As for all other methods, also environmental parameters such as salinity, water temperature, depth and wind strenght and direction were recorded during e-DNA sampling. For the three main sampling periods in spring, summer and autumn of 2022, 90 e-DNA samples were taken. In addition, 30 samples were taken in winter season (End of November 2022). Out of the 120 samples initially taken, 108 samples could be analyzed by Sinsoma GmbH while in 12 samples, filter issues occurred and lab analysis was not possible. For all samples, a general metabarcoding PCR was conducted for verification.

Prior to the sampling of e-DNA at the actual sampling sites in the Baltic lagoons, pre-tests with the filter kits, primer/probe combination and following lab analysis were conducted with tank trials and known abundances of pike. This pre-tests were performed at "Lake Döllnsee" a shallow lake in Brandenburg, Germany. Here, pike were caught with rod and reel angling and stored in 700 litre tanks with different total biomasses of pike (12 kg, 6 kg and 2 kg) and also a control (lake water) was in place and filtration of distilled water was performed as a negative control. After 12, 18 and 24 hours after the placement of pike in the tanks, e-DNA was sampled (N=1 per



**Figure 4: Sampling of surface water for e-DNA analysis (left) and filtration process of mixed water sample with standardized e-DNA kits (right)**

treatment and timepoint) and analyzed as described above. In addition, three samples were taken from lake water and three samples from distilled water as a negative control. All e-DNA related procedures were conducted using single use gloves and cleansing of all containers between sites and areas with distilled water was done to avoid potential contamination effects.

#### 2.2.6 Environmental data

As previously mentioned, a variety of environmental data were collected. This included macrophyte sampling, depth structures of the areas and sites during the three seasons and set depth (e.g. Gillnetting) or depth points (e.g. e-DNA), salinity, oxygen, visibility and wind direction and strength. All water-related parameters (temperature, oxygen and salinity) were measured with a 'WTW Multi 3320 – Multiparameter' measuring device with probes for temperature, oxygen and salinity. Salinity was measured in PSU, temperature in °C and oxygen in mg/L units. Visibility was assessed with a standard Secchi disk. Depth structures were assessed by using an echosounder (Garmin GPS Map 923xsv). All areas were completely scanned and logged by driving by boat in gridlines from north to south and from east to west in close distance to each other. Data acquired were corrected for water levels from official water level measurement station in the specific areas (Roser, 2023). Wind strength and direction data were acquired by a weather report provider ([www.windy.com](http://www.windy.com)) and also assessed at the specific sites by estimation. Macrophyte related data were acquired in each season for each area and site. For each area and each season, 12 transects (6 per site MPA and OA) were used to gather macrophyte data. Each transect was laid from shore to the central point of the sites and for each transect, 10 observations were made in equal distance to one another and transect length was depending on the distance from the spatial extend of the water body. At each observation, macrophyte coverage was either visually estimated or a live-footage underwater camera was deployed. Mean macrophyte coverage was then calculated by interpolation with the data from the echosounder depth structure data (Roser, 2023). All data related to set depth and depth points were acquired by depth measurements with the echosounder.

#### 2.2.7 Aging and growth

As mentioned before, for the two methods that successfully captured pike (angling and gillnetting), approximately 5-10 scales were collected from 290 pike near the dorsal

fin from each individual pike. Out of these 5-10 scales, three scales were chosen, cleaned and placed onto a microscopic glass carrier plate and a scaled high resolution photo was taken under a 'Leica MZ8' microscope. Images were processed with the software 'ImageJ2 (Vers. 2.14.0)'. For each scale, the centre of the scale was marked and a line was inserted from this centre point towards the most distant outside edge of the scale. On this line, the growth rings from each year were visually identified and marked as increments and age at capture was calculated as the amount of identified growth rings. All scales were only processed once and by the same person for consistency and error continuity. This procedure allows a precise measurement of distances between the growth rings. From those data, a size-corrected growth rate calculation was performed from the mean values of the three scales measured per pike. The distance between the yearly increments has a relationship to the growth of the individual in that year, what allows an estimate of the size at a different ages of the individual fish. Back-calculated sizes at different life years ( $L_i$ ) was based on the Fraser-Lee equation (Francis, 1990), which assumes a linear relationship between the length of the fish and the radius of the scales (Scale-proportional hypothesis). Back calculation of lengths ( $L_i$ ) at different ages was performed with the following formula, where  $S_i$  is the scale radius at Age $_i$ ,  $S_c$  is the scale radius at capture,  $L_c$  is the length of the fish at capture and  $c$  is the intercept of the fish length to scale radius relationship regression:

$$L_i = \frac{S_i}{S_c} \times (L_c - c) + c$$

From the resulting back calculated sizes at each life year for each individual fish, the annual growth rate (in mm) was calculated as the difference between each year. Then, following (Berggren et al., 2022), separately for male and female pike, a linear regression was applied to derive the coefficient and intercept describing the relationship between the respective sizes throughout the different life years (Age $_i$ ) and associated growth between the years:

Male pike: Intercept: 416 mm, coefficient: 0.408

Female pike: Intercept: 464 mm, coefficient: 0.453

Based on the aforementioned coefficients, for both genders in pike independently, the predicted length at Age<sub>i</sub> was calculated for all pike sampled, independently of gear, area, site, or season. Then, the difference between the observed back calculated length at Age<sub>i</sub> was compared to the predicted length at Age<sub>i</sub>. This means that the difference in observed and predicted size corrected growth rate was analyzed for each individual pike for all Age<sub>i</sub> observed in that respective individual. The formula for the calculation of size corrected growth rate (Berggren et al., 2022), where  $L_{Age(i)}$  is the length of a pike at Age<sub>(i)</sub>,  $coef_{(m \text{ and } f)}$  are the respective regression coefficients for male and female pike and  $L_{Age(i)+1}$  is the length of the same pike in the following year:

$$L_{Age(i)+1} - coef_{(m \text{ and } f)} \times L_{Age(i)}$$

## 2.3 Statistical Analysis

All statistical analysis and graphical visualisation of results were performed in R studio (Ver. 4.2.2, R Core Team 2022). Statistical models were performed using ‘glm’ and ‘glmm’ (generalized linear models and generalized linear mixed models, (Dobson & Barnett, 2018) and fixed model terms were reported with ‘Anova’ function of the ‘car’ package (Fox & Weisenberg, 2018). Pairwise comparison was performed with ‘Tukey HSD’ from the ‘multcomp’ package (Hothorn et al., 2016).

For CPUE correlation analysis between the different gear types analyzed in this study (angling, gillnetting and e-DNA), the ‘gg scatter’ package was used with the ‘pearson correlation coefficient’ (Sedgwick, 2012). Additionally, for e-DNA analysis, ‘boosted regression trees’ were applied to determine the relative contribution (in %) of predictor variables on the e-DNA concentration (Elith et al., 2008).

For the comparison of the three different gears (gillnetting, angling and e-DNA), the different CPUE’s from all gears were scaled between 0 and 1 by (standardized CPUE, sCPUE) utilizing the following formula (Gibson-Reinemer et al., 2016):

$$sCPUE = \frac{CPUE - \min(CPUE)}{\max(CPUE) - \min(CPUE)}$$

### 2.3.1 Gillnetting

Gillnet CPUE was calculated as the number of captured pike per gillnet (number of pike/100m gillnet). The dependent variable was the number of captured pike and

independent variables were area, season, site and the interaction of area and site to test whether CPUE differed between the areas, sites and seasons. As bycatch rates of common bream (*Abramis brama*) were high in pike gillnetting, the sum of bycatch as individuals per gillnet was included as a variable. Model formula for the 'glm' was:

$$CPUE \sim Area + Season + Site + Area:Site + bycatch$$

### 2.3.2 Angling

For the analysis of angling data, each observation from each angler and session (N=726) were used. The number of captured pike per angler and session was set as the dependent variable. Similar to the gillnet model, area, season and site were set as the independent variables. In addition, and the interaction term between area and site to account for the different protection levels of the areas was included in the model. Fishing time (effort) was included for each angler as an offset-variable to account for each session length. The 'glm' formula was:

$$\begin{aligned} \text{Number of captured pike} &\sim (\text{offset (effort)}) + Area + Season + Site \\ &+ Area:Site \end{aligned}$$

### 2.3.4 e-DNA

For statistical analysis, e-DNA concentrations (copies/μl of DNA extract) were normalized to 1000 ml for all samples where less than 1000 ml were sampled due to filter clogging. This normed concentration was set as dependent variable and explanatory variables were area, season, site, depth (mean water depth of the five subsamples), water temperature, wind strength, salinity, sampling location (whether at gillnet points or windward/leeward shore) and the interaction of season and site. Out of the initial 108 successfully taken samples, there were 13 samples where no pike and no DNA of any other fish species was found. Those samples were excluded from the analysis. Furthermore, the samples taken in winter were too far away in terms of time frame from the main sampling periods and were therefore also excluded. Only 83 e-DNA samples were analysed by using a 'glm' with the following formula:

$$\begin{aligned} \text{normed eDNA concentration} &\sim Area + Season + Site + Season * Site \\ &+ depth + water temperature + wind strength \\ &+ sampling location + PSU \end{aligned}$$

### 2.3.5 standardized CPUE (sCPUE)

In order to compare the performance of the different gears (angling, gillnetting and e-DNA) across the different sites and season the standardized CPUE's (sCPUE) were analyzed with a 'glmm'. The interaction effects of gear and site and gear and season were included to see differences between the gears in the MPA's and OA's and throughout the different seasons, while season itself was set as a random factor. As it is expected that all gears function similar across the areas, area was also included as a random effect. The interaction of area and site as well as area and season were also set as random factors. The model had the following formula:

$$sCPUE \sim Site: Gear + Season: Gear + Gear + Site + (1|Season) + (1|Area) + (1|Area: Site) + (1|Area: Season)$$

### 2.3.6 Correlations of CPUE among gears

For correlations analysis, mean CPUE's were calculated per gear and pooled for each area, site and season combination. Then, the correlations between gears were analysed on a global level across all areas, seasons and sites (N=18 mean observations per gear) and specifically for each season and gear (N=6 per season and gear) and each site and gear (N=6 per season and gear). The pearson correlation coefficient was calculated with the 'stat\_cor' r-package and linear regression (r-package 'geom\_smooth', method 'lm') was used to show correspondence between the mean CPUEs of the different gear types. Means for each gear were calculated from the raw CPUE data as described in section 2.2.

### 2.3.7 Length and age of pike

Multiple analysis regarding the length of pike were conducted. To see the influence of the different mesh sizes on the length of the captured pike, a 'lm' model (linear model) with 'Tukey' pairwise comparison was applied with the following formula:

$$Total\ length\ of\ pike \sim Mesh\ Size\ in\ mm$$

Furthermore, it was analyzed whether mean pike length was influenced by gear type and by the different areas and sites. A 'glmm' model with a poisson distribution and identity-link function was utilized with the following formula:

$$\begin{aligned} \text{Total length of pike} \sim & \text{Area} + \text{Site} + \text{Gear} + \text{Gear: Site} + (1|\text{Area: site}) \\ & + (1|\text{Sex}) \end{aligned}$$

Furthermore, the size distributions were compared between the specific gears and sites using a Kolmogorov-Smirnov test (Berger & Zhou, 2014). Similar to the length of the pike as described above, the age at capture of pike was analyzed depending on the gear type, area and site by using a ‘glmm’ model (gaussian distribution) with the following formula:

$$\begin{aligned} \text{Age at capture} \sim & \text{Area} + \text{Site} + \text{Gear} + \text{Site: Gear} + (1|\text{Area}) + (1|\text{Area: Site}) \\ & + (1|\text{Sex}) \end{aligned}$$

#### 2.3.7 Growth rate of pike

In order to determine differences in growth related to the different areas, sites, and gears, a size-corrected growth analysis was performed using only gill net and angling data. Size-corrected growth in fish is a way to measure growth rates that accounts for differences in the initial size of the fish. This method is particularly useful in ecological and fisheries studies, as it allows for more accurate comparisons of growth rates among fish of different sizes (Berggren et al., 2022). To account for repeated measurements for the same individuals, the individuals were included as a random effect within the model. Also, sex was set as a random effect as growth rate differs between male and female pike (Bregazzi & Kennedy, 1980). The model was computed with a ‘glmm’ with the following formula:

$$\begin{aligned} \text{Growth rate} \sim & \text{Area} + \text{Site} + \text{Gear} + \text{Site: Gear} + (1|\text{Area: Site}) \\ & + (1|\text{lifeyear}) + (1|\text{Individual}) + (1|\text{Sex}) \end{aligned}$$

#### 2.3.7 Condition factor of pike

In order to assess the relative condition factor (Kn, Le Cren, 1951) of the captured pike, a data set from the entire lagoon system was used that was not part of my sampling. With total of 2008 pike with length (mm) and weight (g) data, data were log transformed and the relationship (coefficients a and b) was determined by a linear model (lm):

$$\log(\text{weight}) = \log(a) + b \times \log(\text{length})$$



Coefficients ((log)a=2.73, b=3.15) from the reference data set were then applied to calculate relative condition Kn of pike from this study with the following formula:

$$Kn = \frac{Weight}{a \times Length^b}$$

Then, a 'glm' model was used in order to test the effect of area, site, season, gear and the interaction of gear and site. The model formula was:

$$relative\ condition\ (Kn) = Area + Site + Season + Gear + Site:Gear$$

### 3. Results

In total over all gear types pooled, 386 pike were captured. In the Grabow, most pike were caught (N=159, 41,2%), followed by Ummanz (N=117, 30,3%) and N. and S. (N=110, 28,5%). The majority of pike were captured in spring (N=184, 47,7%), followed by summer (N=120, 31,1%) and autumn (N=82, 21,2%). Most pike were captured in the MPA's (N=285, 73,8%) compared to the OA's (N=101, 26,2%). Experimental angling caught most of the pike (N=266, 68,1%) while gillnetting caught less pike (N=106, 31,9%).

#### 3.1 Angling

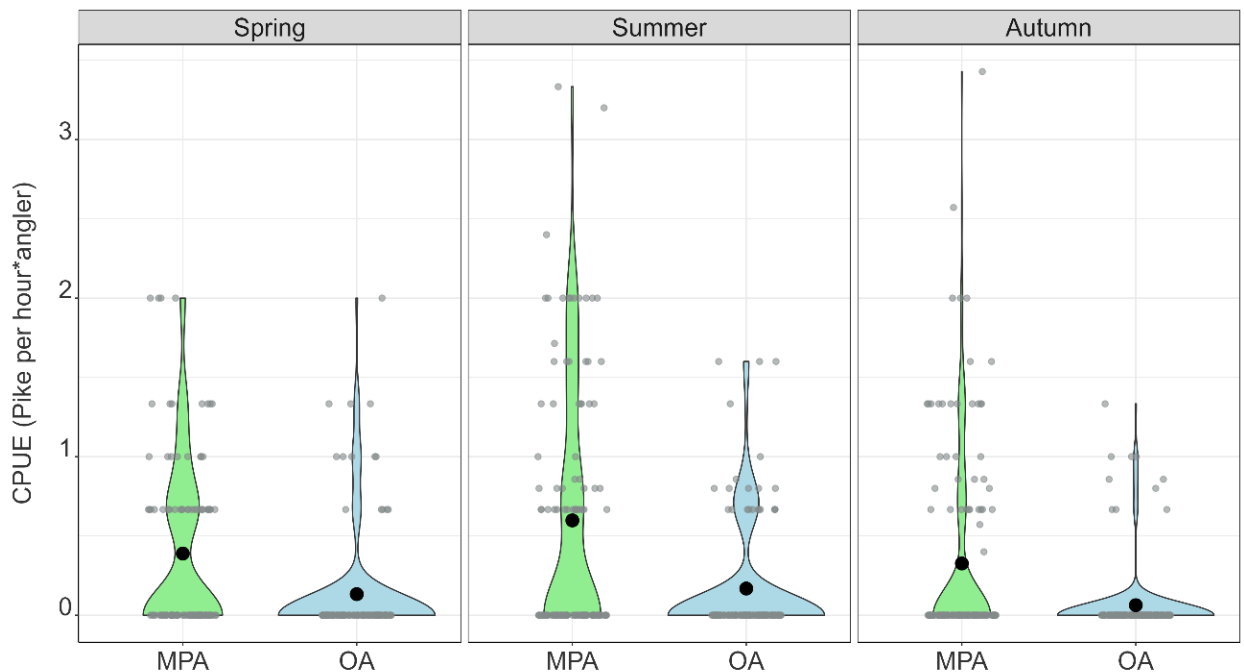
In a total of 40 angling days (963 angling hours), 266 pike were captured with angling.

| Area      | Season | Site | N Pike [Share %] | Mean CPUE $\pm$ SD<br>[pike / h*angler] |
|-----------|--------|------|------------------|-----------------------------------------|
| Grabow    | Spring | MPA  | 32 [12%]         | 0.48 $\pm$ 0.62                         |
|           |        | OA   | 8 [3%]           | 0.12 $\pm$ 0.35                         |
|           | Summer | MPA  | 43 [16,1%]       | 0.81 $\pm$ 0.90                         |
|           |        | OA   | 14 [5,2%]        | 0.26 $\pm$ 0.43                         |
|           | Autumn | MPA  | 21 [7,8%]        | 0.23 $\pm$ 0.44                         |
|           |        | OA   | 0 [0%]           | 0 $\pm$ 0                               |
| Ummanz    | Spring | MPA  | 13 [4,9%]        | 0.31 $\pm$ 0.44                         |
|           |        | OA   | 3 [1,1 %]        | 0.07 $\pm$ 0.24                         |
|           | Summer | MPA  | 28 [10,5%]       | 0.47 $\pm$ 0.73                         |
|           |        | OA   | 3 [1,1%]         | 0.04 $\pm$ 0.19                         |
|           | Autumn | MPA  | 23 [8,6%]        | 0.45 $\pm$ 0.68                         |
|           |        | OA   | 3 [1,1%]         | 0.07 $\pm$ 0.26                         |
| N. and S. | Spring | MPA  | 11 [4,1%]        | 0.30 $\pm$ 0.44                         |
|           |        | OA   | 7 [2,6%]         | 0.20 $\pm$ 0.49                         |
|           | Summer | MPA  | 21 [7,9]         | 0.52 $\pm$ 0.67                         |
|           |        | OA   | 9 [3,3%]         | 0.22 $\pm$ 0.46                         |
|           | Autumn | MPA  | 20 [7,5%]        | 0.33 $\pm$ 0.72                         |
|           |        | OA   | 7 [2,6%]         | 0.13 $\pm$ 0.32                         |

**Table 3: Angling overview of number of captured pike [share in %] and mean CPUE and SD (pike per hour \* angler] across the different areas (Grabow, Ummanz and N. and S.), seasons (spring, summer autumn) and sites (MPA and OA).**

The majority of pike were caught in the MPA's (N=212, 79,7%) compared to the OA's (N=54, 20,3%). Accordingly, mean CPUE  $\pm$  SD (pike per angling hour\*angler) in the MPA's ( $0,43 \pm 0.66$ ) was around four times higher than in the OA's ( $0,11 \pm 0.32$ ), Table 3 gives an overview of the captured pike (N and % share), mean CPUE and SD of the different areas, seasons and sites. Most pike were captured in the Grabow area (N=118, 44,3%) and mean CPUE was also highest ( $0.29 \pm 0.56$ ), followed by Sellin (N=75, 28,2%, mean CPUE  $0.28 \pm 0.55$ ) and Ummanz (N=73, 27,4%, mean CPUE  $0.24 \pm 0.52$ ). In addition, most pike were caught during the summer season (N=118, 44,3%) with also the mean highest CPUE ( $0.38 \pm 0.65$ ) while in spring (mean CPUE  $0.26 \pm 0.47$ ) and autumn (mean CPUE  $0.19 \pm 0.48$ ) less pike were caught (N=74, 27,8% for both).

