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EFFECTS OF TEMPERATURE AND SALINITY ON THE SURVIVAL AND PHYSIOLOGY OF BALTIC *Mytilus* sp. EARLY LIFE-STAGES

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Declaration of Authorship

Hereby, I declare full authorship of the Master thesis and no other than the declared references were used. This document has not been in any other examination procedure from any other institute other than GEOMAR Helmholtz Center for Ocean Research and Christian Albrechts Universtität zu Kiel. Additionally, the printed version is consistent to the version provided on a digital data carrier.

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ABSTRACT

Low salinity conditions of brackish and estuarine coastal systems represent a challenging habitat for marine organisms to survive and develop. Anthropogenic induced climate change is expected to change sea surface patterns, further increasing desalination and elevating temperatures. The Baltic Sea mussel populations are formed by hybrids of *Mytilus edulis* and *Mytilus trossulus*. To date, *Mytilus* populations inhabiting the eastern coast of the basin thrive at the lower limit of their salinity tolerance range. Early life-stages of marine invertebrates are found to be more sensitive to environmental change and extreme variations of abiotic factors. To understand how changes in salinity and temperature will act on Baltic *Mytilus* larvae, development of the full larval stage was monitored in a fully crossed lab experiment with three salinity levels (7, 9 and 11 psu) and two temperatures (12 and 15 °C). Mussels were collected from Ahrenshoop, Germany in May 2017. The combined treatment of 11 psu and 12°C represents the mean natural field conditions during larval season. Rates of survival, growth and settlement of larvae were strongly reduced at salinities of 9 and 7 psu. Higher temperatures alleviated the negative effects of low salinity. After a 67 day period, undeveloped D-stage larvae were still found present in adverse salinity conditions of 7 psu regardless experimental temperature. Physiology of the larvae was directly affected by desalination and temperature variation. Respiration rates increased with decreasing salinities of 9 and 7 psu. In addition, a +3°C temperature increase resulted in elevated respiration rates. The opposite pattern was observed for clearance rates. Lower levels of energy available for growth (scope for growth) of larvae reared under low salinity are a key finding in this study. Combining the adverse conditions of low salinity and temperature elevation of 3°C resulted in decreasing scope for growth with time. Predicted conditions for the Baltic Sea may represent a limiting factor for survival and development of *Mytilus* sp. early life-stages being bottleneck for survival redefining the distribution limits of mussel populations in this area.

Key-words

Baltic Sea, Climate change, Energy budgets, *Mytilus* sp., larval physiology.

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Jennifer Catherine Nascimento Schulze

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1 INTRODUCTION

1.1 Global climate change

Increased levels of fossil fuel burning and other anthropogenic activities during the last decades are leading to a rapid change in the global environment. Since pre-industrial times, the concentration of atmospheric greenhouse gases (GHG) has been elevated by 31% (IPCC, 2014). Carbon dioxide (CO_2) is likely to contribute to 78% of this increase.

Approximately 30% of the emitted CO_2 is absorbed by the ocean, leading to changes in the seawater content of total dissolved inorganic carbon (C_T). While the increase in seawater CO_2 reduces ocean pH levels and leads to acidification, changes in atmospheric CO_2 concentration leads to variations on atmospheric temperature (IPCC, 2014).

Predictions of future changes in environmental conditions caused by climate change are based on several GHG emission scenarios (IPCC SRES, 2000; IPCC, 2014). These scenarios rely on different possible outcomes of demographic and socio-economic development such as changes in the use of industrial technology. These different scenarios are included in climate change models, and allow estimating global temperature increase (Figure 1).

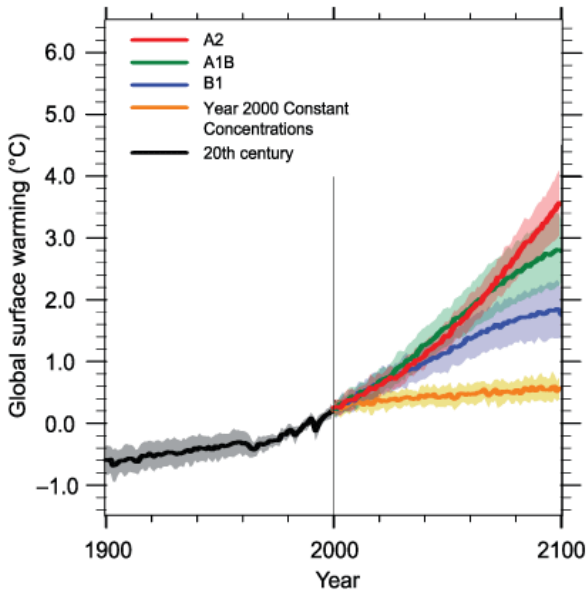

Figure 1: Multi-model global averages of surface warming relative to 1980–1999 (Solid lines) ± standard deviation (shaded area) for the scenarios A2, A1B and B1, as continuations of the 20th century simulations (blue line year 2000). Experiment representing scenario where concentration of GHG was maintained equal as the year 2000 is represented by the orange line (adapted from IPCC, 2007).

Climate change will have a strong impact on ocean sea surface temperature (SST) (Curry et al., 2003; Doney et al., 2012). A temperature increase of 0.6 – 2.0°C in the first 100 m depth layer is predicted by the year 2100 (IPCC, 2013). Besides, during the past 56 years ocean heat content has significantly increased in the upper 2000 m depth layers (Cheng et al., 2017). This trend is more pronounced from 1980's onwards.

Climate change will additionally impact ocean sea surface salinity (SSS) (Curry et al., 2003) by causing variations in the global hydrology cycle. The rise of surface air temperatures due to global warming can cause changes in evaporation and precipitation patterns. Increased net precipitation is expected at high latitudes results in the freshening of seawater (Curry et al., 2003, Boyer et al., 2005). At tropical latitudes, decreased net precipitation and high evaporation levels may result in the elevation of SSS. Predictions are region specific and extremely dependent on ocean circulation as well as on local environmental characteristics.

1.2 The Baltic Sea and regional impacts of climate change

The Baltic Sea is a semi-enclosed brackish water basin connected to the North Sea by the Great Belt and Sound straits in Denmark. The large riverine freshwater input from the surrounding landmasses creates the characteristic vertical and horizontal salinity gradient of the Baltic Sea (Mohrholz et al., 2015). In the north-east regions, average surface salinities are found as low as 4 practical salinity units (psu), slightly increasing at the central portion of the basin, where SSS ranges from 6-8 psu. Higher SSS values of 15-25 psu are present in the south-western Baltic Sea and at the connecting west straits water is 18-33 psu (Krauss et al., 2001; Meier et al., 2006). Although the overall gradient is relatively stable, sporadic strong inflow events from the North Sea can result in inter-annual and regional shifts in the salinity front. Projected changes for this area include higher levels of precipitation, shorter periods with ice cover and lower wind influence. As a result, diminished inflow of saline water from the North Atlantic and lower SSS are expected (Meier et al., 2006; Meier, 2012). A salinity decrease of 1.5-2 (psu) is predicted for the Baltic Sea towards the end of this century (Figure 2) (Meier et al., 2012; Gräwe et al., 2013). Annual SST of the basin is expected to increase between 2°C- 4°C by 2071 - 2100, with stronger effects in the southern areas during the summer months (Helcom, 2013; Meier et al., 2012).

Nevertheless, projected environmental changes for this region are dependent on the simulated outcomes of GHG emission models, global climate models and regional climate

models. Due to a high variability of all factors, uncertainty of predictions must be considered. Opposing outcomes of SSS variation are predicted, those including a slight increase in average salinity, as well as a lowering up to 45% of present values of the Baltic Sea (Meier et al., 2006; 2012).

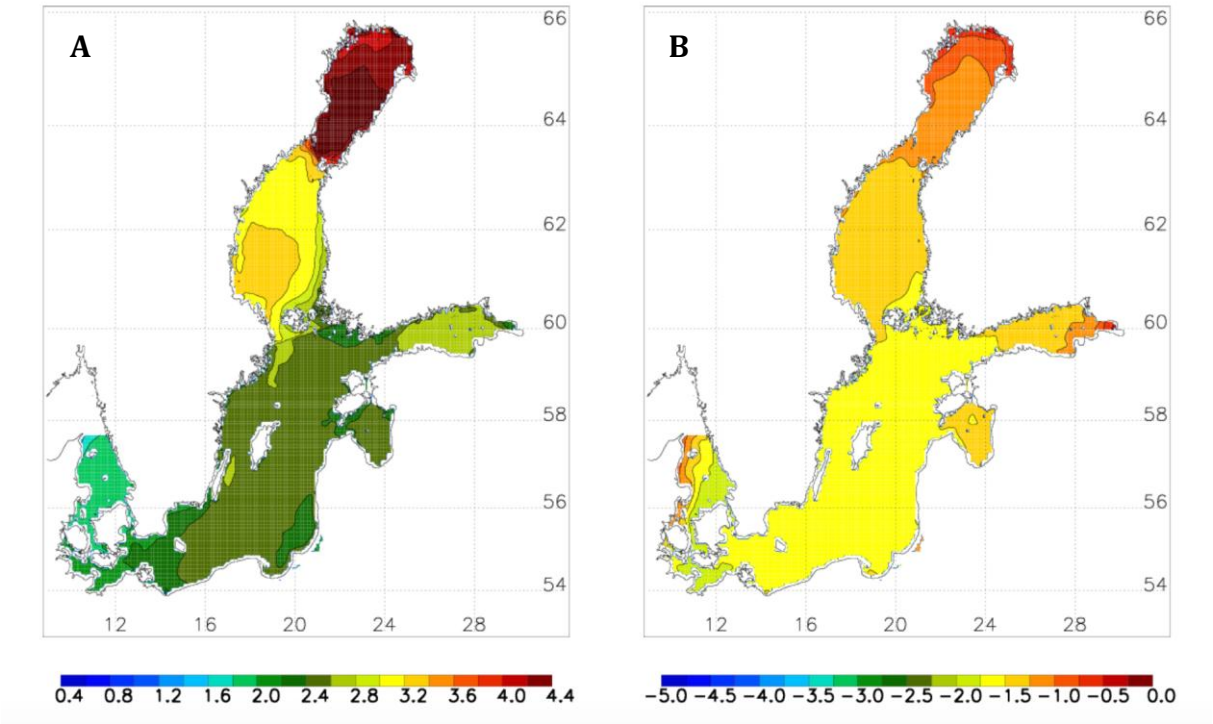

Figure 2: Changes in A) summer mean SST (°C) and B) annual mean SSS in the Baltic Sea (g kg^{-1}) between the years 2069-2098, calculated using the ensemble mean variation of regional climate models (adapted from Meier et al., 2012).

The connection of the Baltic Sea to the North Sea was formed approximately 7500 years ago (Donner et al., 1995). The Baltic is characterized as a young sea recently colonized by marine and fresh water species (Pereyra et al., 2009). Marine organisms inhabiting this region live under osmotic stress due to the brackish water conditions of the basin. Hypoosmotic tolerance is considered as a limiting factor of marine species range expansion (Dahl, 1956) and suggested as an active force leading to the low macrozoobenthos diversity in the Baltic Sea (Elmgren & Hill, 1997; Bonsdorff, 2006). The dispersal capacity, ecological role and phenotypic plasticity of marine organisms towards varying environmental conditions (e.g. abiotic factors) additionally define species distribution patterns.

In the Baltic Sea, the greatest proportion of marine organisms are found inhabiting the more saline waters at the western portion of the basin (Leppäkoski et al. 2002). Desalinization

of this basin as a consequence of climate change may lead to changes in species distribution, decreasing their habitat range (Gräwe et al. 2013).

1.3 *Mytilus* sp. (Linnaeus, 1758) mussels

Mussel species belonging to the genus *Mytilus* are found inhabiting the intertidal and subtidal zones of temperate to sub-polar climates (Figure 3) (Gaitán-Espitia et al., 2016). Mussels are considered a foundation species in the benthic communities of hard bottom environments. The ability to attach to hard substratum by the use of their byssus threads allows these organisms to form dense aggregations or mussel reefs. Common names for these organisms are blue mussel or common mussel (*M. edulis* and *M. galloprovincialis*, Lamark, 1819), and Pacific blue mussel (*M. trossulus*, Gould, 1850). In the North Sea, mussel reefs provide increased habitat structure, allowing other epibionts to attach at the structured shell surface, also providing refuges against predation (Buschbaum et al., 2008). In the Baltic Sea, these organisms represent approximately 90% of the benthic animal biomass (Schiewer, 2008). The ability to filter-feed up to 5 l g dw^{-1} h^{-1} allows the blue mussel to play a major role in nutrient recirculation (Kautsky & Evans, 1987). In addition to their ecological value, this edible bivalve is considered an economically important organism to aquaculture in Europe. The total production of *M. edulis* and *M. galloprovincialis* mussels by means of farming in 2014 was of 475.500 tonnes and worth EUR 438 million (EUMOFA, 2016).

