Coping with extreme temperatures:
Thermal preference and critical lower thermal limits
of the common brown shrimp

(*Crangon crangon*, L.)

Dissertation

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SUMMARY

The common brown shrimp (*Crangon crangon*, L.) is a central component of the Wadden Sea ecosystem and represents one of the most valuable fisheries resources in the North Sea. As for all ectothermic organisms, temperature is considered as a central environmental factor for the common brown shrimp. During the annual cycle, brown shrimp are confronted with a wide range of environmental temperatures with thermal extremes during summer and winter. Still, our understanding of the brown shrimp’s thermal biology as well as the behavioral component of thermal selection in this species is limited. This is the more striking as contemporary shifts in the ocean’s water temperatures as well as future scenarios about climate change raise the question how aquatic ectothermic species like the common brown shrimp will respond to these changes. By investigating the mechanisms of temperature selection and thermal preference behavior as well as the brown shrimp’s thermal capacities towards low temperature, the present thesis aims to contribute to a better understanding of the brown shrimp’s thermal biology.

Chapter I describes the design, construction and evaluation of an annular chamber system for thermal preference experiments on aquatic ectotherms. This system was designed to investigate the mechanisms of temperature selection and thermal preference behavior in the common brown shrimp. Chapter I also introduces a computational approach for automated recording and data analysis of thermal preference experiments in annular chamber systems. By means of this program, an *in silico* comparison of different thermal gradient representations and temperature assignment procedures was conducted, evaluating the effect of spatial resolution on thermal preference estimates. The results of this chapter revealed annular chamber systems to represent a powerful tool for determining thermal preferenda of aquatic ectotherms. Automated data recording and analysis simplified the implementation of thermal preference experiments considerably and allowed for highly resolved thermal preference data for even prolonged experimental trials.

Chapter II aims to establish a methodological framework for thermal preference experiments on the common brown shrimp. This chapter therefore investigates and compares the short- and long-term thermoregulatory behavior of adult common brown shrimp by means of acute and gravitational thermal preference experiments using the annular chamber system. For the acute approach, brown shrimp were acclimated to 5 temperatures between 9°C and 19°C and for the gravitational approach to 3 temperatures within the same range. Following acclimation, thermoregulatory and thermal preference behavior of brown shrimp were studied for 2 h (acute) and up to 48 h (gravitational). The results of these experiments revealed...
brown shrimp to be thermosensitive and perform behavioral thermoregulation. Thus, temperature can be considered as a directive factor in the behavior of this species. Acute preferenda were found to be highly affected by prior thermal acclimation resulting in a final thermal preferendum of 15.9°C. In contrast gravitational preferenda were unaffected by the prior thermal history and brown shrimp selected a uniform thermal preferendum when being exposed to the thermal gradient for at least 20-24h. Compared to the acute preferendum, gravitational temperature preferenda were lower and ranged between 13.5-15.0°C after 24 h and 12.0-14.9°C after 48 h, respectively. No significant difference between the 24 h and 48 h thermal preferendum was detected. Based on the results of this chapter, gravitational thermal preferenda of the common brown shrimp can be obtained after 20 h of gradient exposure, representing a shorter period as reported for other aquatic ectothermic species before.

Chapter III uses the annular chamber system from Chapter I to investigate the seasonal thermal preferenda of the common brown shrimp during a 14 month period. Gravitational thermal preferenda were determined for juvenile and adult, male and female brown shrimp of different size groups, sampled at several localities in the German Wadden Sea. These experiments should reveal whether brown shrimp show distinct seasonal thermal preferenda and whether the final thermal preferendum paradigm holds for the common brown shrimp. The results of these experiments revealed a huge variability in thermal selection of the common brown shrimp. Thermal preference of brown shrimp differed throughout the seasonal cycle with low preferenda during winter and high preferenda during late summer. However, thermal preferenda also differed between the cold winter 2010/11 and the less cold winter 2011/12. Statistical analysis by means of a generalized additive model (GAM) revealed seasonality as well as body size as main determinants of thermal selection. Moreover, the temporal development of thermal preference over time also suggested an effect of cohort on thermal selection. Consequently, brown shrimp did not share a common thermal preferendum and thus thermal selection of the common brown shrimp does not comply with the final thermal preferendum paradigm, representing the first evidence for a marine, invertebrate ectotherm.

In Chapter IV, the lower thermal capacity limits of the common brown shrimp were analyzed by means of laboratory experiments determining the critical thermal minima (CT\text{min}) as well as the critical lethal minima (CL\text{min}). CT\text{min} were analyzed in animals acclimated to 4.0, 9.0 and 14.0°C using a cooling rate of -0.2°Cmin\textsuperscript{-1} whereas CL\text{min} were determined at a cooling rate of -1.0°C day\textsuperscript{-1} without prior acclimation. Both types of critical lower thermal limits were obtained for brown shrimp of different body size, gender and maturation state. CT\text{min} were
significantly affected by acclimation temperature and a positive correlation of acclimation temperature and lower thermal tolerance was identified. Depending on the acclimation temperature, CT_{min} in brown shrimp varied from -1.4°C to 2.5°C. In contrast to acclimation temperature, thermal tolerance just varied slightly with gender and no effect of size or maturation state was identified. As brown shrimp even tolerated the lowest temperature that could be established in the setup, the CL_{min} could not be determined. In the CL_{min} experiments, however, a negative relationship of temperature and reactivity within the range of 7°C and 1°C was observed. When triggered with a single electrical pulse, the number of flicks first increased as temperature decreased. This relationship broke down between 1°C and 0°C where an abrupt drop in reactivity of the shrimp became apparent. The results of this chapter demonstrated a high potential of adaptation towards a wide range of temperatures in brown shrimp including even subzero temperatures. However, low responsiveness at temperatures approaching 0°C also reveals that brown shrimp are adversely affected by low temperatures. In addition, low responsiveness of brown shrimp at low temperatures is of high relevance for scientific surveys, as parts of these surveys are being conducted during the cold winter season.
ZUSAMMENFASSUNG


In Kapitel IV wurde die Toleranz von Nordseegarnelen gegenüber tiefen Temperaturen mittels Kurz- und Langezeitversuchen zur Bestimmung der kritischen thermischen Minima (CT\textsubscript{min}) und der kritischen lethalen Minima (CL\textsubscript{min}) untersucht. Zur Bestimmung der CT\textsubscript{min} wurden Nordseegarnelen bei 4.0, 9.0 und 14.0°C akklimiert. Die CT\textsubscript{min} wurden bei einer Kühlungsrate von -0.2°C pro Tag ermittelt. Die Bestimmung der CL\textsubscript{min} hingegen erfolgte bei einer Kühlungsrate von -1.0°C pro Tag und ohne vorherige Akklimierung. Beide Ansätze wurden für Tiere von unterschiedlicher Größe, Geschlecht und Geschlechtsreife durchgeführt. Die CT\textsubscript{min} waren statistisch signifikant von der Akklimationstemperatur beeinflusst und variierten zwischen -1.4°C und 2.5°C. Das Geschlecht der Nordseegarnelen beeinflusste die CT\textsubscript{min} dagegen nur geringfügig. Körpergröße und Geschlechtsreife hatten keinen nachweisbaren Einfluss. Da die Garnelen die niedrigste Temperatur, die in der Versuchsanlage erzeugt werden konnte zu überdauern vermochten, konnte das CL\textsubscript{min} nicht bestimmt werden. In den Versuchen zur Bestimmung der CL\textsubscript{min} konnte allerdings ein negativer Zusammenhang zwischen der Wassertemperatur und dem Reaktionsvermögen im Bereich von 7°C bis 1°C festgestellt werden. Dieser Zusammenhang brach jedoch unterhalb von 1°C abrupt ab und die Nordseegarnelen zeigten ein stark verringertes Reaktionsvermögen zwischen 1°C und 0°C. Temperaturen unterhalb von 1°C können für Nordseegarnelen deshalb als kritisch angesehen werden. Die Ergebnisse dieses Kapitels bestätigten das große Anpassungsvermögen von Nordseegarnelen gegenüber eines weiten Temperaturbereichs. Zudem wurde gezeigt, dass Nordseegarnelen Minustemperaturen überdauern können. Der negative Zusammenhang von Temperatur und Reaktionsvermögen zeigt jedoch auch, dass Temperaturen unter 0°C einen negativen Einfluss auf Nordseegarnelen haben. Die Erkenntnis des stark verringerten Reaktionsvermögens bei
niedrigen Temperaturen ist von hoher Relevanz für wissenschaftliche Feldbeprobungen, die standardisiert auch im Winter durchgeführt werden.
1. GENERAL INTRODUCTION

1.1 The thermal niche of aquatic ectotherms

Animals are confronted with a variety of interacting environmental factors, biotic as well as abiotic, affecting and controlling for their performance, distribution as well as survival. The concept of the ecological niche (e.g., Grinnel, 1917; Elton, 1927; Hutchinson, 1957; MacArthur and Levin, 1967) describes and accounts for this complexity of interacting environmental factors, comprising the uncountable aspects of an organism’s biology. Hutchinson (1957) defined the ecological niche as an \( n \)-dimensional hypervolume, i.e. the set of biotic and abiotic factors that are typical for and characterize a certain species. Based on this niche theory, two species can only hold the same ecological niche if they are conspecifics (Gause, 1934) and thus conspecifics share common requirements. Within the niche concept of Hutchinson (1957, 1978), niche variables have been defined as chemical or physical environmental variables for which competition is not relevant and that are relatively static, i.e. scenopoetic variables, like temperature, light or humidity. On the other hand, resource-related variables or variables being part of interactions, like food or space, etc., are termed bionomic variables.

Within the \( n \)-dimensional hypervolume of environmental factors, temperature represents a key variable and plays a particularly important role. Following Hutchinson (1957), temperature was considered as a scenopoetic variable. Magnuson et al. (1979) and Roughgarden et al (1981), however, highlighted that animals can indeed compete for temperature like for any other resource via the access to optimum temperatures, with severe consequences for ecological performance and fitness. In this regard, the temperature an organism experiences can either be optimal, suboptimal or inadequate (Tracey and Christian, 1986). Besides its direct consequences on fitness and ecological performance, temperature can act as a directive, controlling, limiting as well as a lethal factor (Fry, 1947; Reynolds, 1977a). The range of temperatures where a given species can exist is represented by the width of the thermal niche (Hutchinson, 1957). This width of the thermal niche can either be expressed as the fundamental or as the realized thermal niche, respectively (Fig. 1.1). Here, the fundamental thermal niche refers to that range of temperatures a species can theoretically survive when competition as well as impacts of other niche variables are absent. In contrast, the realized thermal niche refers to that range of the fundamental niche where an animal can survive including the effects of interactions (Hutchinson, 1957).
Figure 1.1: Thermal dependence of a hypothetical thermal reaction norm. The thermal reaction norm is bound by the lower and the upper critical thermal limits. Grey bars indicate the hypothetical frequency of selected temperatures. Note that the most frequently selected temperature is below the optimum temperature. For further details see text. Redrawn and modified after Martin and Huey (2008).

The fundamental as well as the realized thermal niches are bound by extreme temperatures to the lower as well as well as to the upper range. Here, the bounds of the fundamental thermal niche refer to temperatures an animal can theoretically survive whereas the bounds of the realized thermal niche apply for natural conditions. These bounds are represented by the lower and the upper incipient lethal temperature level (LILT, UILT), the critical thermal minimum and maximum (CT$_{\text{min}}$, CT$_{\text{max}}$) as well as the critical lethal minimum and maximum (CL$_{\text{min}}$, CL$_{\text{max}}$) (Fry, 1947; Jobling, 1981; Lutterschmidt and Hutchison, 1997; Beitinger et al., 2000). Differences among these bounds are mainly related to methodological reasons how these values are being determined (Beitinger et al., 2000; Dallas and Ketley, 2011).
1. GENERAL INTRODUCTION

1.2 Thermal preference and behavioral thermoregulation

In contrast to endotherms, ectotherms lack endogenous heat production (Hickman et al., 2007) and thus body temperature is highly affected by ambient temperature. Due to this external control of body temperature, temperature directly affects physiological rates as well as most life history traits, habitat utilization and distribution of ectothermic organisms (Neill & Magnuson 1974; Perry et al. 2005; Bicego et al., 2007). Even though ectothermic organisms lack the ability for endogenous thermoregulatory mechanisms, they still can modulate their body temperature to some extent. Supposing the ability of thermoreception, ectotherms can use behavior to respond towards environmental temperature by avoiding suboptimal and selecting for optimal thermal conditions. This behavioral mechanism of temperature control is called behavioral thermoregulation. By means of behavioral thermoregulation and when given the choice, ectotherms can control body temperature optimizing for physiological processes and minimize adverse temperature effects through external means.

When provided with a thermal gradient spanning the range of the thermal niche, conspecifics of an ectothermic species will repeatedly select a certain temperature over other temperatures (Fry, 1947; Reynolds and Casterlin, 1979a; Angilletta, 2009). This repeatedly selected temperature has been termed “preferred temperature”, “preference temperature” or “thermal preferendum” (Fry, 1947; Reynolds and Casterlin, 1979a). However, thermal selection will not result in a single preferred temperature as ectotherms like fish have been described to shuttle within the thermal gradient and select temperatures within a certain range instead of preferring one specific temperature. Therefore, temperature preference is most adequately represented as thermal preference zone rather than by a single temperature value (Reynolds 1978; Magnuson et al., 1979).

Thermal preference behavior has been observed in almost all evertbrate and vertebrate aquatic ectotherms, like planarians (Tsukuda and Ogoshi, 1985), crustaceans (e.g., Reynolds and Casterlin, 1979b; Lagerspetz and Vainio, 2006; Tattersall et al., 2012) as well as in fish (e.g., Badenhuizen, 1967; Lafrance et al., 2005; Bertolo et al., 2011). The precision and extent of thermoregulatory behavior can vary drastically among ectotherms as stenothermal organisms tend to select preferred temperatures within a rather limited range of temperatures (Hickman et al., 2007). Eurythermic species, in contrast, can cope with a wide range of temperatures and are characterized by larger thermal preference zones (Zahn, 1961; Hokanson, 1977; Magnuson et al., 1979).

The thermal preference zone is closely connected to the physiological temperature optimum. Jobling (1981) therefore suggested that optimal growth temperature of fish can be determined rather quickly by experimentally determining thermal preference. However,
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Jobling (1981) already noted that thermal selection in ectotherms will result in preferenda that are located slightly below the physiologically optimal temperature. Martin and Huey (2008) related this to the reason that ectotherms are not perfect thermoregulators and since temperatures slightly above the optimum will depress fitness considerably more than temperatures below. Ectotherms will thus prefer a temperature below optimum temperature also accounting for the skewness of thermal performance curves (DeWitt and Friedman, 1979; Martin and Huey, 2008; Angilletta, 2009) (Fig. 1.1).

1.3 The “final thermal preferendum paradigm”

Transferring Hutchinson’s (1957) definition of the overall ecological niche to the thermal niche, conspecifics should share common thermal requirements. Thus, thermal selection of conspecifics should result in a similar thermal preference. This rationale was expressed by Fry (1947) in the final thermal preferendum paradigm (FTPP). Fry (1947) defined the final thermal preferendum (FTP) in a bipartite way. First, the FTP was defined as “...temperature at which the preferred temperature is equal to the acclimation temperature” and second “...temperature at which all individuals will ultimately congregate, regardless of their thermal experience...” (sensu Fry, 1947). It has long been accepted that, within a given species, there is only one species specific FTP. Several recent findings, however, challenge the FTPP as factors like nutritional state (Morgan, 1993; Magee et al., 1999; Pulgar et al., 1999, 2003; van Dijk et al., 2002), ontogeny (McCauley & Huggins, 1979; Lafrance et al., 2005), seasonality (Zahn, 1964; Hesthagen, 1979; Clark & Green, 1991; Mortensen et al., 2007), genetic polymorphism (Petersen & Steffensen, 2003; Behrens et al., 2012), reproductive state (Ihnat & Bulkley, 1984; Roscoe et al., 2010) as well as the application of behavioral thermoregulatory tactics (Bertolo et al., 2011) have been found to influence thermal selection and thus the FTP. Thus, these studies revealed that thermal preference seems to be considerably more variable than initially assumed giving rise to distinct preference states within the realized thermal niche of a certain species.

1.4 Methodological approaches for thermal preference experiments

Laboratory based temperature gradient experiments are an effective way to study thermoregulatory behaviour and preferred body temperature of aquatic ectotherms (Coutant, 1977; McCauley 1977; Angilletta, 2009). In laboratory based experiments, potentially confounding variables within the $n$-dimensional hypervolume can be excluded and the mere effect of temperature on thermal selection revealed. In thermal preference experiments, the
test organisms are provided with a wide temperature range of equally accessible temperatures. The thermal range offered to the organisms should span the whole thermal niche of the species being tested.

A variety of experimental setups has been used to perform temperature preference experiments so far (McCauley 1977). Within these experimental setups, spatial gradients in horizontal (Mathur et al., 1982; Chen and Chen, 1991; Lafrance et al., 2005) and vertical (Marek and Gvozdik, 2012) direction as well as temporal gradients have been established (Neill et al., 1972; Beiting et al., 1975). Systems being used comprised two-chamber devices, radial or roseette devices, linear gradient tanks, crossed-gradient devices, transverse and vertical gradient setups, toroidal temperature gradient devices and annular chamber systems (reviewed by McCauley, 1977; Myrick et al., 2004). Here, it will just be referred to the most classical and frequently used systems of temperature gradient devices, namely linear gradient tanks and shuttleboxes as well as a considerably new approach, i.e. annular chamber systems.

Linear gradient tanks of rectangular shape, also called rectangular raceways, represent the most classical setup for temperature preference experiments (McCauley 1977). In general, rectangular troughs, elongated tanks or even tubes have been used as for this type of system (e.g., Badenhuizen, 1967; Chen and Chen, 1991; Lafrance et al., 2005). Linear gradient tanks have been used in various dimensions and water depths and have been made from different materials, although the majority of studies used troughs made of steel (McCauley, 1977; Mathur et al., 1982; Chen and Chen, 1991; Tepler et al., 2011). The horizontal thermal gradient in troughs and tanks has been established in different ways, indirectly via thermal diffusion from water baths or directly by introducing tempered water into the setup (Mathur et al., 1982; Kivivuori and Lagerspetz, 1990; Chen and Chen, 1991; Lafrance et al., 2005; Bates et al., 2010). Rectangular troughs holding a horizontal thermal gradient represent the most frequently used gradient device for thermal preference studies as they are comparatively easy to construct and maintain (McCauley, 1977). By using this type of setup a variety of preference experiments on aquatic ectotherms such as planarians and worms (Tsukuda and Ogoshi, 1985; Bates et al., 2010), crustaceans (Mathur et al., 1982; Taylor, 1984; Re et al., 2006), molluscs (Diaz et al., 2000, 2011; Tepler et al., 2011) and fish (Badenhuizen, 1967; Hesthagen, 1979; Lafrance et al., 2005) has been conducted so far.