The pike angling CPUE (Figure 5) was significantly different between the sites (post hoc Tukey,  $p=0.001$ ), where CPUE in the MPA was higher than in the OA.

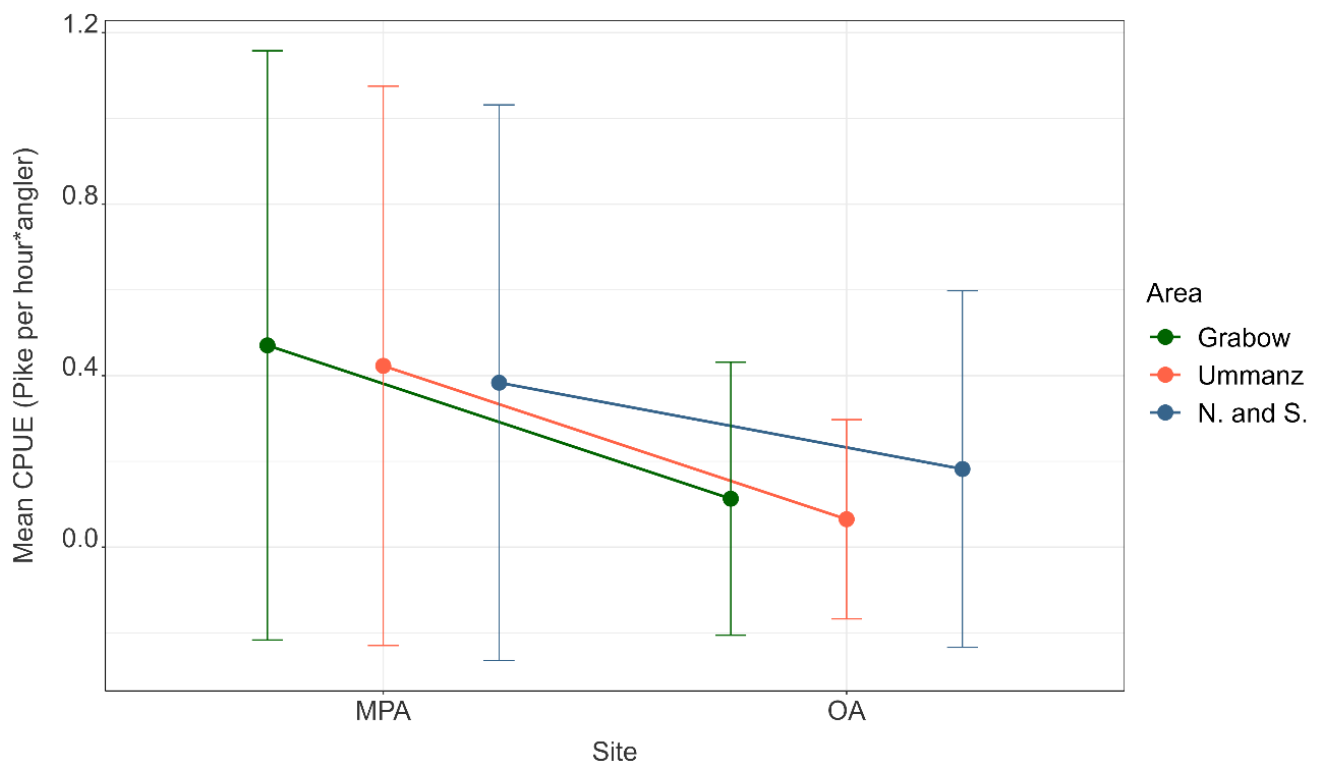


**Figure 5: Violin plots for angling CPUE (pike per hour \* angler) across the different seasons (spring, summer and autumn) and sites (MPA and OA). Black dots show the mean values and small grey dots show raw CPUE data.**

Furthermore, there was a significant difference in CPUE between the seasons ( $p=0.003$ ), where the CPUE in summer was significantly higher than in spring (post hoc Tukey,  $p=0.001$ ) and autumn (post hoc Tukey,  $p=0.001$ ). CPUE did not differ significantly between spring and autumn (post hoc Tukey,  $p=0.38$ ) and between the different areas ( $X^2=3.12$ ,  $Df=2$ ,  $p=0.20$ ). The interaction between site and area was

significant ( $X^2=7.5$ ,  $Df=2$ ,  $p=0.02$ ) indicating varying differences in CPUE in the respective MPA and OA of each area.

Differences in CPUE (figure 6) between the sites was most prominent in the Grabow area (mean CPUE MPA:  $0.47 \pm 0.68$ ; mean CPUE OA:  $0.11 \pm 0.31$ , ratio: 4.1) and in the Ummanz area (mean CPUE MPA:  $0.42 \pm 0.65$ ; mean CPUE OA:  $0.06 \pm 0.23$ , ratio: 7.0). The differences between MPA and OA in Grabow and Ummanz were both significant ( $p=0.001$ ), while the difference in the area N. and S. was less strong, but also significant (mean CPUE MPA:  $0.38 \pm 0.64$ ; mean CPUE OA:  $0.18 \pm 0.45$ , ratio: 2.5,  $p=0.01$ ). The only significantly different OA's ( $p=0.05$ ) were between Ummanz (mean CPUE:  $0.06 \pm 0.23$ ) and N. and S. (mean CPUE:  $0.18 \pm 0.41$ ), where the CPUE was about 3 fold higher than in Ummanz. The mean CPUE in the MPA's was around 2.9 times higher than in the OA's in spring. In summer, the mean CPUE was around 3.6 times higher in the MPA's and in autumn, the mean CPUE was around 5.3 times higher in the MPA's (Figure 5).



**Figure 6: Plots for angling mean CPUE and SD (pike per hour \* angler) across the different areas (Grabow, Ummanz and N. and S.) and sites (MPA and OA).**

### 3.2 Gillnetting

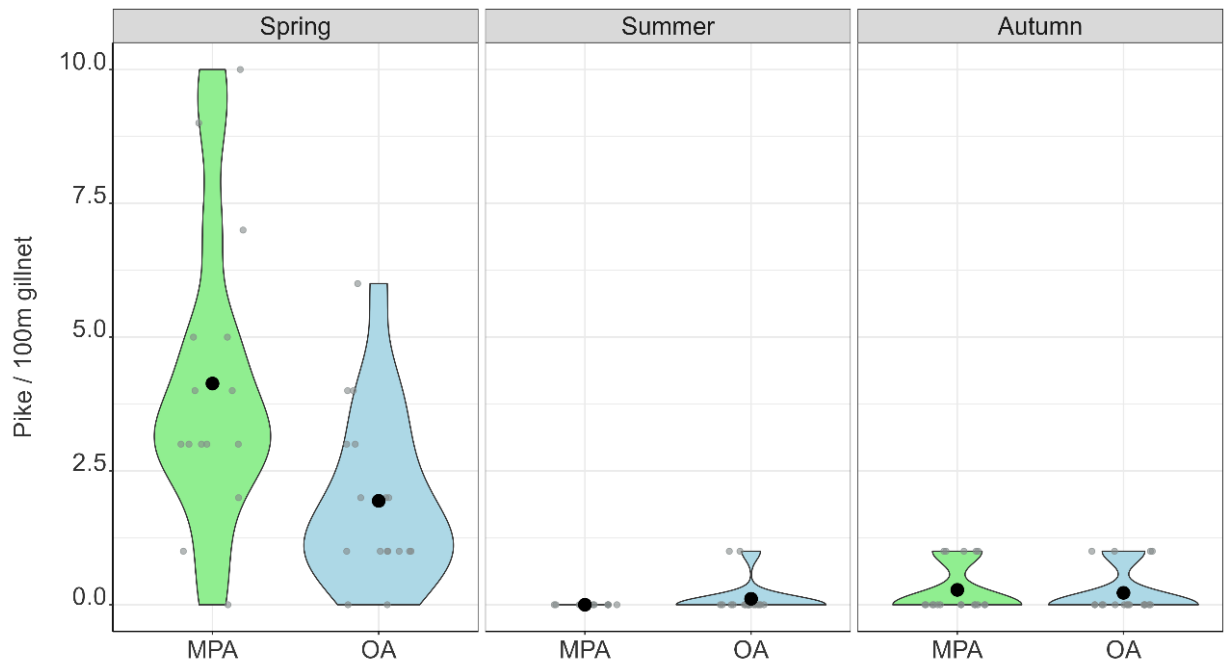
With gillnetting, a total of 106 pikes were captured. There was a very strong seasonal trend in the catch rates, where the majority of pike (N=95, 90%) were captured in spring, while only few pike (N=9, 8,4%) were captured in autumn and only 2 pike in summer (1,8%). There were more pike captured in the MPA's (N=67, 63,2%). Most pike were caught in Ummanz (N=40, 37,7%) and Grabow (N=39, 36,8%), while in N. and S., 27 pikes were captured (25,5%). Table 4 gives an overview of the specific number of pike captured per area, season and site and shows gillnet CPUE (pike per 100m gillnet) and CPUE and number of fish for the bycatch. From the total of 106 captured pike, 103 could be assigned to a specific mesh size.

**Table 4: Gillnetting overview of number of captured pike [share in %] and mean CPUE and SD (pike per 100m gillnet) and bycatch CPUE (fish per 100m gillnet) and sum of bycatch (number of fish) across the different areas, seasons and sites.**

| Area      | Season | Site | N Pike<br>[Share %] | Mean CPUE $\pm$<br>SD<br>[pike / 100m] | Mean CPUE $\pm$ SD<br>bycatch (fish /<br>100m) and sum of<br>bycatch |
|-----------|--------|------|---------------------|----------------------------------------|----------------------------------------------------------------------|
| Grabow    | Spring | MPA  | 26 [26%]            | 4,3 $\pm$ 4.17                         | 7.1 $\pm$ 5.91 / 43                                                  |
|           |        | OA   | 10 [9%]             | 1.6 $\pm$ 1.63                         | 6.3 $\pm$ 2.33 / 38                                                  |
|           | Summer | MPA  | 0 [0%]              | 0 $\pm$ 0.00                           | 4.1 $\pm$ 3.54 / 25                                                  |
|           |        | OA   | 1 [0,9%]            | 0.16 $\pm$ 0.40                        | 2.5 $\pm$ 3.20 / 15                                                  |
|           | Autumn | MPA  | 1 [0,9%]            | 0.16 $\pm$ 0.40                        | 3.1 $\pm$ 4.70 / 19                                                  |
|           |        | OA   | 1 [0,9%]            | 0.16 $\pm$ 0.40                        | 0.83 $\pm$ 0.75 / 5                                                  |
| Ummanz    | Spring | MPA  | 22 [21%]            | 4.4 $\pm$ 1.81                         | 10.6 $\pm$ 4.38 / 53                                                 |
|           |        | OA   | 14 [13,2 %]         | 2.8 $\pm$ 2.16                         | 11.8 $\pm$ 5.26 / 59                                                 |
|           | Summer | MPA  | 0 [0%]              | 0 $\pm$ 0.00                           | 2.8 $\pm$ 1.60 / 17                                                  |
|           |        | OA   | 1 [0,9%]            | 0.16 $\pm$ 0.40                        | 3.6 $\pm$ 1.75 / 22                                                  |
|           | Autumn | MPA  | 2 [1,9%]            | 0.33 $\pm$ 0.51                        | 4.1 $\pm$ 2.13 / 25                                                  |
|           |        | OA   | 1 [0,9%]            | 0.16 $\pm$ 0.40                        | 9.8 $\pm$ 11.51 / 59                                                 |
| N. and S. | Spring | MPA  | 14 [13,2%]          | 3.5 $\pm$ 1.00                         | 5.5 $\pm$ 3.1 / 22                                                   |
|           |        | OA   | 9 [8,5%]            | 1.5 $\pm$ 0.83                         | 6.3 $\pm$ 3.55 / 38                                                  |
|           | Summer | MPA  | 0 [0]               | 0 $\pm$ 0.00                           | 12.5 $\pm$ 5.44 / 50                                                 |
|           |        | OA   | 0 [0%]              | 0 $\pm$ 0.00                           | 3.83 $\pm$ 2.32 / 23                                                 |
|           | Autumn | MPA  | 2 [1,9%]            | 0.33 $\pm$ 0.51                        | 23.1 $\pm$ 8.42 / 139                                                |
|           |        | OA   | 2 [1,9%]            | 0.33 $\pm$ 0.51                        | 4.16 $\pm$ 3.18 / 25                                                 |

Most pike (N=34, 33%) were caught on the 50mm mesh size, 32 pike (31%) on the 60mm mesh size, 28 pike (27,2%) on the 75mm mesh size, 7 pike (6,8%) on the 95mm mesh size and only 2 pike (1,9%) on the 110mm mesh size (Table 5). The effect of season on gillnet CPUE was significant ( $X^2=168$ ,  $Df=2$ ,  $p=0.001$ ), underlining the observational data. CPUE was significantly lower in summer (mean CPUE  $\pm$  SD per 100m gillnet:  $0.058 \pm 0.23$ ; post hoc Tukey,  $p=0.001$ ) and autumn ( $0.25 \pm 0.42$ ; post hoc Tukey,  $p=0.001$ ) compared to spring ( $2.97 \pm 2.44$ ). Summer and autumn did not differ significantly (post hoc Tukey,  $p=0.11$ ).

Also, the effect of site was significant ( $X^2=10.2$ ,  $Df=1$ ,  $p=0.001$ ), where CPUE in the MPA's ( $1.37 \pm 2.40$ ) was significantly higher (ratio: 1.9) than in the OA's ( $0.73 \pm 1.25$ ;  $p=0.04$ , figure 7). The effect of area ( $X^2=3.7$ ,  $Df=2$ ,  $p=0.15$ ) and also the interaction between area and site ( $X^2=1.2$ ,  $Df=2$ ,  $p=0.53$ ) were not significant. In spring, the mean CPUE in the MPA's was around two times higher than in the OA's, while in summer, the CPUE were not different due to low catches. In autumn, the mean CPUE in the MPA's was around 1.2 times higher compared to OA's. The effect of the number of bycatch (number of fish per net, mainly large common bream) was almost significant ( $X^2=4.2$ ,  $Df=1$ ,  $p=0.06$ ), indicating that high bycatch rates reduce the number of captured pike in gillnets. The model had a  $R^2$  of 0.90, therefore explaining 90% of the

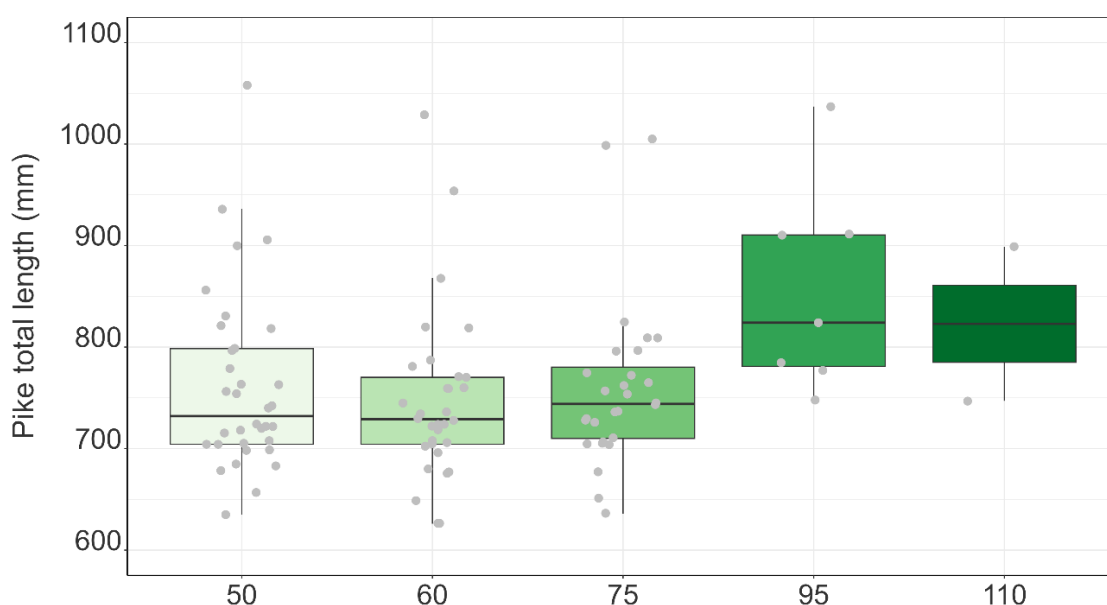


**Figure 7: Violin plots for gillnetting CPUE (pike per 100m gillnet) across the different seasons (spring, summer and autumn) and sites (MPA and OA). Black dots show the mean values and small grey dots show raw CPUE data.**

variation. Furthermore, the mesh size had a significant effect on the size of the captured pike ( $p=0.04$ , Table 5, Figure 8). Significantly larger pike (mean size in mm  $\pm$  SD:  $856 \pm 102.2$ ,  $p=0.02$ ) were captured with the 95mm mesh size compared to the 60mm mesh size ( $746.5 \pm 83.88$ ). Almost significantly different ( $p=0.06$ ) were the pike sizes between the 95mm mesh size and the 75mm mesh size ( $759.5 \pm 82.21$ ) and between the 95mm and 50 mm mesh size ( $761.6 \pm 88.70$ ,  $p=0.06$ ). There were no differences between the other mesh sizes. The correlation between the length of the captured pike and the different mesh sizes (Appendix figure 1) was weakly positive, but significant ( $R=0.21$ ,  $p=0.036$ ), indicating that with larger mesh sizes, larger pike get captured.