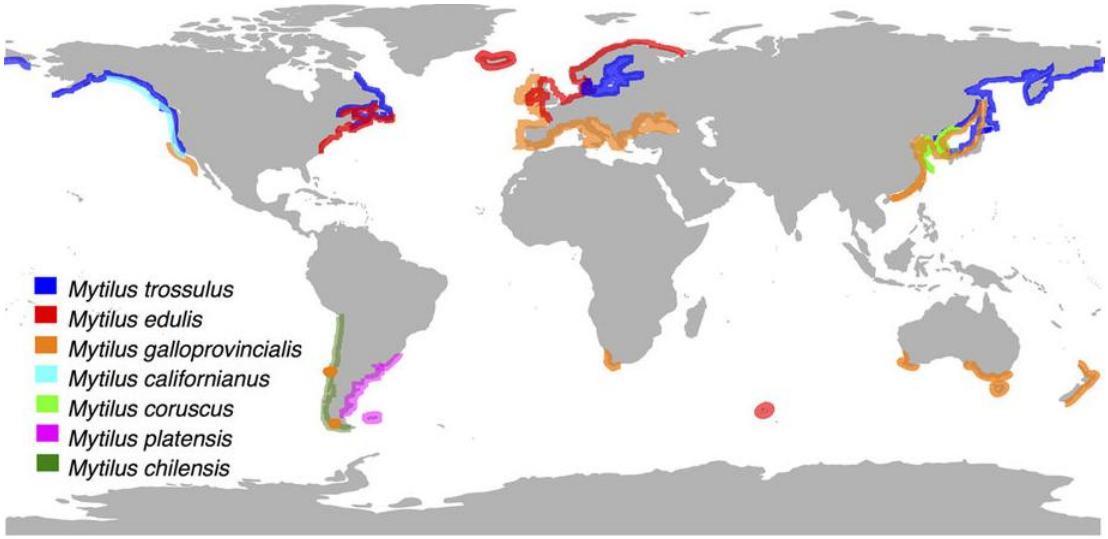

Figure 3: The distribution of genus *Mytilus* in North and South hemispheres. Baltic Sea mussel population (dark blue line) is composed by hybrids of *M. edulis* and *M. trossulus* species (adapted from Gaitán-Espitia et al., 2016).

1.3.1 Mytilus edulis x Mytilus trossulus hybrid zone in the Baltic Sea

In the northern hemisphere, the *Mytilus* species complex is composed of three different taxa, *Mytilus edulis*, *Mytilus galloprovincialis* and *Mytilus trossulus* (Väinölä & Hvilsom, 1991; Väinölä & Strelkov, 2011). Hybrid zones formed by this complex are amongst the most studied in the marine environment (Riginos & Cunningham, 2004).

The currently accepted speciation process hypothesis is that the Pacific *M. trossulus* spread across the North Atlantic Ocean, approximately 3.5 million years ago, after the formation of the Bering Strait (Riginos & Cunningham, 2004). This initial spread resulted in the allopatric speciation between Pacific *M. trossulus* and the North Atlantic *Mytilus* and most likely took place during the early Pliocene (Vermeij, 1991). A following vicariant speciation process led to the formation of *M. edulis* and *M. galloprovincialis* species. A second and more recent invasion of the North Atlantic during the Pleistocene established the Pacific *M. trossulus* populations forming the present hybrid zones (Riginos & Cunningham, 2004). This hypothesis is supported by genetic data from several studies (Varvio et al., 1988; Koehn, 1991; McDonald et al., 1991; Martinez-Lage et al., 2002). Whether the West Atlantic population spread into the East Atlantic or the contrary happened is still unclear (Väinölä & Strelkov, 2011).

To date, the Baltic Sea *Mytilus* population is defined as a hybrid swarm of *M. edulis* and *M. trossulus* (Stuckas et al. 2009). Regional distribution pattern of hybrids at this region is

suggested to be dependent on the salinity gradient of the basin (Figure 4). Mussels characterized as *M. edulis* are more frequently found in the west and more saline portion of the Baltic whereas *M. trossulus* is considered to inhabit the central and eastern less saline waters. This hypothesis was supported by studies describing physiological responses to low salinities; morphometric and allozyme characteristics of the two species (Koehn, 1991; McDonald et al., 1991; Väinölä & Hvilsom, 1991). However, recent findings suggest that no pure *M. trossulus* are found in the Baltic Sea (Stuckas et al., 2009; 2017) and female *M. trossulus*-like mtDNA was found to be substituted by the *M. edulis* mitochondrial genome (Rawson & Hilbish 1998; Quesada et al., 1999). For this reason, Baltic populations will be referred to as *M. edulis*-like and *M. trossulus*-like. Based on allele frequencies at the loci Glu-5`, EFbis, MAL-I and M7 lysine, a decline in *M. edulis* allele frequency from the Western entrance of the Baltic Sea towards Eastern regions is described (Figure 5), with no pure *M. edulis* specimens being identified further east than Ahrenshoop (AHP) on the German island of Zingst in the Southern Baltic Sea (Stuckas et al., 2017). At this specific site, proportion of *M. edulis*, *M. trossulus* and hybrids between the two species was described as 0.8 / 0 / 0.2, respectively.

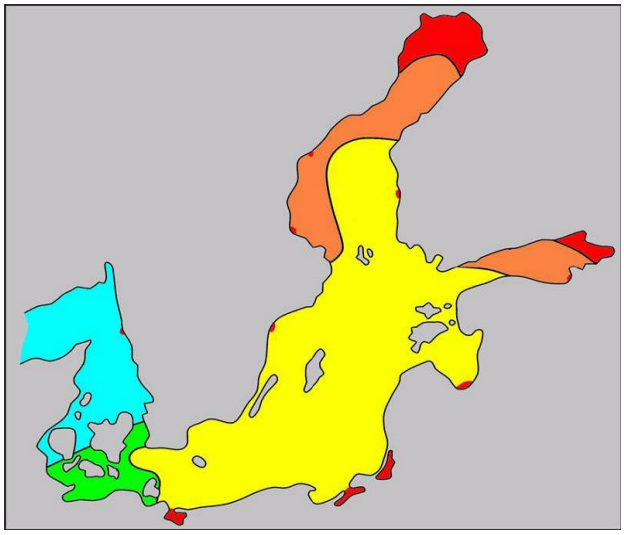

Figure 4: Salinity zones in the Baltic Sea. Salinities ranges are represented by different colors: blue (26-33 psu); green (8-26 psu); yellow (5-8 psu); orange (2-5 psu) and red (0-2 psu) (adapted from Aladin & Plotnikov, 2009).

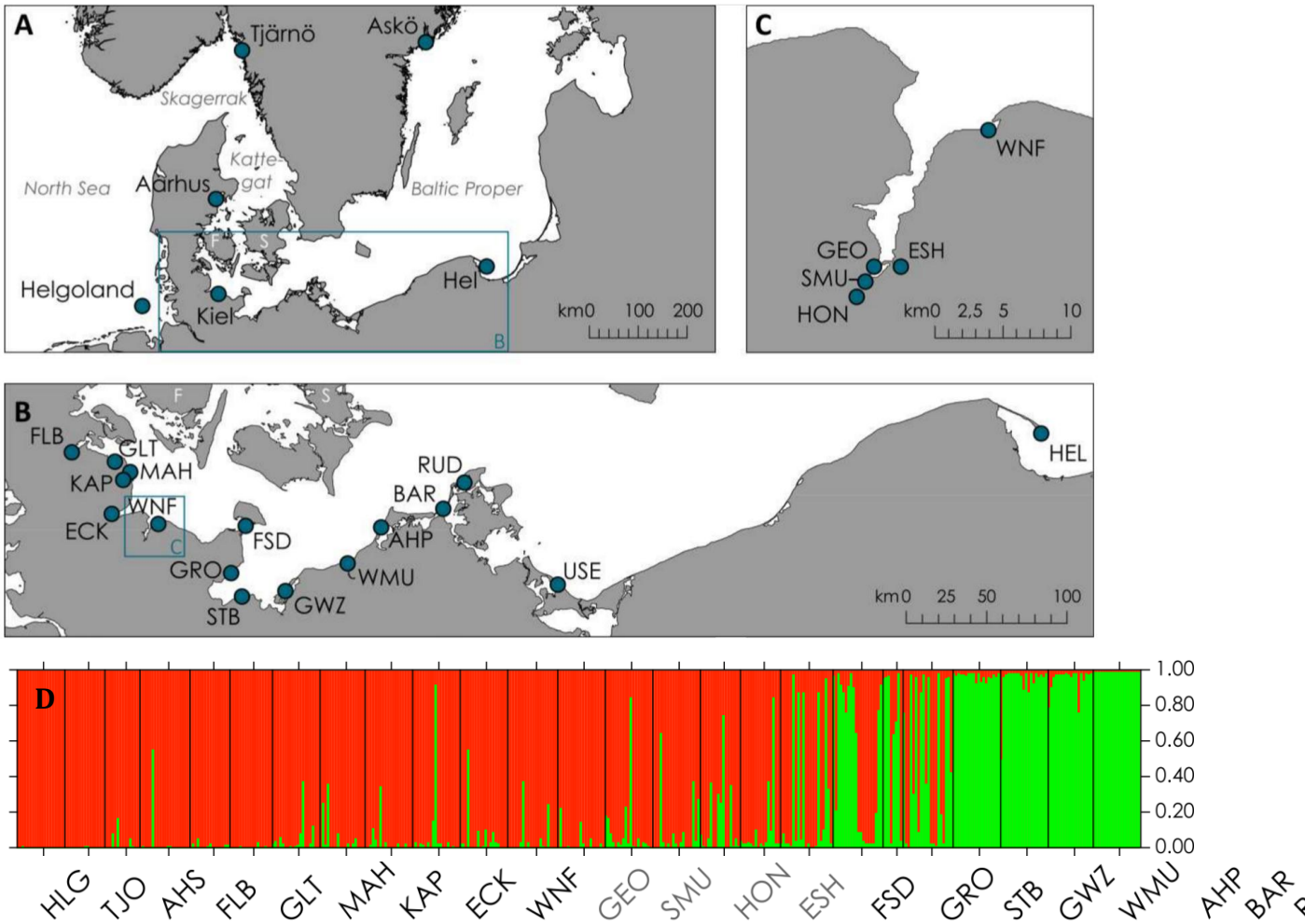

Figure 5: Sampling localities for genetic identification of *Mytilus* sp. at the Baltic Sea. Site label abbreviation A) North Sea population of Helgoland (HGL); B) Flensburg (FLB), Gelting (GLT), Maasholm (MAH), Kappeln (KAP), Eckernförde (ECK), Wendtorf (WNF), Fehmarnsund (FSD), Grömitz (GRO), Steinbeck (STB), Gollwitz (GWZ), Warnemünde (WMU), Ahrenshoop (AHP), Barhöft (BAR), Dranske (RUD), Usedom (USE), Hel (HEL); C) Closer view of Kiel Fjord sites including GEOMAR (GEO), Ship museum (SMU), Hörn (HON), East shore (ESH). D) Population structure using Bayesian inference based on 25 populations distributed along the Baltic Sea southern coast (STRUCTURE plot). Abbreviations represent sampling sites distributed from West (left) to East (right) portions of the basin. Genetically pure reference specimens of *M. trossulus* were obtained from Penn Cove (PCO, North America) and *M. edulis* from Helgoland (HGL, North Sea). Specimen allocation to one of the two clusters red (individuals allocated as *M. edulis*-like) and green (individuals allocated as *M. trossulus*-like), were hybrids are represented by two-colored bars. (adapted from Stuckas et al., 2017).

The highest proportion of admixture between the two species was described at Ahrenshoop (AHP) and its surroundings areas. Also, AHP animals are either classified as predominantly *M. edulis*- like or as predominantly *M. trossulus*-like (Fig. 3), illustrating that both major hybrid types have a high level of fitness under the specific conditions at this site. This geographical zone also represented the starting point were a strong shift towards a higher fraction of *M. trossulus*-like alleles was found following the southwest to southeast coastal zone (Stuckas et al., 2009; 2017). Väinölä & Strelkov (2011) suggest that no strong reproductive barriers in the Baltic Sea are active in maintaining a cohesive *M. trossulus* genome and therefore classify the Baltic *Mytilus* populations as a hybrid swarm. In the following chapters, the mussels inhabiting the *M. edulis* and *trossulus* complex in the Baltic Sea will be referred to as 'Baltic *Mytilus*'.

Shifts in allele frequencies from *M. edulis* to *M. trossulus*-like at different loci coinciding with the lowering salinity gradient from west to eastern waters of the Baltic (Theisen, 1978; Väinola & Hvilsom, 1991; Stuckas et al. 2009) suggest that salinity may act as a selective driving force in this hybrid zone.

Data related to the influence of salinity selection for specific *Mytilus* genotypes in early life-stages in the Baltic Sea is still scarce. Performance of larva and juvenile hybrids in different salinity gradients has been previously investigated in the Western Atlantic (Qiu et al. 2002; Matson et al., 2003) and North Atlantic (Beaumont et al., 2005). Hybrids of *M. trossulus* and *M. galloprovincialis* had higher survival success when reared in salinities of 20 psu than pure *M. galloprovincialis* larvae (Matson et al., 2003), suggesting that *M. trossulus* alleles increased tolerance to hypoosmotic stress. For *Mytilus* populations originally from the North and Baltic Sea, no evidence of changes in tolerance towards low salinity of larvae reared under 20 psu compared with 30 psu was found (Beaumont et al., 2005). Thus, hybrids of *M. edulis* x *M. trossulus* and *M. galloprovincialis* x *M. trossulus* did not differ significantly in survival and growth rates in comparison with non-hybrids. It is important to highlight that the authors of these studies stated that the number of *M. trossulus*-like successful spawners was low and that the experimental results were based on a low number and poor quality of gametes (Beaumont et al., 2005).

Understanding how salinity may impact survival and development of *M. edulis*-like and *M. trossulus*–like early life-stages at the Baltic Sea populations is crucial to comprehend the ongoing speciation processes and to predict future distribution patterns of this hybrid zone.