Shuttleboxes consist of at least two tanks or chambers (Neill et al., 1972; McCauley, 1977), although setups with up to four chambers have been used (Reynolds, 1977b; McCauley, 1977). The chambers of a shuttlebox are connected allowing the test organisms to move
between the different compartments of the system. In contrast to linear gradient tanks, shuttleboxes do not maintain a thermal gradient in a large water volume, but shuttleboxes use a temporal thermal gradient that is actively modulated by the organism’s behaviour instead. Therefore, shuttleboxes are equipped with photocells or laser beams that are either located in the tunnel between the two chambers (Neill et al., 1972; Reynolds et al., 1976; Mortensen et al., 2007) or within each of the tanks (Staaks et al., 1999; Ohlberger et al., 2008) monitoring the movements of the organisms. The monitoring equipment is connected to the cooling and heating devices. Movements between the compartments cause a temperature modulation maintaining a temperature difference between the two compartments which can be repeatedly selected by the fish. Temperature adjustments between the two compartments are then continued until the animal holds its position, indicating that it obtained its preferred temperature (Neill et al., 1972). Shuttleboxes have been mostly used to study thermoregulatory behaviour in fish as fish learn quickly how to control their body temperature by shuttling between the chambers and are fast enough to escape from sub- or supraoptimal temperatures that might arise in shuttleboxes (e.g., Neill et al., 1972; Staaks et al., 1999; Mortensen et al., 2007). Shuttleboxes, however, have also been used for studies on invertebrate ectotherms like crustaceans (Casterlin and Reynolds, 1977; Reynolds and Casterlin, 1979b; Tattersall et al., 2012).

Compared to these more classical systems, annular chamber systems represent a relatively new experimental approach for measuring thermal preferenda of aquatic ectotherms (Myrick et al., 2004). In the past, several approaches using circular setups have been conducted (McCauley, 1977; Kivivuori, 1994), however, the operation principle of annular chambers is considerably different. Myrick et al. (2004) first used an annular chamber system for aquatic ectotherm thermal preference experiments. Since then, several studies determined temperature preference and thermoregulatory behaviour of fish in annular chamber systems (Chen et al. 2008; McMahon et al. 2008; Gräns et al. 2010, 2012; Klimley et al. 2011; Behrens et al. 2012). Based on the original work from Myrick et al. (2004), systems ranging from 0.22 (Chen et al. 2008) to 3 m (Gräns et al. 2010, 2012; Klimley et al. 2011) in total diameter have been used. All these annular chambers being used so far are comprised of four concentric circles of either acrylic or PVC. The circles delineate three separate channel systems within the annular chamber with the outermost channel representing a reservoir channel. Here, tempered water is introduced into the setup. Water then enters into the second outermost channel through perforated walls, which has been termed swimming channel, holding the test organisms (Myrick et al., 2004). From the swimming channel the
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water flows to the outermost channel, i.e. effluent channel, again by perforated walls. The water is then discarded from the system by a centre drain.

Annular chamber systems have been considered advantageous for aquatic ectotherm temperature preference experiments compared to the aforementioned more classical systems (Myrick et al., 2004). This is mainly due to chamber design providing even light intensity, water depth and evenness in flow rate while corners and single points for rheotaxis are absent. Additionally, annular chambers allow to establish wide temperature gradients, covering the temperature range of even eurythermal species (Myrick et al. 2004). In contrast to shuttlebox systems, most annular chambers used so far (Myrick et al., 2004; Chen et al. 2008; McMahon et al. 2008; Gräns et al. 2010; Klimley et al. 2011) lack automation in experimental monitoring, data recording and subsequent analysis of the temperature preference experiments. These shortcomings make thermal preference experiments in annular chamber systems laborious and time consuming, especially when long-term experiments are being conducted.

1.5 The acute and gravitational method for thermal preference experiments

Based on the bipartite definition of the FTPP, thermal preference can be determined by means of two different experimental approaches. Following to the first definition of the FTPP, thermal preference can be determined by means of short-term experiments (Fry, 1947; Richards et al., 1977; Reynolds and Casterlin, 1979a). In this so called *acute* approach, with experiments typically lasting for 1-2 h (Richards et al., 1977; Reynolds and Casterlin, 1979a), thermal preference experiments are conducted with animals that have been acclimated to a set of different temperatures. The preference test should then reveal that respective temperature where acclimation and preference temperature are equal. In contrast and according to the second definition of the FTPP, long-term experiments are conducted to reveal that temperature where the individuals exposed to a thermal gradient will ultimately congregate (Fry, 1947). In this *gravitational* approach, with experiments lasting for up to 96 h, sufficient time for reacclimation within each experimental trial is provided enabling the tested animals to gravitate to their final or ultimate thermal preferendum (Reynolds, 1978; Reynolds and Casterlin, 1979a). The results obtained by the gravitational approach are therefore unaffected by the previous thermal history of the experimental animals and thus no prior acclimation is needed. So far, both methodologies have been widely used and provided substantial information on thermal requirements as well as the basic thermal biology of a
variety of mollusc, crustacean and fish species (e.g., Badenhuizen, 1967; Coutant, 1977; Reynolds and Casterlin, 1979b; Mathur et al., 1982; Diaz et al., 2000; Tepler et al., 2011).

1.6 The common brown shrimp (*Crangon crangon*, L.)

The common brown shrimp (*Crangon, crangon*, L.) is a prevalent species in European coastal marine and estuarine waters. It is a benthic decapod crustacean that typically inhabits muddy and sandy soft-bottom substrates across the sublittoral and eulittoral zone (Tiews, 1970). The brown shrimp is widely distributed along the European coastline, from the White Sea, to the entire North-East Atlantic coast including the North Sea and the Mediterranean as well as the Adriatic Sea and the Black Sea (Tiews, 1970; Campos and van der Veer, 2008). Brown shrimp are also distributed in the Baltic Sea (Tiews, 1970) and have been reported around Iceland (Gunnarson et al., 2007). Apart from its range expansion, the population genetic structure of the common brown shrimp is not well resolved, yet. Population genetic analysis of brown shrimp around Great Britain and the Baltic Sea based on amplification length polymorphism (AFLP) revealed three main populations, i.e. western Britain, eastern English Channel and a Baltic Sea group (Weetmann et al., 2007). Based on allozymes analysis, Bulnheim and Schwenzer (1993) defined four regional groups, namely the North Sea, Baltic Sea, North Atlantic and the Adriatic Sea. A more recent analysis based on a mitochondrial cytochrome-c-oxidase I fragment also revealed four groups (Luttikhuizen et al., 2008). However, this study found restricted gene flow between the Northeastern Atlantic, western Mediterranean, Adriatic as well as the Black Sea. Still, with the toolbox of molecular biology increasing rapidly, new studies might identify a more structured population. Based on the current knowledge, however, the brown shrimp population is rather consistent over large areas and separation of genetically diverse groups is mainly due to oceanographic barriers (Luttikhuizen et al., 2008).

Brown shrimp are able to cover such a wide range of distribution, as they are extremely adaptable towards a wide range of different environmental factors. Brown shrimp are able to cope with a wide range of salinities and have been reported to occur at 0-35 PSU (Havinga, 1930; Boddeke, 1976; Mees, 1994; Mouny et al., 2000). However, at salinities below 5 PSU reproduction is no longer possible (Havinga, 1930; Cieluch et al. 2005) and larval stages suffer high mortalities (Gelin et al. 2001). Brown shrimp are also highly adaptable to a wide range of temperatures. Apart from the low salinities and high temperatures hydrographical restrictions only exist due to low oxygen concentrations (Hagerman and Vismann, 1995).
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Across its distributional range, brown shrimp are exceptionally numerous. In the North Sea, brown shrimp can reach high densities with up to 60-80 individuals per m² (Boddeke et al., 1986; Beukema, 1992). Due to this high abundance, brown shrimp also play an important ecological role and is considered as a keystone species for coastal, marine ecosystems. Brown shrimp represent an important prey for numerous invertebrate species, fish and water birds (Pihl, 1985; van der Veer and Bergmann, 1987). On the other hand, brown shrimp are strong epibenthic predators for numerous epi- and infaunal species (del Norte-Campos and Temming, 1994; Pihl and Rosenberg, 1984; Oh et al., 2001). Besides its ecological role, high abundances in European waters and a high market value make brown shrimp a commercially important fishery resource with annual landing around 30000 metric tons (ICES, 2011).

Brown shrimp have a complex life cycle with several cohorts per year derived from 2-3 main distinct spawning events (Havinga, 1930). Exact assignment to individual cohorts is difficult, as age determination due to the lack of permanent hard structures is impossible (Tiews, 1970). Female brown shrimp carry eggs throughout most of the year, however, abundance of egg-bearing females during fall is low (Havinga, 1930; Kuipers and Dapper, 1984). Spawning takes place both in winter and summer where larvae hatch from winter- and summer-eggs, respectively. Winter spawning is the central spawning period as the population in the next year is mainly derived from winter eggs (Hufnagl and Temming, 2011).

Following hatch, the common brown shrimp passes through five to six larval stages (Gurney, 1982; Criales and Anger, 1986) after which juvenile brown shrimp become benthic at approximately 5 mm total length (Kuipers and Dapper, 1984). The juveniles migrate towards the coast and reach the shallow nursery grounds at 10-20 mm total length, with highest abundances during May and June (Kuipers and Dapper, 1984; Beukema, 1992). When brown shrimp reach approximately 30 mm in body size, they migrate to deeper waters and tidal gullies again before becoming sexually mature at body sizes around 22-43 mm in males and 30-55 mm in female brown shrimp (Lloyd and Yonge, 1947; Boddeke, 1966; Meixner, 1970; Oh and Hartnoll, 2004). With decreasing water temperatures during autumn, brown shrimp migrate to more offshore waters for overwintering (Havinga, 1930; Lloyd and Yonge, 1947; Boddeke, 1976) where winter spawning occurs.

1.7 Temperature and the common brown shrimp

Albeit its wide distributional range, the North Sea is considered as a one of the most important habitats for the common brown shrimp. Currently, climatic driven changes causing
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Oceanic water temperatures to rise affect local communities as has been shown for the North Sea fish assemblage (Perry et al., 2005; Stenevik and Sundby, 2007; Dulvy et al., 2008). It is so far unknown whether these changes will also affect the North Sea brown shrimp population. Indeed, fisheries catch data indicate some alterations in the distributional range of brown shrimp as landings in the southern part of the North Sea off Belgium and France decreased while an increase of landings has been reported from Danish waters (ICES, 2005). Still, it is so far unresolved, whether this is related to a temperature driven northward shift due to temperature increases in the southern North Sea.

Several studies addressed the role of temperature for the common brown shrimp so far. Campos and van der Veer (2008) extending the synopsis on brown shrimp by Tiews (1970) reviewed the extensive amount of field studies on this species. From these studies, Campos and van der Veer (2008) synthesized that brown shrimp hold a thermal range of 6-30°C. Freitas et al. (2007, 2010) used a dynamic energy budget model on respiration rates of brown shrimp and calculated a thermal capacity range from 0-30°C. Based on their model, they further specified 23°C as physiological optimum temperature for brown shrimp. Hufnagl and Temming (2011) analyzed growth rates based on 25 studies on brown shrimp from the North Sea. Here, it was revealed that optimum temperature for growth differs according to the shrimp’s body size. Juveniles of 20-30 mm showed highest growth rates at 23-27°C. In contrast, brown shrimp of 30-60 mm grew best at 18-22°C. Henderson et al. (2006), however, stated that brown shrimp in the Bristol Channel avoid temperatures above 22°C and van Donk and de Wilde (1981) observed high mortalities during acclimation of adult brown shrimp already around 20°C. Recently, Madeira et al. (2012) reported a CT_{max} of 33.8°C for juvenile brown shrimp of the Tagus estuary being in good agreement with the critical upper thermal range of 30-32.5°C derived by Berghahn (1983), who reported that juvenile brown shrimp in the Wadden Sea seem to avoid temperatures above 27°C.

In contrast to the general thermal requirements and tolerance limits, the role of thermal selection and temperature preference behavior have not been considered for the common brown shrimp so far. Thermal preference behavior, however, might be a crucial mechanism affecting brown shrimp distribution as future climate driven changes were projected to increase the ocean’s water temperatures by 2-4°C until the end of the century (IPCC, 2007). Thermal selection and temperature preference behavior might lead the shrimp to deeper or more northerly waters escaping from thermally unfavorable conditions. Thus, a basic understanding of thermal preference behavior is necessary to explain past and predict future changes with regard to global change. Knowledge about thermal preferenda might further be used in individual based bio-physical models of the North Sea brown shrimp population.
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helping to improve management approaches for a sustainable utilization of the North Sea brown shrimp stock.

1.8 Aims and outline of this thesis

The present study aims to contribute to a better understanding of the common brown shrimp’s thermal biology. On the one hand, this thesis investigates the mechanisms of thermal selection and thermal preference behavior, which has not been considered for the common brown shrimp, yet. On the other hand, the lower critical thermal limits were determined to reveal the thermal capacity of brown shrimp towards low temperatures and thus to complete the current knowledge on the brown shrimp’s thermal niche.

To investigate thermal selection and thermal preference behavior of the common brown shrimp, an experimental as well as a methodological framework for thermal preference experiments on this species had to be established. Chapter I therefore describes the design, construction and evaluation of an annular chamber system and gives detailed information on setup and operation of annular chamber systems. It further illustrates certain pitfalls that have to be considered and eliminated when using such systems. Besides, this chapter introduces an automated, computer based approach for data recording and analysis of thermal preference experiments. By means of this, thermal preference experiments should become less time consuming and laborious and allow for prolonged and continuous periods of observation, still, generating highly resolved data in time. In addition, an in silico comparison of different thermal gradient representations and temperature assignment procedures was conducted to evaluate the effect of spatial resolution on thermal preference estimates and facilitate thermal preference experiments in annular chambers for future approaches.

In Chapter II, the annular chamber system was used to investigate the acute and gravitational thermal preference behavior of adult common brown shrimp. Acute and gravitational thermal preferenda were determined to analyze if brown shrimp are thermosensitive and whether temperature can be considered as a directive factor in the behavior of this species. For the acute approach, brown shrimp were acclimated to 5 temperatures between 9°C and 19°C for two weeks and for the gravitational approach to 3 temperatures within the same range. Acute thermal preference should then reveal whether thermal selection of brown shrimp is affected by prior thermal acclimation and whether a final thermal preferendum can be identified. In contrast, the gravitational approach should reveal whether prolonged exposure to a thermal gradient is affected by prior acclimation and
whether gravitational thermal preference result in thermal preferenda that are unaffected by the prior thermal history. These experiments should provide a methodological framework to conduct a seasonal comparison of thermal selection in brown shrimp.

In Chapter III, the annular chamber system was used to investigate thermal preference behavior of brown shrimp throughout the seasonal cycle. Gravitational preferenda for juvenile and adult as well as female brown shrimp of different body size were determined during a 14 month period. Subsequently, generalized additive models (GAM) were used to identify whether brown shrimp hold a final thermal preferendum and therefore test for the validity of the final thermal preferendum paradigm in a marine invertebrate ecotermic species. GAM were also used to test for the effect of several covariates like season, body size and gender on thermal preference. The data provided by this study should generate a data basis for future individual based modeling approaches or approaches where the principles of thermal selection and thermal preference behavior are incorporated into bio-physical models of the North Sea that analyze effects of past, present and future scenarios of climate change on the brown shrimp population.

Chapter IV determines the critical thermal minima (CT$_{\text{min}}$) and the critical lethal minima (CL$_{\text{min}}$) of male and female brown shrimp of different body sizes and maturation states. These laboratory based experiments were conducted to investigate the lower thermal capacity limits of the common brown shrimp and reveal the lower limits of its thermal niche. This study also investigates the responsiveness of brown shrimp at low temperatures by means of a single electrical pulse. The here presented data are highly relevant for scientific surveys assessing total abundance and biomass of the brown shrimp stock as some of these studies are conducted during periods of low water temperatures.

1.9 References

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CHAPTER I

2. Automation and critical evaluation of an annular chamber for aquatic ectotherm temperature preference experiments

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Abstract

1. Annular chambers represent a novel approach for thermal preference experiments in aquatic ectothermic organisms. Most approaches using annular chambers so far lack automation in data recording and analysis, making temperature preference experiments laborious and time consuming.
2. Here, we describe the design and construction of a modified version of an annular chamber system. We conducted extensive tests to improve the systems' functionality and confirm accuracy of the thermal gradient. Additionally, we present an automated MATLAB routine for data recording and analysis of temperature preference experiments using the common brown shrimp (Crangon crangon, L.) as a test organism. Using this automated routine, we performed an in silico comparison of different thermal gradient representations with various complexities to test for the effect of temperature resolution on the accuracy of thermal preference estimates.
3. The here presented annular chamber produced a stable thermal gradient of Δ23°C, ranging between 3°C and 25°C. Automated recording and data analysis facilitated implementation of long-term experiments and allowed the collection of highly resolved preference data. The in silico comparison revealed a more accurate specification of the preference zone with increasing resolution of the temperature gradient. With regard to spatial resolution of the thermal gradient and assignment of position and temperature data, the in silico comparison demonstrated previous approaches to be inappropriate for benthic and passive species.
4. We present guidelines for annular chamber construction and automation of data analysis in these systems, making annular chambers more handy and applicable for a wide range of preference studies. Besides its use for experiments in annular chambers, the principle of the here presented automated MATLAB routine can be applied to a wide range of behavioural and preference studies.