**Table 5: Gillnetting number of pike captured [share in %] and mean size and SD (total length in mm) with different mesh sizes in mm (knot-to-knot).**

| Mesh Size<br>(mm, knot-to-knot) | N Pike [Share %] | Mean size $\pm$ SD<br>(total length, mm) |
|---------------------------------|------------------|------------------------------------------|
| 50                              | 34 [33%]         | $761.6 \pm 88.7$                         |
| 60                              | 32 [31%]         | $747.5 \pm 83.8$                         |
| 75                              | 28 [27,3%]       | $759.5 \pm 82.2$                         |
| 95                              | 7 [6,7%]         | $856.0 \pm 102.2$                        |
| 110                             | 2 [2%]           | $823.0 \pm 107.5$                        |

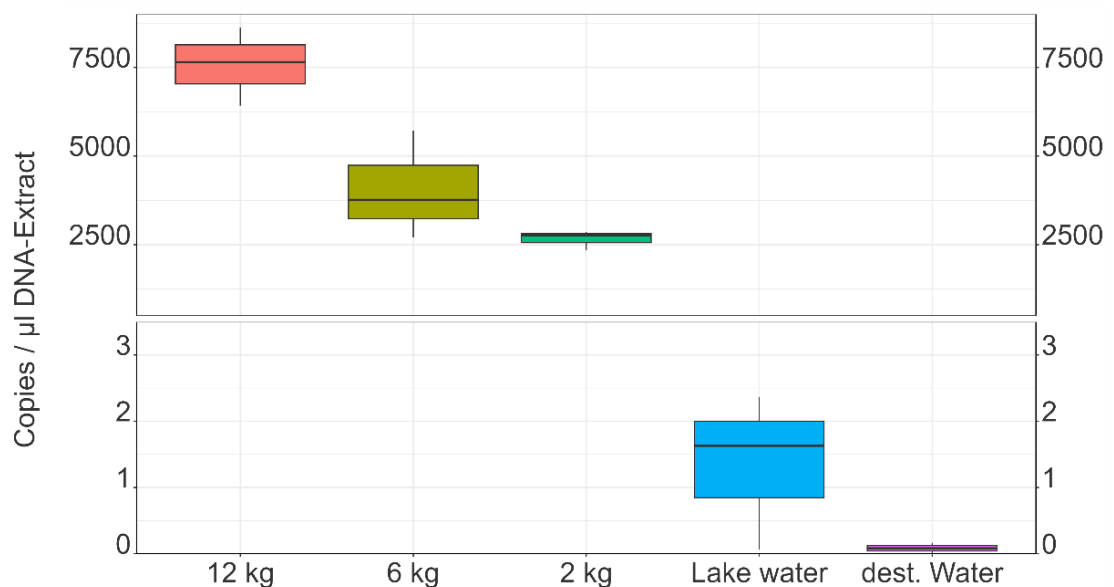


**Figure 8: Box plots for the total length of pike (mm) captured with different mesh sizes (knot-to-knot in mm: 50, 60, 75, 95, 110). Grey dots represent one individual pike.**

### 3.3 e-DNA

#### 3.3.1 e-DNA pretest

There was a very strong relationship between the biomass in the different treatments (12, 6 and 2 kg of pike) and the respective e-DNA concentrations measured along the different time points (after 12, 18 and 24 hours). Highest concentrations were found in the 12kg treatment (mean copies per  $\mu\text{l}$  DNA-extract  $\pm$  SD:  $7567.3 \pm 1104.76$ ), followed by the 6kg treatment ( $4061.6 \pm 1529.03$ ) and the 2 kg treatment ( $2659 \pm 272.52$ ). Lake water samples had mean copies of  $1.35 \pm 1.17$ , while distilled water samples had mean copies of  $0.08 \pm 0.08$ , figure 9. The correlation between the different biomasses and the e-DNA concentration was strongly positive and highly significant ( $R=0.95$ ,  $p=0.001$ ), indicating that with higher biomasses of pike, higher concentrations of e-DNA are measured (Appendix figure 2).



**Figure 9: Box plots for the e-DNA concentration (Copies /  $\mu\text{l}$  DNA-Extract) for the different biomass treatments (12, 6, and 2kg of pike) and for distilled and lake water.**

#### 3.3.2 e-DNA

Similar to the results described in section 2.2 (gillnetting), e-DNA concentrations were strongly variable between the seasons with peaks in spring (mean copies per  $\mu\text{l}$  DNA-extract  $\pm$  SD:  $7.2 \pm 8.50$ ) and lower concentrations in summer ( $1.5 \pm 2.31$ ) and autumn ( $1.8 \pm 2.89$ ). Mean concentrations in the respective MPA's were about 1.5 times higher ( $4.2 \pm 7.43$ ), compared to the OA's ( $2.9 \pm 3.63$ ), but this was mainly driven by the



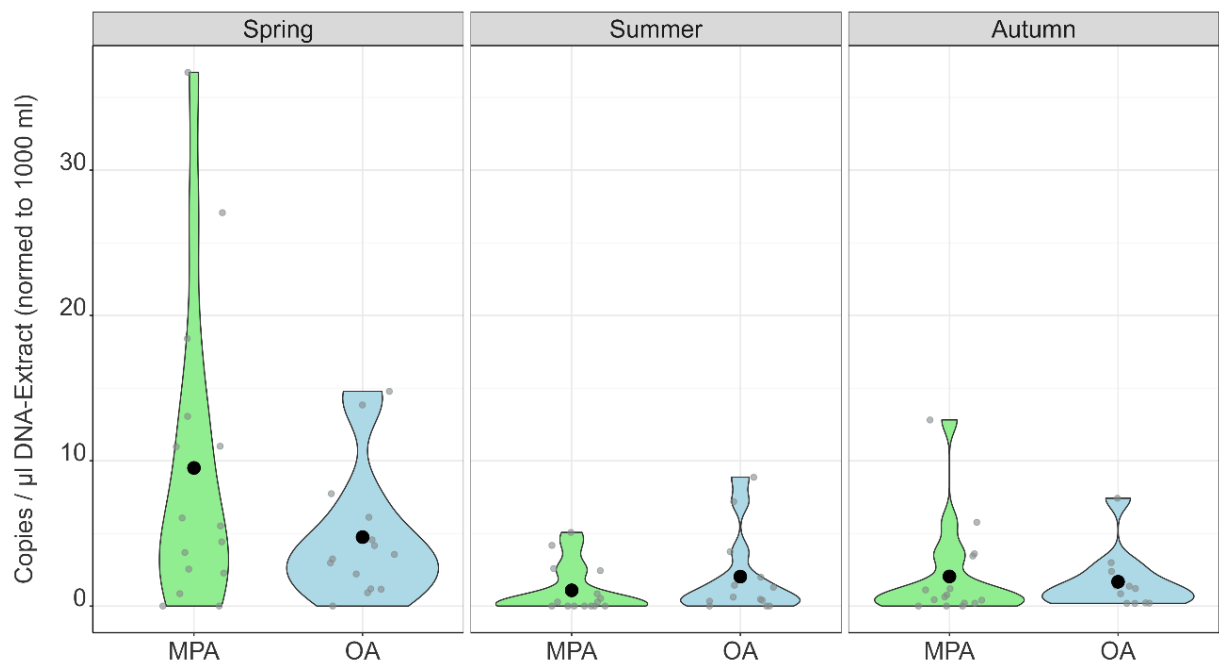
observations in spring (MPA:  $9.5 \pm 10.66$ , OA:  $4.7 \pm 4.56$ , ratio: 2.0). The effect of site was not significant ( $X^2=2.6$ , Df=1,  $p=0.09$ ), but the interaction between site and season was significant ( $X^2=6.5$ , Df=2,  $p=0.03$ ). In spring, the e-DNA concentrations were significantly higher in the MPA's compared to the OA's (post-hoc Tukey,  $p=0.04$ ). In summer, values in the OA's were about double ( $2.0 \pm 2.87$ ) compared to the MPA's ( $1.0 \pm 1.67$ ), but not significant. In autumn, concentrations were higher again in the MPA's ( $2.0 \pm 3.42$ ) than in the OA's ( $1.6 \pm 2.12$ ), but not significantly. Highest mean values were measured in Ummanz ( $5.6 \pm 9.30$ ) and N. and S. ( $3.2 \pm 3.09$ ), while in Grabow, values were the lowest ( $2.0 \pm 3.12$ ). The number of samples with zero concentrations were highest in summer (Table 6).

**Table 6: Overview over the mean e-DNA concentrations an SD (copies per  $\mu$ l DNA Extract), ranges, and number of samples and number of samples with zero [share in %] across the different areas, seasons and sites.**

| Area      | Season | Site | Mean Conc. $\pm$ SD<br>[copies / $\mu$ l] | Range<br>[copies / $\mu$ l] | N samples<br>[Samples with Zero, Share %] |
|-----------|--------|------|-------------------------------------------|-----------------------------|-------------------------------------------|
| Grabow    | Spring | MPA  | $6.36 \pm 5.35$                           | 0.8 - 13.1                  | 5 [0, 0%]                                 |
|           |        | OA   | $2.19 \pm 1.53$                           | 0 - 3.6                     | 5 [1, 20%]                                |
|           | Summer | MPA  | $0.9 \pm 0.97$                            | 0.3 - 2.6                   | 5 [0, 0%]                                 |
|           |        | OA   | $1.2 \pm 1.5$                             | 0 - 3.7                     | 5 [1, 20%]                                |
|           | Autumn | MPA  | $0.36 \pm 0.54$                           | 0 - 1.2                     | 5 [3, 60%]                                |
|           |        | OA   | $1.12 \pm 1.61$                           | 0.2 - 3                     | 3 [0, 0%]                                 |
| Ummanz    | Spring | MPA  | $19.38 \pm 13.01$                         | 3.7 - 36.7                  | 5 [0, 0%]                                 |
|           |        | OA   | $8.62 \pm 5.56$                           | 2.2 - 14.8                  | 5 [0, 0%]                                 |
|           | Summer | MPA  | $0 \pm 0$                                 | 0 - 0                       | 5 [5, 100%]                               |
|           |        | OA   | $0.13 \pm 0.23$                           | 0 - 0.4                     | 3 [2, 66%]                                |
|           | Autumn | MPA  | $0.58 \pm 0.36$                           | 0.2 - 1.1                   | 5 [0, 0%]                                 |
|           |        | OA   | $2.16 \pm 3.52$                           | 0.2 - 7.4                   | 4 [0, 0%]                                 |
| N. and S. | Spring | MPA  | $2.77 \pm 2.91$                           | 0 - 6.1                     | 5 [2, 0%]                                 |
|           |        | OA   | $3.08 \pm 2.5$                            | 0.9 - 6.1                   | 4 [0, 0%]                                 |
|           | Summer | MPA  | $2.33 \pm 2.33$                           | 0 - 5.1                     | 5 [2, 40%]                                |
|           |        | OA   | $3.98 \pm 3.77$                           | 0.5 - 8.9                   | 5 [0, 0%]                                 |
|           | Autumn | MPA  | $5.16 \pm 4.71$                           | 0.2 - 12.8                  | 5 [0, 0%]                                 |
|           |        | OA   | $1.58 \pm 0.54$                           | 1.2 - 2.4                   | 4 [0, 0%]                                 |

The seasonal effect in the e-DNA concentrations were significant ( $X^2=28.48$ , Df=2,  $p=0.001$ ) where spring pike e-DNA concentration was significantly higher than

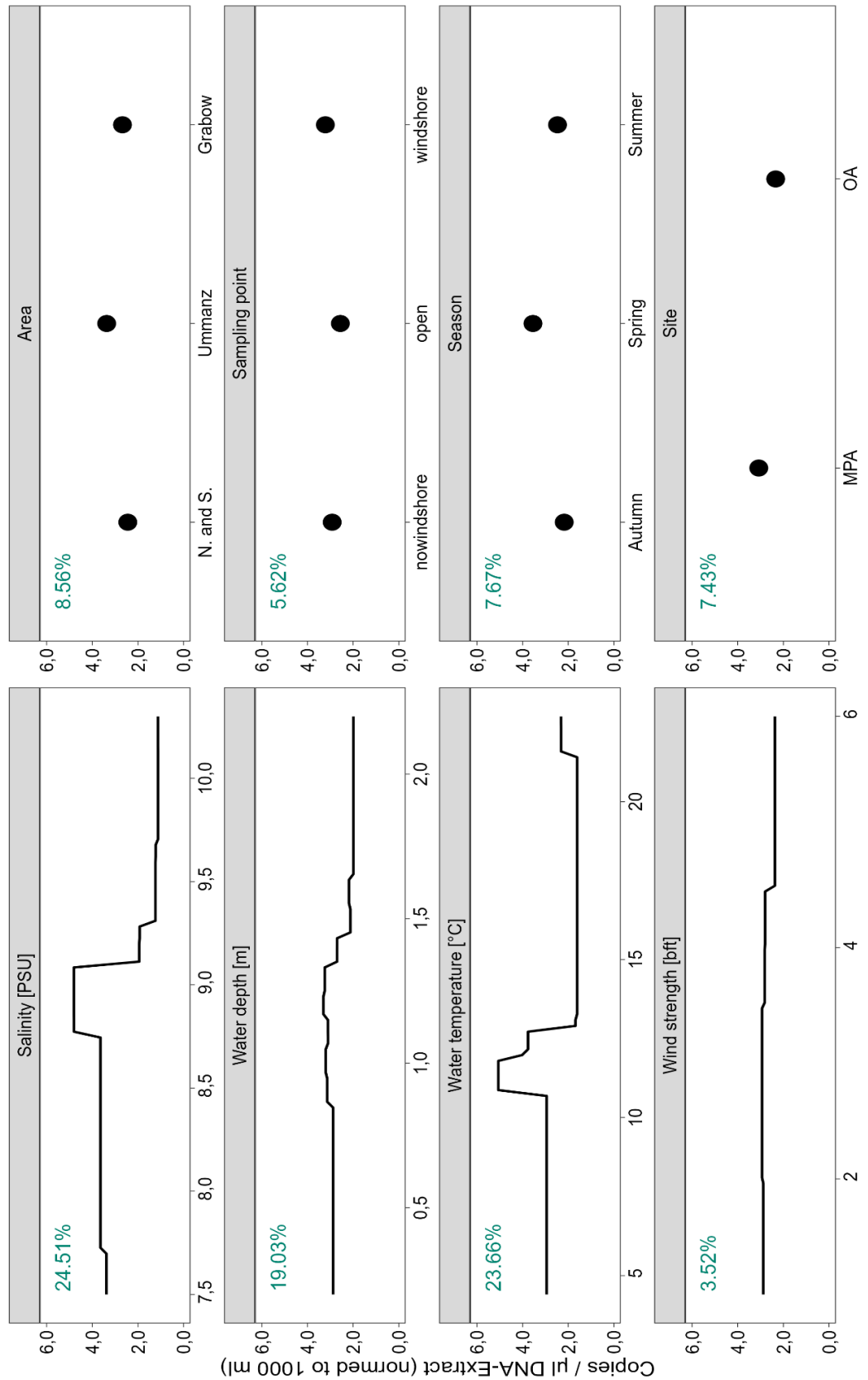
autumn (post hoc Tukey,  $p=0.001$ ) and summer (post hoc Tukey,  $p=0.001$ ). Also, summer and autumn differed significantly (post hoc Tukey,  $p=0.001$ ) with higher concentrations in autumn ( $1.88 \pm 2.89$ ) compared to summer ( $1.51 \pm 2.31$ ). There was no significant effect of area ( $X^2=1.8$ ,  $Df=2$ ,  $p=0.3$ ). The two of the environmental variables wind strength ( $X^2=6.3$ ,  $Df=1$ ,  $p=0.01$ ) and water temperature ( $X^2=18.1$ ,  $Df=1$ ,  $p=0.001$ ) were significant, where higher temperatures and stronger wind increasing e-DNA concentrations. In spring, the mean concentrations in the MPA's were around two times higher compared to the OA's, while in summer, the mean concentrations were around two times higher in the OA's. In autumn, the mean concentrations were around 1.25 times higher in the MPA's compared to the mean concentrations in the OA's. Results are shown in figure 10.



**Figure 10: Violin plots for e-DNA concentrations (copies per  $\mu\text{l}$  DNA Extract) across the different seasons (spring, summer and autumn) and sites (MPA and OA). Black dots show the mean values and small grey dots show raw concentration data.**

The model results were supplemented by boosted regression tree models (BRT's, figure 11). The included environmental parameters such as salinity (24.5%), water depth (19.0%) and water temperature (23.6%) explained the majority of the e-DNA concentrations (sum=67,1%). Wind strength however was only explaining 3.5% of the variation. Area, site and season each only explained less than 10%. Also, if the sample was taken windward/leeward shore or at the gillnet points (open) explained less than 10%. Peaks in e-DNA concentrations were found between 10 to 13 °C water

temperature and 8.7 to 9 PSU. However, salinity and water temperature were moderately and significantly correlated ( $R=0.39$ ,  $p=0.001$ , Appendix figure 3).

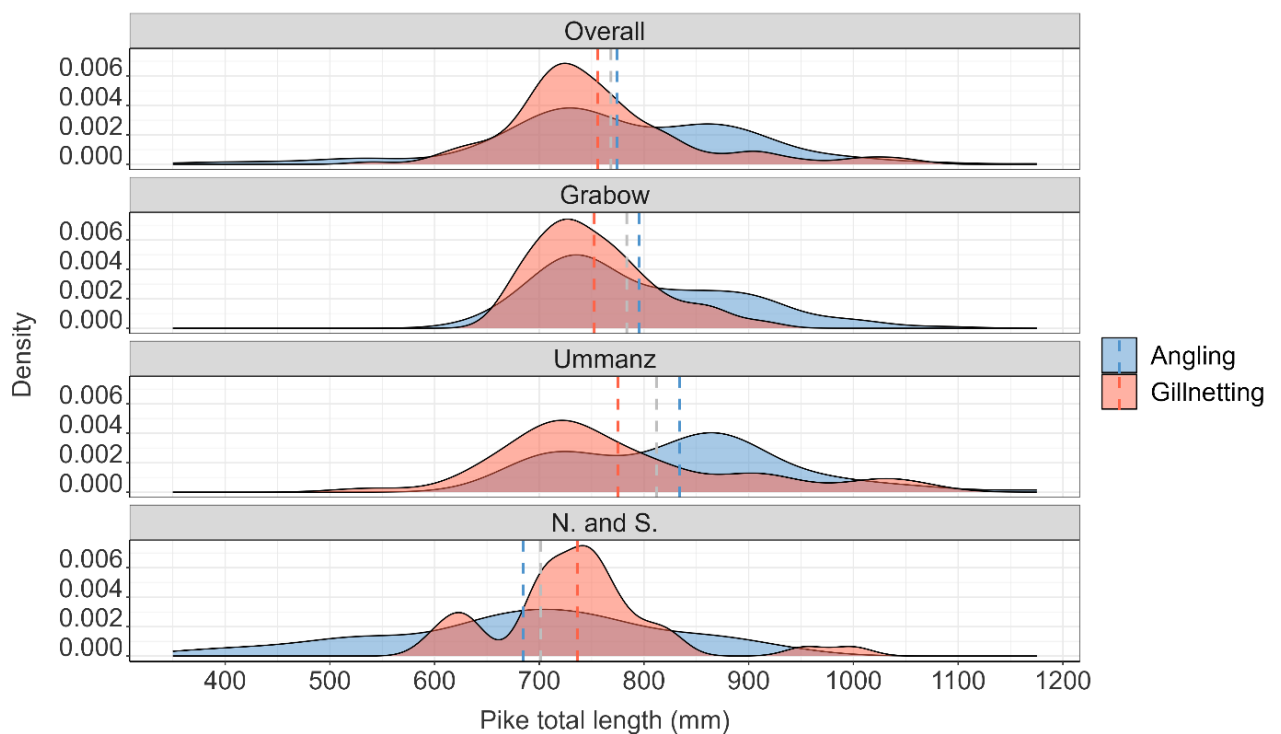


**Figure 11: Overview over the result of the BRT-analysis, where the relative influence of variables on the e-DNA concentration is shown.**

### 3.4 Length, age and growth of pike

#### 3.4.1 Length of pike

Pike size ranged from 350 to 1175 mm and the average length of all sampled pike (N=386) was (mean length in mm  $\pm$  SD) 768mm  $\pm$  114. The mean largest pike were captured in Ummanz (812mm  $\pm$  113), followed by Grabow (784mm  $\pm$  86) and N. and S. (701mm  $\pm$  124). There were no seasonal patterns in pike length (spring: 761mm  $\pm$  88.9; summer: 775mm  $\pm$  139; autumn: 775mm  $\pm$  127). Also, the mean length in the MPA's (771mm  $\pm$  121) was similar to the mean length in the OA's (761mm  $\pm$  95). Angling caught slightly larger individuals (774mm  $\pm$  124) compared to gillnetting (756mm  $\pm$  89), figure 12.