1.4 Abiotic factors: Impact of salinity and temperature on mussel physiology

Temperature and salinity are physical factors known to have a strong influence on physiology and spatial distribution of marine invertebrates (Gosling, 2008; Larsen et al., 2014). As other ectotherms, mussels are not able to maintain their body temperature constant. For these organisms, thermal regulation is dependent on external medium conditions. Increasing temperature elevates molecular vibration and directly modifies velocity of chemical reactions by elevations in kinetic energy (Willmer et al., 2005). Environmental temperature has a strong effect on ectotherms performance (e.g. growth, metabolism, and filtration) (Hochachka & Somero, 2002). Thus, physiological rates can be defined as a function of body temperature, based on thermal reaction norms (Figure 6). The thermal tolerance range (or thermal window) is defined by the range of temperatures supported by an organism, varying among species (Angllietta, 2009). Due to future global warming, marine organisms may have to face conditions at the limit of their thermal windows. This may lead to structural damage in enzymes and proteins and cause a reduction or termination of biochemical reactions. Understanding the basic mechanisms of physiological processes is necessary to clarify how organisms can cope with ecosystem changes.

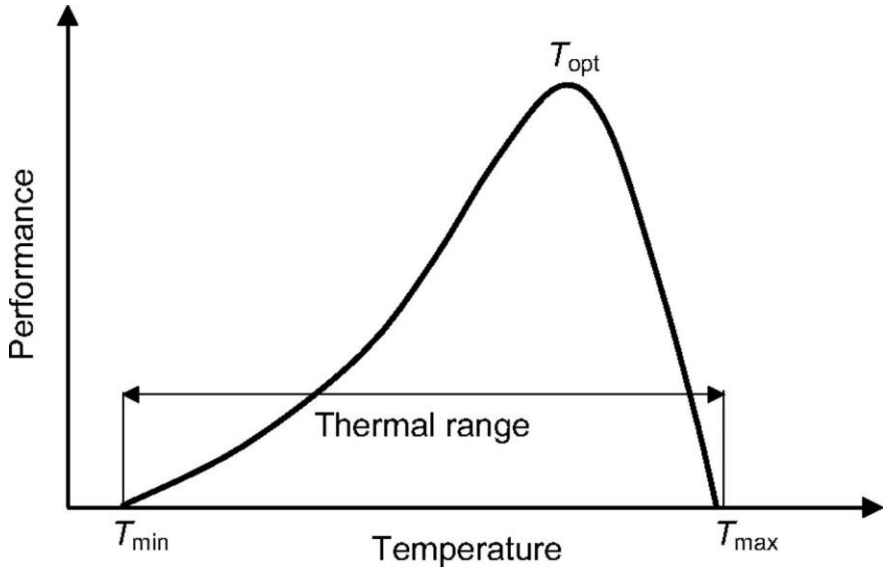

Figure 6: Thermal performance reaction norm of a measured parameter representing organism performance (e.g. growth, respiration rate) in relation to temperature (adapted from Angilletta, 2009). Here, Topt defines optimal temperature that enables performance to reach maximum output. T_{max} (Maximum temperatures) and T_{min} (minimum temperatures) denotes temperatures that enable individual's performance.

In contrast to the steady abiotic characteristics of the open ocean, high salinity fluctuations found in coastal areas and reduced average salinities of estuarine zones are a result of the strong influence of precipitation and river inflows. In these habitats, this abiotic factor plays a key role in limiting species distribution (Gosling, 2008). The capacity of aquatic organisms to regulate or conform intracellular osmolality to the external environment is essential to survive in varying salinity conditions. Reaching osmotic equilibrium prevents drastic changes in cellular volume and functioning of biochemical processes. Low salinity environments require the physiological ability of osmoconformers to lower their intracellular concentration of molecular organic osmolytes (i.e. free amino acids, methylamine compounds and trimethylamine oxide) and/or the regulation of intracellular ion concentrations (Yancey, 1988). Regulation of intracellular ion concentration requires the use of active membrane transport proteins such as Na^+/K^+-ATPases, Ca^{2+}-ATPases, and V-type ATPases and variation in production or excretion of organic osmolytes require allocation of metabolic energy (Willmer et al., 2005). Osmotic stress is considered a major physiological factor limiting marine species distribution in the Baltic Sea (Vuorinen et al., 2015).

Mytilus sp. are characterized as euryhaline organisms able to survive within a salinity range of 4.5 - 40 psu (Westerbom et al., 2002; Bayne, 1976). Like other osmoconformers, they are able to adjust intracellular osmolality following the external environmental gradient, remaining in a slightly hyperosmotic state compared to seawater (Willmer, 1997). Adjustment of cellular osmotic pressure is accomplished by reducing or increasing the intracellular concentration of organic osmolytes (Pierce & Greenberg, 1972). The osmolality of the extracellular fluid (haemolymph) varies with environmental salinity (Henry & Magnum, 1980). When facing rising salinity conditions, an increase in the intracellular content of organic osmolytes is observed (Koehn et al., 1980). The opposite pattern is a result of exposure to decreasing salinities. A decline of the intracellular content of organic osmolytes in gill tissue was found in *M. californianus* exposed to decreased salinity conditions (from 32 to 19 psu) during a 21-day period (Silva & Wright, 1994). Losses were mostly of the nitrogenous compounds taurine (30%) and betaine (40%), suggesting that these organisms rely mostly on adjustment of cellular organic osmolyte content rather than inorganic ions to remain iso-osmotic with the environment. It is suggested that adue to hypoosmotic stress, Baltic mussel populations show differences in morphology, physiology and growth when compared to North Sea populations (Kaustky et al., 1982). Reduction in growth rate and mean size of adults may partly be explained due to increased metabolic expenditure for osmoregulation (Kaustky et al., 1990; Tedengren & Kautsky, 1986, Riisgard et al., 2012).

Whereas biotic factors e.g. intraspecific competition for food and predation of larger individuals, are likely to also contribute to the smaller length of eastern Baltic populations (Westermbom et al., 2002; Larsen et al., 2014). A difficulty to produce calcified shells in salinities lower than 7 psu is also suggested to be a driving factor leading to observed mussel dwarfism towards the eastern areas (Riisgard et al., 2014).

Projected warming and desalination of the Baltic Sea will require marine organisms to adapt to conditions of physiological stress caused by the predicted changes in hypoosmotic conditions. Distribution pattern of Baltic *Mytilus* is dependent on velocity of adaptation to predicted environmental changes.

1.4.1 Effects of salinity and temperature on physiology of early life-stages

Mussels, like most marine invertebrates, undergo a planktotrophic larval phase during their development. They remain in this free-swimming stage for around 3 to 5 weeks, until reaching a mean size of ~300 µm in shell length (Thorson, 1950). After this phase, the larvae settle on suitable substrata and metamorphose (Thorson, 1961). Survival to these initial developmental stages is a decisive factor in population size and distribution, considered as a bottleneck for species abundance (Thorson, 1950). An estimated 99% larval mortality is caused by predation, dispersal to unsuitable environments and extreme physical factors (Bayne, 1976). Survival, development rate and metamorphosis success are dependent on the relationship between physiology and abiotic factors (e.g. temperature, salinity, pH and dissolved oxygen) (Sprung et al., 1984a, 1984b; 1984c; Wang & Widdows, 1991; Manoj-Nair & Appukuntan 2003, Rico-Villa et al., 2010).

Extreme variations in abiotic factors cause physiological stress. The degree of tolerance to the various physicochemical factors differs between species and ontogeny, with early life-stages being more vulnerable to changes (Bayne et al., 1976). In addition, interaction of abiotic factors may enhance or suppress their effect on organisms. A positive correlation between temperature and physiological performances of growth, respiration and food consumption rates in *M. edulis* larvae has been previously described (Sprung, 1984a; 1984b; 1984c). Moreover, lower growth rates were found when rearing *M. edulis* larvae at the limits of their osmotic tolerance range (20 psu and 40 psu) (Brenko & Calabrese, 1969). These results were intensified when combining the experimental salinities with extreme temperature conditions of 25°C and 10°C. Similar interaction effects of temperature and

salinity on larvae growth were also described for the larvae of the brackish water bivalve *Mytilopsis leucophaeta* (Conrad, 1831; Verween et al., 2007).

Baltic *Mytilus* populations already live below their salinity optimum. Predicted local changes in temperature and salinity will increase levels of physiological stress. Adaptation of early life-stages to non-optimal environmental conditions will enable this species to remain within their current geographic distribution range.

1.5 Hypothesis

The aim of this study was to investigate the combined effects of salinity and temperature on the survival and growth physiology of Baltic *Mytilus* larvae, considering realistic environmental conditions and future projections for this region. In addition, we aim to gather further information on how expected changes in abiotic factors may act as driving forces to selection favoring for a specific genotype of the two species of the Baltic Sea mussels, *M. edulis* and *M. trossulus*, that form the hybrid zone. For this, the following parameters were measured and monitored until settlement: survival, settlement success, growth and rates of respiration and clearance during a 67-day exposure to 3 different salinities (11 psu, 9 psu and 7 psu) and 2 different temperatures (12°C and 15°C). Settled spat were collected for species composition analysis and genotyping, performed using a previously developed specific diagnostic marker Glu-5` (Innoue et al., 1995).

In this study, the tested hypotheses are:

- Null Hypothesis: Desalination and temperature increase do not have and effect on survival and physiology of the *Mytilus* sp. larvae.
- Hypothesis 1: Hypoosmotic stress caused by long exposure to low salinity conditions (7 and 9 psu) will lead to reduced survival, growth and settlement rates of larvae compared to those reared in control salinities (11 psu).
- Hypothesis 2: A temperature increase (+3°C) will elevate respiration and clearance rates responses and accelerate growth and development of the larvae reared under 15°C treatments in comparison to those under 12°C.
- Hypothesis 3: Surviving and developing in diminished salinity conditions will require an extra expenditure of metabolic energy. Thus, lower levels of energy available for

growth (i.e. scope for growth) are expected for larvae reared in 7 and 9 psu in comparison to larvae grown in control salinity treatments of 11 psu.

- Hypothesis 4: A antagonistic effect of temperature and salinity is expected. Increased temperature will enhance survival, growth and energy budgets of the larvae while low salinities will lead to the opposite outcome.

2 MATERIAL AND METHODS

2.1 Animal collection and laboratory acclimation

Adult Baltic *Mytilus* (mean shell length 3 cm) were collected from Ahrenshoop, Germany (54°38.6'N, 12°42.7'O, Figure 7) on the 4th of April, 2016. During collection, seawater temperature was 11.3°C, pH 8.18 and salinity 12.4 psu. Water parameters were measured with the conductivity portable meter Cond 3110, WTW. A total of 80 mussels were collected from wooden poles at a water depth of ca. 1m and transported to the laboratory facilities of Helmholtz Center for Ocean Research GEOMAR, Kiel. During transport, animals were placed inside 20 l cool boxes containing seawater oxygenated with battery-operated aerators (Pulsator, Zebco).

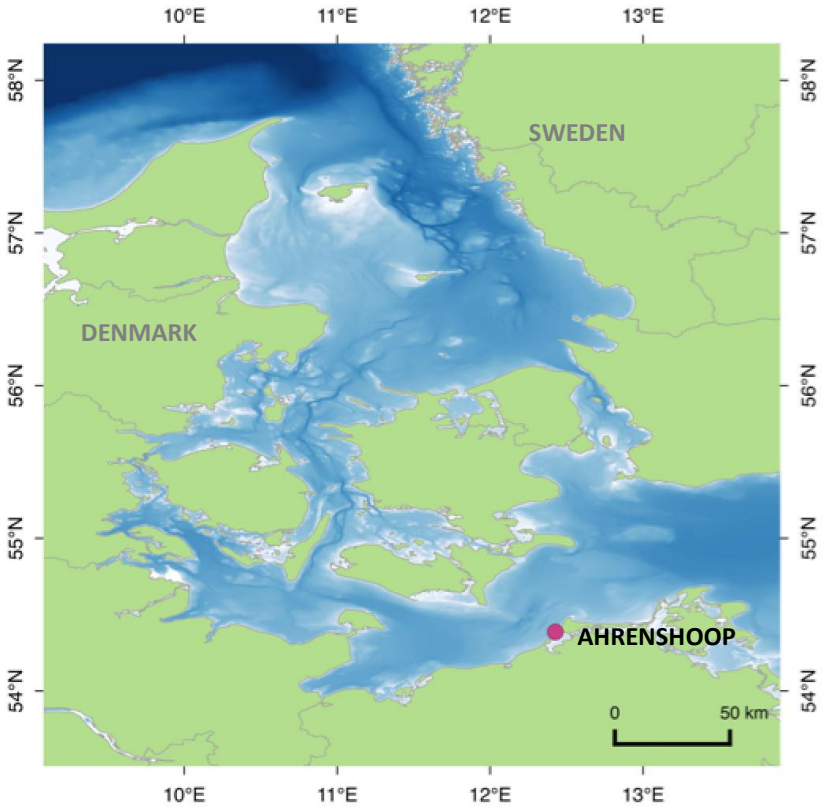

Figure 7: Survey map of adult *Mytilius* sp. sampling site. Red dot represents the sampling location in the Baltic sea (54°38.6'N, 12°42.7'O) of animals used in this study.

Upon arrival, the mussels were transferred to plastic aquaria filled with 20 l aerated 11 psu seawater. Between the years of 2000 and 2014, observed mean SST from Kiel bight of 11.79 ± 0.3°C at a 1.5 m depth was observed during the month of May (Metereological department of Geomar). Mean annual salinity from Ahrenshoop of 11.2 ± 0.6 psu has been

previously observed (Sanders, 2017; personal communication). Thus, for this experiment, control salinity and temperature treatment was defined as 11 psu and 12°C. Animals were kept for a 10-day acclimation period in a 10°C constant temperature room (see chapter 2.4, section Water exchange) with a 12:12 h light/dark period and daily fed with 15 ml *Rhodomonas salina* culture with a density of ~1.3 million cells/ml.