Keywords: behavior, brown shrimp, climate change, thermal preference, thermoregulation, Wadden Sea
2.1 Introduction
Climate change causing ocean temperatures to rise is currently postulated as one of the main drivers in aquatic ecosystems. Several studies highlighted the consequences of increased seawater temperature on the aquatic life, with changes in geographic distribution and abundance as well as aquatic organisms approaching their physiological limits being the most prominent ones (e.g., Parmesan & Yohe, 2003, Perry et al., 2005; Dulvy et al., 2008). Aquatic ectotherms may be especially affected by climatic driven temperature increases, as ambient temperature directly controls body temperature in these organisms. By this, rising seawater temperatures instantaneously act on physiological rates, affecting most life history traits as well as habitat utilization and distribution (Neill & Magnuson, 1974; Perry et al., 2005; Dulvy et al., 2008; Bertolo et al., 2011). By means of behavioural thermoregulation, however, ectothermic organisms are able to actively control and modulate body temperature, in turn optimising for physiological processes in a heterogeneous thermal environment (Fry, 1947; Reynolds & Casterlin, 1979a; Bicego et al., 2007).
Laboratory based temperature gradient experiments are an effective way to study thermoregulatory behaviour and preferred body temperature of aquatic ectotherms (McCauley 1977). Different methodological approaches have been used so far, with the majority of studies adopting rectangular troughs (Mathur et al., 1982; Lafrance et al., 2005; Tepler et al., 2011) and shuttle-box systems (Neill et al., 1972; Reynolds & Casterlin, 1979b; Staaks et al., 1999; Mortensen et al., 2007) on numerous vertebrate as well as invertebrate aquatic ectothermic species (McCauley, 1977). However, these classical systems have certain drawbacks inherent to their design. The rectangular shape as well as the presence of corners can induce a site-specific bias towards corners or to the ends of the apparatus, especially in thigmotatic species (Badenhuizen, 1967; Bevelhimer, 1996; Dillon et al., 2009). In rectangular systems, currents along the trough might differ providing various points of rheotaxis (McCauley, 1977). The presence of cover, differences in light intensity and pressure that come along with vertical thermal gradients might affect temperature selection as well (McCauley, 1977; Myrick et al., 2004). Additionally, shuttleboxes are not suitable for slow moving species or organisms that may not be able to learn how to behaviourally control the temperature within the experimental system (Kivivuori, 1994; Lagerspetz & Vainio, 2006; Ohlberger et al., 2008).
In contrast to these classical systems, annular chambers represent a new methodological approach for aquatic ectotherm thermal preference studies (Myrick et al., 2004). Annular chambers are considered to be advantageous to more classical systems as they circumvent certain confounding variables present in the aforementioned setups (Myrick et al., 2004). In
annular chambers, light intensity is even, water depth and flow rate are constant and most important is that, due to the annular shape of the swimming channel, thigmotactic cues like corners are absent. In annular chambers wide temperature gradients can be established, covering the temperature range of even eurythermal species (Myrick et al., 2004). Additionally, chamber design is rather flexible and can be individually modified and scaled to meet ones specific experimental requirements.

Custom made annular shaped preference chambers, either based on acrylic or PVC, have been used in several studies on fishes so far (Myrick et al., 2004; Chen et al., 2008; McMahon et al., 2008; Gräns et al., 2010; Klimley et al., 2011; Behrens et al., 2012). Based on the original work from Myrick et al. (2004), systems ranging from 0.22 (Chen et al., 2008) to 3 m (Gräns et al., 2010; Klimley et al., 2011) in total diameter were utilized. Besides the annular shape of the apparatus, however, chamber features and functioning in these studies differed considerably. Temperature monitoring, spatial resolution of the gradient as well as allocation of the test organisms and respective temperature assignment was accomplished differently and at varying complexity. Indeed, the spatial resolution of temperature assignment might influence precision in thermal preference zone determination. This particularly applies to mobile benthic species that are closely associated with the substratum. In contrast to fish that continuously move in a thermal gradient, benthic organisms that gravitate to a respective temperature will remain at a certain position (Hesthagen, 1979; Behrens et al., 2012). Even slight discrepancies in temperature allocation will therefore prompt blurred assignment of the frequented temperatures.

The annular shaped design has proved successful for thermal preference studies in fishes, however, its suitability for invertebrate species like crustaceans has not been evaluated, yet. The objective of the present study was to reproduce and improve an annular chamber based on the original work of Myrick et al. (2004), compiling the information on chamber construction and handling from subsequent studies (Chen et al., 2008; McMahon et al., 2008; Gräns et al., 2010; Klimley et al., 2011; Behrens et al., 2012). As most of the previously used annular chambers lack automation in recording of animal position and temperatures (Myrick et al., 2004; Chen et al., 2008; McMahon et al., 2008; Gräns et al., 2010; Klimley et al., 2011), we established an automated routine for data recording and analysis of thermal preference experiments in MATLAB. The aim of this automated routine was to reduce the presence of an observer for experimental monitoring and data acquisition as well as analysis of thermal preference experiments. Thus thermal preference experiments should become less time consuming and laborious and result in prolonged and continuous periods of observation generating highly resolved data in time. We finally compared five
different approaches on thermal gradient representation and temperature assignment in *silico* using the common brown shrimp (*Crangon crangon*, L.) as an invertebrate test organism.

### 2.2 Materials and Methods

#### 2.2.1 Annular chamber system

The annular chamber system was a modified version of the setup originally described by Myrick et al. (2004) with a total diameter of 145cm and further dimensions as specified (Fig. 2.1, Tab. 2.1). In contrast to the original system, the chamber for the present study was made of concentric polyvinyl chloride (PVC) walls mounted on a glass base using SikaFlex (Sika Germany GmbH, Stuttgart, Germany). Due to the sedentary and bottom associated living of brown shrimp, the holes for water intake were placed below the prospective water surface rather than above as described in Myrick et al. (2004). The holes were drilled at 1.1cm distance each, along four shifted rows over the whole respective height of the water column. By this, we intended to assure a smoothed water inflow into the swimming channel (SC) and avoid thermal stratification. To allow for observation during day and night, the area below the SC was illuminated by 24 equally spaced infrared LEDs (SFH 485 P, 880 nm, OSRAM).

Temperated water was provided from five reservoirs containing water of 3, 9, 14, 19 and 26°C. By these temperature steps, a thermal gradient of ~5°C increments between each of the 8 compartments could be achieved. Hot water (14, 19 and 26°C) was obtained by 3kW immersion heaters (RY330, Redring Electric LTD, Peterborough, UK) and electrical titanium heating rods (600 W, Schego, Offenbach am Main, Germany). Heaters were regulated by temperature sensors (Pt100 RTD temperature probe, JUMO GmbH & Co. KG, Fulda, Germany) connected to an electronic thermostat (Jumo eTRON M, JUMO GmbH und Co KG, Fulda, Germany) keeping temperatures at the respective set value ± 0.2°C. Water was cooled (3°C and 9°C) via the central in-house cooling unit (EUWAB24KAZW1, DAIKIN Airconditioning Germany GmbH, Unterhaching, Germany) charging two titanium heat exchangers (VT04 CD16, GEA Ecoflex, Sarstedt, Germany). The outflow of the heat exchangers was controlled by Pt100 thermocouples (Pt100 Class B sensor, RS Components GmbH, Mörfelden-Walldorf, Germany) connected to a PID process controller (4100+, West Control Solutions, Kassel, Germany). The PID controller regulated a three-way control valve (three-way control valve type 323, Belimo Automation AG, Hinwil, Switzerland) via a modulating rotary actuator (LR24A-SR, Belimo Automation AG, Hinwil, Switzerland) to keep
temperatures at the respective temperature ± 0.2°C. Water was distributed at 3.5 L min⁻¹ (2.0-5.0 L min⁻¹ in the evaluation phase) to each of the 8 compartments of the reservoir channel, resulting in ~100% SC volume exchange min⁻¹ of the SC. For chamber evaluation, test runs were conducted at 6 different flow rates, i.e. 50, 70, 90, 110, 130 and 150% of SC volume exchange min⁻¹ with the thermal gradient established. Dye tests were conducted at all flow rates to control for water flow throughout the SC. Cooled air was injected below the SC to avoid water condensation below the coldest compartment.

Figure 2.1: Schematic illustration of the annular preference chamber setup. a) Top view of the annular preference chamber and water delivery system. (1) water inlet, (2) spherical valve, (3) on-off temperature control switch, (4) heater, (5) pump, (6) reservoir tank, (7) level sensor, (8) divider, (9) center drain, (10) circle d, (11) circle c, (12) circle b, (13) circle a, (14) temperature sensor, (15) PID controller, (16) Pt100 temperature sensor, (17) three-way control valve, (18) heat exchanger water inlet, (19) heat exchanger coolant inlet, (20) heat exchanger coolant outlet, (21) heat exchanger. Light blue lines indicate water and dark blue lines coolant pipes. b) Schematic 3D illustration of the annular preference chamber system. (1) temperature sensor, (2) swimming channel outflow holes, (3) swimming channel inflow holes, (4) v-notch, (5) center drain, (6) circle a, (7) circle b, (8) circle c, (9) circle d, (10) divider, (11) compartment wall, (12) glass base, (13) wooden base.

The temperature gradient in the SC was monitored by 32 equally spaced temperature sensors (DS1820-LC, B+B Thermo-Technik GmbH, Donaueschingen, Germany), attached to
the outer wall of the SC at mid-water depth and connected to a digital USB-thermometer (TLOG64-USB, B+B Thermo-Technik GmbH, Donaueschingen, Germany). Temperature was recorded every 15 sec and visualized in real-time using the PC-Datalogger Software (PC-Datalogger, B+B Thermo-Technik GmbH, Donaueschingen, Germany). Perpendicular to the center of the annular chamber a mirror was mounted at 45°, deflecting the SC to a camera (EcoLine TV7002, ABUS Security-Center GmbH & Co. KG, Affing, Germany) equipped with a daylight filter (SKR FIL 093, Joseph Schneider Optische Werke GmbH, Bad Kreuznach, Germany) and the CAT-filter removed. The camera was connected to a video monitor to allow for continuous surveillance of the setup. To achieve an even and diffuse illumination of the SC, 8 cold cathode tube lights (350V, 2.4W, 6mA, Conrad Electronics, Hirschau, Germany) were mounted in equal distances on a circular PVC frame suspended 1.5m above the experimental chamber. The whole setup was surrounded by a 2m curtain to exclude outside light and avoid any disturbance during the experiments. We conducted initial test runs using brown shrimp without a thermal gradient to check for a potential tank bias.

Table 2.1: Dimensions of the annular chamber system.

<table>
<thead>
<tr>
<th></th>
<th>diameter (cm)</th>
<th>height (cm)</th>
<th>channel width (cm)</th>
<th>water level (cm)</th>
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<tbody>
<tr>
<td>circle a</td>
<td>145</td>
<td>15</td>
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<tr>
<td>circle b</td>
<td>125</td>
<td>15</td>
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<td>circle c</td>
<td>95</td>
<td>5.5</td>
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<td>-</td>
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<tr>
<td>circle d</td>
<td>75</td>
<td>4.5</td>
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<td>reservoir channel (ab)</td>
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<td>10</td>
<td>7</td>
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<td>swimming channel (bc)</td>
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<td>effluent channel (cd)</td>
<td>-</td>
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</table>

2.2.2 Evaluation of the annular chamber

As the proportions of the present chamber deviated from the setup originally described by Myrick et al. (2004), the system was thoroughly evaluated for evenness in flow rate (flow meter, mn 7.5, 0.04 – 10 m/s, Höntzsch GmbH, Waiblingen, Germany), illumination (LI-250A light meter, LI-COR, Lincoln, Nebraska, USA) and development of the thermal gradient. Flow rate was determined at mid water depth at 3 points per compartment, whereas light intensity was measured in the SC center of each compartment taking the 15sec average per measurement. Each measurement was repeated three times. Temperature measurements on the thermal gradient were conducted by a thermocouple (TS-NTC202 temperature
sensor, B+B Thermo-Technik GmbH, Donaueschingen, Germany; calibrated with a Technoterm 9500, Testoterm KG, Lenzkirch, Germany) fixed to a vertically adjustable custom made PVC rack. For temperature measurement, the SC was divided into 64 virtual segments in radial direction. Thirty-two of the transects were in line with the 32 mounted DS1820-LC temperature sensors, whereas the other 32 segments were located between two adjacent sensors. Temperature measurements were conducted according to a fixed, circular grid throughout the SC consisting of 64 x 3 x 3 nodes (eight compartments including eight transitions between the compartments x seven transects per compartment x three water depths x three positions in radial direction) and replicated three times. Temperature measurements with the TS-NTC202 thermocouple were synchronized to the measurements by the mounted DS1820-LC sensors, recording temperature every 2 sec. This resulted in two temperature matrices (TS-NTC202 thermocouple grid and mounted DS1820-LC sensors) that were used to calculate the deviance between the mounted sensors and the grid measurements. The resultant matrix was then used to calculate a spatially resolved 64 x 3 x 3 temperature grid based on the temperature measurements by the mounted DS1820-LC sensors (Fig. 2.2b).

2.2.3 Automation of data recording and analysis
Temperature and image data were recorded using a custom made MATLAB program including the Image Acquisition Toolbox. Temperature data were retrieved from the PC-Datalogger software every 60 sec synchronized to image acquisition. Compilation and assignment of image and temperature data was conducted off-line by a second custom made MATLAB program including the Image Processing Toolbox (see supplementary information for sample code). As a prerequisite for automated analysis, the outer and inner margins of SC were vectorized by means of two ellipses enclosing the annular shaped SC, i.e. the arena for observation (see supplementary information, lines 173-239). We initially assumed a circular shape of the SC in the acquired image, however, the deflection due to the huge size of the mirror added some amount of distortion to the image. Temperature sensor positions and the coordinates of the temperature grid were integrated into this vectorization as well (see supplementary information, lines 260-264 and 120-139).
For off-line analysis of thermal preference experiments, an array of acquired image and temperature data from each experiment was loaded to the program successively (see supplementary information, lines 33-43). The background, recorded at light and dark prior to the experiment (see supplementary information, lines 145-171) was subtracted from the
respective target image, obtaining an image that just contained the difference of both pictures, i.e. the experimental animals. Upon conversion to a binary image, an image erosion and dilation was applied and the resulting blobs were filtered based on their size to remove false positive detections (see supplementary information, lines 271-342). The geometrical center of gravity (approximately mid body position) for each experimental animal was determined and the position within the SC specified according to its xy-coordinates (see supplementary information, lines 321-370). Subsequently, each single animal was assigned to the closest node of the circular temperature grid and the respective temperature ascribed accordingly (see supplementary information, lines 372-442). This procedure was conducted for the whole image and temperature data array, storing position and temperature information of the experimental animals into a consecutive data array which was exported to MS-Excel (see supplementary information, lines 444-460).

2.2.4 In silico comparison of thermal gradient representations

We conducted an in silico comparison of four different approaches of thermal gradient representation and temperature assignment to test for the effect of spatial temperature resolution on thermal preference estimates. By this we intended to potentially reduce the amount of effort for data monitoring and acquisition in prospective studies using annular chamber systems for thermal preference or preference testing on any other environmental factor. The four approaches were derived from previously published studies on annular chambers and temperature monitoring and assignment procedures therein (Myrick et al., 2004; McMahon et al., 2008; Gräns et al., 2010; Klimley et al., 2011).

In approach (1) we used the temperature recordings provided by the evenly spaced DS1820-LC temperature sensors, mounted to the outer wall of the SC (sensor mode). The sensor based mode has been repeatedly applied in annular thermal preference studies (Myrick et al., 2004; Gräns et al., 2010; Klimley et al., 2011; Behrens et al., 2012). For approach (2) we assumed a continuous and linear thermal gradient between the hottest and coldest temperature in the SC, representing the quasi perfect state of the gradient (continuous mode). For this approach, the hottest and coldest temperatures were determined by means of the temperature sensors and all further temperatures were interpolated at uniform intervals of 0.67°C in 64 steps around the whole SC. In approach (3), the temperatures of the five reservoirs were used to create discrete temperature fields for each of the 8 compartments throughout the SC (discrete mode). This approach provided an example for a low spatial resolution of the temperature gradient. All three approaches were compared to approach (4),
i.e. a temperature grid with 64 x 3 x 3 nodes covering the whole SC (grid mode). However, as the shrimp exclusively stayed at the bottom of the SC, we just used the bottom layer for temperature assignment, i.e. 64 x 1 x 3 nodes. We assume the grid scenario to be the best representation of the real state of thermal gradient. The three other scenarios were compared against the grid mode in silico, to ultimately identify the most accurate approach.

2.2.5 Experimental animals and protocol
The common brown shrimp was used as test organism, as this species is a key component for the North Sea coastal ecosystem, a habitat with documented response attributed to climatic-driven changes and contemporary shifts in water temperature (Lotze et al., 2005; Perry et al., 2005; Dulvy et al., 2008; Reise & van Beusekom, 2008). Besides its ecological importance, the common brown shrimp is a highly valuable fishery resource (ICES 2011). The brown shrimp represents an ideal test organism for the system, being both highly mobile and tolerating a wide thermal range (Campos & van der Veer, 2008). As brown shrimp occur at high densities in the field, an approach where multiple animals could be tested within one experimental trial was needed (Richards et al., 1977).

Brown shrimp for the experiments were caught by the research vessel FFS Solea in January 2011, off the Isle of Helgoland (54°20'N, 007°22'E) at 37 m depth. On board of FFS Solea, animals were kept in an aerated tank with surface water flow-through until arrival in Cuxhaven, approximately 5 h post catch. Shrimp were transferred to continuously aerated tanks and transported to the laboratory facilities of the Institute of Hydrobiology and Fisheries Science, University of Hamburg, Germany. Here, animals were maintained in 1 m³ circular tanks at 8 ± 0.5°C with aerated artificial seawater of 30 PSU. The tanks were connected to the in-house temperature controlled recirculating water system with a foam fractionator and a moving bed biofilter. Upon two days of acclimation, shrimp were sorted to the nearest 5 mm total length and transferred to separate temperature-controlled circular tanks and maintained at 8 ± 0.5°C. The brown shrimp were fed dry feed (Marico Advance, Coppens International, Helmond, Netherlands), live Artemia nauplii (SEPArt, Inve Aquaculture, Dendermonde, Belgium) and chopped herring and sprat pieces to apparent satiation every day. Twenty four hours prior to each experiment, 10 animals from one respective size class were dip-netted from the holding units. Sex was then determined based on the appendices of the first and second endopodite (Tiews, 1954) and the brown shrimp were transferred to a separate holding unit with temperature conditions as stated above to minimize handling stress before the experiment. Experiments were started at the following day between 7 and 8 am in the
morning. Brown shrimp were released into the SC with the temperature gradient being established at that segment corresponding to the temperature the shrimp were maintained. Data acquisition was started and animals were left undisturbed throughout the whole experiment. After 20h of exposure to the thermal gradient, temperature preference was analyzed from the last 3h period. To avoid pseudoreplication, the preferred temperature of brown shrimp within one run was calculated as the mean of the median selected temperatures of each single shrimp in one experimental trial (Mathur & Silver, 1980; Karlsson et al., 1984). Three successive trials were conducted using 4.5, 5.5 and 6.5 cm female brown shrimp to test the automated analysis procedure. For each trial, the four different in silico temperature allocation procedures were performed.