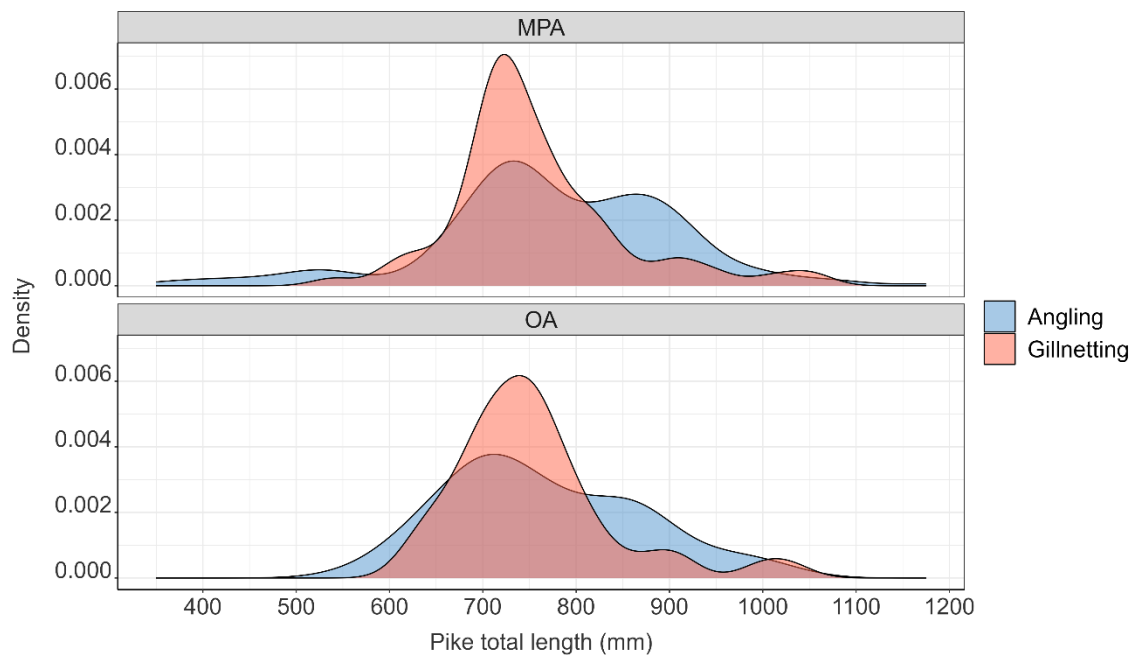


**Figure 12: Density plots of the size distribution of captured pike overall and depending on the gear across the different areas. Coloured dashed lines show mean sizes for the respective gear. Grey dashed line shows mean for from both gears per area.**

There was no significant difference in mean length between the sites ( $X^2=0.1$ , Df=1,  $p=0.7$ ) and the two gears multimesh gillnets and angling ( $X^2=0.8$ , Df=1,  $p=0.3$ ). Also, the interaction between site and gear was not significant ( $X^2=0.23$ , Df=1,  $p=0.6$ ). However, pike differed significantly in length between the areas ( $X^2=18.4$ , Df= 2,  $p=0.001$ ). In N. and S., pike were significantly smaller compared to Grabow (post hoc

Tukey,  $p=0.001$ ) and Ummanz (post hoc Tukey,  $p=0.002$ ). There was no significant difference between Grabow and Ummanz (post hoc Tukey,  $p=0.8$ ).

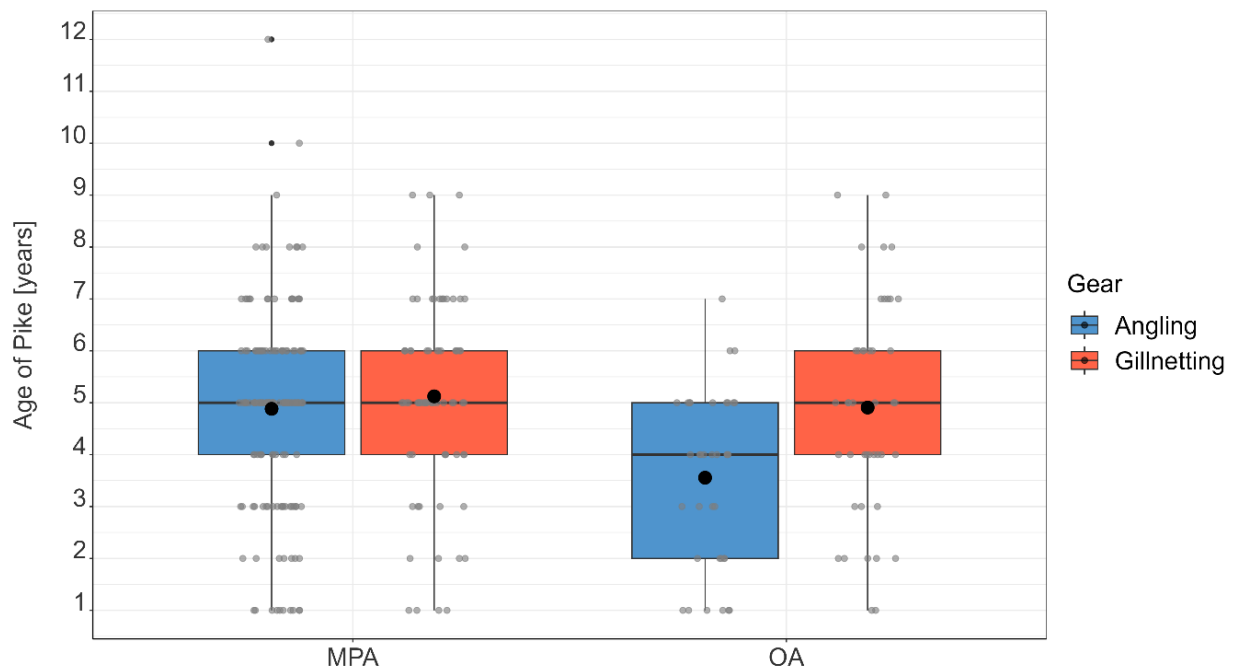
In contrast to the mean length, the Kolmogorov-Smirnov test showed, that the size distributions differed significantly between gears (figure 12) overall ( $p=0.001$ ) and also between gears in all three areas respective (Grabow:  $p=0.02$ , Ummanz:  $p=0.001$ , N. and S.:  $p=0.02$ ). Size distribution of pike did not differ between the sites, when taking both gears together ( $p=0.28$ ), but in the MPA, the size distribution differed significantly ( $p=0.003$ , figure 13) between the two gears. When looking at the distributions as shown in figure 12 and 13, angling in general caught more smaller pikes (<600 mm), but especially in the area N. and S. Furthermore, angling also caught a higher frequency of larger pike (>800 mm) compared to gillnetting. Gillnetting size distribution was more dome shaped and peaking around 700 to 750 mm size, while angling size distribution was more widespread across the across the size spectrum.



**Figure 13: Density plots of the size distribution of captured pike overall and depending on the gear across the sites.**

### 3.4.2 Age of pike

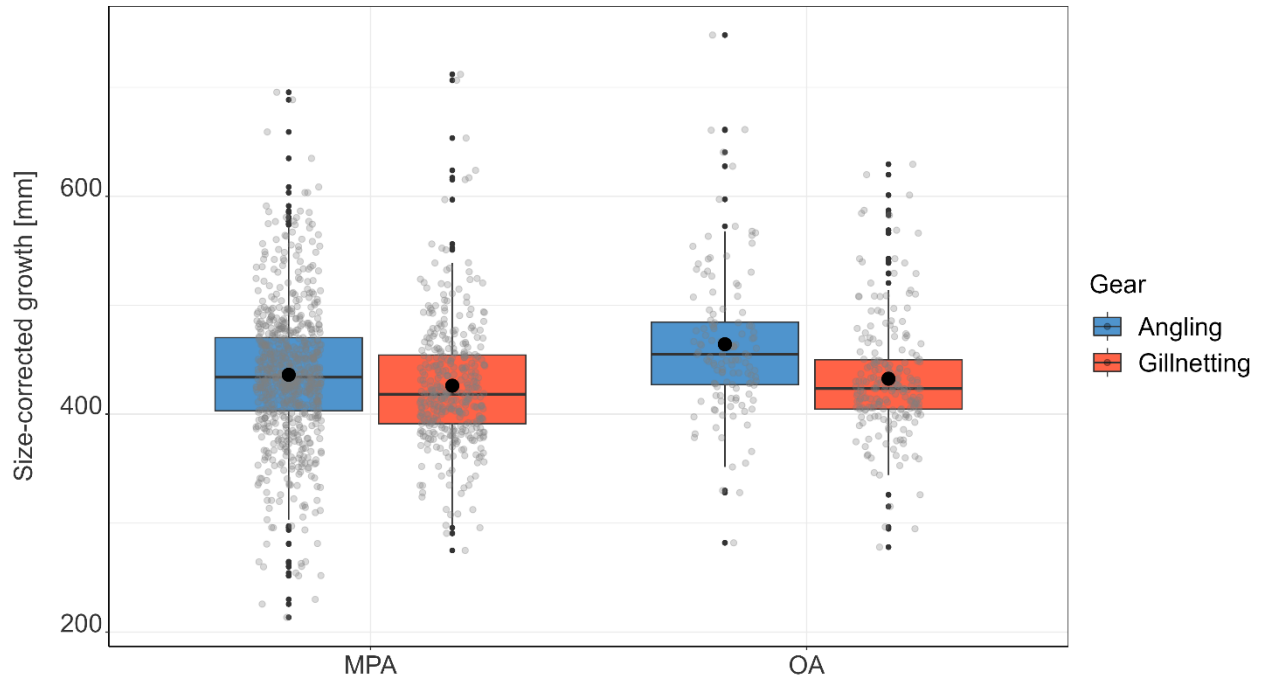
The average age of all sampled pike (N=290) was (mean age in years  $\pm$  SD)  $4.7 \pm 1.9$  years. On average, the oldest pike were captured in Ummanz ( $5.3 \text{ years} \pm 2.0$ ), followed by Grabow ( $4.9 \text{ years} \pm 1.4$ ) and N. and S. ( $3.9 \text{ years} \pm 2.3$ ). Pike in the MPA's were older ( $4.9 \text{ years} \pm 1.9$ ) compared to the OA's ( $4.2 \text{ years} \pm 2.0$ ). Pike from gillnetting were slightly older ( $5.0 \text{ years} \pm 1.9$ ) than the pike captured with angling ( $4.6 \text{ years} \pm 1.9$ ). The effect of site was significant ( $X^2=8.9$ , Df=1,  $p=0.002$ ) and fish from the OA's were significantly younger compared to the MPA's (post hoc Tukey,  $p=0.002$ ), which would be expected for an exploited stock. While the effect of gear on pike age in the sample was not significant ( $X^2=0.3$ , Df=1,  $p=0.5$ ), the interaction effect between site and gear was significant ( $X^2=7.0$ , Df=1,  $p=0.008$ ), where fish captured with angling in OA's were significantly younger than fish captured with gillnetting in the OA's ( $p=0.004$ ), while the age did not differ between the gears in the MPA's ( $p=0.9$ ), figure 14. The effect of area was not significant ( $X^2=5.1$ , Df=2,  $p=0.07$ ).



**Figure 14: Box plots for the age of captured pike across the sites (MPA and OA) and depending on the gear (angling and gillnetting). Black dots show the mean values and small grey dots show individual pike.**

### 3.4.3 Growth of pike

The effect of site on the size corrected growth rate of pike was significant ( $X^2=10.8$ ,  $Df=1$ ,  $p=0.001$ ), indicating that the growth of fish in the MPA (mean growth and SD:  $432.9 \text{ mm} \pm 57.4$ ) was lower than in the OA ( $453.5 \text{ mm} \pm 56.1$ ).



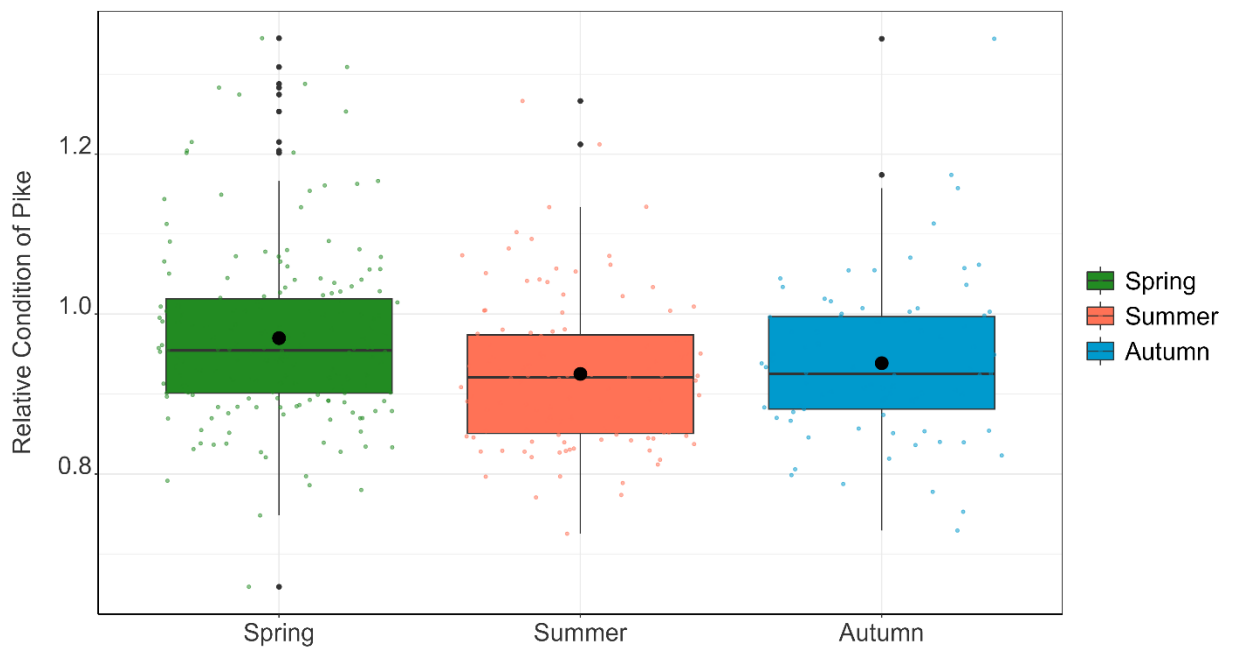
**Figure 13: Box plots for the size corrected growth of captured pike across the sites (MPA and OA) and depending on the gear (angling and gillnetting). Black dots show the mean values and small grey dots show individual pike.**

The main effect of gear was not significant ( $X^2=0.2$ ,  $Df=1$ ,  $p=0.6$ ). However, the interaction between site and gear was significant ( $X^2=3.6$ ,  $Df=1$ ,  $p=0.04$ ). The difference in size-corrected growth rate of pike captured by angling in the MPA ( $433.5\text{mm} \pm 59.0$ ) and OA ( $473.4\text{mm} \pm 59.7$ ) was significant ( $p=0.001$ ), while there was no difference ( $p=0.9$ ) in growth from gillnet pikes between the MPA ( $431.7\text{mm} \pm 54.6$ ) and the OA ( $436.8\text{mm} \pm 47.3$ ). Also, the growth rate revealed by angling in OA was significantly higher compared to the growth rate revealed by gillnetting in the MPA ( $p=0.03$ ) as shown in figure 15. The effect of the area on the growth rate was not significant ( $X^2=1.9$ ,  $Df=2$ ,  $p=0.38$ ).



#### 3.4.4 Condition of pike

From all pike captured (N=386), the relative condition (mean relative condition  $\pm$  SD) was  $0.95 \pm 0.10$  and ranged from 0.65 to 1.34. The highest mean relative condition was found in pike from N. and S. ( $0.96 \pm 0.08$ ), followed by Grabow ( $0.94 \pm 0.09$ ) and Ummanz ( $0.93 \pm 0.12$ ).