2.2 Experimental setup

A total of thirty 2 l Duran glass bottles containing the larvae were placed in steal water baths model Haake SWB25, Thermo Scientific. Water temperatures was set and maintained using a heated recirculator Thermo Electron DC10, Thermo Scientific (Figure 8, B). Each water bath contained 4 bottles. Larvae were exposed to a fully cross-experimental design of 2 different temperatures: 12°C (Control) and 15°C (a +3°C increase treatment); and 3 different salinities: 7 psu, 9 psu and 11 psu, resulting in 6 different treatments. For the 15°C treatments, a daily increase in temperature of 1.5°C was applied to the tanks during the first 48 hours of the experiment. To reach the lower salinity treatments, salinity was decreased to 9 psu by stepwise addition of deionized water during the first 24 hours being followed by a total water exchange lowering salinity to 7 psu. After the acclimation phase, temperature and salinity were kept constant throughout the experiment. Seawater was gently aerated using an air pump and plastic tubing connected to 10 mL pipettes. The larval experiment lasted for 11 weeks until the first settlement was observed.

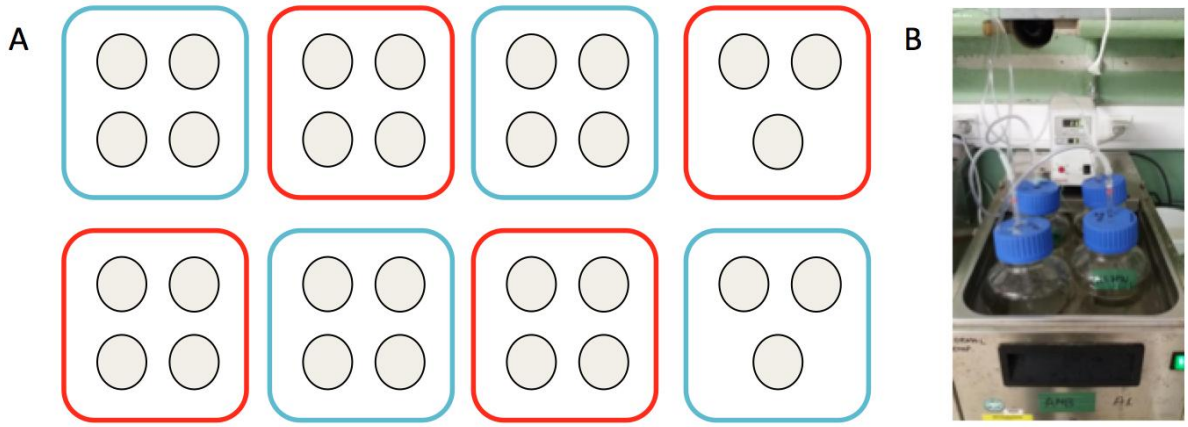

Figure 8: Experimental setup. A) Position of tanks inside the water baths. Red lines represent the water baths with the temperature of 15°C and blue squares represent water temperature at 12°C. Circles represent the 2 l glass bottles containing the *Mytilus* larvae. In total, thirty 2 l

glass bottles where used (6 treatments with 5 replicates each). B) Picture of the water baths containing four 2 l glass bottles used during the experiment.

2.3 Spawning and Fertilization

Adults were spawned by placing mussels individually inside 50 ml plastic beakers containing 11 psu seawater filtered with a 0.2 µm pore size filter, inside water baths. The water temperature was slowly elevated from 10°C to 15°C within ca. 20 minutes using aquarium heaters (Eheim 3619). Mussels started to spawn within ca. 3 hours. If spawning did not start within this period, water temperature was further increased to 20°C. Gender was identified visually under stereomicroscope (Leica model MDG41) and gametes were collected in individual beakers. After spawning was completed, adults were removed from beakers, flash frozen in liquid nitrogen and stored at -20°C for further genetic analysis. The eggs from each female mussel were added separately into a new beaker containing 80 ml of 11 psu filtered sea water (FSW). Eggs were gently homogenized with a plastic Pasteur pipette every 5 minutes to avoid damage during sinking and densities were determined under the microscope with 3 x 10 µl samples. Similarly, for each individual male sperm density of five sub samples was measured using the Spectophotometer nanodrop Nd-1000 and the NanoDrop software version 3.7.1 (peQLab Biotechnologie GmBH) using a precalculated concentration curve. Six females and 6 males that successfully spawned were used for fertilization, performed in individual crosses by mixing sperm solution from one sire and eggs from one dam in a ratio of 100:1, generating 6 individual families. After ca. 30 minutes, fertilization success of each family was determined under a microscope. Percentage of viable eggs (VE) was calculated by expression VE = (n^o of fertilized eggs presenting polar body development/ n^o of unfertilized eggs) x 100). For the experiment, all families were pooled. For this purpose, 4000 embryos from each of all 6 families that had reached a two-cell stage (or further stage) were transferred into 2 l experimental glass bottles filled with aerated FSW with an initial density in experimental units was 12 embryos / ml.

2.4 Maintenance

Water Exchange: Water exchanges were conducted 2 x weekly. FSW at each salinity was prepared 24 h before and left aerated overnight at 10^{o}C. Prior to the exchange, water

temperature was adjusted to experimental conditions of 12°C and 15°C with the aid of aquarium heaters. The experimental tanks were then emptied by gently passing water though a 65µm mesh filter to retain the larvae. The mesh was always kept submerged inside a 2L beaker which protected larvae from air exposure acting as a source of additional stress. Filters containing the larvae were gently rinsed back into the tanks with FSW and freshly prepared seawater was added.

Salinity preparation: To reach the salinities of 11 psu, 9 psu and 7 psu, seawater from Kiel Fjord (mean salinity of 16 psu) was filtered with a series of 50, 20 and 5µm filters and subsequently sterilized with a 0,2µm filter and UV light (Strahler UV-C Water sterilizer 500). Kiel Fjord water was then mixed with de-ionised H_2O (dH_2O) until the desired salinity was reached. Mixture with dH_2O causes an over proportional decline of seawater alkalinity which was thus adjusted to conditions encountered in Ahrenshoop (1858.9 µmol kg^{-1}) by addition of $NaHCO_3$ from a 1M stock solution (Sanders, 2017; personal communication).

Monitoring of abiotic conditions: Levels of seawater ammonium concentrations in the experimental tanks were weekly monitored using the JBL NH_4 aquaria kit. Concentrations were maintained below 0.05 mg/L throughout the experiment. Temperature and salinity in tanks and water baths were measured daily with a conductivity portable meter.

Feeding: Larvae were fed daily from the 3rd day post fertilization (DPF) onwards with live microalgae cultured at the GEOMAR institute facilities. Algae were cultured at conditions of 20°C and 24h light period with seawater enriched with f/2 medium (Guillard, 1975) in *Rhodomonas* sp. cultures and Walne's solution (Walne, 1966) in *Isochrisis galbana* cultures. Salinity of microalgae cultures was brought down stepwise until 9 psu and used in all experimental treatments. Until 16 DPF, *I. galbana* was added daily at a concentration of 15000 cells mL^{-1}. *Rhodomonas* sp. was then used as the main source of food, added daily at a density of 3000 cells ml^{-1}. The density of the microalgae culture was measured daily with flow cytometer (BD Accuri C6, Biosciences) using pre-defined gating set manually to the two specific algae species with the BD Accuri C6 plus software (Biosciences).

2.5 Growth and Survival

Larval survival and size were determined throughout the experiment. On every third experimental day, a 5 ml volume was sub-sampled using a 10 ml pipette, placed in a 20 ml glass well and concentration of living larvae was determined using a Stereo Microscope (Leica MDG41). Survival was expressed as percentage (n^o of surviving larvae ml^{-1} / initial number of larvae ml^{-1} x 100). Larvae were fixed with 10 µL of a 4% paraformaldehyde (PFA) solution buffered to pH 8.0 using 5M NaOH, which was added to the glass well. Larvae were concentrated in the center of the well and pictures were taken with a colour camera (Leica DFC310FX). Shell length (SL) was determined by the maximum length in µm from anterior to posterior distance, using ImageJ software 1.48v. Fifteen larvae from each replicate were measured from each sampling point. Shell growth rates were calculated as µm d^{-1}.

2.6 Settlement success

At 69 DPF, settlement of larvae was measured by counting the number of individuals that settled on the sides of the experimental tanks. For this, individual tanks were emptied, gently scraped along the sides and bottom with a sponge to remove the settled larvae from the walls and rinsed onto a 65µm mesh filter. This method was previously tested and demonstrated to not damage a significant number of spat. Settled larvae were counted under the microscope and afterwards placed back into experimental tanks. Results are expressed in settlement percentage ((n^o of settled larvae / initial no of larvae) X 100).

2.7 Larval Clearance Rate

Following each water exchange, larval clearance rate (CR) was determined. After measuring the density of the microalgae culture used for feeding, a known number of cells was added to all bottles containing larvae and 6 control tanks (one for each treatment) without larvae. After adding the microalgae, water was gently mixed and samples were collected from all tanks using a pipette and transferred into 500 µl Eppendorf tubes. Cell concentrations of the seawater were measured on two time points: the first 30 minutes after addition and gentle mixing of the tanks (t0) and after a ~15 hour period (t1). Cell concentrations of all samples were measured in 20 µl duplicates using a flow cytometer (BD Accuri C6, Biosciences) at

pre-defined gates specifically chosen for the microalgae species. The CR was calculated according to: log (t0 microalgae concentration – log (t1 microalgae concentration) / total number of larvae X volume (µl) X time). Final CR was defined as μl^{-1} $individual^{-1}$ $hour^{-1}$ (from Riisgard, 2001).

2.8 Respiration rate

Respiration rate (RR) was measured as oxygen consumption twice a week using a multichannel respirometer (SDR SensorDish Reader, Presens Precision Sensing, Regensburg, Germany). The system's setup consists of 24 custom made (Eydam, Kiel) gas tight glass vials containing an average volume of ~700 µl. Each of the vials contains a sensor spot of an oxygen sensitive luminescent dye located on the bottom side. The oxygen-sensitive indicator dye is excited using blue LED and the luminescent decay time is measured by the reader plate (OxoDish) positioned under each of the spots. Oxygen concentrations were calculated with the SDR Sensordish reader software version SDR_v4.0.0. A user-defined calibration using air-saturated water and deoxygenated water (prepared using a 1% sodium sulfite solution) as water references was performed prior to measurements, following the SDR SensorDish Instruction manual (Presens Precision Sensing, 2016). The measurements were performed at experimental temperatures of 12^{o}C and 15^{o}C setting up the vials inside a fridge (Bomann KSW34). Seawater was prepared in advance (see chapter 2.2. Experimental setup) and was additionally sterilized with a 0.2 µm mesh filter and kept under measurement conditions for ca. 30 minutes in order to adjust its temperature. To minimize the effects of feeding and digestion on larval metabolic rates, animals used for respiration measurements were always sampled from the experimental units prior to feeding. After slowly mixing the experimental bottles, larvae were collected by sampling of ~25 ml water samples from each replicate, passed through a filter with 20 µm mesh size. Larvae were rinsed with freshly filtered seawater and the mesh was kept submersed inside a glass vessel, maintaining favorable conditions for the larvae. To remove the animals from the mesh, water was mixed with a pipette, re-suspending the larvae in the water column and a 1 ml volume was transferred into each respiration vial, which was subsequently closed. Vials without larvae served as controls. Oxygen consumption measurements lasted overnight and oxygen concentration always remained above 40% air saturation throughout the measuring interval. Following the measuring period, larvae were rinsed from the vials with FSW, and larval density was

measured. Samples were fixated with 4% PFA solution and stored at -80^{o}C. Photographs of larva were taken and shell lengths was measured as described above.

Larval respiration rate was calculated from the linear decline of the oxygen concentration after subtraction of the oxygen decline in control vials during measuring interval. The final respiration rate is expressed as pmol O_2 h^{-1} $larvae^{-1}$.

2.9 Scope for growth calculation

For the calculation of scope for growth (SfG) during larval life stage, metabolic and feeding rates measured on the same DPF were converted to energy equivalents (EE) by using a factor of 484 µJ $nmol^{-1}$ O_2, (Gnaiger, 1983). Energy uptake of larvae was calculated using the measured clearance rate, while energy loss was calculated from measured respiration rates. Larval carbon assimilation efficiency (CAE) of 57.95% was estimated based on published data for larval stages of the bivalves *Crassostrea virginica* and *Mercenaria mercenaria* of 53.6% and 62.2% respectively, both fed with *I. galbana* (Reinfelder et al., 1994). Carbon uptake was calculated for a mean carbon content of *I. galbana* of 12.7 pg/cell (see table 1), which was converted to energy equivalents using 1,34 10^5 µJ $µgC^{-1}$ (Renaud et al., 2011 in Stumpp et al., 2011). Thus, SfG was calculated from energy input (CR x n^{o} of *I. galbana* (cells/µl) x C content (cell) x CAE (µg) x 1,34 10^5 µJ $µgC^{-1}$) and subtraction of respiratory energy loss (O_2 nmol $individual^{-1}$ * 484µJ $nmol^{-1}$ O_2), thus, SfG = energy input – energy loss.

Table 1: Carbon content of Isochrysis galbana in pg/cell used to calculate the SfG in this study.