2.2.6 Data analysis
Thermal preferenda were calculated as the mean of the median selected temperatures as well as the first and third quartiles, representing the upper and lower limits of the thermal preference zone (Magnuson et al., 1979). Data analysis was conducted in R (R Development Core Team, 2011) using the car (Fox and Weisberg, 2011) and pgirmess (Giraudoux, 2011) packages. Assumptions of normality and homogeneity of variances were determined by means of Shapiro-Wilk’s test and Levene’s test, respectively. If assumptions were confirmed, an ANOVA otherwise a Kruskal-Wallis ANOVA was conducted. Tukey tests or multiple comparison tests were used for post-hoc testing, respectively. During chamber evaluation, we tested whether the SC was illuminated evenly. Based on pretrials at different flow rates (50-150% SC volume exchange min⁻¹), we tested whether a vertical thermal stratification occurred in the SC and validated if the temperature gradient was homogenous in radial direction. We also tested whether brown shrimp showed any site preference in the absence of a thermal gradient.

2.3 Results

2.3.1 Annular chamber and setup evaluation
The presented version of an annular chamber system consistently produced a stable 3-25°C temperature gradient (Fig. 2.2), covering the full thermal niche of the common brown shrimp. Temperatures at individual positions in the SC were considerably stable, varying ±0.2°C. However, the wide thermal gradient of Δ23°C throughout the SC evoked subsequent problems. In the initial phase of setup evaluation, experimenting with different flow rates of 50% to 100% of SC volume exchange min⁻¹, we observed a considerable amount of thermal
stratification in the SC (Kruskal-Wallis ANOVA, p<0.01). Warm water masses spread into surface layers of adjacent compartments, whereas the cold water expanded along the bottom. By increasing flow rates up to 150% SC volume exchange min$^{-1}$ thermal stratification could be eliminated (Kruskal-Wallis ANOVA, p>0.05). High flow rates, however, made the gradient unsteady and volatile, especially at the transition zones between adjacent reservoir compartments as reported previously (McMahon et al., 2008). Additionally, brown shrimp used for successive test runs to determine a potential tank bias without a thermal gradient, appeared to be disturbed by flow rates >110% SC volume exchange min$^{-1}$, as they did not come to rest, continued to swim up and down and tried to escape from the setup.

Following Chen et al. (2008) we mounted small radial dividers (15 x 4 cm) between the 8 compartments. In the present study, the dividers were immersed into the top 1 cm of the water column of the SC (Fig. 2.1). By this we were able to block the proliferation of warm surface water, counterbalancing the thermally induced shearing forces due to the high temperature differences at narrow space. Successive dye tests confirmed an even and linear, radial flow through the SC, with the shrimp not showing any avoidance of dividers in subsequent test runs at 100% SC volume exchange min$^{-1}$. The small dividers promoted an

Figure 2.2: Schematic illustration of the temperature profile in the temperature preference chamber. a) Temperature profile within the swimming channel at mid-channel position. b) Top view of the spatial temperature distribution as determined by means of the temperature grid. Black dots in (b) indicate grid nodes. For illustration purposes the thermal gradient is rotated clockwise by 90° compared to Fig. 2.1a.
even and smooth temperature transition throughout the SC (Fig. 2.2) and eliminated vertical thermal stratification at 100% SC volume exchange min⁻¹ (Kruskal-Wallis ANOVA, p>0.05). At 100% SC volume exchange min⁻¹, current velocities were below detectable limits at all locations. Illumination throughout the SC ranged from 0.149-0.175 W*m⁻², but differed not significantly (one-way ANOVA, p>0.05).

2.3.2 Brown shrimp behaviour
Brown shrimp did not show any site preference in the SC when the thermal gradient was absent (one-way ANOVA, p>0.05). Shrimp dispersed equally throughout the setup showing moderate activity and alternate times of rest. However, there was a slight preference towards the outer and inner walls of the SC indicating positive thigmotaxis in brown shrimp. At 100% SC volume exchange min⁻¹, shrimp exclusively remained at the bottom of the SC showing predominantly pacing locomotion. If swimming, shrimp stayed close to the bottom of the SC as well.

Shrimp released into the thermal gradient behaved considerably different and showed marked differences in individual behaviour. While some shrimp quickly found a target area in the SC and shuttled within a narrow temperature range, others continued to cruise throughout the SC for an extended time period while exploring the whole thermal gradient. Animals entering temperatures >18°C performed U-turns or successively increased locomotor speed until favourable thermal conditions were reattained. However, following 3-5 hours of exposure to the gradient, all animals ended up in a restricted temperature range of ±5°C. The subsequent time was characterized by intermediate shuttling behaviour, with short excursions throughout the SC and subsequent return to the previously frequented temperature areas.

2.3.3 Automation of thermal preference experiments
The automated monitoring of the annular chamber experiments allowed us to record highly resolved temperature preference data as demonstrated by three test runs using 4.5, 5.5 and 6.5 cm female brown shrimp (Fig. 2.3). During the experiments, observer time could be reduced to occasional controls of setup functioning, without permanent presence to record temperatures and animal positions in the apparatus. Using approach (4), i.e. the grid mode, these test runs revealed thermal preference zones for 4.5 cm female brown shrimp ranging from 4.7-7.9°C and 6.2-8.2°C for 5.5 cm females. Female brown shrimp of 6.5 cm selected 4.1-7.7°C. In general, the preferred temperature zone at dark was slightly more variable than
at light. However, the preference zone (median ± 1st and 3rd quartile) narrowed towards the end of the experiments in all groups.

Automated analysis and data association of position and temperature records within MATLAB worked reliably for all three size classes. When testing the 4.5 cm size class, however, automated analysis was more error-prone than for 5.5 and 6.5 cm shrimp, mainly due to size-related limitations in object detection. Error rates for automated object detection ranged from 2% (6.5 cm) to 10% (4.5 cm). We faced two major problems in object detection. First, brown shrimp showed a slight preference towards the outer and inner walls of the SC. By approaching the inner wall, shrimp became occluded by entering the dead angle of the system. Although the shrimps were still visible in the acquired image, the program parameters specified for image processing to avoid false positive detection excluded these shrimp from the data array. This could be adjusted by proper parameterization to some part, but for 4.5

**Figure 2.3:** Thermoregulatory behaviour of female brown shrimp of (a) 4.5, (b) 5.5 and (c) 6.5 cm within the annular chamber system. Boxes include 1st and 3rd quartiles. Whiskers denote 95% of the data range. Shaded areas correspond to observations during scotophase.
cm individuals, this procedure ultimately reached an end. Secondly, having a more or less narrow thermal preference zone, shrimp accumulated at certain areas of the annular chamber, causing distinct objects to merge. However, a manual inspection of the recorded thermal preference data could be achieved within minutes for each single trial to correct for these deficiencies in the automated analysis.

2.3.4 In silico comparison of thermal gradient representations
For in silico comparison, approach (4) (grid mode) represented the reference state as this scenario represented the thermal gradient most accurately. The in silico comparison of the four gradient representations (Fig. 2.4) revealed slight differences in the estimated median preferred temperatures of up to ~1°C between the scenarios (Fig. 2.5, Tab. 2.2). The 1st and 3rd quartiles confining the thermal preference zone differed with up to ~1.6°C (Fig. 2.5, Tab. 2.2). In general, the thermal preference zone became narrower with increasing resolution of the thermal gradient. However, approach (1) (sensor mode) with 32 equally spaced temperature sensors produced a similar thermal preference zone as approach (3) (discrete mode), using a low spatial resolution of the thermal gradient (Fig. 2.4, Tab. 2.2). In contrast, approach (2) (continuous mode), assuming a linear temperature gradient between the hottest and coldest temperature in the SC was comparable to approach (4) (grid mode), correcting each sensor value at a particular position within the SC. Here, the median temperature preferenda were considerably lower with narrower thermal preference zones than in approach (1) (sensor mode) and approach (3) (discrete mode).

2.4 Discussion
2.4.1 Annular chamber setup and functioning
The general setup of the annular chamber for the present study was straightforward. Basic craftsmanship and CAD software tools are sufficient to design and construct an annular chamber in approximately 6-8 weeks of working time. However, we identified certain pitfalls that have to be considered and eliminated when constructing and using such systems. These problems refer to chamber dimensioning, thermal stratification and water flux through the SC. In the first study using an annular chamber system, Myrick et al. (2004) highlighted the chamber design to be rather flexible and stated that chamber dimensions could be easily modified to meet ones specific experimental requirements. The total diameter could be in- or decreased and the SC width and depth be modified to account for experimental animal size
(Chen et al., 2008; McMahon et al., 2008; Gräns et al., 2010). Indeed, total diameter and chamber size are directly linked to the thermal gradient width that can be established within the SC (Chen et al., 2008). For eurythermic species, as in the present study, SC width has to be increased accordingly to provide the full temperature range of the thermal niche. In large setups, however, small specimens are hard to detect due to constraints in camera resolution and object detection. Additionally, size and total chamber volume are directly linked to heating and cooling capacity for the overall setting. This is of particular importance when continuously high flow rates of temperated water are required, especially for long lasting gravitational thermal preference experiments. For the here presented system, a total of ~12.3 kW was needed to create and maintain a thermal gradient as described.

Previous studies using annular chambers highlighted constant and radial flow-through providing a non-thermally stratified temperature gradient in the SC (Myrick et al., 2004; Gräns et al., 2010; Klimley et al., 2011; Behrens et al., 2012). We were therefore surprised by the high amount of thermal stratification in the initial test runs in our system. Thermal stratification was not omnipresent in the SC, but marked temperature differences between surface and bottom water developed especially within the coldest compartment, with up to

**Figure 2.4:** Schematic illustration of *in silico* temperature gradient resolution for four spatially resolved temperature scenarios. a) discrete mode, b) continuous mode, c) sensor mode. Black dots in (c) indicate sensor positions. For illustration purposes the thermal gradient is rotated clockwise by 90° compared to Fig. 2.1a.
\( \Delta 4^\circ C \) at no more than 5.5 cm water depth. For the initial test runs, we started with 50% flow rate, i.e. 50% SC volume exchange min\(^{-1}\), following McMahon et al. (2008) and gradually increased flow rate in 10% steps with subsequent temperature measurements. High flow rates eliminated thermal stratification in our system, but produced volatile temperature gradients. Subsequent dye tests confirmed vast backwash and eddy formation as described by McMahon et al. (2008). Additionally, we found flow rates generated at >110% SC volume exchange min\(^{-1}\) to disturb the shrimp as they did not come to rest, continued to swim up and down in the SC and even tried to escape from the setup. In contrast, low flow rates increased stratification as residence time was increased and an insufficient amount of mixing occurred in the SC. In the present system with small dividers (Fig. 2.1) immersed into the SC, flow rate could be kept at \(~100\%\) while thermal stratification was non-significant. In contrast to previous setups (Chen et al. 2008), these dividers were just immersed into the upper 1 cm water layer to block the shearing forces in the surface water. However, this also reduced the spread of the cold bottom water. Still, the test animals did not show any avoidance of these dividers while shuttling in the SC.

Table 2.2: Thermal preference zone, median preferred temperature and 25% and 75% quartiles for four different spatial temperature gradient representations as derived by the in silico analysis with brown shrimp (Crangon crangon) in a 3-25°C gradient.

<table>
<thead>
<tr>
<th>Mode</th>
<th>Median</th>
<th>25%</th>
<th>75%</th>
</tr>
</thead>
<tbody>
<tr>
<td>grid mode</td>
<td>6.3</td>
<td>4.9</td>
<td>7.9</td>
</tr>
<tr>
<td>continuous mode</td>
<td>6.1</td>
<td>4.9</td>
<td>7.6</td>
</tr>
<tr>
<td>discrete mode</td>
<td>7.5</td>
<td>3.91</td>
<td>9.3</td>
</tr>
<tr>
<td>sensor mode</td>
<td>7.2</td>
<td>4.17</td>
<td>9.1</td>
</tr>
</tbody>
</table>

2.4.2 Automation of data recording and analysis

Automation of image and temperature data recording allowed for highly resolved preference data for an extended experimental period in the present study. Most setups used for thermal preference experiments so far, except for shuttleboxes, relied on an observer recording temperatures and positions of the test animals within the setup (e.g., Myrick et al., 2004; Chen et al., 2008; Diaz et al., 2011). Video or image recording with subsequent data analysis facilitated the analysis of temperature preference experiments in previous studies, especially for prolonged gravitational preference tests (McMahon et al., 2008; Gräns et al., 2010; Klimley et al., 2011). Still, data analysis and assignment of individual temperature and position data for large datasets is laborious and time consuming. Without automated
analysis, huge amounts of video or image footage have to be inspected and position data assigned to the respective temperatures manually. By means of the here presented MATLAB program, data analysis of thermal preference experiments in annular chamber systems could be conducted automatically. Just minor adjustments of the presented MATLAB program are needed (see supplementary information) to adapt the automation routine to other annular chambers. Additionally, the basic principle of position and temperature assignment - or assignment of any other factor - can be transferred to other types of experimental systems as well.

Basically, the MATLAB program performed a simple object detection with subsequent position and temperature assignment based on an annular shape of the arena. We did not apply dynamic background subtraction but used two separate background images (day and night) instead. Additionally, we omitted a tracking module in our program. Due to this, mergence and occlusion of objects was of minor importance, as it was not necessary to detect and follow each individual separately but simple object detection was sufficient. Mergence and occlusion of objects could be partly counterbalanced by a proper parameterization of the automated routine (see supplementary information, lines 290-291). The remaining errors in object detection and temperature assignment could be corrected by a manual inspection of the data exported to MS EXCEL.

Apart from these technical reasons, tracking was omitted in the present approach as the whole group of animals within each trial was treated as one single experimental unit and the mean of the individual medians calculated accordingly. Individual tracking of multiple test organisms generating multiple preference values within the same run would be problematic for statistical reasons (McCauley, 1977). The number of degrees of freedom would be
overestimated, resulting in type I errors as shown for statistical analysis of thermal preference experiments before (Mathur & Silver, 1980; Karlsson et al., 1984). However, our computer program can in fact be used for tracking applications in future studies, as the coordinates of the detected objects are extracted by means of the MATLAB routine off-line (see supplementary information, lines 397-341). For a single object, a slight modification of the provided code would be necessary to accomplish tracking for future applications.

2.4.3 Brown shrimp behaviour
Shrimp showed typical behavioral patterns in the SC, with ortho- and klinokinesis while exploring the thermal gradient. Following a period of acclimation and exploratory movements, however, the shrimp settled within a restricted range of temperatures. Ortho- and klinokinetic behavior revealed thermosensitivity of brown shrimp (Lagerspetz & Vainio, 2006) and showed brown shrimp to be able to thermoregulate behaviorally. The temperature the brown shrimp experienced during husbandry seemed to be of minor importance with regard to the thermal preference zone. On average, brown shrimp selected considerably colder temperatures compared to acclimation temperature. This is potentially due to the prolonged period the shrimp were exposed to the thermal gradient allowing for reacclimation and the shrimp to gravitate towards their final or ultimate thermal preferendum (Fry, 1947; Reynolds, 1978). The thermal preferenda just varied slightly between the three tested size groups, with the lower limit of the temperature preference zone differing as much as $\Delta 1.1^\circ C$ between 4.5, 5.5 and 6.5 cm female brown shrimp. The upper limit was more consistent, with a standard deviation of 0.25°C. However, the presented preferenda should be treated with caution and should not be used to draw conclusions on thermal preference in the common brown shrimp, as we just tested three size classes without replication. Indeed, the annular shape of the present setup can be considered as a prerequisite to study thermal behavior in brown shrimp in future studies, as we observed a positive thigmotactic behavior towards the outer and inner margin of the SC. In a rectangular setup, this positive thigmotaxis could affect thermal selection by an end of tank bias as has been previously reported (Badenhuizen, 1967; Bevelhimer, 1996).

2.4.4 In silico comparison of thermal gradient representations
Temperature preference is most adequately described as a preference zone instead of a single temperature value (Reynolds, 1978; Magnuson et al., 1979). To account for exploratory and shuttling behavior, the median with the 1st and 3rd quartile are commonly
used to describe the thermal preference zone including potential skewness of selected temperatures (Magnuson et al., 1979; Martin & Huey, 2008). Hence, spatial resolution of the thermal gradient and correct temperature assignment are fundamental tasks in thermal preference testing. This was evaluated and confirmed by means of different temperature gradient representations, using different temperature matrices at varying resolution and complexity. All four gradient approaches were found to represent the median adequately, however, the widths of the temperature preference zones differed considerably. The deviations among the four different approaches might partly be due to the specific behaviour of the shrimp. In contrast to demersal or pelagic fish, benthic organisms as shrimp once gravitated settle and do hardly move between shuttling phases. Even slight deficiencies in temperature assignments will therefore accumulate and cause an under- or overestimation of the selected temperature massively.