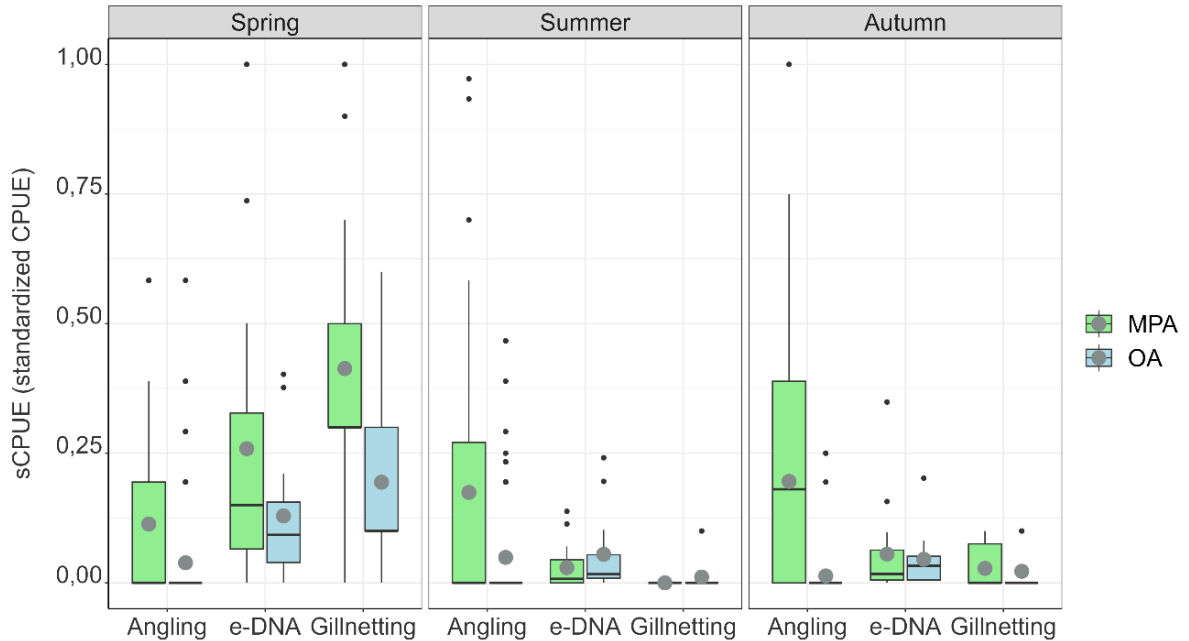


**Figure 16: Box plots for the size relative condition (Kn) of captured pike across the seasons. Black dots show the mean values and small coloured dots show individual pike.**

Fish from the MPA's ( $0.94 \pm 0.10$ ) were less well conditioned compared to fish from the OA's ( $0.96 \pm 0.10$ ). Condition varied between the season and highest values were found in spring ( $0.96 \pm 0.10$ ), while summer ( $0.92 \pm 0.09$ ) and autumn ( $0.93 \pm 0.09$ ) were relatively indifferent. Furthermore, the condition of pike from gillnetting was higher ( $0.97 \pm 0.12$ ) compared to pike captured with angling ( $0.93 \pm 0.09$ ). The only significant effect was season ( $X^2=6.0$ ,  $Df=2$ ,  $p=0.04$ ), where the condition of pike was significantly higher (post hoc Tukey,  $p=0.03$ ) in spring ( $0.96 \pm 0.10$ ) compared to summer ( $0.92 \pm 0.09$ ), figure 16. The effects of site ( $X^2=0.57$ ,  $Df=1$ ,  $p=0.4$ ), gear ( $X^2=1.2$ ,  $Df=1$ ,  $p=0.2$ ) and the interaction of the two ( $X^2=0.47$ ,  $Df=1$ ,  $p=0.5$ ) were not significant. The effect of area was near significance ( $X^2=5.9$ ,  $Df=2$ ,  $p=0.051$ ). Relative condition of pike from Ummanz was significantly lower (post hoc Tukey,  $p=0.04$ ) compared to N. and S.

### 3.5 Standardized CPUE (sCPUE)

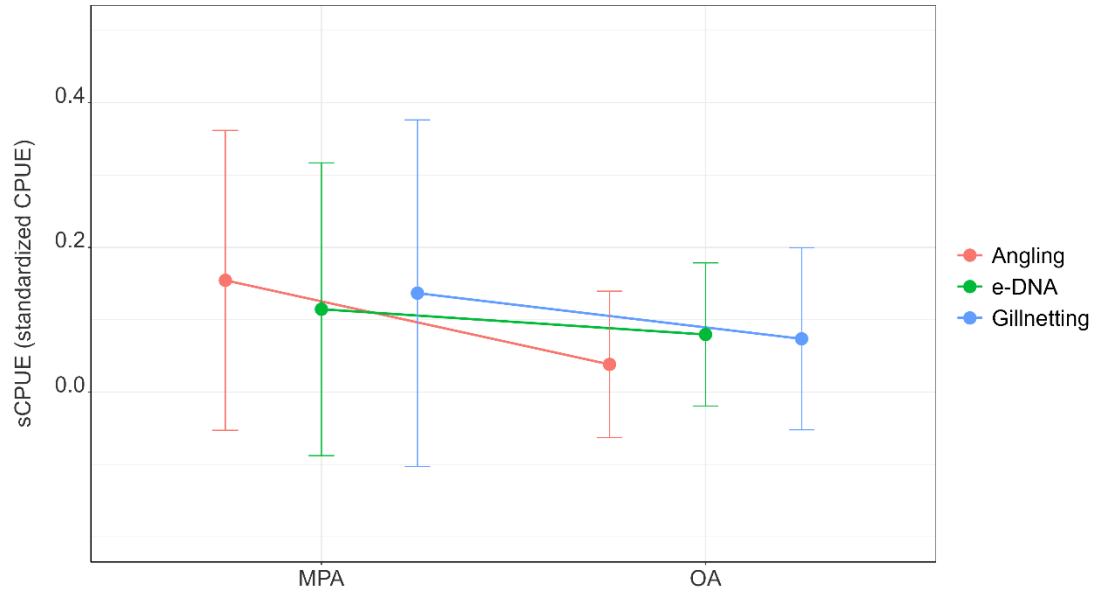
For the standardized CPUE (sCPUE), the effect of site was significant ( $X^2$ , Df=1,  $p=0.001$ ). For all gears combined, the sCPUE was significantly lower (post hoc Tukey,  $p=0.001$ ) in the OA's (mean sCPUE  $\pm$  SD:  $0.04 \pm 0.10$ ) compared to the MPA's ( $0.14 \pm 0.2$ ). Also, the effect of gear was significant ( $X^2=13.1$ , Df=2,  $p=0.001$ ).



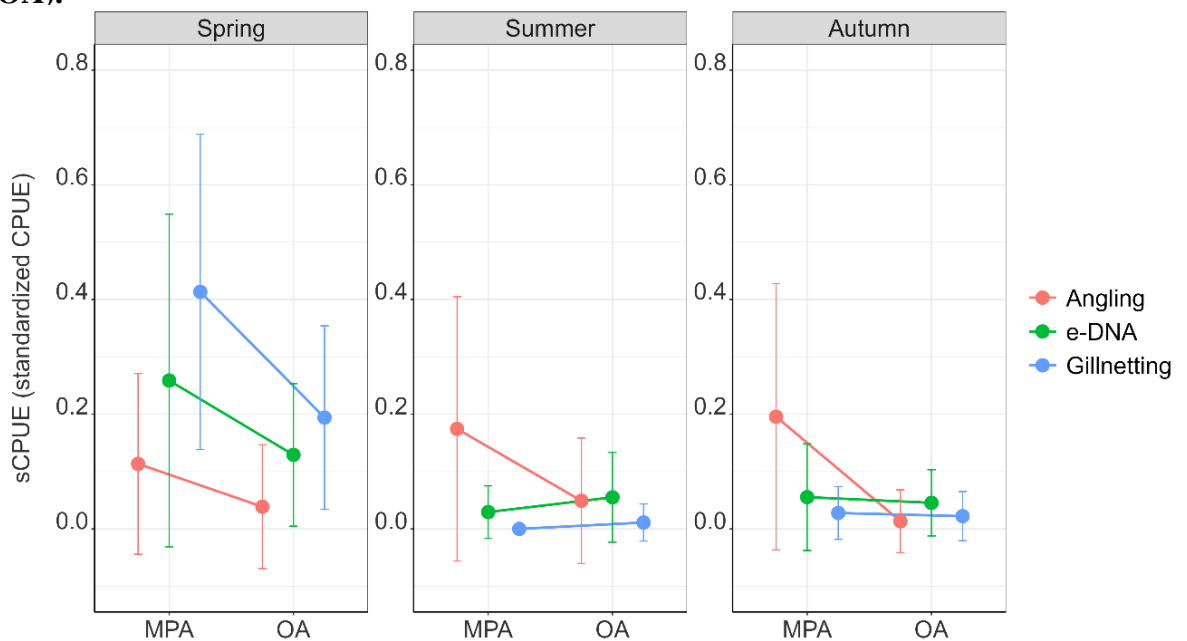
**Figure 17: Box plots for standardized CPUE (sCPUE) across the different seasons (spring, summer and autumn) and sites (MPA and OA) for the three gears (angling, gillnetting and e-DNA). Large grey dots indicate mean values.**

Gillnetting ( $0.10 \pm 0.19$ ) was significantly different (post hoc Tukey,  $p=0.005$ ) from angling ( $0.10 \pm 0.17$ ) and also angling and e-DNA ( $0.09 \pm 0.16$ ) were significantly different (post hoc Tukey,  $p=0.02$ ). In addition, the interaction between gear and season was highly significant ( $X^2=94.7$ , Df=6,  $p=0.001$ ). As seasonality within the respective gears is already analysed within each chapter for the specific gears, only the significant differences between two different gears are presented here. Angling sCPUE in spring ( $0.07 \pm 0.13$ ) was significantly lower compared to gillnetting ( $0.29 \pm 2.44$ ,  $p=0.001$ ) and e-DNA ( $0.19 \pm 0.23$ ,  $p=0.003$ ) in spring. In summer, the sCPUE of gillnetting ( $0.005 \pm 0.02$ ) was significantly lower ( $p=0.001$ ) compared to angling ( $0.11 \pm 0.19$ ), while the sCPUE for e-DNA ( $0.04 \pm 0.06$ ) was significantly higher ( $0.01$ ) than for gillnetting. In autumn, the sCPUE for angling ( $0.10 \pm 0.19$ ) was significantly higher compared to gillnetting ( $0.025 \pm 0.04$ ,  $p=0.001$ ) and e-DNA ( $0.05 \pm 0.07$ ,  $p=0.02$ ), figure 17.

The interaction effect of site and gear was near significance ( $X^2=94.7$ ,  $Df= 2$ ,  $p=0.06$ ) and some of the specific gear and site effects were significant. For angling, the sCPUE in the MPA's ( $0.15 \pm 0.20$ ) was significantly higher compared to the sCPUE of eDNA ( $0.07 \pm 0.09$ ,  $p=0.02$ ) and gillnetting ( $0.07 \pm 0.12$ ,  $p=0.01$ ) in the OA's. Angling sCPUE in the OA's ( $0.03 \pm 0.10$ ) was significantly lower compared to the sCPUE of gillnetting ( $0.13 \pm 2.23$ ,  $p=0.003$ ) and e-DNA ( $0.11 \pm 0.20$ ,  $p=0.03$ ) in the MPA's (Figure 17, 18 and 19).



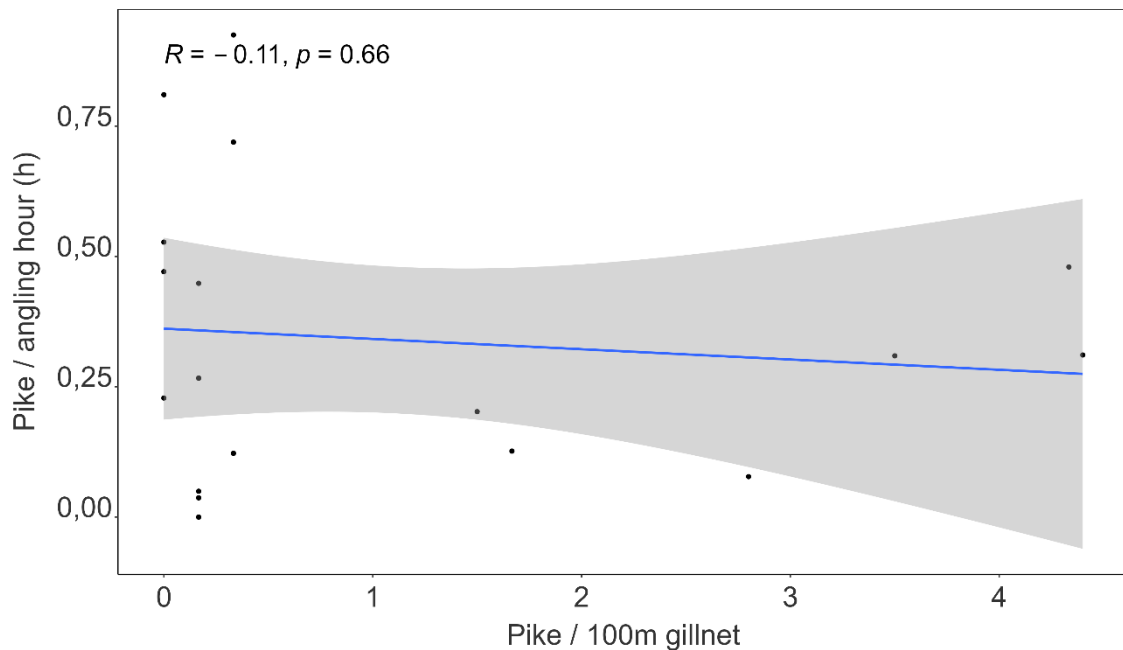
**Figure 18: Plots for mean standardized CPUE (sCPUE) ± SD for the three gears (angling, gillnetting and e-DNA) pooled for all seasons and across the sites (MPA and OA).**



**Figure 19: Plots for mean standardized CPUE (sCPUE) ± SD for the three gears (angling, gillnetting and e-DNA) across the three seasons and sites (MPA and OA).**

### 3.6 Correlations between gears

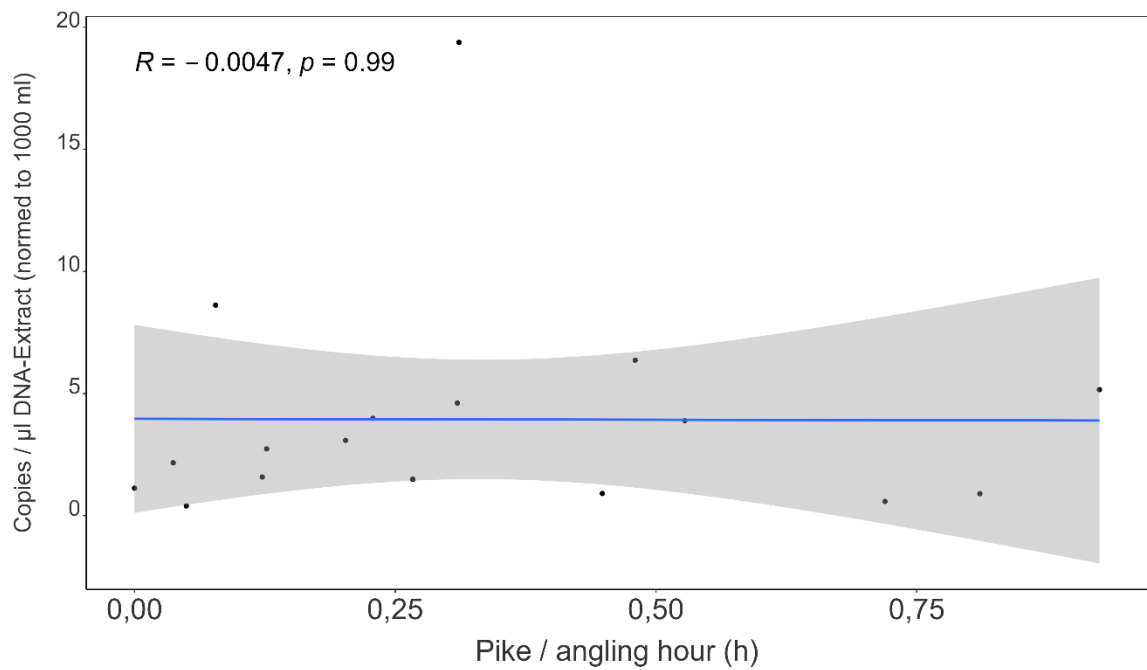
The global correlations (across all areas, seasons and sites) between angling in comparison to gillnetting and e-DNA were close to 0, indicating no correlation between the CPUE's of different gears and angling. For angling and gillnetting (Figure 20), the pearson correlation coefficient (R) was -0.11 and not significant ( $p=0.66$ ).



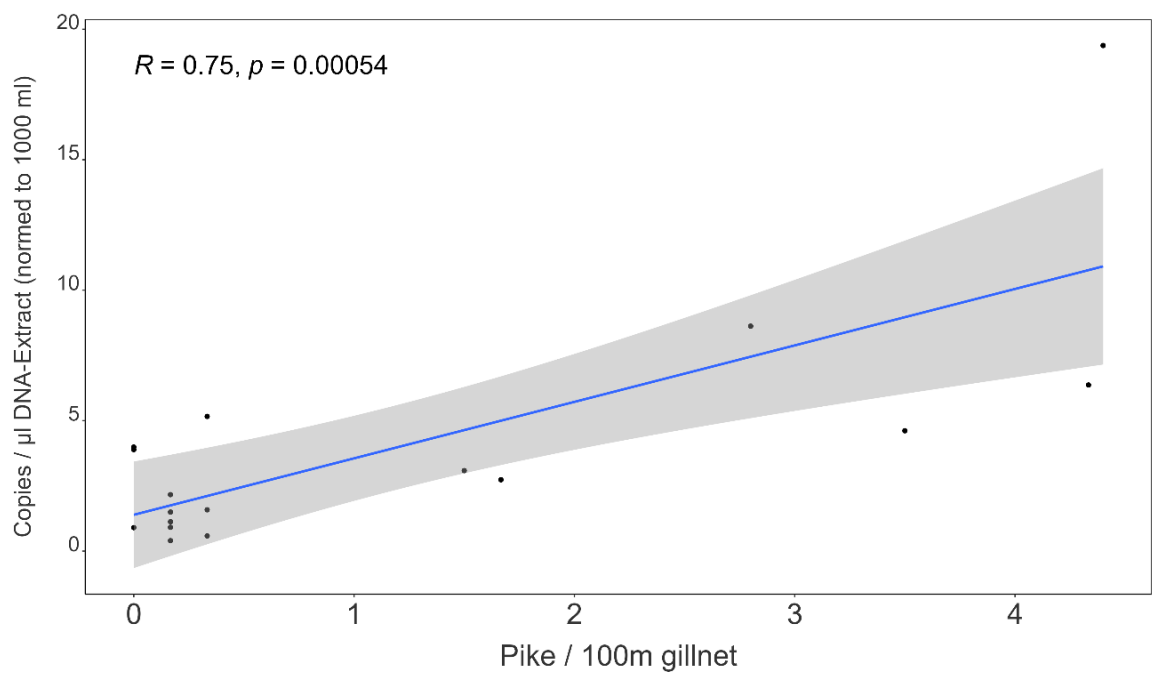
**Figure 20: Global correlation plot between angling (pike per hour \* angler) and gillnetting (pike per 100m gillnet) CPUE with pearson correlation coefficient and significance (R and p value).**

Very similar to angling and gillnetting, there was no correlation between angling and e-DNA ( $R=0.004$ ,  $p=0.99$ ) as shown in figure 21. Therefore, high (or low) angling CPUE do not correspond to equivalently high (or low) relative abundance measures collected by gill netting or eDNA.

In contrast to the correlations between angling and e-DNA and gillnetting, the correlation between gillnetting and e-DNA was highly significant ( $p=0.001$ ) and strongly positive ( $R=0.75$ ) as shown in figure 22.