Maximum C content (pg/cell)	Cultured Temperature (°C)	Reference
13	20	Ylenia Carotenuto et al., 2002
6-13	23	Perez-Morales et al., 2015
6.97	16	Montagnes et al., 1994
23.8	25	Ishiwata et al., 2013

2.10 Single larvae DNA Extraction

DNA extractions from single settled individuals were performed following Zhan et al. (2008, with modifications). Briefly, spat from each replicate were rinsed with modified isotonic phosphate-buffered saline (PBS) (0.14 M NaCl, 0.003 M KCl, 0.01 M Na_2HPO_4, 0.002 M KH_2PO_4, pH adjusted with concentrated HCL to pH 7.5) and transferred separately with a pipette into 200 µl PCR tubes and stored at -20^{o}C for further analysis. For DNA extraction, 20 µl of lysis buffer (LoTEPA lysis buffer - low concentration of Tris-HCl, EDTA and Proteinase K; Tris-HCl 3 mm, pH 7.5; EDTA 0.2 mm, pH 7.5; Proteinase K 0.5 mg/ml, for details see Zhan et al., 2008) was added to each tube and placed in a programmed FlexCycler block (Biozym, Block assembly T48) for 90 minutes at 56^{o}C and 10 minutes at 95^{o}C for followed by 10 minutes of cooling at 4^{o}C. DNA was extracted from 16 individuals from each replicate or all individuals remaining alive when survival was lower.

2.11 Genotypic assessment

Samples were genotyped at the nuclear locus Glu-5' (Inoue et al., 1995). The foot adhesive protein gene (Glu-5') shows changes in length at the variable region in different *Mytilus* species (Innoue et al., 1995). Thus, it serves as a highly diagnostic locus used to distinguish between the species *M. galloprovincialis*, *M. edulis* and *M. trossulus* (Innoue et al.,1995; Rawson et al., 1996; Wood et al., 2003; Riginos & Cunningham, 2005; Kijewski et al., 2006; Kijewski et al., 2011). Mussels *M. edulis*, *M. trossulus* and *M. galloprovincialis* can be differentiated based on natural PCR product length variation of 180, 168 and 126 base pairs respectively (Innoue et al., 1995).

DNA fragments were amplified in a polymerase chain reaction following the modified protocol from Inoue et al., 1995 using 1 µl of extracted DNA from single larvae, 5 µl of Multiplex PCR Master Mix (Multiplex PCR Kit, Qiagen), 5 pmol of each primer (table 2) and 3 µl of double distilled water in a total reaction volume of 10 µl. The reaction was performed using a Veriti 96-well fast thermal cycler (Applied Biosystems Inc, California, USA). The forward primer was labelled with the fluorescent dye Atto 565 (Red colour). For amplification, DNA was denatured at 95°C for 15 minutes, followed by 36 cycles of 94°C for 30 sec, 57°C for 90 sec and 72°C for 60 sec. Finally, a 30 min elongation step at 60°C was performed and kept at 4°C.

Table 2: Primer sequences for the molecular marker Glu-5' used in this study.

Marker	Sequence	Author	Provider
Glu-5'			
Forward: Me15_F (Atto 565)	5'-CCAGTATACAAACCTGTGAAGA-3'	Inoue et al., (1995)	Eurofins Genomics (Ebersber, Germany)
Reverse: Me16_R	5'-TGTTGTCTTAATAGGTTTGTAAGA-3'		

Subsequently, length variations of Glu-5' were analysed through automated capillary gel electrophoresis, 1µL of each PCR product was mixed with 8.75µL of Hi-Di formamide (Thermofischer Scientific, Schwerte, Germany) and 0.25µL of LIZ 500 dye standart (Thermofischer Scientific, Schwerte, Germany) in a 96-well plate. Samples were denatured at 95°C for 2 min in a PCR thermal cycler (FlexCycler T48, Analytik Jena). Samples were then sequenced using a Genetic Analyser (ABI 3130 Genetic Analyser, Applied Biosystems Inc, California, USA). Resulting allelic lengths were analysed using the genotyping software GeneMarker AFLP V1.91 (Biogene Ltd, Kimbolton, UK). Characteristic lengths for the marker Glu-5' found in this study were 181 bp for *M. edulis* and 168 bp for *M. trossulus*. The 1 bp deviation from the original allelic length of *M. edulis* found in this study may result from using different equipment and primer batches for analyses.

2.12 Statistical analyses

All statistical analyses and graphical illustration were performed using Rstudio (Version 1.0.44). Two-way ANCOVA analyses were applied to compare the different physiological rates (larval survival, growth and respiration) in relation to salinity, temperature and their interaction using DPF or SL as a co-variable. Two-way ANOVA analysis was applied to test for effects of salinity and temperature factors on the settlement success of the larvae at 69 DPF. Settlement data were analysed after square root transformation. Kruskal-Wallis H test was used to test the difference in performance of clearance rate, SfG and the proportion of hybrids between the different experimental treatments. The Shapiro–Wilk test was used to check that the data were normally distributed and the Levene's test and Fligner-Killeen test were used to check for homogeneity of variances. A Tukey HSD test was the post hoc test applied following the statistical analyses. A P value of < 0.05 was assumed as the significance level for all analyses. To provide clear information, two-way ANCOVA and two-

way ANOVA results are listed in tables within the results section. For all figures, horizontal lines represent calculated standard error.

3 RESULTS

3.1 Effect of temperature and salinity on larval survival

A significant decrease in larval relative density with DPF occurred in all cultures until settlement was observed (Table 3). The slope of regression enabled relative percentage survival estimation in data normalized to DPF (Figure 9). Larvae that were unable to metamorphose and settle consequently died or delayed development remaining at larval D-stage. A drop of relative density caused by mortality (in treatments combining 9 psu and 12°C; 9 psu and 15°C; 7psu and 12°C; 7 psu and 15°C) and larval settlement activity (in treatments combining 11 psu and 15°C) is observed in the experimental tanks from 36 to 46 DPF. A negative impact of low salinity ($p<0.001$) resulted in significantly lower survival of spat reared in 7 and 9 psu at both temperature treatment conditions. Temperature increase of 3°C accelerated settlement levels of larvae reared in 11 psu as well as mortality rate of larvae reared in 9 psu treatments. In extreme low salinity conditions, an antagonistic effect of increased temperature elevating larval survival levels was observed in this experiment.

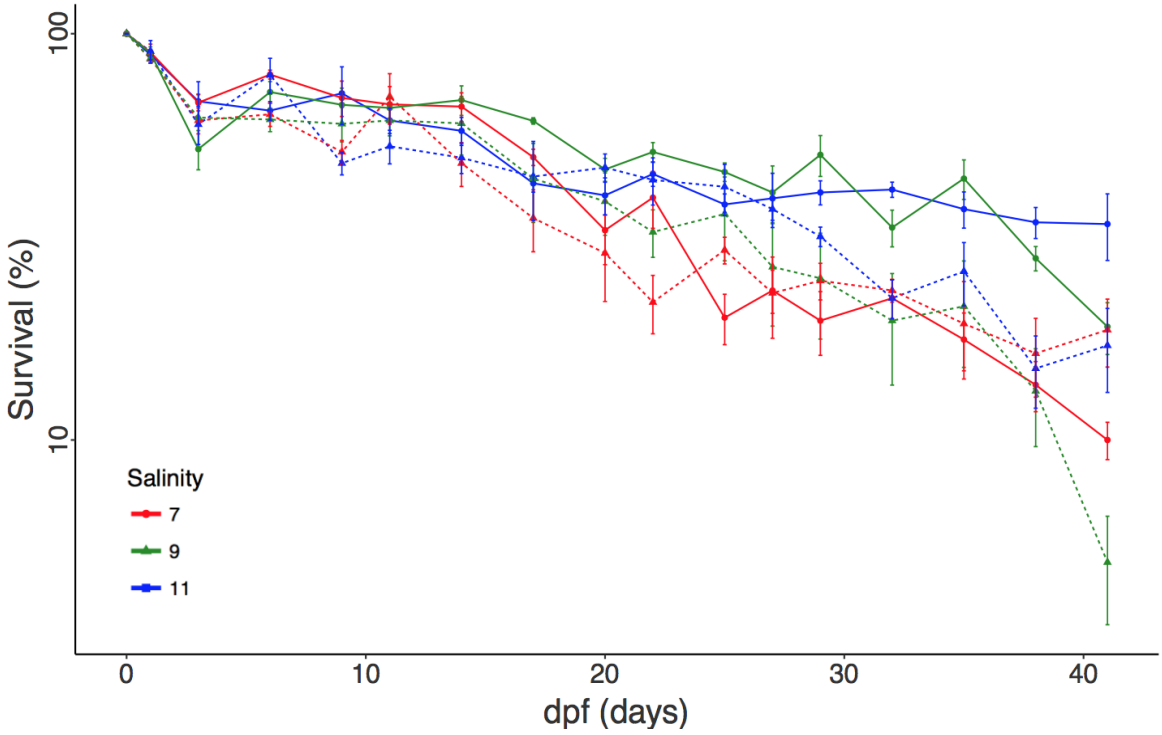

Figure 9: Relative density of larvae in 5 replicate cultures under the different treatments of temperature: control temperature (solid lines, circles) and high temperature (dotted lines, triangles) and salinity of 7 psu (red lines) 9 psu (green lines) and 11 psu (blue lines) over DPF.

Table 3: Two-way ANCOVA analyses results of survival of Baltic *Mytilus* larvae reared under different salinities (7 psu, 9 psu and 11 psu) and temperatures (12°C and 15°C) conditions. Here larval relative density normalized to days post-fertilization (DPF) are represented.

Response:Survival					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr (>F)**
DPF	1	304385	304385	1647.736	**<0.001**
Temperature	1	6753	36.555	38	**<0.001**
Salinity	2	3302	1638	8.868	**<0.001**
Temperature:Salinity	2	1260	630	3.410	**0.034**
Residuals	558	103079	185		

3.2 Effect of temperature and salinity in settlement success

Settlement of *Mytilus* sp. larvae was impacted by temperature and salinity ($p<0.05$). A synergistic effect of increased temperature was found in this experiment. Settlement success was significantly higher in larvae reared at 15°C compared to 12°C treatments (Figure 10). Salinity had a negative impact on larval settlement and lower settlement levels were found in larvae reared at 9 and 7 psu treatments. The mean percentage of larvae able to settle at 7 psu treatments after 69 DPF was 0.02 ± 0.01 % (12°C) and 0.3 ± 0.1 % (15°C) from the initial estimated number of 24000 individuals added at each experimental tank. These values were significantly lower compared to number of settled larvae reared at 11 psu with 0.78 ± 0.38 % (12°C) and 4.47 ± 0.9 (15°C). Settlement success of larvae reared at 7 and 9 psu did not differ significantly between the temperatures treatments. Treatment combining low salinity (7 psu) and control temperature (12°C) had the highest number of live D-staged larvae in the end of experimental period (1.55 ± 0.6 %) surpassing the percentage of settled organisms. Presence of undeveloped larvae was less pronounced in other treatments, not reaching values higher than 0.24 ± 0.1 % (7 psu, 15°C).

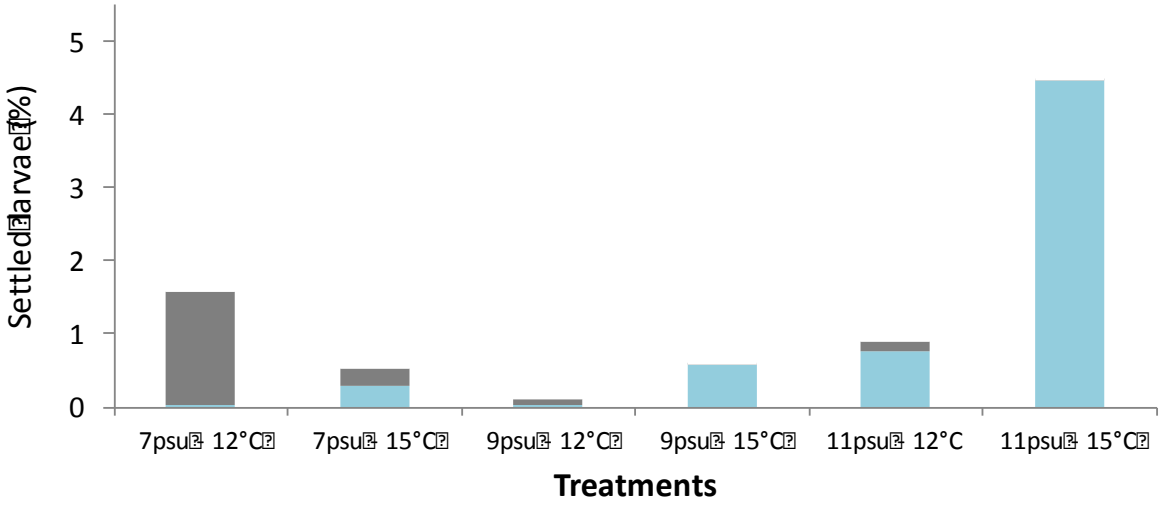

Figure 10: Settlement success in percentage of Baltic *Mytilus* larvae in 5 replicate cultures under the different treatments of temperature (12°C and 15°C) and salinities (7 psu, 9 psu and 11 psu. Percentage of settled larvae is represented by blue bars and D-stage larvae by grey bars.

Table 4: Two-way ANOVA analyses results of settlement success of Baltic *Mytilus* larvae reared under different salinities (7 psu, 9 psu and 11 psu) and temperatures (12°C and 15°C) conditions.

Response: Settlement					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Temperature	1	813.2	813.2	24.30	**<0.001**
Salinity	1	1443.1	1443.1	43.12	**<0.001**
Temperature:Salinity	1	235.6	235.6	7.04	**0.018**
Residuals	16	535.5	33.5		

3.3 Effect of temperature and salinity on larval growth

Temperature, salinity and their interaction had a significant effect on larval growth in this study ($p<0.05$). Growth measured in SL in µm of larvae reared under 15°C temperature treatments increased significantly in all salinities ($p<0.01$). A significant negative impact of low salinity on growth was observed ($p<0.001$). Specimens with significantly shorter SL were found in low salinity treatments (7 psu) independent of the temperature treatment. Maximum SL was reached by the treatment presenting high temperature (15°C) and control salinity (11

psu) (250,60 ± 5,82 µm) whereas minimum SL were presented by larvae reared in treatment of 7 psu combined with 12°C (145,66 ± 2,99 µm).