We chose approach (3) (discrete mode), i.e. eight homogenous temperature fields within the SC assigned by the reservoir temperatures, as an example for a coarse spatial resolution of the thermal gradient. Interestingly, this coarse representation of the temperature gradient delivered comparable estimates of the temperature preference zone as approach (1) (sensor mode) which is commonly applied in annular chamber systems (Myrick et al., 2004; Gräns et al., 2010; Klimley et al., 2011; Behrens et al., 2012). Nevertheless, approach (1) (sensor mode) was found to represent the thermal gradient inadequately when compared to reference state for the thermal gradient, i.e. approach (4) (grid mode) which represented the thermal gradient most accurately. This might be due to the slight inhomogeneity of the temperature field in radial direction that has been reported as a common problem in annular chamber systems (Myrick et al., 2004; McMahon et al., 2008). Apart from that, too few sensors might have been used in the present study and sensor spacing might have been too extensive in relation to the test organism’s size. Previous studies counterbalanced inhomogeneity of the temperature field in radial direction by means of two rows of temperature sensors (Gräns et al., 2010; Klimley et al., 2011). In these studies, the sensors were mounted on the outer as well as the inner wall of the SC and the temperature per transect was calculated as the mean of the closest pair of radial sensor. However, using the same numbers of temperature sensors as in approach (1) (sensor mode) the aforementioned issues could be counterbalanced by means of approach (4) (grid mode) in the present study. To our opinion, the grid mode represented the thermal gradient most accurately. In approach (4) (grid mode), a temperature grid of 64 x 1 x 3 nodes was integrated into the SC allowing for precise temperature measurements as well as a high spatial resolution of the thermal gradient. The estimated thermal preference zones revealed equivalent results as in approach
(2) (continuous mode), where a continuous and linear thermal gradient at uniform intervals of 0.67°C in 32 steps between the hottest and coldest temperatures in the SC was interpolated. For future approaches in annular chamber systems, the approach presented here (4) (grid mode) is therefore recommended to assure a high resolution of the thermal gradient allowing for precise temperature assignment by means of a manageable amount of temperature sensors. However, once the thermal gradient is specified and its continuity and stability confirmed, approach (2) (continuous mode) can be used to avoid the extensive use of sensors.

Overall, automation of data recording and image analysis facilitated thermal preference experiments and reduced the amount of effort considerably, allowing for more complex and comprehensive thermal preference studies in the future. The presented automated analysis can easily be projected to other environmental variables like salinity, ammonia or oxygen concentration, turbidity, etc. Different types of sensors can be integrated into this network to monitor gradients in annular chambers. Additionally, once the gradient is specified and its stability confirmed, a modified version of approach (2) (continuous mode) can be used to assign animal position to respective gradient values. By this, preferenda for a variety of environmental variables can be determined in annular chamber systems.

2.5 Acknowledgements

The authors thank Thomas Neudecker and the crew from the research vessel FFS Solea for providing life brown shrimp for this study. Carmen Czerwinski and Wiebke Bretting are thanked for data validation and laboratory assistance. We thank Marc Hufnagl for his helpful comments on the MATLAB script. This study was partly funded by the Cluster of Excellence “Integrated Climate System Analysis and Prediction” (CiSAP) of the University of Hamburg. We also acknowledge the constructive comments of two anonymous reviewers on an earlier version of this manuscript.

2.6 References


2.7 Supplementary Information

% PROGRAM DESCRIPTION
% MATLAB routine for automated analysis of temperature preference experiments
% using annular chamber systems
% Requires MATLAB 2010b or higher and the Image Processing Toolbox (IPT)
% Being experimental, this script comes with absolutely no warranty or
% liability for accuracy, injury or damages.

% Code structure:
% 1. select directory for image and temperature data
% 2. read temperature data from txt file, correct and reshape data and store
% 3. in matrix
% 4. process background image for image subtraction
% 5. fit ellipse to chamber margins, specify sensor positions and borders
% 6. of temperature fields
% 7. read and process target image, subtract background and plot image with
% 8. within swimming channel
% 9. write data to .xls file

clear all
fclose all
close all

%% 1. select directory for image and temperature data

FolderImages = uigetdir('YOUR DIRECTORY','CHOOSE IMAGE FOLDER');
ImageDataNames = dir(FolderImages)
ImageDataNames = ImageDataNames(3:end);

FolderTempData = uigetdir('YOUR DIRECTORY','CHOOSE TEMPERATURE DATA FOLDER');
TempDataNames = dir(FolderTempData)
TempDataNames = TempDataNames(3:end);

%% 2. read temperature data from txt file, correct and reshape data and store

for kk = 1 : size(ImageDataNames)
    txtData = [FolderTempData,'\',TempDataNames(kk).name];
    fid = fopen(txtData,'r');
    TempData = textscan(fid,'%s');
    fclose all;
    TempData = TempData {1,1}(1:end);
    TempData = TempData';
    TempData = reshape(TempData,18,[]);
end

StrucData(1,:) = TempData(15,[1:37]);
StrucData(3,:) = TempData(4,[2:38]);

% sensors IDs are assigned according to their position in the annular chamber:
% compartment 1 -> 5 = warm -> cold
51
% x_1 = first sensor in compartment x
52
% a = left semicircle; b = right semicircle
53
SensorOrder = ['5_2 ';'5_1 ';'4a_4';'4a_3';'4a_2';'4a_1';'3a_4';'3a_3';
54
'a_2_1';'2a_4';'2a_3';'2a_2';'2a_1';'1_1 ';'1_2 ';'1_3 ';'1_4 ';
55
'2b_1 ';'2b_2 ';'2b_3 ';'2b_4 ';'3b_1 ';'3b_2 ';'3b_3 ';'3b_4 ';'4b_1 ';'4b_2 ;
56
'4b_3 ';'4b_4 ';'5_4 ';'5_3 '];
57
for k=1:size(SensorOrder)
58
% find possible matches for string between sensor IDs and sensor names from temperature txt and write sensor
59
% ID to matrix
60
Final_Data(k,1) = StrucData(1,strmatch(SensorOrder(k,:), StrucData(1,:)));
61
% find possible matches for string between sensor IDs and sensor names from temperature txt and write %
62
temperature data to matrix
63
Final_Data(k,5) = StrucData(3,strmatch(SensorOrder(k,:), StrucData(1,:)));
64
end
65
clear k
66

TempReal = cell2mat(Final_Data(:,5));                                      % transform temperature data from cell to matrix
67
Correct = [0.3; 0.2; 0.25; 0.25; 0.18; 0.26; 0.22; 0.35; 0.32; 0.16; % deviation of sensor temperature data as
68
determined by sensor calibration
69
0.22; 0.5; 0.12; 0.18; 0.12; 0.21; 0.08; 0.18; 0.06; 0.25; 0; 0.2;
70
0.2; 0.2; 0.38; 0.21; 0.21; 0.18; 0.06; 0.38; 0.38];
71
TempReal = [TempReal-Correct];                                             % correct for temperature deviation of sensors
72

% calculate 'virtual' sensors from two adjacent 'real' sensors
73
maxSO = length(SensorOrder);
74
TempVirtual(2:(maxSO),1) = (TempReal(1:maxSO-1,1)+TempReal(2:maxSO,1))/2;
75
TempVirtual(1,1) = (TempReal(1,1)+TempReal(maxSO,1))/2;
76
clear maxSO;
77
TempReal = num2cell(TempReal);                                           % convert numeric to cell
78
TempVirtual = num2cell(TempVirtual);                                      % convert numeric to cell
79

% sensor order for cell array
80
SensorOrder2 = {'5_2      ';'5_1      ';'4a_4     ';'4a_3     ';'4a_2     ';
81
'4a_1     ';'3a_4     ';'3a_3     ';'3a_2     ';'3a_1     ';2a_4     ';
82
'2a_3     ';'2a_2     ';'2a_1     ';'1_1     ';'1_2     ';'1_3     ';
83
'1_4     ';2b_1     ';2b_2     ';2b_3     ';2b_4     ';'3b_1     ';
84
'3b_2     ';3b_3     ';3b_4     ';4b_1     ';4b_2     ';4b_3     ';
85
'4b_4     ';5_4     ';5_3     '};
86
% assignment of virtual sensor names
87
SensorOrderVirt = {'5_2v    ';'5_1v    ';'4a_4/5_1 ';'4a_3v    ';'4a_2v    ';
88
'4a_1v    ';'3a_4v    ';'3a_3v    ';'3a_2v    ';'3a_1v    ';2a_4v    ';
89
'2a_3v    ';'2a_2v    ';'2a_1v    ';'1_1v    ';'1_2v    ';'1_3v    ';
90
'1_4v    ';2b_1v    ';2b_2v    ';2b_3v    ';2b_4v    ';3b_1v    ';
91
'3b_2v    ';3b_3v    ';3b_4v    ';4b_1v    ';4b_2v    ';4b_3v    ';
92
'4b_4v    ';5_4v/5_3v'};
93

% assignment of virtual sensor names
94
Sensors = horzcat(SensorOrderVirt, SensorOrder2); % concatenate sensor names horizontally
95

% spatial correction of temperature fields by a 64 x 1 x 3 node temperature grid
96
% read ASCII-delimited file of spatial temperature correction data
97
CorrectGrid = dlmread('YOURFILE-FOR-CORRECTION.txt', '	');
98
% select bottom layer of 3-Dimensional grid: temperature fields close to outer wall of swimming channel, mid and
99
% inner wall
100
omi_bottom = CorrectGrid(:,1:3);
101
TemperatureCorr = [TempAll TempAll TempAll];
102

% spatial correction of temperature fields by a 64 x 1 x 3 node temperature grid
103
% read ASCII-delimited file of spatial temperature correction data
104
CorrectGrid = dlmread('YOURFILE-FOR-CORRECTION.txt', '	');
105
% select bottom layer of 3-Dimensional grid: temperature fields close to outer wall of swimming channel, mid and
106
% inner wall
107
omi_bottom = CorrectGrid(:,1:3);
108

% spatial correction of temperature fields by a 64 x 1 x 3 node temperature grid
109
% read ASCII-delimited file of spatial temperature correction data
110
CorrectGrid = dlmread('YOURFILE-FOR-CORRECTION.txt', '	');
111
% select bottom layer of 3-Dimensional grid: temperature fields close to outer wall of swimming channel, mid and
112
% inner wall
113
omi_bottom = CorrectGrid(:,1:3);
114

% spatial correction of temperature fields by a 64 x 1 x 3 node temperature grid
115
% read ASCII-delimited file of spatial temperature correction data
116
CorrectGrid = dlmread('YOURFILE-FOR-CORRECTION.txt', '	');
117
% select bottom layer of 3-Dimensional grid: temperature fields close to outer wall of swimming channel, mid and
118
% inner wall
119
omi_bottom = CorrectGrid(:,1:3);
120

% spatial correction of temperature fields by a 64 x 1 x 3 node temperature grid
121
% read ASCII-delimited file of spatial temperature correction data
122
CorrectGrid = dlmread('YOURFILE-FOR-CORRECTION.txt', '	');
123
% select bottom layer of 3-Dimensional grid: temperature fields close to outer wall of swimming channel, mid and
124
% inner wall
125
omi_bottom = CorrectGrid(:,1:3);
126
TemperatureCorr = [TempAll TempAll TempAll];
TemperatureCorr = cell2mat(TemperatureCorr);

CorrBottom(:, :) = [omi_bottom + TemperatureCorr]; % spatial correction of temperature fields

% corrected temperatures fields close to outer wall of swimming channel
CorrBottomOuter(:, 1) = (num2cell(CorrBottom(:, 1)))';
CorrBottomMid(:, 1) = (num2cell(CorrBottom(:, 2)))';
CorrBottomInner(:, 1) = (num2cell(CorrBottom(:, 3)))';

SensorNr = (1:64);
SensorNr = num2cell(SensorNr)';

Final_Data = [Sensors spacer spacer spacer TempAll spacer CorrBottomOuter spacer CorrBottomMid spacer CorrBottomInner spacer SensorNr];
Final_Data = Final_Data';

%% 3. process background image for image subtraction

% read in background image (annular chamber without animals)
IM = imread('YOUR BACKGROUND IMAGE.jpg');
IM_center = IM;
h = fspecial('motion'); % create predefined 2-D filter: 'motion'
IM_background = imfilter(IM_center, h); % apply filter to image

level = graythresh(IM_center); % compute global image threshold
bw1 = im2bw(IM_background, level); % convert image to binary image, based on predefined threshold

% morphological operation 'majority' (Sets a pixel to 1 if five or more pixels in its 3-by-3 neighbourhood are 1s; otherwise, it sets the pixel to 0) on binary image
bw1 = bwmorph(bw1, 'majority', 30);
bw1 = bwareaopen(bw1, 120); % morphologically open binary image to remove small objects (<=120 pixels)

% remove reflections resulting from illumination
% find connected components in binary image (i.e. light spots and swimming channel)
concomp_bw1 = bwconncomp(bw1);
labeled = labelmatrix(concomp_bw1); % create label matrix from bwconncomp structure
numPixels = cellfun(@numel, concomp_bw1.PixelIdxList); % number of elements (i.e. pixels) and number of pixels therein
[biggest, idx] = max(numPixels); % identify biggest element according and define element within numPixels
idxVec = (1:concomp_bw1.NumObjects); % array for number of identified elements
for i = idxVec
    bw1(concomp_bw1.PixelIdxList{i})= 0; % fill light spots
end

clear concomp_bw1 labeled numPixels biggest idx idxVec

%% 4. fit ellipse to chamber margins, specify sensor positions and borders of temperature fields

s = regionprops(double(bw1), 'centroid'); % identify center of swimming channel
Center = cat(1, s.Centroid);

x = Center(1, 1); % x-coordinate of object center
y = Center(1, 2); % y-coordinate of object center
r1 = 299; % radius of circle for outer margin of outer swimming channel wall
r2 = 286; % radius of circle for inner margin of outer swimming channel wall
r3 = 217; % radius of circle for inner margin of inner swimming channel wall
r4 = 224; % radius of circle for outer margin of inner swimming channel wall
xc = 0.0001:1; % vector for ellipse

[x3] = double(x+r3*cos(2*pi*xc)); % x-coordinates, circle for inner margin of inner swimming channel wall
[y3] = double(y+r3*sin(2*pi*xc)); % y-coordinates, circle for inner margin of inner swimming channel wall
[x4] = double(x+r4*cos(2*pi*xc)); % x-coordinates, circle for outer margin of inner swimming channel wall
[y4] = double(y+r4*sin(2*pi*xc)); % y-coordinates, circle for outer margin of inner swimming channel wall
\[ x_2 = \text{double}(x+2r\cos(2\pi xc))' \]  
% x-coordinates, circle for inner margin of outer swimming channel wall
\[ y_2 = \text{double}(y+2r\sin(2\pi xc))' \]  
% y-coordinates, circle for inner margin of outer swimming channel wall
\[ x_1 = \text{double}(x+r\cos(2\pi xc))' \]  
% x-coordinates, circle for outer margin of outer swimming channel wall
\[ y_1 = \text{double}(y+r\sin(2\pi xc))' \]  
% y-coordinates, circle for outer margin of outer swimming channel wall
\[ \text{circle1} = [x_3, y_3] \]  
% xy-coordinates circle for inner margin of inner swimming channel wall
\[ \text{circle2} = [x_4, y_4] \]  
% xy-coordinates circle for outer margin of inner swimming channel wall
\[ \text{circle0} = [x_2, y_2] \]  
% xy-coordinates circle for inner margin of outer swimming channel wall
\[ \text{circleout} = [x_1, y_1] \]  
% xy-coordinates circle for outer margin of outer swimming channel wall

% calculate coordinates of inner ellipse
\[ b = \text{Center}(1,2) - \text{min}(y_3) \]  
% calculate distance of co-vertex from center in x direction, inner ellipse
\[ a = \text{max}(x_4) - \text{Center}(1,1) \]  
% calculate distance of co-vertex from center in y direction, inner ellipse
\[ c = \sqrt{a^2 - b^2} \]  
% calculate distance of vertex from center, inner ellipse
\[ x_{fli} = \text{Center}(1,1) - c \]  
% x-coordinate left focus of inner ellipse
\[ y_{fli} = \text{Center}(1,2) \]  
% y-coordinate left focus of inner ellipse
\[ x_{fri} = \text{Center}(1,1) + c \]  
% x-coordinate right focus of inner ellipse
\[ y_{fri} = \text{Center}(1,2) \]  
% y-coordinate right focus of inner ellipse
\[ t = \text{linspace}(0,2\pi,5000) \]  
% number of ellipse dots
\[ x_e = ((x_{fli} + x_{fri})/2 + (x_{fri} - x_{fli})/(2c)*a\cos(t) - (y_{fri} - y_{fli})/(2c)*b\sin(t))' \]  
% x-coordinates of inner ellipse
\[ y_e = ((y_{fli} + y_{fri})/2 + (y_{fri} - y_{fli})/(2c)*a\cos(t) + (x_{fri} - x_{fli})/(2c)*b\sin(t))' \]  
% y-coordinates of inner ellipse

% calculate coordinates of outer ellipse
\[ aa = \text{max}(x_1) - \text{Center}(1,1) \]  
% calculate distance of co-vertex from center in x direction, outer ellipse
\[ ba = \text{Center}(1,2) - \text{min}(y_2) \]  
% calculate distance of co-vertex from center in y direction, outer ellipse
\[ ca = \sqrt{aa^2 - ba^2} \]  
% calculate distance of vertex from center, outer ellipse
\[ x_{flo} = \text{Center}(1,1) - ca \]  
% x-coordinate left focus of outer ellipse
\[ y_{flo} = \text{Center}(1,2) \]  
% y-coordinate left focus of outer ellipse
\[ x_{fro} = \text{Center}(1,1) + ca \]  
% x-coordinate right focus of outer ellipse
\[ y_{fro} = \text{Center}(1,2) \]  
% y-coordinate right focus of outer ellipse
\[ x_{e} = ((x_{flo} + x_{fro})/2 + (x_{fro} - x_{flo})/(2c)*aa\cos(t) - (y_{fro} - y_{flo})/(2c)*ba\sin(t))' \]  
% x-coordinates of outer ellipse
\[ y_{e} = ((y_{flo} + y_{fro})/2 + (y_{fro} - y_{flo})/(2c)*aa\cos(t) + (x_{fro} - x_{flo})/(2c)*ba\sin(t))' \]  
% y-coordinates of outer ellipse

% vector, center x-coordinate
\[ \text{orix} = \text{repmat}((\text{Center}(1,1)),5000,1) \]  
% vector, center y-coordinate
\[ \text{oriy} = \text{repmat}((\text{Center}(1,2)),5000,1) \]  

% vector for radial orientation
\[ \text{radial} = (0:0.0720144:360)' \]  
% matrix containing radial orientation, x-coordinate center, y-coordinate center and xy-coordinates of outer and inner ellipse
\[ \text{EllipInOut} = [x_e,y_e,x_{e},y_{e}] \]  

% quarter-wise arrangement of ellipse coordinates
\[ \text{quad1} = \text{flipud}(\text{EllipInOut}(2501:3750,:)) \]  
\[ \text{quad2} = \text{sortrows}(\text{EllipInOut}(1251:2500,:),2) \]  
\[ \text{quad3} = \text{flipud}(\text{EllipInOut}(1:1250,:)) \]  
\[ \text{quad4} = \text{flipud}(\text{EllipInOut}(3751:5000,:)) \]  
\[ \text{EllipInOut} = \text{cat}(1,\text{quad1},\text{quad2},\text{quad3},\text{quad4}) \]  