**Figure 21: Global correlation plot between angling CPUE (pike per hour \* angler) and e-DNA concentrations (copies per µl DNA-Extract) with pearson correlation coefficient and significance (R and p value).**



**Figure 22: Global correlation plot between e-DNA concentrations (copies per µl DNA-Extract) and gillnetting CPUE (pike per 100m gillnet) with pearson correlation coefficient and significance (R and p value).**

The correlations on seasonal level between angling and gillnetting (Appendix figure 4) were mixed. For spring, the correlation was strongly positive ( $R=0.74$ ) and almost significant ( $p=0.08$ ). In summer, the correlation was strongly negative ( $R=-0.86$ ), but not significant ( $p=0.14$ ). In autumn, the correlation was moderately positive ( $R=0.61$ ), but not significant ( $p=0.2$ ). When correlating angling and gillnetting separately for each site (MPA and OA, Appendix Figure 5), angling and gillnetting were negatively and almost significantly correlated ( $R=-0.61$ ,  $p=0.07$ ), while there was no correlation in the OA's ( $R=0.04$ ,  $p=0.99$ )

The correlation between angling and e-DNA in spring was weakly positive and not significant ( $R=0.2$ ,  $p=0.71$ ), while in summer, there was no correlation ( $R=0.04$ ,  $p=0.94$ ). In autumn, the two gears were moderately positively, but not significantly correlated ( $R=0.48$ ,  $p=0.34$ ) as seen in Appendix figure 6. For the correlation between angling and e-DNA separately for each site (Appendix figure 7), the correlation in the MPA's was moderately negative and not significant ( $R=-0.5$ ,  $p=0.22$ ), while there was no correlation in the OA's ( $R=0.07$ ,  $p=0.85$ ).

The seasonal correlations between gillnetting and e-DNA (Appendix figure 8) were variable. While the correlation in spring was strongly positive, but not significant ( $R=0.67$ ,  $p=0.15$ ), the correlation in summer was strongly negative, but also not significant ( $R=-0.64$ ,  $p=0.24$ ). In autumn, the correlation between gillnetting and e-DNA was slightly positive, yet not significant as well ( $R=0.34$ ,  $p=0.51$ ).

In contrast to the seasonal correlations between gillnetting and e-DNA, both correlations in the MPA's ( $R=0.72$ ,  $p=0.04$ ) and OA's ( $R=0.8$ ,  $p=0.01$ ) were strongly positive and significant (Appendix figure 9).

## 4. Discussion

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In this thesis, a comparison of five different sampling gear types was conducted in their ability to derive relative abundance estimates and inferences such as age, growth, size and condition of pike in the Baltic lagoons around the island of Rügen, Germany. Methodologically, the study was performed by comparing three different areas with each other in different seasons in order to capture possible biases related to seasonal effects on catchability. Each of the three compared areas consisted of a site with a certain degree of protection against predominantly recreational fishing efforts, and a comparable, geographical close, open access site, which were open to all forms of commercial and recreational fishing. This contrast was made under the assumption that pike abundance in protected areas would be systematically greater and thereby examine which gear type was able to track this abundance difference systematically over time. From the five gear types and sampling methods that were applied in the Baltic lagoons within this thesis, three gears/sampling methods - angling, gillnetting and pike-specific e-DNA - provided analysable data, while longlining and the ROUV did not provide sufficient data for further analysis. In line with hypothesis 1 (H1), those gears and sampling methods showed differences, although to varying extents, in relative abundances between the investigated protected and unprotected sites on an annual average. But the abundance estimates from the respective gears and sampling methods were strongly varying throughout the seasons and sites. Hypothesis 2 (H2) could therefore not be fully accepted as only e-DNA and gillnetting were positively and significantly correlated with each other on an annual basis. Other correlations between the respective gears and sampling methods were non-existent or only evident in specific sites and/or seasons. In favour of hypothesis 3 (H3), angling was consistently showing strong differences in CPUE between the protected and unprotected sites in all seasons, while gillnetting and e-DNA showed similar strong differences only in spring. In summer, gillnet catches were basically non-existent and e-DNA concentrations were showing higher concentrations in the unprotected sites. In autumn, gillnetting CPUE and e-DNA concentrations showed the same degree of difference (around 1.2 times higher in the protected site), but in angling, this ratio was around 5.3 times higher in the protected sites. In line with H3, angling was the only gear that performed consistently in all seasons. Hypothesis 4 (H4) was found partly true. Indeed, angling captured larger and faster growing pike compared to gillnetting and this finding was especially true for the fish captured by angling in the unprotected

sites. However, the condition of the pike was not depending on the capture gear, but solely on season. Therefore, the second part of hypothesis of H4 (gillnetting will capture better conditioned pike) was not found true.

#### Protected and unprotected sites and pike abundance

Under the often-supported assumption that areas with a certain degree of protection against fishing pressure hold higher abundances of fish (Edgar et al., 2007; Sciberras et al., 2013), all gears and sampling methods (angling, gillnetting and e-DNA) showed significantly higher catch rates in the investigated protected sites (MPA) compared to the unprotected sites (OA), but the magnitude of those differences was strongly varying throughout the different seasons. In spring, all three gears and sampling methods showed the same degree of significant differences between the sites (ratio: 2.0-2.9). In the two other seasons, this similarity in the ratios between the gears along the protected and unprotected sites was not observed. Only angling showed significant differences (ratio in summer: 3.6, ratio in autumn: 5.3) between the sites, while gillnetting and e-DNA showed rather similar values between the sites ranging from ratios from 0.1 to a 1.25 in summer and autumn.

For gillnetting, very low catch rates were observed in summer and autumn, while the catch rates in spring were high. The lack of pike catches outside of spring make a comparison with the two other gears for summer and autumn difficult, if not impossible. Many other studies, that investigated the effectiveness of gillnets and other passive gears to capture pike in different seasons have reported similar observations, where the catch rates were notably increased during the spring season (Neumann & Willis, 1995; Olsson, 2019; Pierce, 2012). As a passive gear type, gillnet catches depend on small-scale movement patterns of fish (Brandt, 1984; Hubert et al., 2012). Pike within the studied lagoon system show increased movement and aggregation patterns during spawning seasons, which usually falls into the spring month of March till May at water temperatures between 9.8 to 13.8 °C (Craig, 2008; Dhellemmes et al., 2023; Frost & Kipling, 1967, Lukyanova et al., 2023; Nilsson, 2006). The spawning related increased movement patterns of pike is likely the reasons for the high catch in gillnetting in spring. In contrast to my findings, Pierce (2012) has argued for gillnetting in summer month being the best way to represent abundance of pikes in small lakes in Minnesota. Given that, it is very likely that the effectiveness of gillnets to capture pike can be seasonally variable and thus is depending on more factors than



simple season and associated water temperature levels. Such factors, for example, are the type of ecosystem investigated, morphological characteristics of the water body, size structures of pike and the density of pike in a given area (Pierce et al., 2010). Furthermore, the gillnet effort in this thesis was much lower (only set once per season and area) compared to other studies, where gillnetting was conducted over multiple days with more nets per km<sup>2</sup> of surface area of the investigated water bodies (Pierce & Tomcko, 2003; Pierce et al., 2003; Pierce et al., 2010). Effort (in terms of gillnet sampling days and number of nets deployed) in the present thesis might therefore been just too low in order to counter the low catchability of pike outside the spawning season. In this thesis, gillnetting was only functional in spring and in this season, gillnetting showed a difference in CPUE, which was around 2.1 times higher in protected sites compared to the unprotected sites.

The same ratio (around 2.0) between protected and unprotected sites in spring was revealed by e-DNA concentrations. Also similar to gillnets, e-DNA concentrations were peaking in spring, where the concentrations were around 5 times higher compared to the remaining seasons (summer and autumn). The peak in spring is likely the result of pike spawning activity itself. It is well known that e-DNA concentrations are extremely high during the spawning period as a result of the release of eggs and sperm into the water column (Ogonowski et al., 2022; Tsuji & Shibata, 2021). Peaks of e-DNA observed in this thesis were measured between 10 to 13 °C water temperature, which is in line with Nilsson (2006). In the other seasons, the ratio between the sites revealed by e-DNA were weaker compared to spring (ratio in summer: 0.5, ratio in autumn: 1.25).

A large issue within this thesis regarding e-DNA is the spatial connection between the respective sites in the areas Grabow and Ummanz. In those two areas, the sites are bordering each other and are not clearly separated water bodies. It is therefore possible, that e-DNA concentrations in the respective sites are more similar due to a mixing of the water, making a clear distinction between the sites more difficult and diluted. In oceanic environments, currents can transport e-DNA as far as 600 km in a week (Thomsen et al., 2012). For coastal bay like areas, Yamamoto et al. (2016) suggests that e-DNA concentrations reflect fish biomass best within a 150 m radius and for lakes it is suggested, that detection is limited to 30 to 50 m (Dunker et al., 2016). The extend of spatial distribution of e-DNA from its source to sampling point is therefore

highly variable and potentially non-linear and many studies that looked at the spatial dispersion of e-DNA as a result of flow and currents in combination with other factors affecting e-DNA concentration (e.g. degradation) reported complex, system-specific responses, that need further investigations (Harrison et al., 2019; Hinlo et al., 2018; Laporte et al., 2020, Rourke et al., 2022). In this study, we sampled e-DNA on the leeward and windward shores of the respective sites, but there was no evidence that e-DNA accumulated here. No measurements of currents or flow regimes in the areas were performed during this thesis and thus, no statements regarding the influence of currents, causing a potential mixing, can be made.

In addition, e-DNA distributions in the water are often heterogeneous and patchy (Eichmiller et al., 2014; Jane et al., 2015; Takahara et al., 2012, Wilcox et al., 2016). Capturing this spatial pattern and to derive reliable estimates about fish abundance will require appropriate spatial coverage, high amounts of filtered water and sufficient number of samples (Furlan et al., 2016; Moyer et al., 2016). While having a similar amount of filtered water per sample as in the present thesis, Ogonowski et al. (2022) covered a much larger area with subsamples and had a larger volume of water, that was later pooled and sampled upon. This might be the better approach in order to capture this patchy distributions of e-DNA concentrations, but the authors sampled only during spawning season and the efficiency of other e-DNA protocols for pike outside spawning season remains to be clarified.

Under controlled conditions, the correlation between e-DNA concentrations and known abundances of pike has been found to be very strong (Karlsson et al., 2022). The controlled experiment performed during the present study supported that and also showed that the technical aspects of the e-DNA protocol such as the filtering process and PCR-analysis are able to capture pike-DNA. However, those aforementioned strong correlations derived from controlled experiments are often not as clear under natural conditions, where a wide range of biotic (e.g. fish distribution and density) and abiotic factors (e.g. currents, salinity) can influence the e-DNA concentrations in the water (Rourke et al., 2022).

Therefore, it remains challenging to derive reliable relative abundance estimates of pike with e-DNA protocols in natural environments. Also spring season, where high levels of e-DNA concentrations are recorded, remains an issue as concentrations are peaking due to the release of gametes into the water. This most certainly masks

concentrations that might possibly reflect actual abundances (Rourke et al., 2022; Tsuji & Shibata, 2021) and baseline levels of e-DNA concentrations need to be derived from appropriate sampling outside from spawning season (Rourke et al., 2022). Given the issue of close proximity of sites in two areas investigated within this thesis and potential mixing of water, evening out concentrations, it is difficult to derive fine scaled spatial differences in e-DNA concentrations. This limits the applicability of e-DNA for assessments as performed within this thesis, but e-DNA might still be a useful tool for identifying potential spawning areas and times for pike (Tsuji & Shibata, 2021).

In contrast to gillnetting and e-DNA, angling showed strong differences between the protected and unprotected site consistently over all seasons and the magnitude of differences were most pronounced in angling (ratio in spring: 2.9, ratio in summer: 3.6, ratio in autumn: 5.3; mean ratio: 3.9). Many studies suggest angling has the potential to track abundances of pike quite well (Arlinghaus et al., 2016; Bergström et al., 2022; Eklöf et al., 2023; Niemi et al., 2023; Tibblin et al., 2023).

Yet, abundances might be underestimated with lure angling in fished areas (=OA's) due to lure avoidance (timidity) as a consequence of fishing effort or past fisheries selection of lowly vulnerable genotypes or by learning (Arlinghaus et al., 2017; Beukema, 1970; Heino et al., 2015; Kuparinen et al., 2007; Lucas et al., 2023). The key question that arises then is to what extent differences between the respective sites are explained by actual abundance differences or by timidity. The majority of studies investigating pike abundances and pike catch rates in protected and non-protected areas concluded, similar to the results of this thesis, that the catch rate in areas with some degree of protection against fishing pressure are much higher compared to fished areas, ranging from 2.5 times to six times higher catch rates in the protected zones, depending on the protection status (Bergström et al., 2007; Bergström et al., 2022; Börjessen et al., 2022; Edgren, 2005; Eklöf et al., 2023).

This is in line with the findings along the gradient of protection in the three areas investigated within this thesis, where the magnitude of catch rates was corresponding well with the degree of protection. Fishing (including angling) can reduce the abundance of fish by harvesting individuals from the stock, leading to size and age truncated stocks, abundance decline, and selection of certain traits that lower vulnerability to fishing (Lewin et al., 2006; Lewin et al., 2019). More generally,

however, and the absence of fishing effort is typically leading to increased densities fish in areas protected by fishing (Côté et al., 2001; Garcia-Rubies et al., 2013; Guidetti et al., 2014).

Angling catch rates should then be higher in protected areas and hence be a suitable tool to track pike abundance. Yet, angling-induced timidity is difficult to rule out. Increased hook shyness will accentuate the differences in catch rates in protected and unprotected areas, which can lead to extremely biased inferences about protection effects on fish abundance as shown in past studies. For example, while catch rates by angling were found to differ in protected and unprotected sites in a coastal fish in Spain, camera-based assessment showed that the studied species showed no abundance differences, but simply an alteration of vulnerability to ingest angling bait (Alós et al., 2013; Alós et al., 2015).

There is very strong evidence that passive gears (including angling and gillnetting) are strongly selective towards certain traits of fish. A clear pattern, supported by various studies, is that those gears are capturing more bold or aggressive, more active and faster growing individuals from the overall population (Biro & Post, 2008; Klefoth et al., 2017; Philipp et al., 2009). If the fish with a higher vulnerability are then removed in a harvest orientated fishery, those behavioural phenotypes can be significantly reduced, altering the overall variation in behaviour and associated vulnerability of the entire population over time (Mittelbach et al., 2014; Olsen et al., 2012).

This mechanism is often described as fisheries-induced evolution, where less active and less aggressive fish are favoured (Alós et al., 2019; Arlinghaus et al., 2016; Hilborn & Walters, 1992). But even without harvest (e.g. catch-and-release angling), individual fish within a generation might show reduced catchability as a result of learning to avoid angling baits (Beukema, 1970; Czapla et al., 2023). For pike, this selectivity pattern has also been observed, where the more active pike are more vulnerable to angling gear (Kobler et al., 2009; Monk et al., 2021). Pike are also known to become hook shy and learn from past captures, especially when they captured with lures (Beukema, 1970; Lucas et al., 2023). To derive the magnitude of influence of the aforementioned mechanisms on the comparison of relative abundance of pike between the investigated protected and unprotected areas investigated within this thesis, it might be a suitable approach to compare the catch rates of angling with the two other gears (gillnetting and e-DNA). On an annual average, angling showed a four times

higher catch rates in the protected sites, while gillnetting and e-DNA (in spring) showed only a two-fold higher catch rate in the protected sites compared to the unprotected sites. Then, the effect of timidity would be equally strong as the actual abundance difference between the protected and unprotected sites revealed by the two other gears. However, large uncertainties for gillnetting and e-DNA remain and limit their applicability to derive “true” abundance estimates such as the low catchability of gillnets outside the spring season or the potential mixing of waters between the sites in the case of e-DNA.

For the visual observations with the ROUV, which were only performed in spring, the lack of observed pike was probably due to the low visibility levels (0.6-1.3 m) in this season. Visual methods strongly depend on good visibility (Bozec et al., 2011; Figuroa-Pico et al., 2020). The water visibility was lowest in spring compared to summer and autumn and did not allow proper visual estimation of pike. Besides turbidity, flight reactions of pike away from the approaching ROUV might also be a possible explanation for the low detectability, but this cannot be derived from the ROUV observations in this study as no pike were observed fleeing the approaching ROUV. Raoult et al. (2020) found ROUVs to be an effective tool for fish assessment in marine environments as fish did show reduced flight reactions compared to divers. But in the case of the present thesis, turbidity probably was the issue in combination with only deploying the ROUV in one season, what results in too less effort.

For longlining, catching no pike was most probably a result of the low number of hooks that were deployed in the vast lagoon sampling sites, especially the short length of the longlines (only 30 m) and the limited number of hooks (5 hooks per longline). These limitations resulted in a rather low effort for this gear type compared to the size of the studied areas. In some commercial pike fisheries, including the lagoons, longlines are a regularly used gear. Typically, longlines in small-scale coastal fisheries can have lengths of several hundred meters (Løkkeborg et al., 2010), covering much more area than the longlines used within this study. Additionally, being a passive gear type, the catch rate in baited long lines depends on fish actively swimming towards the gear and ingesting the bait (Brandt, 1984). Given the fact that pike are a rather sedentary and low abundance species (Dhellemmes et al., 2023; Diana, 1980; Eklöv et al., 1997; Karaas & Lehtonen, 1993), the small spatial coverage of the used longlines within this thesis likely did not create enough encounters between pike and the longlines.

### Age, size, growth and condition

Within this thesis, we found that the age of the captured pike was significantly lower in the unprotected sites compared to the protected site. Age truncation is very typical for exploited fish stocks and the higher mean age of fish in the protected sites indicates a lower removal rate compared to the unprotected sites in this thesis (Barnett et al., 2017; Edgren, 2005; Lewin et al., 2006).

In this thesis, the difference in age was strongly associated with the gear. Angling in the unprotected sites revealed significantly younger fish compared to angling in the MPA's, while the age revealed by gillnetting did not differ. Interestingly, the mean size of fish was not different between the sites and between the gears. Growth rates explained this observation as pike in the MPA's were slower growing. But also, this effect was strongly driven by the gear type. The growth rate of pike captured by angling was higher in the OA's compared to pike captured by angling in the MPA, while there was no difference in growth rate of pike captured by gillnetting between the sites.