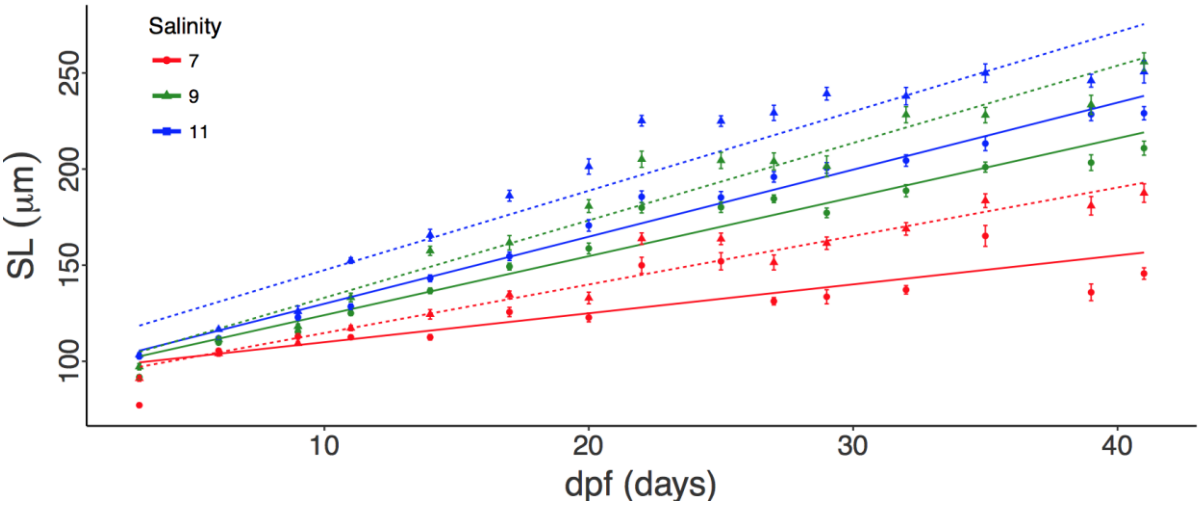

Figure 11: Mean growth in shell length (µm) of larvae in 5 replicate cultures under the different treatments of temperature: control temperature (filled lines, circles) and high temperature (dotted lines, triangles); and salinity of 7 psu (red lines) 9 psu (green lines) and 11 psu (blue lines) over DPF.

Total growth in SL differed significantly within the temperature and salinity treatments (Figure 12). Growth levels were positively correlated with + 3°C temperature increase ($p<0.05$) regardless of salinity larvae were submitted to. A negative impact of salinity ($p<0.01$) was also observed resulting in significantly lower values of TG of larvae reared in 7 psu treatments when compared to 9 and 11 psu.

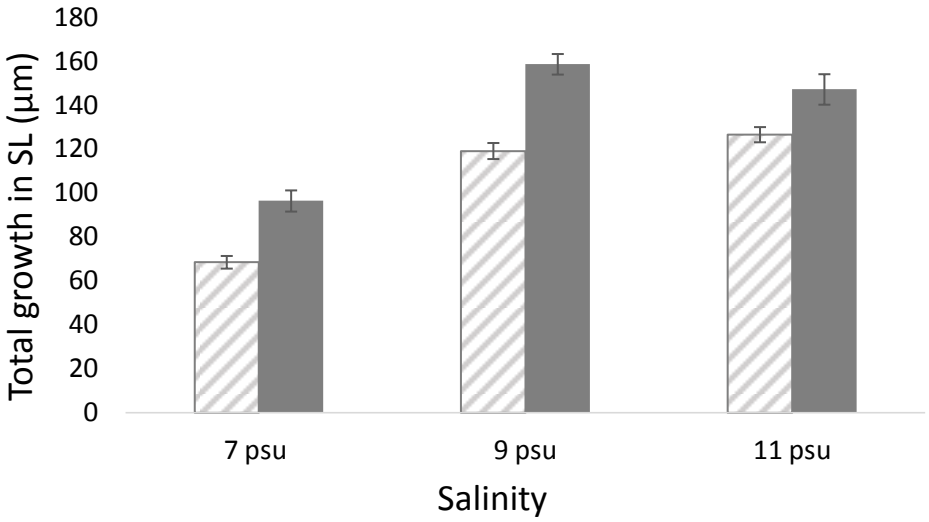

Figure 12: Mean total growth in SL (µm) µm of in 5 replicate cultures under the different treatments of temperature: 12°C (patterned bars) and 15°C (solid bars) and salinity: 7, 9 and 11 psu. Error bars indicate standard error (n=5).

Table 5: Two-way ANCOVA analysis results of measured physiological parameters in Baltic *Mytilus* larvae reared under different salinities (7 psu, 9 psu and 11 psu) and temperatures (12°C and 15°C) conditions. Here larval growth (SL) relative to DPF is represented.

Response: SL					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
DPF	1	8153776	8153776	11264.42	**<0.001**
Salinity	1	2015381	1007690	1392.12	**<0.001**
Temperature	2	560982	560982	775.00	**<0.001**
Temperature:Salinity	2	31632	15816	21.85	**<0.001**
Residuals	6016	4354693	724		

3.4 Effect of temperature and salinity on larval respiration rate

In this study, larval RR were significantly affected by temperature and increased by SL (Table 6). While no significant impact of temperature on RR of larvae reared under 9 and 7 psu was observed in this experiment ($p>0.05$), control salinity conditions (11 psu) showed a significant increase in RR in 15°C (Figure 13). Salinity did not impact RR of larvae reared under 15°C. However, decreasing salinities led to increased RR of larvae reared under 12°C.

RR of larvae reared under 7 psu treatment were higher and increased oxygen consumption in relation to SL was observed compared to the other salinity treatments.

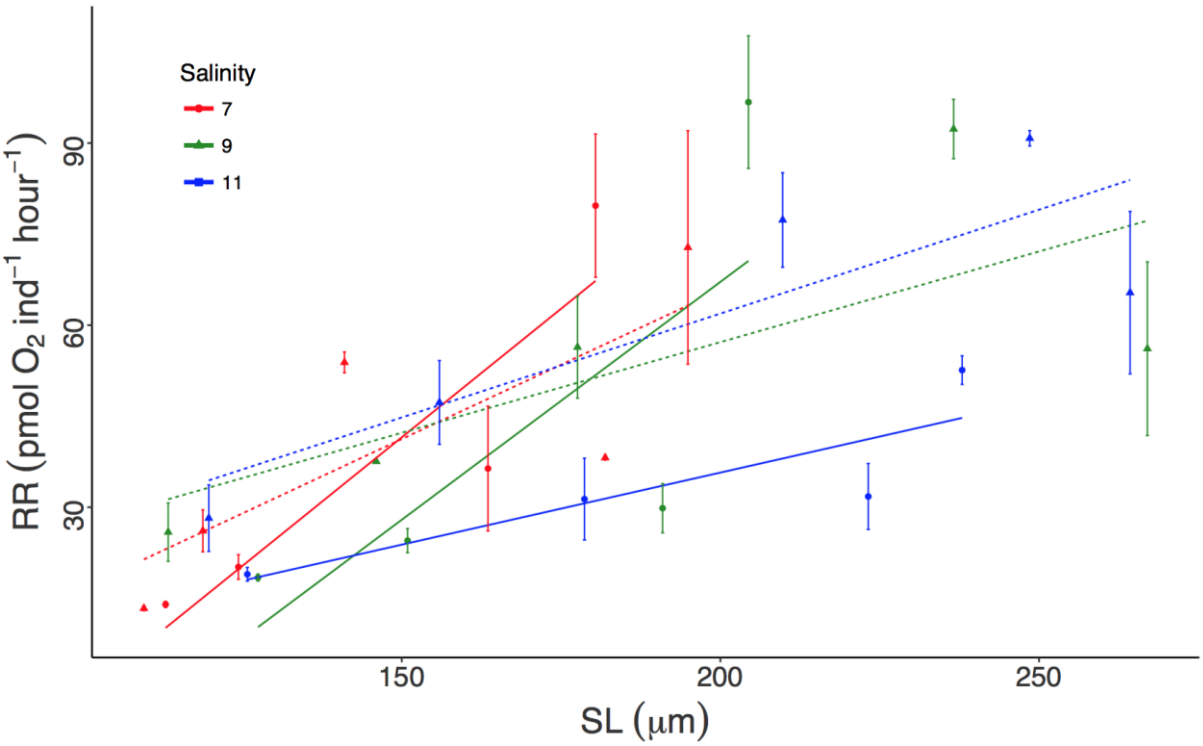

Figure 13: Mean respiration rate (pmol O_2 ind^{-1} $hour^{-1}$) of larvae in 5 replicate cultures under different treatments of temperature: control temperature (solid lines, circles) and high temperature (dotted lines, triangles); and salinity of 7 psu (red lines) 9 psu (green lines) and 11 psu (blue lines) over SL (µm).

Table 6: Two-way ANCOVA analyses of measured respiration rates in Baltic *Mytilus* larvae reared under different salinities (7 psu, 9 psu and 11 psu) and temperatures (12°C and 15°C) conditions. Here RR relative to SL (µm) is represented.

Response: Respiration					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
SL	1	26178	26178	68.120	**<0.001**
Temperature	1	2142	2142	5.573	**<0.001**
Salinity	2	959	479	1.247	0.293
Temperature:Salinity	2	2204	2.867	48.967	0.063
Residuals	71	189.267	0.02666		

3.5 Effect of temperature and salinity on larval clearance rate

In this study, temperature and salinity had a significant effect ($p>0.05$) on larval CR (Figure 14). No significant effect of increased temperature on larvae reared under 7 psu treatments was found ($p > 0.05$). In contrast, a synergistic effect of increased temperature was observed in spat in 9 and 11 psu ($p< 0.05$). An antagonistic effect of increased temperature and low salinity (7 psu) leading to decreasing levels of CR of larvae reared under this treatment was observed. Negative effect of low salinity resulted in significantly lower CR of larvae reared under 7 psu salinity conditions compared to those in 9 and 11 psu. No significant difference was found in treatments of 9 psu and 15°C and 11 psu 12°C ($p>0.05$).

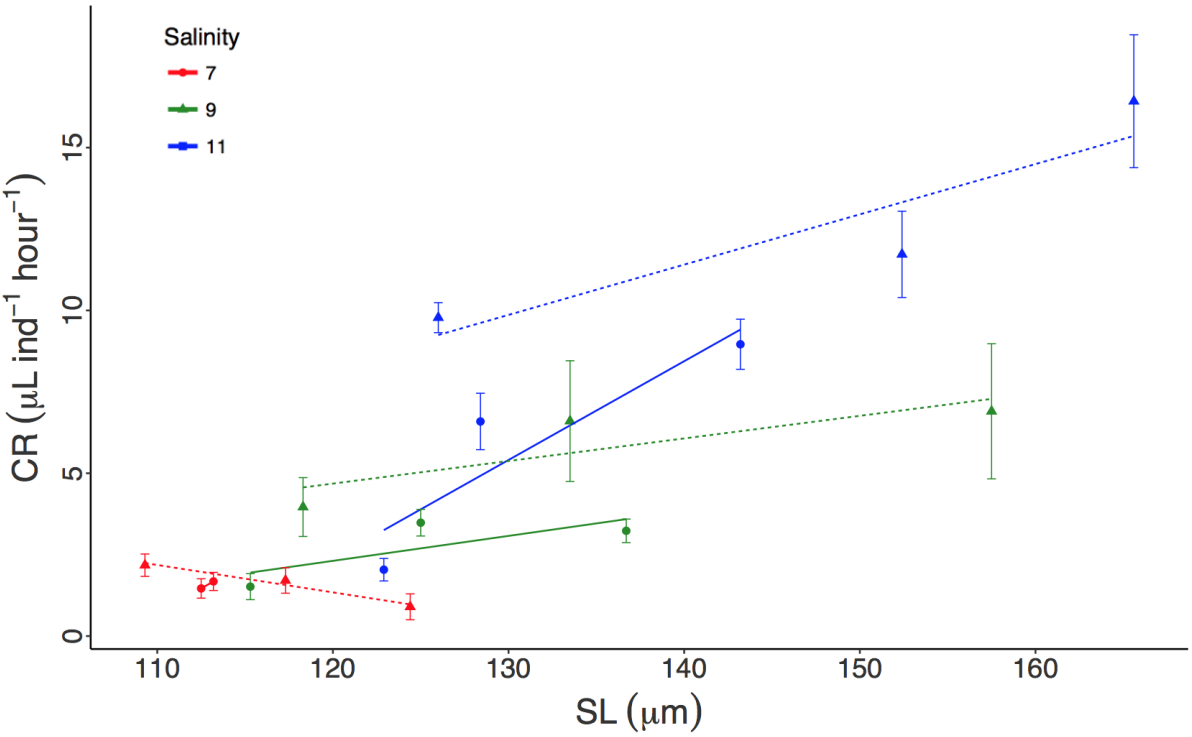

Figure 14: Mean clearance rate (µL ind^{-1} hour^{-1}) of larvae in 5 replicate cultures under the different treatments of temperature: control temperature (solid lines, circles) and high temperature (dotted lines, triangles); and salinity 7 psu (green line) 9 psu (blue line) and 11 psu (red line) over SL (µm).

3.6 Effect of temperature and salinity on Scope for Growth (SfG)

Scope for Growth was significantly impacted by salinity and interaction of this with temperature ($p<0.05$) (Figure 15). In low salinity treatment (7 psu) and antagonistic effect of

temperature elevation was observed, resulting in a declining level of energy available for growth to larvae reared under this treatment. Levels of SfG were higher at 11 psu treatments compared to rates found at 7 and 9 psu. No effect of temperature increase on CR of larvae reared under 11 psu salinity was observed. Nevertheless, a synergistic effect of increased temperature leading to higher CR of larvae reared under 9 psu was found in this study.