% matrix containing radial orientation, x-coordinate center, y-coordinate center and xy-coordinates of outer and inner ellipse
\[ \text{SCDim} = [\text{radial},\text{WidthSC},\text{DistOri}] \]  

% matrix to specify radial temperature fields
TempFieldx = ((EllipInOut(:,6)) - (EllipInOut(:,2))) + x;
TempFieldy = ((EllipInOut(:,7)) - (EllipInOut(:,3))) + y;
PositionTempField = [radial TempFieldx TempFieldy];

% coordinates for temperature probes | beginning of temperature field | end of temperature field
RawSensorPosition = dlmread('YOUR_SENSOR-POSITION.txt', '	');
SensorPosition = (PositionTempField((RawSensorPosition),1))';
TempFieldStart = (PositionTempField((RawSensorPosition(:,2)),1))';
TempFieldEnd = (PositionTempField((RawSensorPosition(:,3)),1)) - 0.0001';

% add sensor positions, temperature field start and end to Final_Data
Final_Data(2,[1:64]) = num2cell(SensorPosition(1,[1:64]));
Final_Data(4,[1:64]) = num2cell(TempFieldStart(1,[1:64]));
Final_Data(3,[1:64]) = num2cell(TempFieldEnd(1,[1:64]));

filename = [FolderImages,'\',ImageDataNames(kk,1).name];
IMg = imread(filename);
IMOriginal = IMg;
IMg(:,:,2) = IMg(:,:,1);
IMg(:,:,3) = IMg(:,:,2);
IMg = rgb2gray(IMg);
IM = rgb2gray(IM);
level = graythresh(IM_background);
bw = im2bw(IM_background,level);
bw = bwmorph(bw, 'majority', 30);
bw = bwareaopen(bw, 120);
level = graythresh(IM);
bw = im2bw(IM, level);
bw = bwmorph(bw, 'majority', 30);
bw = bwareaopen(bw, 120);
b = regionprops(double(bw), 'centroid');
centroids = cat(1, b.Centroid);
IMg = rgb2gray(IMg);
IM = rgb2gray(IM);
level = graythresh(IM);
bw = im2bw(IM, level);
bw = bwmorph(bw, 'majority', 30);
bw = bwareaopen(bw, 120);
level = graythresh(IM);
bw = im2bw(IM, level);
bw = bwmorph(bw, 'majority', 30);
bw = bwareaopen(bw, 120);
b = regionprops(double(bw), 'centroid');

IMg = uint8(bw).*(IMg);
IM = uint8(bw).*(IM);
IM = IM - IMg;
IM = im2bw(IM, level);
IM = im2bw(IM, level);
IM = im2bw(IM, level);
```matlab
IMAGE_labeled = bwlabel(bw,8); % label connected components in 2-D binary image
centroids = regionprops (IMAGE_labeled,'Centroid'); % find center of the objects, i.e. animals in swimming channel
number_of_objects = size(centroids,1); % number of identified objects

scrsz = get(0,'ScreenSize'); % specify figure size
figure('Position', [1 scrsz(3) scrsz(4)]) % specify screen location
centroids_Crangon = cat(1,centroids.Centroid); % concatenate coordinates of identified objects
imshow(IMOriginal,'InitialMagnification','fit'), axis on; % show image
hold on;

% plot centroids
if (size(centroids_Crangon,2)>0);
    plot (centroids_Crangon(:,1),centroids_Crangon(:,2),'b+');
    for (oc =1:number_of_objects)
        text (centroids_Crangon(oc,1),centroids_Crangon(oc,2),
        num2str(oc),'Color',[1 1 1]);
    end
    filename2=[tolower('Centroids_')+ImageDataNames(kk,1).name,'.jpg']; % save image with plotted centroids as .jpg
    saveas(gcf,filename2);
end
hold off

%% 6. assign object coordinates to position in annular chamber
ObjectPos = centroids_Crangon;
for k=1:number_of_objects
    if (ObjectPos(k,1) < Center(1,1)) && (ObjectPos(k,2) < Center(1,2)) || (ObjectPos(k,1) > Center(1,1)) && (ObjectPos(k,2) > Center(1,2))
        ObjectPos(k,3)= atand((abs(ObjectPos(k,1) - Center(1,1))) / (abs(ObjectPos(k,2) - Center(1,2))));
    else
        ObjectPos(k,3) = atand((abs(ObjectPos(k,2) - Center(1,2))) / (abs(ObjectPos(k,1) - Center(1,1))));
    end
% correction for swimming channel sector and eccentricity of ellipse and image axes
    if ObjectPos(k,2) < Center(1,2) && ObjectPos(k,1) > Center(1,1)% downright quarter of swimming channel
        ObjectPos(k,4) = ObjectPos(k,3) + 180 - 0.64815;
    elseif ObjectPos(k,2) < Center(1,2) && ObjectPos(k,1) < Center(1,1)% bottom left quarter of swimming channel
        ObjectPos(k,4) = ObjectPos(k,3) + 90 + 0.64815;
    elseif ObjectPos(k,2) > Center(1,2) && ObjectPos(k,1) < Center(1,1)% top right quarter of swimming channel
        ObjectPos(k,4) = ObjectPos(k,3) + 270 + 0.64815;
    else
        ObjectPos(k,4) = ObjectPos(k,3) - 0.64815; % top left quarter of swimming channel
    end
end

% calculate distance from object to center
ObjectPos(:,5) = sqrt((ObjectPos(:,1) - Center(1)).^2 + (ObjectPos(:,2) - Center(2)).^2);
for k=1:number_of_objects
    for l=1:length(Dist_CenterRad)
        if (ObjectPos(k,4) > (SCDim(l,1)-0.0702)) && (ObjectPos(k,4) < (SCDim(l,1) + 0.0702))
            ObjectPos(k,6:8) = (SCDim(l,1:3));
        end
    end
end

clear k
```

CrangonPos_final(:,1:5) = ObjectPos(:,1:5);

% write swimming channel sector IDs (1:3) (outer 1, mid 2, inner 3) to matrix
for k=1:number_of_objects
    if (ObjectPos(k,5) > ObjectPos(k,8)) && (ObjectPos(k,5) <= (ObjectPos(k,8)+1/3*(ObjectPos(k,7))))
        CrangonPos_final(k,6)=3;
    elseif (ObjectPos(k,5) > (ObjectPos(k,8)+1/3*(ObjectPos(k,7))) && (ObjectPos(k,5) <= (ObjectPos(k,8)+2/3*(ObjectPos(k,7))))
        CrangonPos_final(k,6)=2;
    else
        CrangonPos_final(k,6)=1;
    end
end

clear k

% write sensor numbers to matrix
Final_Data2(:,1:2) = cell2mat(Final_Data(3:4,:)');            % write start and end of temperature fields to new matrix
Final_Data2(:,3) = cell2mat(Final_Data(13,:)');
for k = 1:number_of_objects
    for n = 1:length(Final_Data2(:,1))                                % identify respective temperature field
        if (ObjectPos(k,4) <= (Final_Data2(n,1)) && (ObjectPos(k,4) >= (Final_Data2(n,2))))
            CrangonPos_final(k,7) = (Final_Data2(n,3));
        end
    end
end
for k=1:number_of_objects
    if CrangonPos_final(k,7) == 0
        CrangonPos_final(k,7) = 1;
    end
end

clear k n

% 7. assign spatially corrected temperature according to object coordinates within swimming channel
SensNr=(1:64)';
MatCorrBottomOuter(:,1) = (CorrBottom(:,1));
MatCorrBottomOuter = cat(2,SensNr,MatCorrBottomOuter);
MatCorrBottomMid(:,1) = (CorrBottom(:,2));
MatCorrBottomMid = cat(2,SensNr,MatCorrBottomMid);
MatCorrBottomInner(:,1) = (CorrBottom(:,3));
MatCorrBottomInner = cat(2,SensNr,MatCorrBottomInner);

% write spatially resolved temperature data of identified objects to matrix
for k=1:number_of_objects
    for m=1:length(SensNr)
        if CrangonPos_final(k,7) == MatCorrBottomInner(m,1) && CrangonPos_final(k,6) == 3
            CrangonPos_final(k,8)= MatCorrBottomInner(m,2);
        elseif CrangonPos_final(k,7) == MatCorrBottomMid(m,1) && CrangonPos_final(k,6) == 2
            CrangonPos_final(k,8)= MatCorrBottomMid(m,2);
        elseif CrangonPos_final(k,7) == MatCorrBottomOuter(m,1) && CrangonPos_final(k,6) == 1
            CrangonPos_final(k,8) = MatCorrBottomOuter(m,2);
        end
    end
end

range1 = sprintf('A%i',(kk));                                              % specify columns in excel worksheet
range2 = sprintf('C%i',(kk)); % specify columns in excel worksheet
range3 = sprintf('B%i',(kk)); % specify columns in excel worksheet
PicName = {ImageDataNames(kk,1).name};

% write data to MS-Excel
xlswrite('YOURFILENAME.xls',PicName,1,range1); % write image name to column A
xlswrite('YOURFILENAME.xls',kk,1,range3); % write number to column B
xlswrite('YOURFILENAME.xls',CrangonPos_final(:,8)',1,range2); % write selected temperatures to column C,...,kk

% clear variables before loop is restarted
clear all
close all
end
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3. Acute and gravitational thermal preference of the common brown shrimp (Crangon crangon, L.)

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Abstract

The common brown shrimp (Crangon crangon, L.) is a central component of the Wadden Sea ecosystem. As for all ectothermic organisms, temperature is a critically important environmental factor for C. crangon, having vast impacts on physiology, behavior and distribution. However, little is known about the thermal biology of C. crangon as well as the behavioral component of thermal selection in this species. To investigate whether brown shrimp are thermosensitive and perform behavioral thermoregulation, the short- and long-term thermoregulatory behavior of female C. crangon was investigated by means of acute and gravitational temperature preference experiments. For the acute approach, brown shrimp were acclimated to 5 temperatures between 9°C and 19°C for two weeks and for the gravitational approach to 3 temperatures within the same range. Hereafter, thermal preferenda were determined in an annular preference chamber. Acute and gravitational thermal preference experiments revealed C. crangon to be thermosensitive and perform behavioural thermoregulation. In the acute approach, a positive correlation of acclimation temperature and preferred temperature was observed. The final thermal preferendum using the acute method was determined as 15.9°C. In the gravitational approach, the rhythm of preferred temperatures was heavily modulated by the photoperiod with C. crangon selecting temperatures more precisely during the scotophase compared to the photophase. However, determined at dark exclusively, no effect of acclimation temperature on gravitational thermal preference after 24 and 48 h was observed. Gravitational temperature preferenda ranged between 13.5-15.0°C after 24 h and 12.0-14.9°C after 48 h, respectively, and did not differ significantly. Based on the findings from the gravitational thermal preference experiments, 24 h of gradient exposure can be considered sufficient to obtain gravitational thermal preferenda that are unaffected by the prior thermal history the test organisms experienced. The here
determined thermal preference values were higher than the average temperature experienced by *C. crangon* in the field. Still, thermal preferenda were considerably lower than previously reported optimum temperatures for *C. crangon*. This could partially be related to ontogenetic or differences among populations.

Keywords: annular chamber, brown shrimp, climate change, ectotherm, temperature preference, thermoregulation
3.1 Introduction
Temperature is considered as a central abiotic environmental factor, profoundly affecting and driving aquatic ecosystems. Environmental temperature is of critical importance for aquatic ectothermic organisms, as ectotherms do not possess the ability of endogenous thermoregulation (Fry, 1947; Bicego et al., 2007). Thus, the temperature in the surrounding directly operates on body temperature, affecting almost all aspects of an ectotherm’s physiology, behavior and ecology (Fry, 1947; Huey and Stevenson, 1979; Bicego et al., 2007; Angilletta, 2009). Supposing the ability of thermoreception, however, ectotherms can use behavior to respond towards environmental temperature by avoiding suboptimal and selection for optimal thermal conditions (Neill and Magnuson, 1974; Lagerspetz and Vainio, 2006; Angilletta, 2009). This thermoregulatory behavior allows ectotherms to actively modulate body temperature in a heterogeneous thermal environment, optimizing physiological processes and minimizing disadvantageous temperature effects through external means (Neill, 1979; Beitinger and Fitzpatrick, 1979; Angilletta, 2009).

Based on Fry’s bipartite definition of the final thermal preferendum (Fry, 1947), thermoregulatory behavior and thermal preferenda of aquatic ectotherms can be revealed by means of two experimental methodologies. Both methodologies rely on laboratory based temperature gradient experiments. As the final thermal preferendum was defined as the (1) “temperature at which the preferred temperature is equal to the acclimation temperature” and (2) “temperature at which all individuals will ultimately congregate, regardless of their thermal experience…” (Fry, 1947), short and long-term experiments can be conducted to elucidate behavioral thermoregulation (Richards et al., 1977; Reynolds and Casterlin, 1979a; Jobling, 1981). Short-term approaches, i.e. acute thermal preference tests, use pre-acclimated test animals that are exposed to a thermal gradient for a reduced period of time. The thermal preference for each acclimation temperature is determined within the first two hours the animals have been introduced into the test apparatus (Richards et al., 1977; Reynolds and Casterlin, 1979a). Subsequently, the final thermal preferendum using the acute method is determined graphically, assigning the temperature where preference equals acclimation temperature among the different acclimation groups (Fry, 1947; Richards et al., 1977; Reynolds and Casterlin, 1979a). For long-term tests, i.e. gravitational thermal preference tests, the experimental organisms are subjected to a thermal gradient until a stable thermal preferendum is reached. Gravitational thermal preference is usually obtained 24-96 hours after the animals were introduced into a thermal gradient (Richards et al., 1977; Reynolds and Casterlin, 1979a). In contrast to the acute method, temperature selection in gravitational preference tests should be unaffected by previous thermal acclimation as well.
as the individual thermal history of the test organisms. In the gravitational approach, sufficient time for reacclimation is provided enabling the tested animals to gravitate to their final or ultimate thermal preferendum (Reynolds and Casterlin, 1979a). So far, both methodologies have been widely used and provided substantial information on thermal requirements as well as the basic thermal biology of a variety of molluscs, crustaceans and fishes (e.g., Badenhuizen, 1967; Hesthagen, 1979; Reynolds and Casterlin, 1979b; Mathur et al., 1982; Diaz et al., 2000; Tepler et al., 2011).

The outcomes of the acute and gravitational approach differ to some extent. Acute preference tests result in a single so called crossover-preferendum, i.e. where preference equals acclimation temperature (Reynolds, 1978). Thermal preference, however, should be considered as a preference zone rather than a single temperature value as aquatic organisms tend to select a range of temperatures (Reynolds, 1978; Magnuson et al., 1979). Acute thermal preference tests therefore merely reveal the effect of prior acclimation and can be used to assess the magnitude of the thermal acclimation capacity of an organism, e.g. via the acclimation response ratio (ARR) (Claussen, 1977) or the direction of the temperature-preference relationship (Johnson and Kelsch 1998). In contrast, gravitational preference tests reveal a zone of preferred temperatures that represents thermal selection under natural conditions more realistically. This temperature preference zone typically spans 2-4°C (Magnuson et al., 1979; Golovanov, 2006). As gravitational preference is not affected by the prior thermal history, this method is a suitable tool to study the effects of a variety of factors on temperature preference, e.g. seasonal and gender related differences, effects of scoto- and photophase or physiological state (Golovanov, 2006). Several studies suggested 24-96 h for receiving gravitational temperature preference (e.g., Richards et al., 1977; Reynolds and Casterlin, 1979a; Golovanov, 2006). However, the time when the final or ultimate preferendum is attained might differ between species and has therefore to be evaluated individually.

The common brown shrimp is a demersal, decapod crustacean species inhabiting a wide area of distribution along the European coast from the White Sea, the eastern Atlantic coast, the Baltic and the Mediterranean Sea to the Black Sea (Tiews, 1970; Campos and van der Veer, 2008). Within the Wadden Sea, which is considered as its main area of distribution, the common brown shrimp occurs at high densities and represents a key species for the ecosystem being an important prey for crustacean species, fishes and birds (Pihl, 1985; van der Veer and Bergmann, 1987) as well as an epibenthic predator of epi- and infaunal species (del Norte-Campos and Temming, 1994; Pihl and Rosenberg, 1984; Oh et al., 2001). In addition, brown shrimp support a commercially important fishery with annual landings of
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~30000t over the last decade (ICES, 2011). Brown shrimp are highly mobile and perform extended tidal, daily and seasonal horizontal as well as vertical migrations that are mainly driven by temperature (Havinga, 1930; Boddeke, 1976; Campos and van der Veer, 2008). Recently, considerable changes in brown shrimp abundance and distribution have been observed. In the southern part of the North Sea off Belgium and France landings are decreasing while an increase of landings has been reported from Danish waters (ICES, 2005). This potentially indicates a temperature driven northward shift of the population as has been previously documented for other marine species (Perry et al., 2005). However, the thermal biology of the common brown shrimp is poorly known which impedes the assessments of climate driven changes on this species. Information on thermal requirements and temperature preferenda are limited and mainly based on thermal reaction norms concerning growth (Meixner, 1969; Hufnagl and Temming, 2011a, 2011b), respiration (van Donk and de Wilde, 1981) and field observations that lack a clear separation of correlated and opposing abiotic as well as biotic factors (Havinga, 1930; Lloyd and Yonge, 1947; Campos and van der Veer, 2008). The behavioral component of brown shrimp’s response towards temperature has not been considered, yet. Indeed, it is unknown whether brown shrimp perform behavioral thermoregulation and if temperature selection is affected by prior thermal acclimation.

To obtain a better understanding of the biology of the common brown shrimp and to evaluate the potential effects of climatic driven changes on this species, basic knowledge concerning its thermal biology as well as the ability of thermoreception and behavioural thermoregulation are essential. The objective of the present study was therefore to determine whether adult female brown shrimp are thermosensitive and thermoregulate behaviourally using an annular chamber system (Myrick et al., 2004; see also Chapter I). By this, we further intended to set a methodological framework for future thermal preference studies on the common brown shrimp.