Both, angling and gillnetting are also selective towards more active fish and faster growing fish (Carlson et al., 2007; Edeline et al., 2007; Kobler et al., 2009; Lucas et al., 2023), but based on the results of this thesis, even faster growing pike were captured by angling, especially in the unprotected sites. While in gillnets, the encounter with the gear is mainly driven by movement patterns (in terms of swimming activity), a capture event in angling depends on more factors than simply encountering the gear and in angling, this is the willingness to ingest the bait (Lennox et al., 2017). The willingness to ingest the bait is a result of the internal state of an individual fish and this can be related to hunger, aggression level and metabolism rate. In muskellunge (*Esox masquinongy*) for example, Bieber et al. (2023) and Bieber et al. (2024) have shown that faster growing and more aggressive fish require more energy and therefore require a higher food intake, what results in increased angling vulnerability. This would explain why angling catches even faster growing fish compared to gillnetting as found within this study. But growth rate differences in this study were not solely driven by the gear type, but also by the site (unprotected and protected). Given the high likelihood that removals and angling efforts were consistently lower in the investigated MPA's in this study, the distribution of traits is not or less truncated in comparison to the fished sites, were either past efforts and removals favoured less active and less

aggressive fish, that are less vulnerable and more timid to angling or show hook avoidance though learning (Arlinghaus et al., 2016; Beukema et al., 1970; Diaz-Pauli et al., 2017).

Then, angling in the OA would capture especially the remaining, fast growing and vulnerable fish as the evolutionary favoured less active and less aggressive fish are underrepresented, while in the MPA's, also slower growing fish might be vulnerable to angling due to the lack of timidity, impacting the growth rates measured in the MPA's. The difference in growth rate of pike revealed by angling between the site might also be further influenced by density-dependent effects. Growth rates in fish are generally density dependent, where individuals grow faster at intermediate stock sizes compared to maximum carrying capacity (Lorenzen et al., 2002; Lorenzen et al., 2008; Walters & Post, 1993). Then, based on the evidence that pike are more abundant in the investigated MPA's, growth rates would be lower in the MPA's compared to the OA, also if pike would show the same vulnerability between the sites. This would also explain that pike captured by angling in the OA's were younger at similar sizes as they grow faster.

While there was no difference in the mean sizes of captured pike between the sites and the gears, the size distribution differed between angling and gillnetting. Angling captured higher frequencies of larger and also smaller pike compared to gillnetting. For gillnetting, the size of the captured fish is strongly dependent on the applied mesh sizes. Single mesh sizes usually show a dome-shaped size selectivity and with larger mesh sizes, usually larger fish are captured. (Hamley & Regier, 1973; Hubert et al., 2012). In this thesis, pike size also increased with increasing mesh size, but the number of captured pike decreased. This is in line with other studies, that applied multimesh gillnets for pikes in lakes (Pierce, 2012; Pierce et al., 1994).

In comparison to angling, gillnets failed to capture smaller pike (<600 mm), but also angling did not capture this size class very efficiently, but still more frequently. (Pierce et al. (1994) applied various mesh sizes ranging from 19 to 51 mm (knot-to-knot) in lakes and has shown, that smaller pike (<600 mm) are more efficiently captured with mesh sizes much smaller than 51 mm. The smallest mesh size applied within this thesis (50 mm knot-to-knot) was the largest mesh sizes applied by the authors. Given that, the gillnet configuration applied within this thesis with rather large mesh sizes most likely did not capture smaller pike very well.

Besides the strong size selectivity of gillnets in dependency of the mesh sizes, the underrepresentation of small pike in this study might also be related to size-dependent behaviour. Small individuals face a higher risk of predation (by conspecifics or other larger predators) and often seek refuge in highly structured habitats (Anders Nilsson, 2006; Chapman & Mackay, 1984; Eklöv & Persson, 1996) to avoid predation. Highly structured habitats (e.g. reed belts, dense macrophytes) are impossible to sample with gillnets (Goffaux et al., 2004; Zajicek & Wolter, 2018), creating a mismatch between the preferred habitats of smaller pike and the gillnet deployment locations, what results in low catches of small pike. Furthermore, the growth rate of pike in the Baltic lagoons is very fast, reaching around 400 mm after one year (Rittweg et al. 2023a). At the time, where gillnets show the highest catchability (in spring spawning time), age 1 pike of the past year class are already quite large, creating another mismatch. It remains to be investigated, if smaller mesh size configurations in combination with more gillnet effort (higher number of gillnets deployed) could improve the representation of smaller pike and lead to higher catch rates outside of spring.

Angling did capture smaller pike more efficiently compared to gillnetting. Predatory fish and the size of prey (or lure size) they can ingest is related to the length and gape of the individual and pike are known to ingest prey or lures half of their body size (Arlinghaus et al., 2008; Mittelbach & Persson, 1998). While gillnetting was not catching pike smaller than 600 mm efficiently due to the aforementioned mesh size limitations, pike smaller than 600 mm are very capable of ingesting lure sizes up to 200mm, if not even more (Arlinghaus et al., 2008). This likely explains the higher frequency of smaller pike captured by angling in contrast to gillnetting. Gillnetting captured also large pike, but in much lower numbers compared to gillnetting, what is very likely the result of rather low effort of gillnetting.

The condition of the pike captured within this thesis was not depending on the gear or the protection status, but on season. In spring, the condition was significantly increased. Other studies have found the condition of pike to be increased in this season as the result of the formation of gonads and associated weight increases, especially in female pike (Craig, 1995; Frost & Kipling, 1967; Raat, 1988). However, gillnets usually capture better conditioned fish (within one certain mesh size) in contrast to other gears but that depends strongly on the capture mechanism (Kipling, 1957). Pike can be captured in a gillnet in two ways: by wedging and entangling (Pierce et al.,



1994; Thomas; 2019). Wedging is, when the pike is held tight around the body. Entangling is, when the pike is getting stuck in the net with the teeth or the jawbone, what can result in a bimodal size-selectivity of gillnets (Hubert et al., 2012). In this thesis, it was not recorded how pike were captured in the gillnets (entangled or wedged) but entangling allows also large pike to be captured with comparably small mesh sizes and will also capture fish independently of the condition (Pierce, 2012; Pierce et al., 1994). In line with Hubert et al. (2012), in this thesis also large pike were captured with smaller mesh sizes as a result of entangling most likely.

### Limitations

A large uncertainty of this thesis is related to unknown fishing efforts in the investigated areas and associated protected and unprotected sites. Although efforts were monitored during the fieldwork in 2022, allowing only a limited view on harvest and efforts, no actual data could be obtained regarding commercial and recreational efforts and removals of pike in the past. Especially the lack of information of commercial activities in the protected sites in the past is critical as this might have altered abundance, behaviour and size structure of the pike. Another issue is the geographical connection between the sites, especially in the areas Ummanz and Grabow. Although pike are relatively sedentary, it cannot be excluded that pike are alternating between the geographical close sites, what might dilute the effects from the respective sites. The geographical closeness might have also severely impacted the e-DNA concentration due to a potential mixing of the water between the sites. Gillnets were only effectively capturing pike in spring, while catchability was very low in summer and autumn. This limits the explanatory power of this gear for annual statements of relative abundance in the respective sites. In addition, information regarding age, size and growth of pike were only obtained by angling and gillnetting. Both gears are selective towards more active and faster growing fish, while slower growing and less active pike are very likely underrepresented within in this thesis. Therefore, we likely overestimated the size structure and growth rate of the overall pike population in the studied system.

## Conclusion

To conclude, this study showed that the efficiency of various fishing gears (e-DNA, angling and gillnetting) to assess pike populations in the Baltic lagoons is strongly variable across different seasons. Based on the results presented in this thesis, angling was the only gear that was consistently applicable over all seasons, while gillnetting was severely impacted by low catchability of pike outside of spring. e-DNA is a potential useful tool for assessing spawning times and locations, but for this study design, where fine scaled geographical differentiation between MPA and OA in close proximity to each other were made, e-DNA is not applicable due to mixing of water between the sites.

However, on an overall scale, all gears showed differences in pike abundance between the MPA'S and OA's, although to different degrees. Angling showed the strongest differences and this differences is most likely the result of actual abundance differences, but also due to timidity of pike in the OA's. Quantifying the impact of timidity is a key factor to incorporate in angling based assessments and will require the application of a second gear, that is more immune to timidity and can reliably give an estimate about abundance. Gillnetting might have the potential in doing so, but adaptations and improvements, especially regarding effort, are needed to counter the low catchability of pike outside of spawning season. e-DNA might also be a suitable tool in quantifying timidity, but that would require adequate sampling of clearly separated water bodies, where no mixing of the waters is possible.

When deriving insights on size structure and growth rates of pike populations, one must be aware that different gear types are selecting for certain traits of fish within a population. Gillnetting and angling are very selective towards faster growing pike, where in angling, this selectivity is even stronger. Especially in unprotected sites, where either past removals have altered the distribution of "personalities" over time or pike are avoiding hooks by learning, or both, insights on growth rates and size structures of the population are biased, when derived from angling and gillnetting.

Although the bias in angling, this gear might be the best tool for estimating adult pike abundance in the studied lagoon system, but sampling over multiple days is required to cover day-to-day variability in catch rates. Also, sampling with angling should be conducted in spring and autumn as pike might show reduced angling catchability in

the spawning season. Gillnetting was not a good tool in those seasons, but in spring, gillnetting was very effective, even when sampling only once per area. Higher efforts with more gillnets over multiple days might counter the low catchability of pike in summer and autumn, but what effort is required in detail remains to be investigated. e-DNA assessments of relative abundance remain overall difficult as the relationship of abundance and measured e-DNA concentrations is mitigated by many factors such non-linear spatial distribution of DNA in the water, environmental parameters (e.g. salinity and temperature) and hydrological processes. Nevertheless, for monitoring spawning related activities of pike such as spawning time, locations and also the spawning stock, gillnetting and e-DNA in spring could be more useful compared to angling.

Pike remains to be a very difficult fish species to sample in general and all of the applied gears in this study were biased. Those biases influence the insights gained from the acquired samples by each of the respective gears. Researchers must be well aware of those biases and apply caution, when making statements on population-level metrics such as age and size structures or growth rates. Portt et al. (2006) stated, that the applied gear and sampling protocol must be precisely tailored to the research or management question. If the goal is to track the relative abundance of adult pike in the Baltic lagoons, angling can be recommended. But already when making statements about growth rates for example, angling data are severely biased as a result of the strong selectivity and will overestimate aspects such as growth rates on population level. If the research question is focused on small pike, angling and gillnetting are both inappropriate gears for the lagoons. Seining and explosives might be the better tools in sampling young of the year pike (Lappalainen & Urho, 2006), but for the latter, permissions for sampling are most likely impossible to acquire. A large issue that remains is how to deal with the fraction of pike, that are less vulnerable to passive gears as a result of timidity. Those fish are also part of the population but are most likely underrepresented in angling and gillnetting based assessments. Other gears such as beach seining for example might increase the capture rate of those fish, but this remains to be proven with future research, which also incorporates a comparison of traits (growth rate, age and size structure) with fish from other gear than gillnetting and angling.

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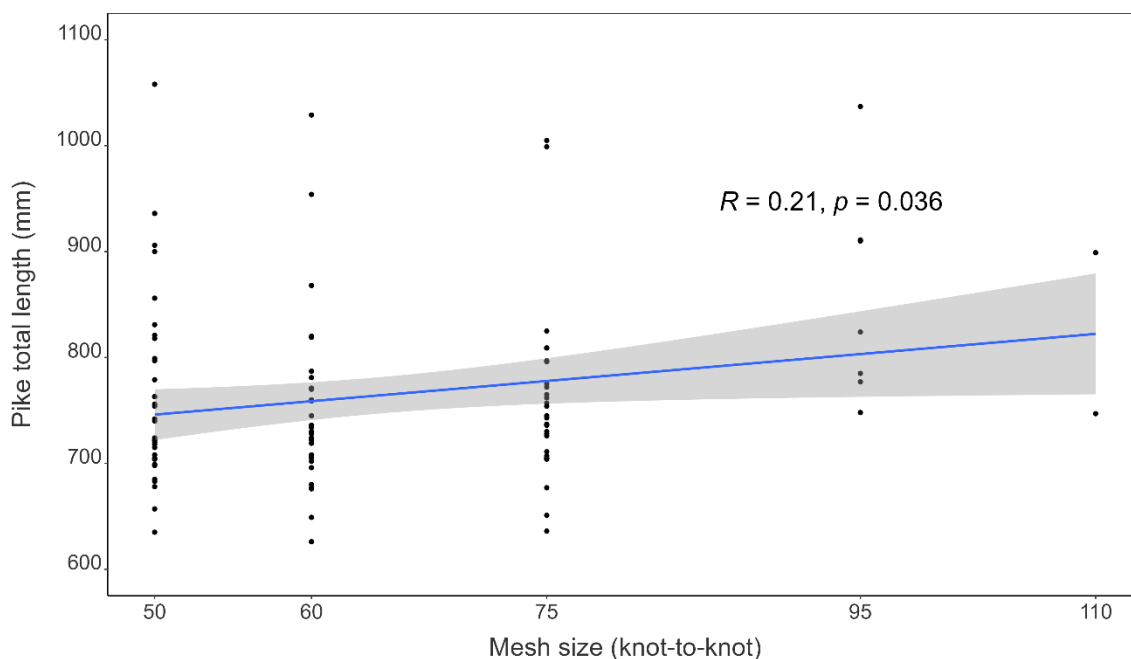
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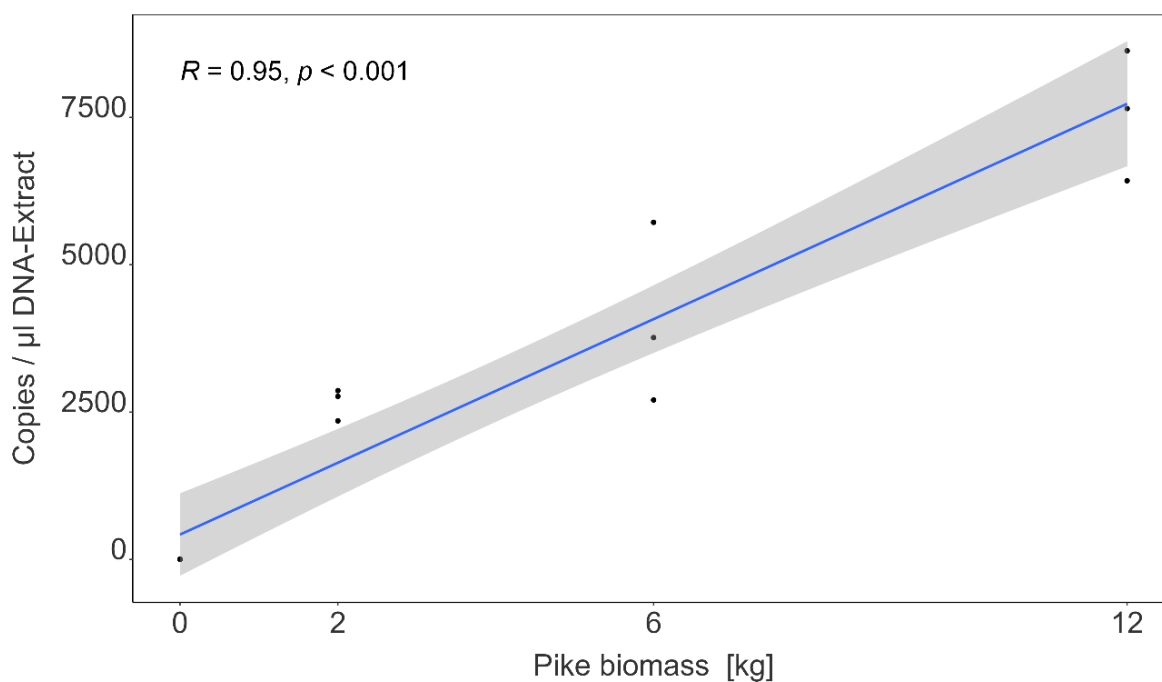
*“Das Boddenhechtprojekt hat mein Leben verändert“*

*-Jörg Schütt-*

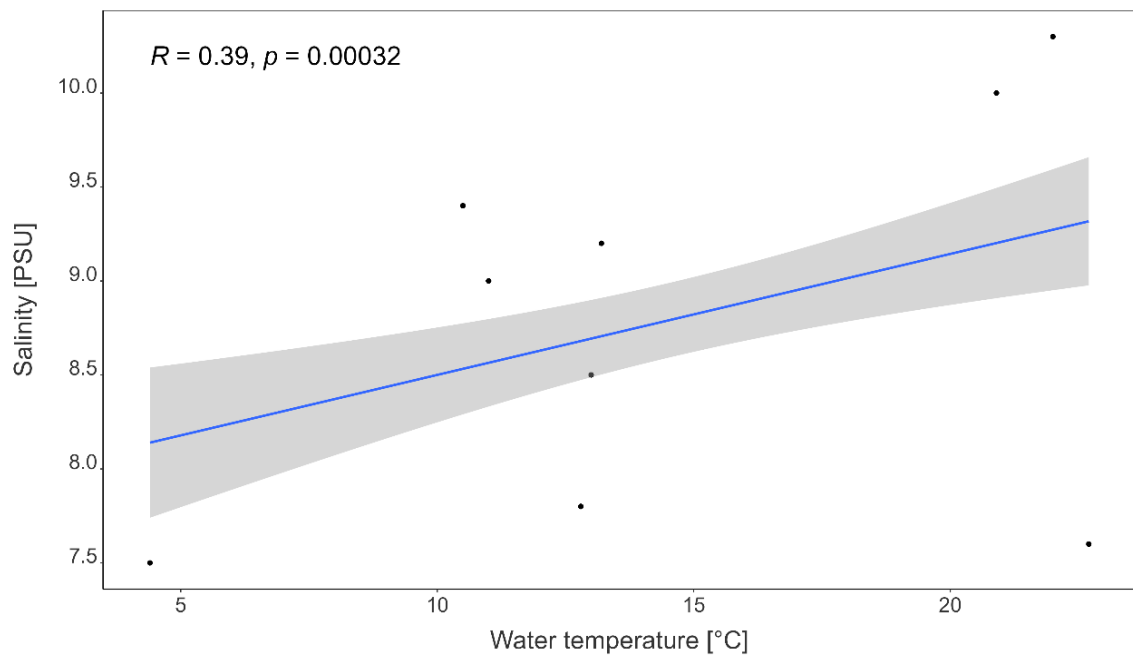
## Appendix



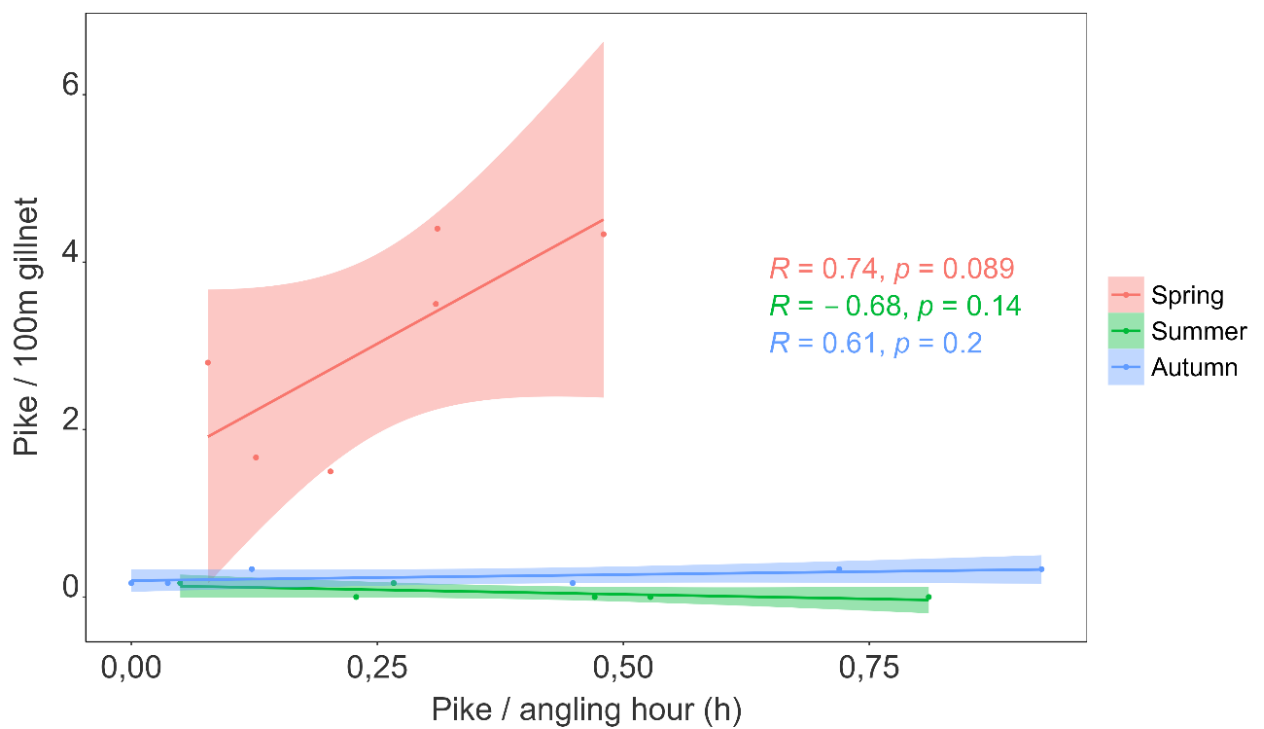
**Appendix figure 1: Correlation between pike length (in mm) and mesh size ((knot-to-knot) with pearson correlation coefficient and significance (R and p value).**