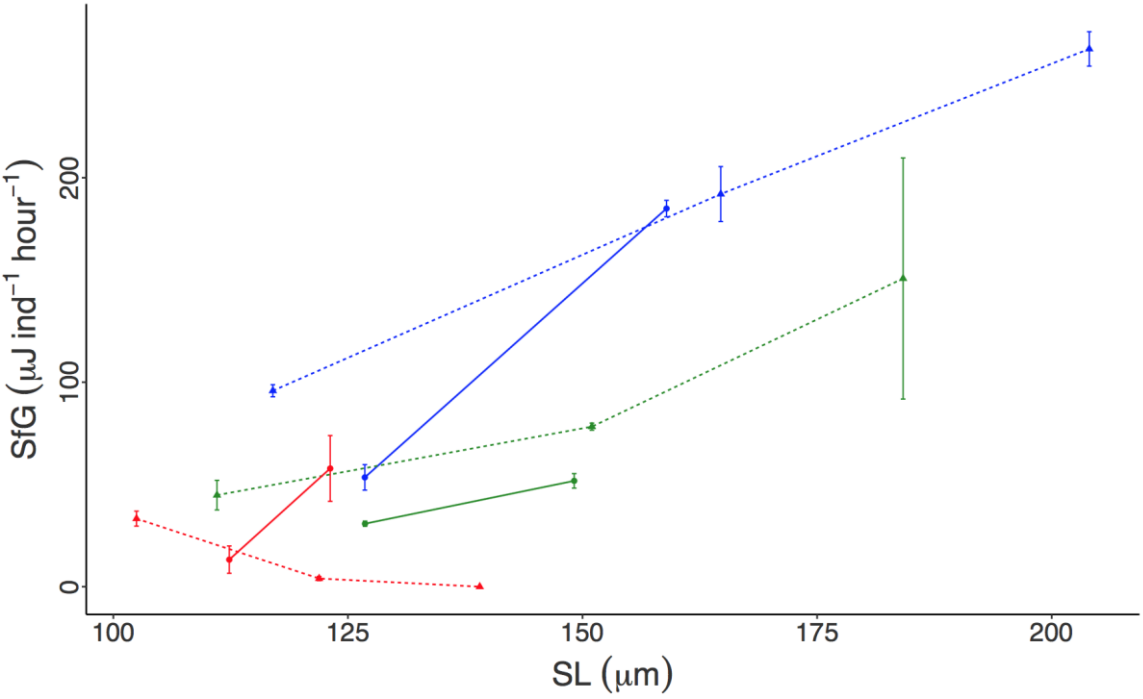

Figure 15: Scope for Growth (µJ ind^{-1} hour^{-1}) of larvae in 3 replicate cultures under control the different treatments of temperature: control temperature (circle marks) and high temperature (triangle marks); and salinity 7 psu (green line) 9 psu (blue line) and 11 psu (red line) over SL (µm).

3.7 Genotypic assessment of settled larvae

In this study, settled spat was genotyped at a single nuclear locus, Glu-5'. No changes in species proportion between the different experimental treatments was found ($p>0.05$). Results suggest that temperature and salinity did not play a strong role in genotypic selection of Baltic *Mytilus* larvae at this specific marker. As a result, from the total 321 genotyped individuals; 91,8% of the young spat was identified as hybrids of *M. edulis* and *M. trossulus* at this specific locus.

4 DISCUSSION

The main objective of this study was to investigate how the changes of salinity and temperature predicted for the future Baltic Sea will influence survival and settlement success of Baltic *Mytilus* larvae. To obtain a better mechanistic understanding of the impacts of abiotic stress on larval fitness, variation in energy available for growth (SfG) was calculated by combining changes in processes responsible for energy intake (clearance rate) and aerobic metabolism (respiration rate).

Sensitivity and tolerance to thermal and osmotic stress is species and population specific, being additionally dependent on the specific life stage. Previous studies have observed that larvae of *M. edulis* from the North Sea can develop and survive at salinities as low as 14 psu (Bayne, 1965). However, 100% mortality of *M. edulis* spat reared under identical salinity conditions from the Western North Atlantic populations has been described (Qiu et al., 2002). This study focused on the effects of changes in abiotic factors on a mussel population adapted to the Baltic Sea conditions.

4.1 Combined effect of temperature and salinity on larval survival and settlement

My experimental results suggest that the projected changes in temperature and salinity will have a negative impact on the survival of Baltic *Mytilus* larvae inhabiting the region of Ahrenshoop. Larval mortality increased with decreasing salinity reaching levels from 60 to 90% at the time of first settlement. The observed survival rates are comparable to literature (Table 7). A 99% mortality rate of free-swimming larvae is observed in the field (Thorson, 1946; Bayne, 1965). The low survival success is related to predation, dispersal and exposure to varying physical factors.

Table 7: Mean percentage survival of *Mytilus* larvae reared under different temperature and salinity conditions.

Species	Salinity (psu)	Temperature (°C)	Time (DPF)	Survival (%)	Reference
M. edulis	28	10	50	40	Rayssac et al., 2010
	28	17	30	80	Rayssac et al., 2010
	30	19	33	73	Lapota et al., 1993
M. trossulus	28	10	50	70	Rayssac et al., 2010
		17	30	70	Rayssac et al., 2010
Mytilus sp.	30	19	28	30	Pernet et al., 2003
	16	19	21	20	Thomsen et al., 2017
Baltic *Mytilus*	7	12	35	17.6	Nascimento-Schulze, 2017
		15		19.3	(present study)
	9	12	35	44	Nascimento-Schulze, 2017
		15		21.3	
	11	12	35	37	Nascimento-Schulze, 2017
		15		26	

At 35 DPF, settlement activity had not occurred in larvae at any of the experimental treatments in my experiments. At this time point, the observed percentage survival rates (mean ± SE) of larvae acclimated to salinities of 9 and 11 psu combined with control temperature (12°C) were 44 ± 4.9% and 37 ± 3.8% respectively. Larvae reared under 7 psu and control temperature had significantly lower survival rates (17.7 ± 2.8%). No difference in survival with increased temperature was observed in larvae raised under combined treatment of 7 psu and 15°C (19.3 ± 5.2%). Antagonistic effect of increased temperature on survival rates at 35 DPF was found at both salinities of 9 and 11 psu (21.3 ± 6.2%; 26 ± 4.6%). A possible explanation for larvae raised under control salinity treatment is that increased temperature resulted in the acceleration of larval development, initiating settlement activity earlier. Thus, the lower number of free-swimming larvae in the water column led to a diminished density count in experimental tanks. In addition to larval mortality, a 30% to 50% mortality at metamorphosis is expected for mussels (Helm et al., 2004). The percentage of successfully settled individuals in 11 psu treatments (>4.5%) fits the previous described information. Larvae reared under 9 psu salinity had a low settlement success and < 0.5% of initial number of larvae could metamorphose. This pattern was more pronounced in the 15°C treatment. Results suggest that a decrease in 2 psu acted strongly during this final larval period in 9 psu treatments, and larvae were not able to metamorphose and successfully

continue to develop. In contrast to the other salinity treatments, animals exposed to 7 psu salinities were still found in an under-developed phase (D-stage) until 67 DPF.

Food availability has a significant influence on larval survival, growth and development. Throughout this experiment, larvae were fed daily with cultures of *I. galbana* (15000 cells ml^{-1} d^{-1}). After 26 DPF larvae began to be fed with *Rhodomonas sp.* (3000 cells ml^{-1} d^{-1}). Prior to daily microalgae addition, complete food depletion was not observed. Remaining cell concentrations of 8000 ± 706 cell ml^{-1} of *I. galbana* and 1525 ± 73.8 cell ml^{-1} of *Rhodomonas* sp. after 24h period in tanks were estimated from clearance measurements. Thus, throughout this experiment, food was never totally consumed before further addition. Minimum values of chlorophyll a content in tanks estimated from remaining number of *I. galbana* cells were found between 1.5 ± 0.0002 µg l^{-1} to 2.11± 0.0002 µg l^{-1} (Valenzuela-Espinoza et al., 2002). Kiel Fjord monitoring shows that maximum concentration of chlorophyll a ranges from 3.5 to 8 µg l^{-1} during summer months (Shi et al., 2014). Food availability in this experiment at any time was roughly comparable to that in the natural habitat in terms of quantity. Using single microalgae species as a food source may have not provided complete nutrition quality. Nevertheless, previous studies using single species cultures of *I. galbana* (Bayne, 1965; Sprung, 1984b; Lazo & Pita, 2012) and *Rhodomonas* sp. (Ventura et al., 2016) as a food source did not show a negative impact on *Mytilus* sp. larval development. Moreover, invertebrate larvae can utilize amino acids and monosaccharides from the water column during development as an additional source of energy uptake (Stephens, 1969; Jaeckle and Manahan, 1989). Thus, the lower growth rates found in larvae reared in 7 psu cannot be attributed to a lack of energy uptake and low levels of energy available for development.

In this study, a negative impact of salinity on survival of young Baltic *Mytilus* was observed. Regarding the high number of settled organisms found at 11 psu 15°C treatment, it is assumed that a drop in survival rates from 37 DPF onwards is caused by initiated settlement activities. Survival rates of larvae reared under 7 psu and 9 psu were significantly lower when compared to 11 psu treatments. A negative interaction of elevated temperature and low salinity was observed for the 9 psu 15°C treatment compared to 9 psu control temperature. This result could reflect the higher larval settlement success found in this treatment. In addition, the increased speed of development at elevated temperature could further influence the high mortality of larvae raised under 9 psu and 15°C. A possible explanation is that the larvae facing conditions of 7 and 9 psu had higher levels of energy allocated towards maintenance due to hypoosmotic stress. The low number of animals able to settle in the 9 psu treatments (<0.05%) suggests that larvae suffered high mortality during metamorphosis.

Insufficient levels of available energy to complete the process could explain these observations.

In this study, a negative impact of salinity on settlement and metamorphosis of young Baltic *Mytilus* was observed. The ability to metamorphose is strongly dependent on the availability of energy sources in the environment and reserves accumulated throughout larval life. During metamorphosis, larvae are unable to ingest food for a 2 to 8 day period and rely on energy reserves accumulated during the larval phase (Bayne, 1976). Among the several ongoing changes, the prodissoconch II shell is transitioned to the adult shell (Waller, 1981). A steady increase in *M. edulis* inorganic content due to shell formation is observed throughout development (Sprung, 1984a). A significant increase in inorganic content in combination with a decrease in absolute content of proteins, carbohydrates and lipids was observed during metamorphosis of the oyster *Ostrea edulis* (L.) (Labarta et al., 1998). The accumulation of energy reserves during larval development is considered one of the factors contributing to larval ability to metamorphose (Haws et a., 1993).

A decrease in settlement success was observed in larvae reared under low salinity treatments (7 psu). In addition, our results show that at 7 psu, larvae remained at the D-stage until at least 67 DPF. The experiment was terminated at this point, but non-metamorphosed individuals were still found alive in experimental tanks. This result was more pronounced for the larvae reared under the 12°C treatment. Marine invertebrates are able to delay metamorphosis in the absence of environmental cues or under adverse conditions (Pechenik et al., 2006). Prolongation of larval phases in *Mytilus sp.* up to 40 days at low temperatures has been previously described (see Bayne, 1965). Free-swimming organisms found in experimental tanks at 7 psu and 12°C at 67 DPF were transferred during a 21-day period to conditions of 11 psu. A mean settlement success of 4.42 ± 2.12 % of the initial number was found after this exposure period. These observations suggest that even though in low numbers, the larvae have a potential to settle if environmental conditions reach levels of favorable salinity. Nevertheless, this potential to further settle with varying abiotic conditions was only briefly observed in this study. Further investigation with proper quantification is thus needed to reveal true response of settlement potential towards varying salinity conditions.

Experimental results suggest that future abiotic changes in environmental conditions will most likely result in a negative outcome on larval survival and settlement. Lowering salinity had a negative impact on survival and settlement rates. An interaction of low salinity and increased temperature (+ 3°C) resulted in poor results of larval survival and settlement rates.

As predicted in hypothesis 1, reduced salinity acted negatively on survival, growth and settlement rates of larvae. Experimental results of larvae psu agree with hypothesis 2 predictions so that larval physiological rates of growth and development were accelerated due to increased temperature treatment. Nevertheless, when reared under intense hypoosmotic stress conditions of 7 psu, increased temperature does not act strongly on larval physiological rates of clearance and respiration and no significant difference in responses was observed regarding these responses.

Understanding how abiotic factors affect larval physiology may clarify the previously discussed results. The effects of desalination and warming in different metabolic compartments will be discussed in the following chapter.

4.2 Combined effect of temperature and salinity on growth, respiration and clearance rates and Scope for Growth (SfG)

Scope for growth can be defined as the proportion of absorbed energy available for anabolic processes such as tissue and shell growth. In this study, SfG increased with time in all treatments but for the larvae reared under 7 psu and 15°C conditions. In this treatment, a negative interaction of low salinity and increased temperature resulted in a decreasing SfG in relation to increasing shell length. Moreover, a negative impact of desalination was found, and SfG was significantly lower in low salinity treatments of 9 and 7 psu compared to control salinity treatments. A + 3°C in temperature did not affect SfG of animals submitted to control salinities. Decreasing SfG in response to increased levels of CO_2 has been previously reported for larvae of the sea urchin, *S. purpuratus* (Stumpp et al., 2011). Experimental results suggest that hypoosmotic stress increases energy consumption for body maintenance. In addition, it was speculated that future predicted changes in salinity and temperature may strongly challenge Baltic *Mytilus* larval development.