3.2. Materials and Methods

3.2.1 Animal sampling, maintenance and acclimation
Brown shrimp were sampled at the coast off Büsum (54°07'09''N, 8°51'43''E) at low tide using a push net (2 mm mesh size) in approximately 1 m water depth. Post catch, the animals were transferred to a well aerated water tank with 1:1 artificial sea water (30 PSU) and natural sea water of the sampling location to promote acclimation to husbandry conditions during transport (approximately 2 h) to the laboratory facilities of the Institute of
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Hydrobiology and Fisheries Science, University of Hamburg. Here, brown shrimp were transferred to a 1 m³ circular tank with aerated artificial seawater of 30 PSU connected to an in-house temperature controlled recirculating water system equipped with a foam fractionator and a moving bed biofilter. Upon 1 day of acclimation, animals were sorted to the nearest 5 mm total length (TL) and sex was determined based on the appendices of the first and second endopodite (Tiews, 1954). Female brown shrimp of 5 cm TL were transferred to separate circular holding units connected to the recirculating water system. Subsequently, water temperature was slowly adjusted to obtain the final acclimation temperatures of 9.0 ± 0.1, 11.5 ± 0.1, 14.0 ± 0.05, 16.5 ± 0.1, 19.0 ± 0.1, 21.5 ± 0.2 and 24.0 ± 0.2°C in each respective tank. During thermal acclimation for 14 days, shrimp were fed dry feed (Marico Advance, Coppens International, Netherlands), live Artemia nauplii (SEPArt, Inve Aquaculture, Belgium) and chopped herring and sprat pieces to apparent satiation every day. Animals were maintained at 10:14 L:D photoperiod following Meixner (1969).

3.2.2 Annular chamber system

Acute and gravitational thermal preference tests were conducted in an annular shaped thermal preference chamber described in Chapter I. In brief, the annular chamber used for the present study had a total diameter of 145 cm, holding a 15 cm wide swimming channel of 5.5 cm water depth (Fig. 3.1). Water was distributed at 3.5 l min⁻¹ to each of the 8 outermost compartments of the annular chamber, i.e. reservoir channels, resulting in ~100% water exchange of the swimming channel min⁻¹. Warm water (14, 19 and 25°C) was obtained by 3 kW immersion heaters (RY330, Redring Electric LTD, Peterborough, UK) and electrical titanium heating rods (600 W, Schego, Offenbach am Main, Germany). Cold water (3°C and 9°C) was obtained via the central in-house cooling unit (EUWAB24KAZW1, DAIKIN Airconditioning Germany GmbH) charging two titanium heat exchangers (VT04 CD16, GEA Ecoflex, Sarstedt, Germany). All temperatures were kept at their respective set value ± 0.2°C.

Thirty-two equally spaced temperature sensors (DS1820-LC, B+B Thermo-Technik GmbH, Donaueschingen, Germany) were mounted on the outer wall of the swimming channel at mid-water depth and connected to a digital USB-thermometer (TLOG64-USB, B+B Thermo-Technik GmbH, Donaueschingen, Germany). Temperature was recorded every 15 sec and visualized in real-time using the PC-Datalogger Software (PC-Datalogger, B+B Thermo-Technik GmbH, Donaueschingen, Germany). Within the swimming channel, a stable thermal gradient of 3-25°C was established (Fig. 3.1, Fig. 3.2). Eight cold cathode tube lights (350 V,
2.4 W, 6mA, Conrad Electronics, Hirschau, Germany) for even and diffuse illumination were mounted in equal distances on a circular PVC frame suspended 1.5 m above the experimental chamber. To allow for observation during day and night, the area below the swimming channel was illuminated by equally spaced infrared LEDs (SFH 485 P, 880 nm, OSRAM). Perpendicular to the center of the preference chamber, a mirror was mounted at 45°, deflecting the swimming channel to a camera (EcoLine TV7002, ABUS Security-Center GmbH & Co. KG, Germany) equipped with a daylight filter (SKR FIL 093, Joseph Schneider Optische Werke GmbH, Bad Kreuznach, Germany) and the CAT-filter removed. To allow for continuous surveillance of the test organisms and data recording, the camera was connected to a video monitor. The whole setup was surrounded by a white curtain to avoid any disturbance during the experiments.

![Schematic illustration of the annular temperature preference chamber.](image)

**Figure 3.1:** Schematic illustration of the annular temperature preference chamber. a) Overhead view on the annular chamber system with (1) swimming channel, (2), reservoir channel, (3) divider, (4) center drain, (5) innermost circle, (6) second innermost circle, (7) second outermost circle, (8) outermost circle, (9) temperature sensor, (10) glass base. b) Top view of the spatial temperature distribution as determined by means of the temperature grid. Black dots in (b) indicate grid nodes used to improve spatial resolution of the thermal gradient.

### 3.2.3 Experimental protocol

The acute and gravitational temperature preference tests were conducted after 14 days of thermal acclimation and replicated three times for each acclimation temperature. For each experiment, 10 brown shrimp from one respective acclimation temperature were introduced into the swimming channel with the thermal gradient being established. The shrimp were placed at that section corresponding to their respective acclimation temperature. Acute
thermal preference tests of 9.0, 11.5, 14.0, 16.5 and 19.0°C acclimated brown shrimp lasted for 2 h, whereas brown shrimp acclimated to 9.0, 14.0 and 19.0°C for gravitational preference experiments were left in the swimming channel for up to 48 h. The acute preference tests were conducted at dark exclusively starting at 8 pm. Gravitational preference tests were started between 6 and 7 am and brown shrimp were remained in the annular chamber for 48 h under a 10:14 L:D photoperiod. The 24 and 48 h gravitational thermal preferenda were retrieved within a 3 h period between 5 and 8 am at the next as well the following day.

**Figure 3.2:** Temperature distribution within the swimming channel of the annular chamber system. Temperatures per segment were considerably stable and varied ±0.2°C.

For acute and gravitational preference tests, images of the swimming channel and temperature data of the thermal gradient were recorded automatically. Data were derived every 15 sec for the acute and every minute for the gravitational thermal preference experiments throughout the whole trial. A custom programmed MATLAB routine was used for automated analysis of each experiment offline (see Chapter I). In this MATLAB routine, a temperature matrix was integrated into the swimming channel and the individual position data for each animal assigned to the respective temperatures at each location (for details see Chapter I, Fig. 3.1b).
3.2.4 Data analysis
Acute and gravitational thermal preferenda were calculated as the median selected temperature during the respective period of analysis. The upper and lower limits of the thermal preference zone were represented by the 1st and 3rd quartiles (Magnuson et al., 1979). The final thermal preferendum derived by the acute method was defined as that temperature where preference equals acclimation temperature (Fry, 1947). The temperature preference zone derived by the gravitational method was calculated as the interquartile range of the median selected temperatures.

Data were checked for normality and homogeneity of variance by means of a Shapiro-Wilks test and Levene's test, respectively. One way Analysis of Variance (ANOVA) were conducted to test whether acclimation temperature affected acute and gravitational temperature selection and to reveal if thermal preferenda from the acute and the gravitational method differed. Post-hoc testing was conducted by means of Tukey's HSD tests. All statistical analyses were conducted with the R statistical software (R Development Core Team, 2011) using the car (Fox and Weisberg, 2011) and pgirmess (Giraudoux, 2011) packages.

3.3. Results
Acute thermal preference of brown shrimp was significantly affected by acclimation temperature (one-way ANOVA, p<0.001). Within the range of acclimation temperatures, thermal preference of brown shrimp increased with increasing acclimation temperature between 9.0°C and 14.0°C (Tab. 3.1, Fig. 3.3). Brown shrimp acclimated to 9.0°C and 11.5°C selected a wider range of temperatures, with the distribution of temperature selection being more skewed than in the 14.0, 16.5 and 19.0°C acclimation group. Above 14.0°C, thermal preference leveled off and the animals acclimated to 16.5°C and 19.0° selected similar temperatures as did the 14.0°C acclimation group. Thermal selection in brown shrimp acclimated to 9.0°C was significantly lower compare to the remaining group, except for the 11.5°C group (TukeyHSD, p<0.001). Thermal preferenda between 11.5, 14.0, 16.5 and 19.0°C acclimated brown shrimp did not differ significantly (TukeyHSD, p>0.05). Based on the temperature preferenda derived for the five acclimation temperatures (Fig. 3.3), the final thermal preferendum using the acute method was calculated as 15.9°C.

During the acclimation period, mortality was considerably different between the seven acclimation temperatures. Mortality at 9.0, 11.5, 14.0, 16.5°C was ~10% but increased to >50% in the 19.0°C acclimation group. At 21.5 and 24.0°C, all animals died during the two week acclimation period.
In the gravitational approach, acclimation to 9.0, 14.0 and 19.0°C did not significantly affect thermal preference in brown shrimp after being subjected to a thermal gradient for 24 h (one-way ANOVA, p>0.05) and 48 h (one-way ANOVA, p>0.05) (Fig. 3.4). However, thermal preferenda after 24 h were slightly higher compared to 48 h post release into the gradient. Still, thermal preferenda after 24h and 48h did not differ significantly (one-way ANOVA, p>0.05). The thermal preference zone as determined by the median selected temperatures after 24 h was 13.4-15.0°C, representing a range of $\Delta 1.6^\circ$C. Upon exposure for 48 h, the preference zone slightly decreased to 12.0-14.9°C. Thermal selection after prolonged exposure became less precise as indicated by the higher range of $\Delta 2.9^\circ$C in median selected temperatures.

![Figure 3.2: Acute thermal preference of female adult brown shrimp acclimated to temperatures of 9.0, 11.5, 14.0, 16.5 and 19.0°C. Boxes include the 25% and 75% quartiles of selected temperatures. Error bars denote 95% of the data range. The diagonal line represents the line of equality, i.e. where acclimation temperature and preferred temperature are equal.](image)

During the experiments for the gravitational approach with the brown shrimp being subjected to the thermal gradient for up to 48 h, brown shrimp were observed to thermoregulate behaviorally (Fig. 3.5). During the first 10 h, brown shrimp shuttled intensively throughout the thermal gradient as indicated by an interquartile range of $\sim 10^\circ$C. At the second phase of the
first scotophase, shuttling decreased and temperatures were selected more precisely and remained stable for ~10 h. Here, the interquartile range was reduced to ~5-6°C. Compared to the first photophase, brown shrimp selected higher temperatures during the scotophase. Shuttling became again more pronounced during the second photophase, as indicated by an increased interquartile range, and brown shrimp selected lower mean preferred temperatures. During the second scotophase, again, higher temperatures were selected and thermal selection became more precise. However, temperatures selected during the second scotophase were lower compared to the first one.

**Figure 3.4:** Gravitational thermal preferenda of female adult brown shrimp acclimated to 9.0, 14.0 and 19.0°C after 24 and 48 h of gradient exposure. Boxes include 25% and 75% quartiles of selected temperatures. Error bars denote 95% of the data range.

### 3.4. Discussion

The mechanisms controlling and affecting the thermal behavior of crustaceans are still not well understood as specific thermoreceptors have not been identified in this subphylum, yet (Ache, 1982; Lagerspetz and Vainio, 2006). Several studies on thermoregulatory behavior, thermal preference and avoidance, however, revealed crustaceans to be thermosensitive (e.g., Reynolds and Casterlin, 1979b; Mathur et al., 1982; Diaz et al., 2002; Re et al., 2006).
In their review, Lagerspetz and Vainio (2006) compiled the findings from behavioral studies and on the effect of temperature on motor activity of crustaceans and found indications for thermal sensitivity of crustaceans in the range of 0.2-2.0°C. The results from the present study also clearly demonstrated thermostensitivity and thermoregulatory behavior in the common brown shrimp.

3.4.1. Acute thermal preference

In the acute thermal preference tests, thermal acclimation was found to modify thermoregulatory behavior in brown shrimp as has been shown for other decapod crustaceans before (e.g. Hall et al., 1978; Taylor, 1984; Diaz and Bückle, 1993; Hernandez et al., 1995; Diaz et al., 2002). The effect of acclimation on preference temperature has so far been found to vary considerably between aquatic ectothermic species. Positive and negative relationships as well as absence of any effect of thermal acclimation on temperature preference have been described in aquatic ectotherms (Mathur et al., 1982; Nelson and Hopper, 1982; Johnson and Kelsch, 1998; Perez et al., 2003; Reyes et al., 2011). In the present study, a positive effect of acclimation temperature on thermal preference of brown shrimp was identified (Fig. 3.3).

Johnson and Kelsch (1998) related the differences in temperature-preference relationships of fishes to the annual thermal cycle the animals experienced. Species that are subjected to a relatively high annual temperature cycle exhibited positive responses, whereas stenothermic species, subjected to moderate annual temperature cycle, showed no effect towards acclimation or even a negative temperature-preference relationships (Hernandez et al., 1995; Johnson and Kelsch, 1998; Perez et al. 2003; Reyes et al., 2011). Consequently, the positive temperature-preference relationship observed in the common brown shrimp can be related to and reflects the high annual temperature cycle this species is subjected to (Campos and van der Veer, 2008).

As acclimation temperature increased from 9.0 to 14.0°C, brown shrimp selected increasing median preferred temperatures. However, only when acclimated to 9.0°C, the median selected temperature was significantly different compared to animals that were acclimated to temperatures above 11.5°C. This revealed that the acclimation response at 9.0°C was most pronounced compared to the remaining acclimation temperatures potentially indicating a physiological temperature threshold in the brown shrimp’s metabolism at temperatures around 9°C. Still, animals acclimated to 9.0°C showed the widest range of selected
temperatures compared to the remaining groups. This was also true for the 11.5°C acclimation group, albeit to a lesser extent compared to animals acclimated to 9.0°C.

**Figure 3.5**: Thermoregulatory behavior of adult female brown shrimp in a thermal gradient of 3-25°C during a 48 h day-night cycle. Error bars include 25% and 75% quartiles of selected temperatures. Gray bars close to the x-axis denote scotophase.

As acclimation temperature exceeded the final thermal preferendum of 15.9°C, thermal preference did not increase with acclimation temperature any further and brown shrimp acclimated to 16.5°C and 19.0°C selected similar temperatures. Median selected temperatures of 16.5 and 19.0°C acclimated brown shrimp even decreased slightly compared to the 14.0°C acclimation group. This potentially indicates that the prior acclimation had either no or rapidly lost its effect. Apart from that, the break in the temperature-preference relationship might also imply that brown shrimp of the here investigated body size (~5cm TL) reach their upper bound of the thermal preference zone when temperatures approach 16.5°C. Following Jobling (1981), who found the final thermal preferendum to be highly correlated to the optimum temperature for growth, temperatures close to 16.5°C might exceed the physiological temperature optimum suggesting the onset of adverse temperature effects. This is additionally supported by the temperature related
mortality rates observed during the acclimation phase. At 9.0, 11.5, 14.0, 16.5, mortality was low (~10%) but increased to >50% in the 19.0°C acclimation group. Acclimation to temperatures higher than 19.0°C resulted in 100% mortality during the two week acclimation period. Even in repeated trials to acclimate brown shrimp to 21.5 and 24.0°C, all animals died within the two week period. Therefore, thermal preference experiments with 21.5 and 24.0°C acclimated animals could not be conducted and the acute thermal preference response could only be tested for five instead of seven different temperatures as we initially intended. However, by means of the five different acclimation temperatures, we were able to define the point where acclimation temperature equaled preference temperature (Fry, 1947). Acclimation to 21.5 and 24°C would thus not have any additional effect on the here identified final thermal preferendum determined by the acute method.

The acute final thermal preferendum of 15.9°C was far below previously reported temperature optima and thermal tolerance limits of brown shrimp (Campos and van der Veer, 2008; Freitas et al., 2007, 2010; Madeira et al., 2012). Campos and van der Veer (2008), extending the synopsis on brown shrimp by Tiews (1970) and reviewing numerous field studies on brown shrimp, reported an optimal temperature range of ∆15°C for adult brown shrimp ranging up to 20°C. In contrast, the present study suggests the onset of adverse temperature effects already starting at temperatures above 16.5°C. Freitas et al. (2007, 2010), using a dynamic energy budget model, calculated 23°C as the optimum temperature for brown shrimp and reported a thermal tolerance range of ∆30°C. Hufnagl and Temming (2011b), comparing 25 studies on the effect of temperature on growth rates in brown shrimp, revealed highest growth at 23-27°C in 20-30 mm juveniles and 18-22°C in adult shrimp of 40-60 mm. Recently, Madeira et al. (2012) determined 33.8°C as the critical thermal maximum for brown shrimp. However, most of these previous studies used and considered smaller specimens compared to the present study (Freitas et al., 2007, 2010; Madeira et al., 2012), with juvenile brown shrimp holding higher thermal requirements and tolerance limits compared to adults (Campos and van der Veer, 2008). For adult brown shrimp van Donk and de Wilde (1981) reported high mortalities at 25°C within a 12 h experimental period and high mortality during acclimation to 20°C. As observed in the present study, van Donk and de Wilde (1981) stated that prolonged acclimation at temperature >20°C was not possible for adult brown shrimp. During ontogeny, the optimum temperature range decreases and becomes narrower as the age of brown shrimp increases (Campos and van der Veer, 2008). These ontogenetic differences in thermal requirements have also been ascribed to the spatial separation of juveniles and adults as well as male and female brown shrimp in the field, especially during the summer season (Havinga, 1930; Broekema, 1942; Lloyd and
Yonge, 1947). Therefore, the acute preferenda derived from the present study might in fact be representative for adult shrimp only, reflecting this ontogenetic change in thermal requirements of adult common brown shrimp (Campos and van der Veer, 2008). When compared to the findings by Hufnagl and Temming (2011b), the acute thermal preferendum determined in the present study was still below optimum growth temperatures of adult brown shrimp. However, it has been previously reported that thermal preferenda are usually lower than physiological thermal optima (Martin and Huey, 2008). This has been related to ectotherms being not perfect thermoregulators and that temperatures slightly above the optimum will depress fitness considerably more than temperatures slightly below. This is also considered as the reason why thermal reaction norms are usually skewed (Martin and Huey, 2008). Still, population or cohort specific differences might further account for the discrepancy in thermal preference determined in the present study compared to previous studies, as these factors have been previously shown to affect thermal reaction norms in brown shrimp (Campos et al., 2009; Hufnagl and Temming, 2011a, 2011b).