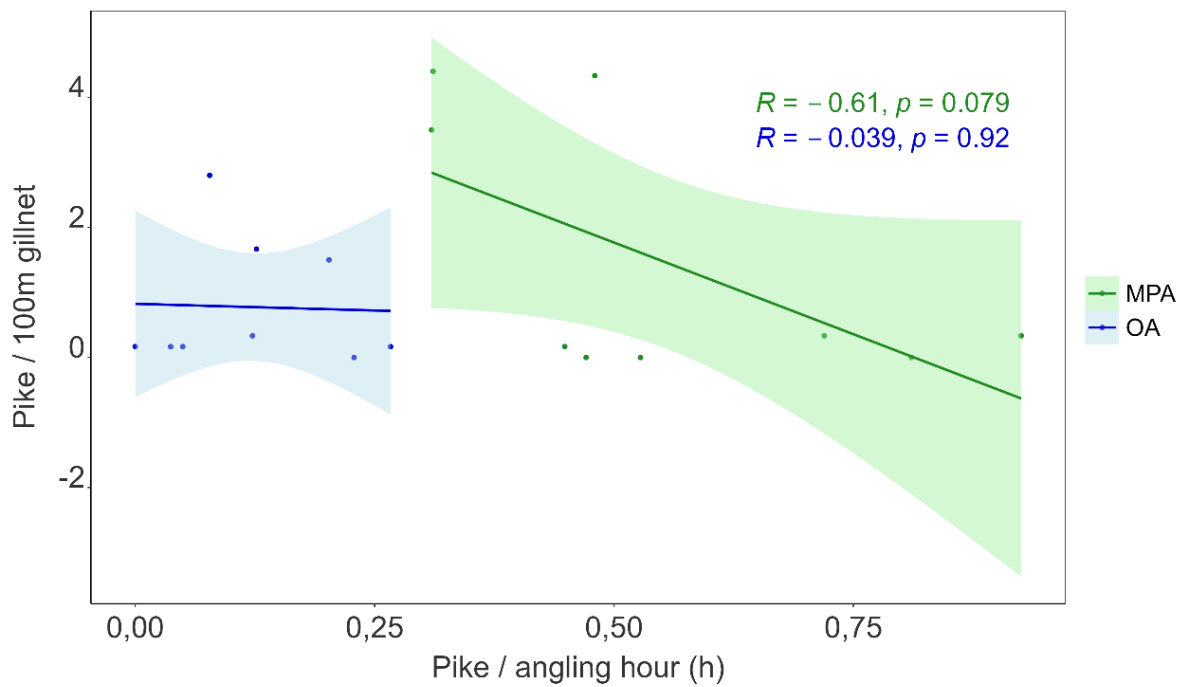
**Appendix figure 2: Correlation plot between e-DNA concentrations (copies per µl DNA-Extract) from pretest at lake Döllnsee and pike biomass with pearson correlation coefficient and significance (R and p value).**



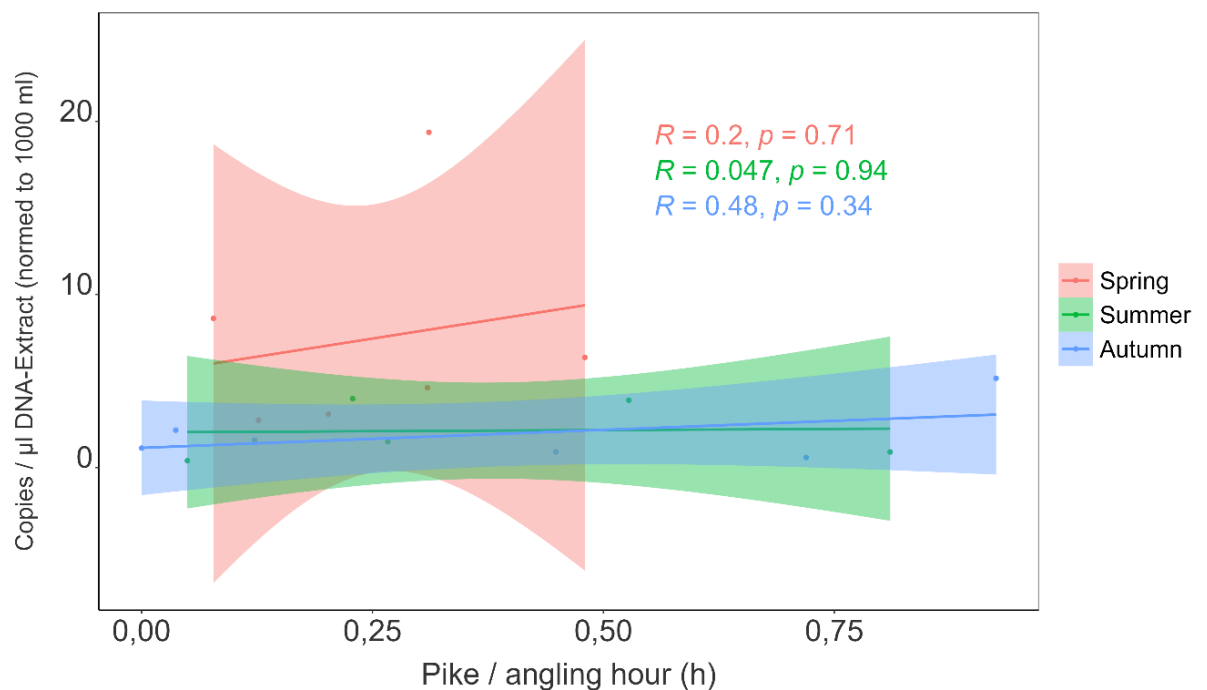
**Appendix figure 3: Correlation plot of salinity (PSU) and water temperature (°C) with pearson correlation coefficient and significance (R and p value).**



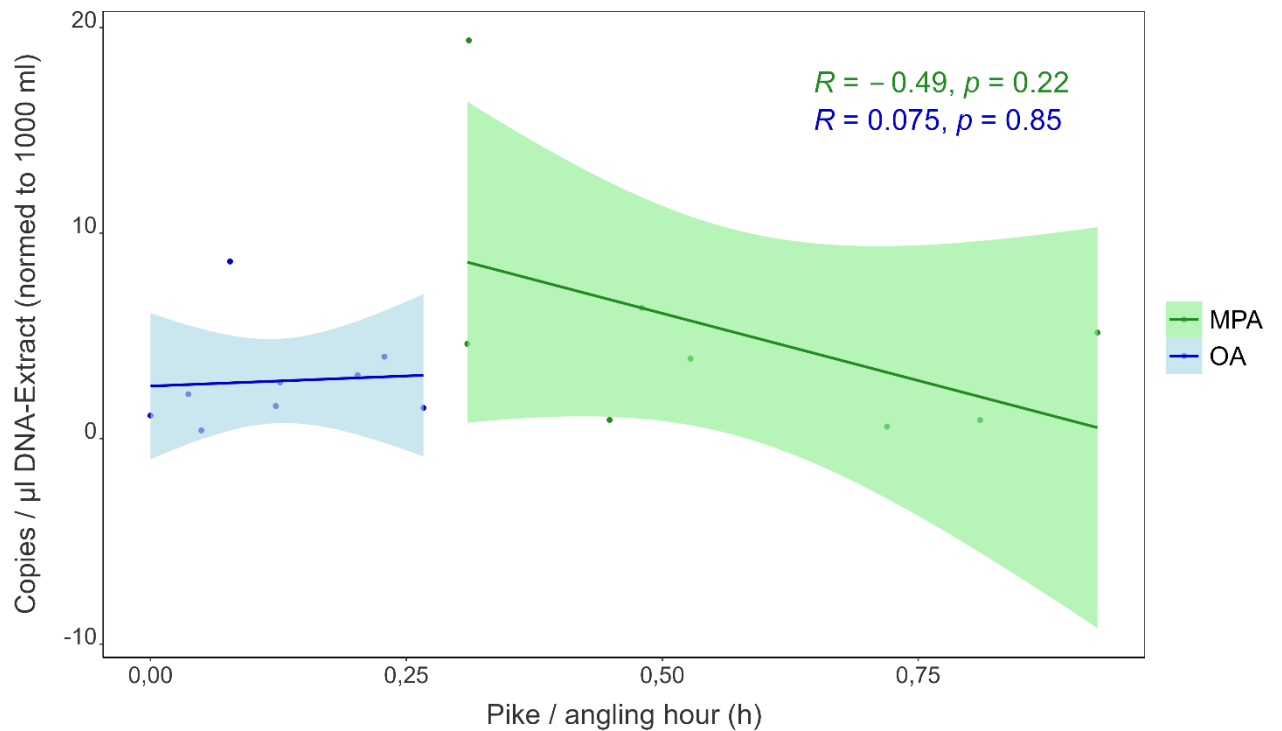
**Appendix figure 4: Seasonal correlation plot of gillnet CPUE (pike per 100m gillnet) and angling CPUE (pike per hour \* angler) with pearson correlation coefficient and significance (R and p value).**



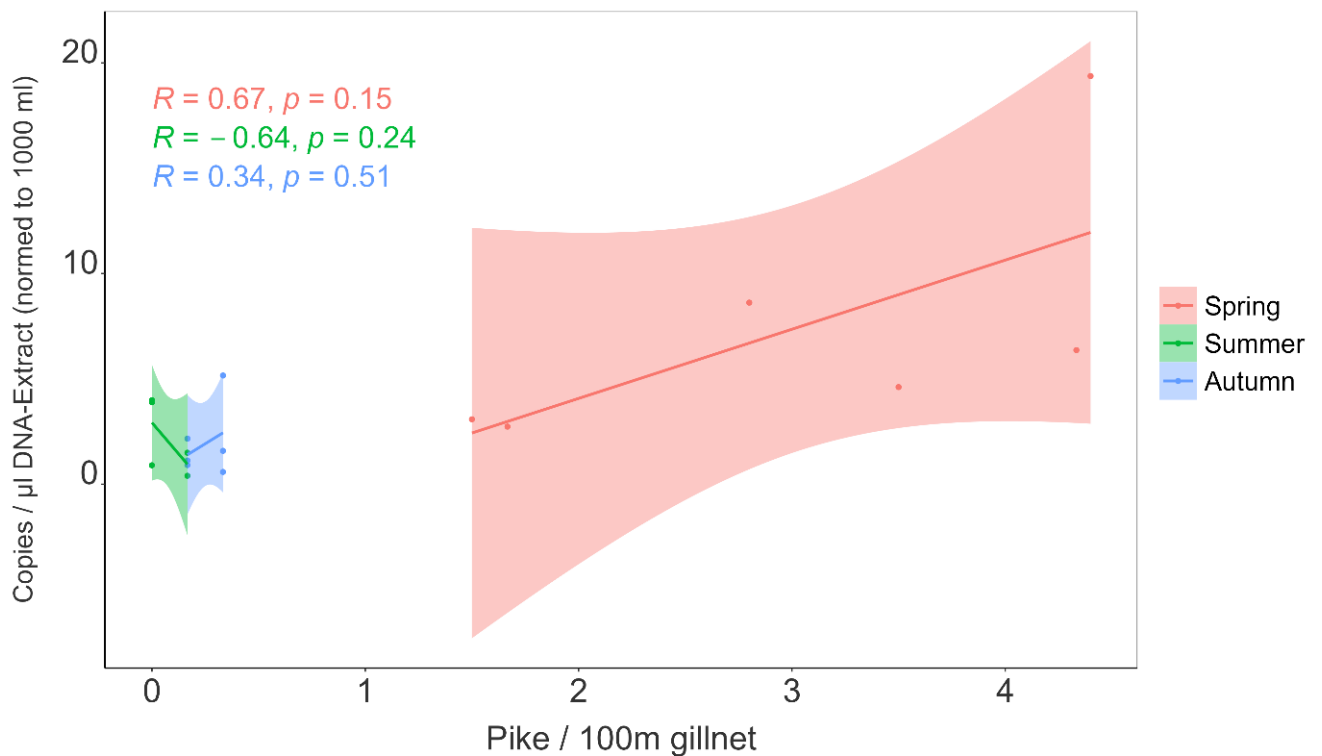
**Appendix figure 5: Site (MPA and OA) correlation plot of gillnet CPUE (pike per 100m gillnet) and angling CPUE (pike per hour \* angler) with pearson correlation coefficient and significance (R and p value).**



**Appendix figure 6: Seasonal correlation plot of e-DNA concentrations (copies per µl DNA-Extract) and angling CPUE (pike per hour \* angler) with pearson correlation coefficient and significance (R and p value).**

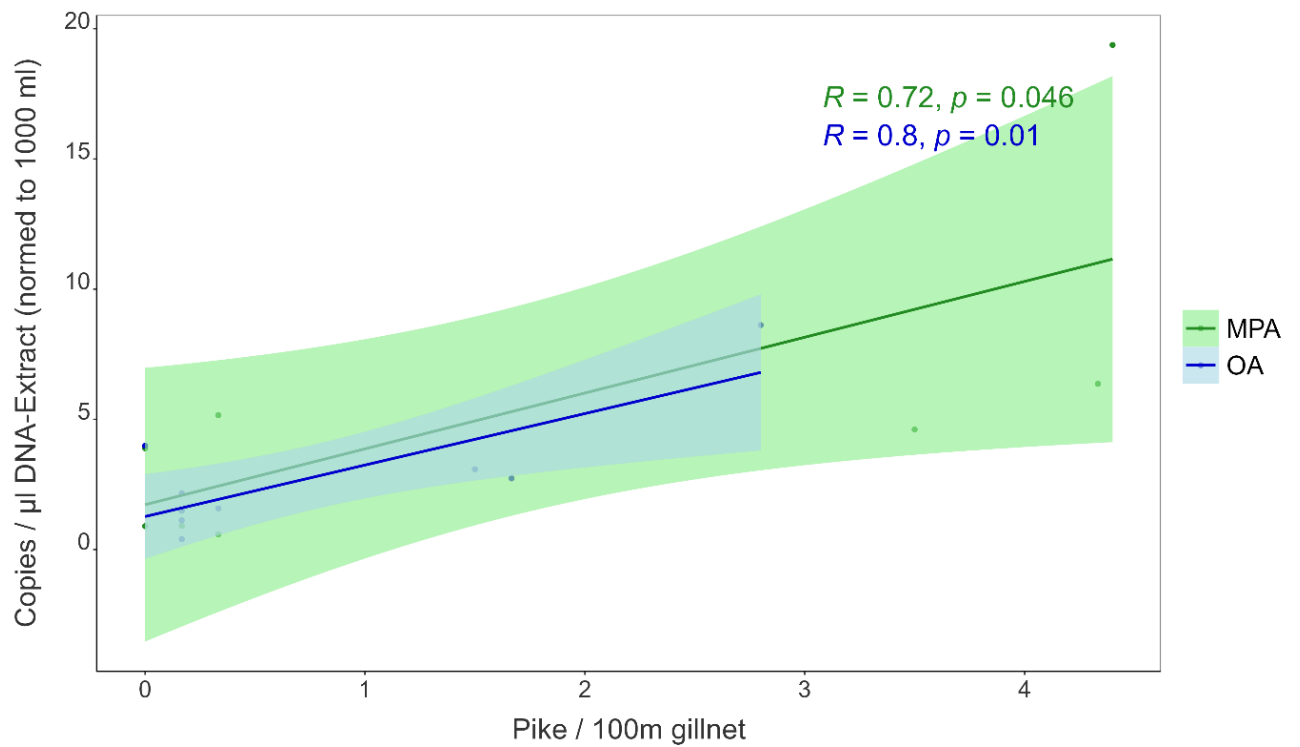


**Appendix figure 7: Site (MPA and OA) correlation plot of e-DNA concentrations (copies per  $\mu\text{l}$  DNA-Extract) and angling CPUE (pike per hour \* angler) with pearson correlation coefficient and significance (R and p value).**



**Appendix figure 8: Seasonal correlation plot of e-DNA concentrations (copies per  $\mu\text{l}$  DNA-Extract) and gillnetting CPUE (pike per 100m gillnet) with pearson correlation coefficient and significance (R and p value).**





**Appendix figure 9: Site (MPA and OA) correlation plot of e-DNA concentrations (copies per  $\mu$ l DNA-Extract) and gillnetting CPUE (pike per 100m gillnet) with pearson correlation coefficient and significance (R and p value).**

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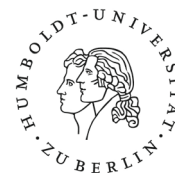
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