When organisms are exposed to temperatures found within their thermal tolerance range, growth positively increases with elevating temperatures until the optimal level is reached (Angilletta, 2009). The increasing temperature accelerates enzymatic processes and elevates physiological performance. Functional compartments of the energy budget e.g. energy input (ingestion and absorption) and output (respiration and excretion) are thus positively increased in this scenario (Beiras et al., 1994).

In this study the positive influence of elevated temperature enhanced growth rates within the different salinity treatments. Larvae reared under 15°C had significantly higher

growth rates than those reared under temperatures of 12°C. Similar effect of temperature on growth rates of *M. edulis* larvae (Sprung et al., 1984a) and other bivalves (e.g. *Venepuris pullastra*, Loosanoff and Davis, 1963; *Mercenaria mercenaria*, Pérez et al., 1973; and *Ruditapes decussatus* (L.), Beiras et al., 1994) have been previously described.

Experimental results demonstrate that desalination significantly lowered growth rates of larvae reared under 7 psu. This trend was significantly alleviated at 9 and 11 psu treatments. A decrease in final SL of larvae reared under low salinity treatments compared to control was observed. Final SL of larvae grown under 7 psu was ~70µm smaller than found in control salinity. Agreeing to our results, a retardation of larval growth has been found in different marine invertebrate taxa when subjected to low salinity conditions (e.g. Nell & Holliday, 1998, Montoroy et al., 2014).

In addition, experimental data revealed a steeper slope and significantly increased RR of larvae reared under 7 psu salinity treatments in comparison to larvae reared at salinities of 9 psu and control. Higher routine metabolic rates (RMR) found in larvae reared under 15°C conditions are most likely a reflection of increased metabolic activity influenced by temperature elevation. Larval RR observed in this study are comparable to literature previously describing *M. edulis* larvae (Hamburger et al., 1984) and bivalves such as *C. gigas* (Garcia-Esquivel et al., 2001). Interestingly, an increase in temperature reduces the detrimental effects of salinity. As elevated temperatures increase developmental pace, larvae reduce their time in this vulnerable stage, and are less affected by additional abiotic stressors (Havenhand, 1993). The alleviation of negative effects of abiotic stressors such as pH and salinity on marine larvae by temperature elevation is reviewed in Preslawski et al. (2014). Nevertheless, a decrease in tissue mass of Baltic *Mytilus* juveniles grown in low salinity conditions of 7 psu compared with individuals of the same SL grown in 16 psu was previously described (Sanders et al., 2015; in preparation). Elevated RR of larvae reared 7 psu conditions could reflect the higher tissue mass in relation with SL of individuals. Thus, further analysis of O_2 consumption in relation to body mass is needed to gain a greater understanding of effects of hypoosmotic stress on early life-stages of Baltic *Mytilus*.

The ability to osmoconform allows marine organisms to maintain their intracellular composition close to osmotic equilibrium with the surrounding environment (Willmer et al., 2009). External salinity gradients have a direct effect on intracellular ionic distributions and across membrane electrical potentials, consequently affecting cellular functioning (Livingstone et al., 1979). When facing long-term exposure to reduced salinity conditions of 8 psu, adult mytilids can decrease intracellular concentrations of organic osmolytes and ions

(Willmer, 1978). In addition, an increased intracellular concentration of K^+ was documented suggesting that mussels can actively regulate specific ions. In contrast, mussels are not able to regulate extracellular fluids (haemolymph) content of most organic osmolytes and ions (Thomsen et al., 2010). Active intracellular ion regulation is enabled by specific ATP consuming transmembrane proteins such as the Na^+/K^+-ATPase, Ca^{2+}-ATPase, and V-type ATPase. The active pumping through cellular membranes is energy consuming process that requires ATP and contributes to the costs of maintenance metabolism. Thus, hypoosmotic stress may lead to metabolic upregulation to reach increased energetic demand (Wood et al., 2016) increasing levels of energy input or allocating available energy from different compartments (e.g growth and reproduction) (Neufeld & Wright, 1996). The activity of the enzyme Na^+/K^+-ATPase represented up to 40% of *in vivo* respiration rates of the sea urchin, *Strongylocentrotus purpuratus* pluteus larvae, suggesting that ion regulation is a costly energetic process in marine invertebrate early life-stages (Leong & Manahan, 1997).

For mussel populations inhabiting the Baltic Sea, the energetic cost of osmoregulation has been suggested to increase with decreasing salinities (Maar et al., 2015). The developed Dynamic Energy Budget Model (DEB) estimates that in 8 psu salinity, osmoregulation represents between 74% and 84% of the total metabolic costs. As a result, an increase in metabolic cost under hypoosmotic stress up to 3.8 (Tedengren & Kaustky, 1987) or 4.7 (Maar et al., 2015) times is expected. In addition, the DEB model suggests that available metabolic energy for growth is allocated to cover enhanced osmoregulation, leading to decreased specific growth rates of mussels, as observed in this present study. In this model, the increase in energy requirement is assumed to be caused by regulation to hypoosmotic stress. Nevertheless, other physiological processes (e.g. calcification) could be equally responsible for such increase in energy use.

Lower calcium and inorganic carbon supply leads to reduced larval growth rates in hypoosmotic environments (Thomsen et al., 2015). Uptake of Ca^{2+} and dissolved inorganic carbon from seawater allows larvae to calcify. Marine bivalves begin to calcify during early-life developmental stages and the first larval shell (prodissoconch I) composed of $CaCO_3$ is formed within initial 48 hours. This shell provides support for internal structures including the velum, and malformation affects the ability to feed (Lucas & Rangel, 1983). The ability to develop prodissoconch I is limited by low concentrations of Ca^{2+} and dissolved inorganic carbon in seawater. Salinity variations in the Baltic Sea result in a linear decline of $[Ca^{2+}]$ and total inorganic carbon seawater content along the southeastern coast of the basin. In Kiel Fjord (mean salinity of 16 psu), seawater $[Ca^{2+}]$ varied from 3.6 – 7.7 mM, whereas at eastern

areas of Usedom (mean salinity of 7 psu) values were found between 1.5 – 3.2 mM (Thomsen et al., 2017 submitted). Excluding Gulf of Bothnia region, calculated levels of inorganic carbon $[HCO_3^-]/[H^+]$ were above the limiting threshold of 0.13. Larval populations of *Mytilus* sp. from Kiel Fjord and the eastern Baltic are suggested to be calcium limited at $[Ca^{2+}]$ concentrations lower than 3 mM (Thomsen et al., 2017, submitted). Lower $[Ca^{2+}]$ led to decrease in shell length and inability to form a complete prodissoconch I, compromising larval performance.

The bivalve $CaCO_3$ shell is formed by biomineralization processes (Lowenstam and Weiner, 1989) and details of mechanism functioning are not yet fully understood. It is currently accepted that mantel epithelium and hemocytes are responsible for the transport of crystalline carbon calcium (Mount et al., 2004). Additionally, these cells secrete the organic matrix protein where the inorganic part of the shell is formed. Mussel larvae shell is produced by a group of specialized ectodermal cells that transform into the mantle epithelium. A disordered amorphous calcium carbonate precursor (ACC) has been hypothesized to be excreted via exocytoses, transported and integrated to the organic matrix (Mount et al., 2004; Weiner & Addadi, 2011). The ACC can stay in this precipitate state or crystallize into specific calcium carbonate polymorphs (Weiss et al., 2002).

Throughout this experiment, larvae were exposed to low salinity conditions of 7 and 9 psu, where $[Ca^{2+}]$ concentration are suggested to negatively affect calcification processes. The formation and growth of shell requires energy and thus, the low levels of calcium and inorganic carbon found present in the different regions of the Baltic Sea seawater may enhance the cost of calcification. Shifts in the energy budget to cover calcification costs could be a possible outcome. This shift nicely explains the decreased levels of SfG and the lower growth performance of larvae found with decreasing salinities in this experiment. In addition, increasing levels of RR with decreasing salinity treatments were found in this study. These results could reflect the elevation of RMR to cover the increased expenses of calcification and osmoregulation processes.

In this study, larval CR was significantly affected by temperature and salinity. Results were comparable in scale to previous studies using North Sea *M. edulis* larvae (Sprung et al., 1984c). A positive effect of increased temperature (+ 3°C) enhancing CR was found for larvae reared under 9 and 11 psu. Temperature did not significantly change the CR of larvae reared under 7 psu. CR differed significantly between salinity treatments, and was negatively affected by lowering salinities. During larval development, the velum structure is responsible for feeding activity, collecting the food particles suspended in the water column. A string

relationship between larval size and ability to filter feed was previously described for *C. fecunda* by Klinzing & Pechenik (2000). The smaller sizes of larvae reared under 7 psu leading to reduced velum surface area could explain the decline larval CR found in this study. In addition, structural damage of the cilia could be a second contributing factor. Evidence that bivalves avoid osmotic damage of gill frontal ciliary mechanism by closing their valves has been previously reported for adults (Davenport & Fletcher, 1978), suggesting adverse effects of low salinity on this functional structure. Increase in CR correlated with SL was previously described for *M. edulis* larvae (Sprung, 1984c). In the present study, this increase was less pronounced. Constant exposure to low salinities damaging the velum structure of the young larvae may have lead to the lower CR found in this study.

The lower levels of SfG of larvae reared in 7 psu treatments in comparison to 9 and 11 psu agree with tested hypothesis 3. An antagonistic relationship between high temperatures and low salinity on larval energy budgets was not observed within my experiment for all tested salinities (hypothesis 4).

4.3 Genetic identification of larvae

Favourable selection towards *M. edulis*-like or *M. trossulus*-like genotypes as a consequence of lowered salinity conditions was investigated by identifying settled and metamorphosed post-larvae using specific markers developed for these species.

In the present study, Baltic *Mytilus* larvae were identified using a single genetic marker. As a result, more than 90% of genotyped individuals were scored as hybrids at the Me15/16 locus. Adult *M. trossulus*-like collected from the eastern south Baltic coast at Gdansk, Poland, genotyped at the Me15/16 locus were classified mostly as hybrids between *M. edulis* and *M. trossulus* (Beaumont et al., 2005). These results are not according to recent findings from Stuckas et al. (2017) describing a proportion of pure *M. edulis*, hybrid and *M. trossulus* of 0.8 / 0.2 / 0 respectivetly at Ahrenshoop, Germany. This proportion decreased to 0.58 / 0.32 / 0 at Hel, Poland. Further analyses using a greater number of markers are needed to provide a clearer picture of the effect of salinity acting upon selection of mussels at the Baltic Sea. Moreover, results in this study are highly dependent on the genotype of adults used to form the parental pairs. Due to time constraints, the analyses of parents' genetic background and the identification of settled spat with multiple diagnostic markers can only be performed in late 2017. These analyses will be performed in collaboration with Dr. Heiko Stuckas, Loreen Knoebel and Dominique Zeus at Senckenberg Dresden. Thus, the effect of salinity and

temperature acting as selection force towards specific genotypes of Baltic *Mytilus* asks for further analyses before any conclusions can be reached.

4.4 Conclusion

In summary, our laboratory experiments suggest that a decline of 2 psu and 4 psu in salinity had a negative impact on larval survival, growth and settlement success when compared to control salinity (11 psu). In addition, the lowering experimental salinities led to elevated respiration rates and decreased clearance rates of the larvae. This impacted directly on the surplus amount of energy available for growth beyond that required for maintenance (Scope for Growth, SfG) of the young mussels. Larvae raised under increased temperature conditions (15°C) and low salinity treatments (9 psu and 7 psu) showed a strong decrease in SfG. The negative effect of hypoosmotic stress in levels of SfG was less pronounced in control temperature treatments (12°C). The experimental results showed that a 3°C increase in temperature may accelerate the performance of growth, respiration and clearance processes such as survival and settlement success.

Results from the present experiment show evidence that changes in salinity and temperature projected to the end of the 21st century, may strongly impact larval survival and settlement of Baltic Sea *Mytilus* populations. Exposure to low salinities will demand higher allocation of metabolic energy to intracellular osmoregulation processes. In addition, the lower levels of [Ca^{2+}] and modified carbonate chemistry of less saline seawater have an impact on growth and calcification processes of the larvae (Thomsen et al., 2017, submitted).

It has been suggested that drift routes of Baltic *Mytilus* larvae have an average dispersal distance of 10-30 km, occasionally reaching 100 km (Stuckas et al., 2017). Experimental results show that larvae can maintain themselves undeveloped up to 67 dpf under adverse conditions of salinity (7 psu). After this period, when transferred to favourable salinity conditions (11 psu), a small portion of the larvae could perform metamorphosis and settle. Assuming Baltic *Mytilus* larvae developing in the field are also capable of delaying metamorphosis, the short dispersal distance would not allow larvae to reach a suitable settlement site.

It is known that environmental temperature can substantially influence the processes of gametogenesis and spawning in bivalves. Also, synchronized gonad maturation observed in field is strongly influenced by several environmental physical factors including seasonal temperature patterns (Giese and Pearse, 1974). Finally, spawning is triggered when specific

temperature is reached (Loosanoff & Davis, 1963). Thus, shifts in seasonal reproduction patterns of *Mytilus* populations submitted to increased SST may allow organisms to overcome negative effects of warming. The ability to shift seasonal patterns has also been observed in different taxa, e.g. early spring bloom of Baltic Sea phytoplankton communities submitted to a 6°C increase in seawater temperature (Lewandowska & Sommer, 2010).

It is likely that future environmental conditions of the Baltic Sea will play a key role on distribution and abundance of *M. edulis*-like and *M. trossulus*-like in this region.

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