### 3.4.2 Gravitational thermal preference
In contrast to the acute tests, acclimation temperature did not affect gravitational thermal preference after 24 and 48h, coinciding with the second definition of the final thermal preferendum paradigm (Fry, 1947). Gravitational thermal preferenda were slightly lower compared to acute preferenda but did not differ significantly (Fig. 3.4). When compared to the acute preference tests, brown shrimp selected temperature with similar precision in the gravitational tests as expressed by the narrow thermal preference zones of 13.5-15.0°C and 12.0-14.9°C following 24 and 48 h in the thermal gradient. The range of the thermal preference zone of $\Delta 1.6^\circ$C and $\Delta 2.9^\circ$C following gradient exposure for 24 h and 48 h, respectively, coincides with the findings of thermal sensitivity of crustaceans (Lagerspetz and Vainio, 2006) as well as previous reports on the breadth of temperature preference zones in general (Magnuson et al., 1979; Golovanov, 2006). There is fairly good agreement between results obtained by the acute and the gravitational approach (Richards et al., 1977; Reynolds, 1978; Jobling et al., 1981; Diaz et al., 2007; Gonzalez et al., 2010), albeit Richards et al. (1977) and Reynolds (1978) noted that results from both methodologies differ to some extent. Other studies found no difference in thermal preference between acute and gravitational tests for fish and crustacean species (Diaz and Buckle, 1993; Hernandez et al., 1995; Perez et al., 2003; Diaz et al., 2007). Badenhuizen (1967) found thermal preferenda for the acute and gravitational method to differ in tilapia that
selected higher temperatures after being subjected to a thermal gradient for an expanded time period. In contrast, de Vlaming (1971) and Reynolds and Thomsen (1974) observed a decline in preference temperature when fish were left in the gradient for up to 4 days. In the present study, a slight decline in preferred temperature after prolonged gradient exposure was observed for brown shrimp as indicated by the thermal preference zones. Brown shrimp selected a narrow thermal zone of 13.4-15.0°C after 24 h. Following 48 h of gradient exposure, the upper range of the median selected temperature range remained almost identical but shrimp also selected slightly cooler temperatures and the lower range of the median selected temperatures decreased to 12.0°C. This decline, however, was marginal and might be related to nutritional correlates due to prolonged exposure to the thermal gradient without feeding (Magee et al., 1999; Despatie et al., 2001; van Dijk et al., 2002).

Apart from thermal acclimation, which had no effect on gravitational preference, we found thermal selection in brown shrimp to be highly affected by the light cycle (Fig. 3.5) as thermal preferenda during the photophase were considerably lower than at dark. Additionally, the 48 h gravitational preference trials revealed thermal selection of brown shrimp to be more precise during dark compared to light-phases. Due to this reason as well as the nocturnal activity of brown shrimp (Tiews, 1970; del Norte-Campos and Temming, 1994), thermal preference of brown shrimp should preferably be determined at dark. This was also the reason why we conducted acute thermal preference tests at dark exclusively. We initially expected a greater variability in thermal selection during the scoto- than the photophase as brown shrimp were reported to be more active during night than day (del Norte-Campos & Temming, 1994). Therefore higher activity should increase shuttling and therefore expand the thermal preference zone at night. In contrast, the here observed increased shuttling during light might in fact be caused by the experimental conditions in the annular chamber system. During day, brown shrimp usually burry in the substratum and remain covered until dusk especially at clear water to avoid predators (Tiews, 1970). However, sediment could not be provided in the current experimental setup as this would interfere with object recognition as well as the high flow rate to avoid thermal stratification. During night, brown shrimp leave their shelter and the absence of sediment disturbing influence of the experimental system is considered to be of less importance. Thermal preferenda derived during the scotophase should therefore be unaffected by the experimental conditions in the annular chamber system.

Based on the findings from the 48 h gravitational approach, the threshold when brown shrimp obtain gravitational thermal preference could be clearly identified. Following a period of intense shuttling during the first 10 h, brown shrimp selected a narrow thermal zone during
the first scotophase. Indeed, consistency and the precise temperature selection during a 10 h period in this first scotophase indicated that shuttling behavior ceased and thermal selection reached a terminal value. This endpoint might indicate that the shrimp reached their final thermal preference that was only interrupted as the second photophase started where intense shuttling set in again. At the beginning of the second scotophase, shuttling decreased. However, shuttling during the second scotophase was higher compared to the first. This, however, might be related to nutritional correlates and brown shrimp searching for food that was more pronounced in the second scotophase as they were not fed during the experiments. Consequently, 48h of gradient exposure are probably too long and 24 h can be considered as sufficient to determine the final thermal preferendum in the common brown shrimp in future studies.

3.4.3 Ecological significance
Based on the findings from the present study, it still remains speculative whether the previously reported northward shift of brown shrimp (ICES, 2005) can be considered as just an occasional expansion of the distributional range as reported for brown shrimp off the coast of Iceland (Gunnarsson et al., 2007) or as a permanent shift reflecting avoidance of high seawater temperatures. The here determined preferenda suggest that brown shrimp have to cope with temperatures below their preference during the most time of the year. Acute and gravitational thermal preferenda were considerably higher compared to the mean annual North Sea temperature (ICES, 2012). The lack of an acclimation response at temperatures above 16.5°C as well as the high mortalities in temperatures ≥19.0°C, however, indicates that higher temperatures can have drastic consequences for brown shrimp, at least for large brown shrimp used in the present study. To ultimately reveal potential effects of current and future climatic driven changes on the common brown shrimp, an extended experimental approach, determining seasonal and size specific preferenda, the interaction with food as well as the effects of extreme temperature events for shorter periods is needed. These data could then be integrated into spatially resolved numerical models of the North Sea to evaluate the effects of future projections of expected temperature increases on brown shrimp distribution.

3.5 Acknowledgements
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3.6 References


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ACUTE AND GRAVITATIONAL THERMAL PREFERENCE

Sage, Thousand Oaks, California, USA.


CHAPTER III

4. Seasonal variability in preferred temperature reveals invalidity of the “final thermal preferendum” paradigm for a marine, invertebrate ectotherm

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Abstract

1. Ectothermic organisms rely on behavioral thermoregulation to modulate body temperature and the final thermal preferendum (FTP) paradigm (FTPP) postulates that a given species holds a species-specific FTP. However, the FTPP has been challenged by a considerable amount of recent findings on vertebrate ectothermic organisms, indicating that the FTP is not as static as originally postulated but can be modulated by various factors. In contrast to vertebrates, evidence from invertebrate ectotherms concerning the validity of the FTPP is far more limited.

2. To test for the validity of the FTPP in a marine invertebrate ectothermic species, we determined thermal preferenda of the common brown shrimp (Crangon crangon, L.) throughout a 14 month period. Thermal preferenda for females and males of different size groups were measured in an annular chamber system using the gravitational method for thermal preference determination.

3. The results of our study revealed that brown shrimp selected a wide range of temperatures and did not show a single or a constant thermal preferendum. Thermal preference was modulated during the 14 month time course following a seasonal cycle suggesting the existence of distinct seasonal thermal preference zones. Brown shrimp selected low temperatures during winter, however, we observed a great difference in preferred temperature between a cold and a relatively mild winter. Highest thermal preferenda were observed after field water temperatures exceeded the annual temperature peak in early autumn.

4. Besides season, body size of brown shrimp significantly affected thermal selection and small individuals selected higher temperatures compared to large ones. However, we
observed a significant interaction of body size and the temperature the shrimp were caught. We also found evidence that cohort identity as well as female reproductive state may affect thermal preference.

5. The results of our study reveal, that thermal selection in brown shrimp is not static and that the FTP can be modulated by various factors, representing the first evidence for a marine, evertedate ectotherm. Therefore, thermal selection of the common brown shrimp does not comply with the FTPP.

Keywords: annular chamber, climate change, final thermal preferendum, thermoregulation
4.1 Introduction

The majority of water breathing organisms is ectothermic, meaning that their body temperature is regulated through external means. In contrast to endothermy, ectothermy implies that body temperature is not determinate and derived from internal heat production but closely follows the temperature in the surrounding (Hickman et al., 2007). Being totally subjected to the ambient thermal conditions, environmental temperature therefore heavily impacts virtually all aspects of an ectotherm’s behavior and physiology and is thus considered as the most critical environmental factor for these organisms (Fry, 1947; Huey & Stevenson, 1979; Bicego et al., 2007; Angilletta, 2009).

Even though ectothermic organisms lack the ability for endogenous thermoregulatory mechanisms, they can still modulate body temperature to some extent. When given the choice, temperature will exert its directive effect (Fry, 1947; Reynolds, 1977) and ectotherms can use behavior to actively select a certain temperature within a heterogeneous thermal environment (Fry 1947; Hutchison & Maness, 1979; Bicego et al. 2007). By means of this behavioral thermoregulation, ectotherms can control body temperature optimizing for physiological processes and minimize adverse thermal effects.

It has long been accepted that behavioral thermoregulation of ectotherms will result in a final thermal preferendum (FTP) that is typical for a given species. This so called final thermal preferendum paradigm (FTPP) was originally defined by Fry (1947) and constitutes that temperature where all individuals of a certain species will ultimately aggregate, regardless of the thermal history they experienced beforehand. However, an increasing number of studies on behavioral thermoregulation of vertebrate aquatic ectotherms challenge the FTPP as several factors have been shown to affect thermal selection. Thermal preference was found to be altered throughout the seasonal cycle resulting in lower thermal preferenda during winter compared to summer (Zahn, 1964; Hesthagen, 1979; Clark & Green, 1991; Mortensen et al., 2007). Ontogeny, with juvenile specimens holding higher thermal preferenda compared to adults was also shown to affect thermal selection (McCauley & Huggins, 1979; Lafrance et al., 2005) as well as feeding and nutritional state (van Dijk et al., 2002; Pulgar et al., 2003), where starving or specimen fed a reduced ration selected colder temperature compared to well fed ones (Morgan, 1993; Magee et al., 1999; Pulgar et al., 1999; Despatie et al., 2001). Besides this, genetic polymorphism (Petersen & Steffensen, 2003; Behrens et al., 2012), reproductive state (Ihnat & Bulkley, 1984; Roscoe et al., 2010) as well as the application of behavioral thermoregulatory tactics (Bertolo et al., 2011) have been found to influence thermal selection and thus the FTP.
In contrast to the findings obtained from studies on vertebrate aquatic ectotherms, evidence with regard to the general validity of the FTP from invertebrate aquatic ectotherms like crustaceans is still limited. Several studies investigated thermoregulatory behavior in aquatic crustaceans, revealing crustaceans to be thermosensitive (reviewed in Lagerspetz & Vainio, 2006). Laboratory based experiments using the acute method, i.e. short-term experiments to determine thermal preference, revealed thermal preferenda of various crustaceans species to be affected by previous thermal acclimation (e.g., Mathur et al., 1982; Diaz et al., 2002; see also Chapter II). In long-term laboratory experiments, i.e. the gravitational method, differences in thermal selection between photo- and scotophase have been detected (Bückle et al., 1994; Taylor, 1984) whereas other studies found no such differences (e.g., Reynolds & Casterlin, 1979; Taylor, 1984; Gonzalez et al., 2010). However, studies on the effects of season, gender or ontogeny on thermal selection or studies combining these factors to test for the validity of the FTPP have - to our knowledge - not been conducted for crustacean species, yet.

The common brown shrimp (Crangon crangon, L.) is an ideal candidate to test for the validity of the FTPP in an invertebrate aquatic ectothermic species. Brown shrimp hold a wide thermal range (Campos & van der Veer, 2008) potentially allowing for shifts in its FTP throughout the seasonal cycle. Seasonal variability in thermal preferenda might also explain the extended seasonal migrations reported for brown shrimp (Boddeke, 1976) with higher temperatures experienced during summer and lower during the winter season. On the other hand, the seasonal migration might imply that brown shrimp track a certain temperature in the field and hold a final thermal preferendum within a narrow range. Besides seasonality and as derived from an extended amount of field studies, horizontal as well as vertical distribution differs during the brown shrimp’s life cycle (reviewed in Campos and van der Veer, 2008). Juveniles are known to inhabit warm and shallow coastal areas, whereas adult brown shrimp move to deeper and less warm waters (Ehrenbaum, 1890; Havinga, 1930; Lloyd and Yonge, 1947; Tiews, 1970; Boddeke et al., 1986). These ontogenetic differences have been ascribed to distinct thermal requirements and tolerance limits among life stages (Campos & van der Veer, 2008). However, this could also be related to differential thermal preferenda indicating that brown shrimp do not share a species specific FTP but that preference varies throughout ontogeny. By means of the aforementioned field studies it was further revealed that horizontal distribution in brown shrimp also varies according to gender. Small females are known to migrate far into the shallow, warm coastal regions, whereas males and adult females stay in the tidal gullies (Havinga, 1930) suggesting an effect of gender and maturation state on thermal preference as well.
With regard to the findings from these previous studies, the present study aimed to determine whether brown shrimp hold a species-specific FTP as originally postulated by Fry (1947) and if the FTPP is valid for this invertebrate ectothermic species. We tested this by means of laboratory based experiments using an annular chamber system and applying the gravitational method for thermal preference determination throughout a 14 month experimental period.

4.2 Materials and Methods

4.2.1 Brown shrimp sampling and maintenance

Brown shrimp were sampled at different stations in the German Bight as well as the German Wadden Sea between 2011 and 2012 (Fig. 4.1, Tab. 4.1). Sampling during winter 2011 and 2012 was conducted on board the research vessel FFS SOLEA. The winter stations (WS1 and WS2 for 2011 and WS3 and WS4 for 2012) were sampled using a 7 m beam trawl equipped with a 20 mm mesh size (stretched mesh) and no tickler chain. Due to the low temperatures in the winter of 2010/11, stations WS1 and WS2 had to be located further offshore compared to WS3 and WS4 in 2012.

Table 4.1: Names, positioning and depth of the sites where brown shrimp were sampled. Temperature at the sampling sites was determined right after sampling.

<table>
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<th>Station Name</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Depth (m)</th>
<th>Temperature (°C)</th>
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<td>7.867</td>
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<td>3</td>
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<tr>
<td>RS4</td>
<td>54.267</td>
<td>8.367</td>
<td>11</td>
<td>17.5</td>
</tr>
<tr>
<td>RS5</td>
<td>54.173</td>
<td>8.642</td>
<td>10</td>
<td>9.9</td>
</tr>
</tbody>
</table>

*Seasonal variability of temperatures at SWRS is given in Fig. 4.2. SWRS was sampled 18 times during the 14 months.

From March to December 2012, brown shrimp were sampled at the coast off Büsum (54°07’09”N, 8°51’43”E) at low tide using a push net (2 mm mesh size) in approximately 1 m water depth. Post sampling, water temperature and salinity were determined in 1 m water depth. The station in Büsum served as a shallow water reference station (SWRS). Sampling in Büsum was conducted at regular intervals, roughly every 2 weeks. In total, the station in
Büsum was sampled 18 times for this study. The station in Büsum has been used for sampling live brown shrimp for several studies in our group already (Hufnagl et al., 2010; Hufnagl & Temming, 2011a; Perger & Temming, 2012, see also Chapter II) and is characterized by a sharp increase from a tidal gully that rises to a shallow mud flat. The station is further known for its easy accessibility and high abundance of brown shrimp of various size classes during the most time of the year.

Figure 4.1: Locations of sampling sites. Stations WS1 and WS2 were sampled during winter 2011. Stations WS3 and WS4 were sampled in winter 2012. RS1-RS5 were sampled from April-November 2011. SWRS served as a shallow water reference stations was sampled 18 times from March-December 2011.

During May-November 2011 and in addition to the SWRS, brown shrimp were sampled at five random stations (RS1-RS5) in the Wadden Sea by a commercial shrimp trawler. The random stations were sampled to obtain live brown shrimp from higher water depth compared to the SWRS. On the commercial trawler, shrimp were sampled using a commercial, 7 m beam trawl equipped with a 20 mm mesh size (stretched mesh). At both, FFS Solea as well as the commercial shrimp trawler, shrimp for our experiments were derived from an extra haul that just lasted 15 minutes. The beam trawls were raised slowly and shrimp removed from the net carefully. Water temperatures were measured at the end of each trawl. On board the FFS Solea brown shrimp were kept in an aerated tank with surface water flow-through until arrival in Cuxhaven, approximately 5 h (2011) and 2 h (2012) post catch. At the
commercial shrimp trawler, brown shrimp were stored in a cooling room and covered with wet cloth. Brown shrimp were transferred to continuously aerated tanks and transported to the laboratory facilities of the Institute of Hydrobiology and Fisheries Science, University of Hamburg, Germany. Here, brown shrimp were transferred to a 1 m³ circular tanks with aerated artificial seawater of 30 PSU connected to an in-house temperature controlled recirculating water system equipped with a foam fractionator and a moving bed biofilter. Upon acclimation, one day for animals sampled at the SWRS and 2 days for animals sampled at the WS and RS, respectively, animals were sorted to the nearest 5 mm total length (TL). Brown shrimp were then transferred to three separate temperature controlled, circular holding units, maintaining three size groups (3.5, 5.0 and 6.5 cm TL; 4.0, 5.5 and 7.0 cm TL; 4.5, 6.0 and 7.5 cm TL) within one tank. The circular holding units were connected to the temperature controlled recirculating system as described above. Temperature (mean ± SD) was kept constant and just slightly varied during the 14 month experimental period (11.7 ± 1.06°C). Brown shrimp were maintained at 10:14 L:D photoperiod following Meixner (1969) and were fed dry feed (Marico Advance, Coppens International, Helmond, Netherlands), live Artemia nauplii (SEPArt, Inve Aquaculture, Dendermonde, Belgium) and chopped herring and sprat pieces to apparent satiation every day. We used brown shrimp for just two weeks post sampling as we revealed dry weight condition to decrease after 4 weeks of husbandry (data not shown).

4.2.2 Thermal preference experiments
Thermal preference was measured in an annular chamber system holding a thermal range of 3-25°C (see Chapter I). During the whole period of this study, the range of the gradient was kept constant. The chamber used in the present study was a modified version of the annular chamber originally specified by Myrick et al. (2002). The here used version of the annular chamber has been described in detail in Chapter I. Twenty four hours prior to each experiment, 10 animals from one respective size class and of one sex were dip-netted from the holding units. Sex was verified based on the appendices of the first and second endopodite (Tiews, 1954) and the brown shrimp were maintained in a fourth circular holding unit as described above until the experiment was started. Experiments were started at the following day between 7 and 8 am in the morning. Brown shrimp were released into the swimming channel (SC) with the temperature gradient being established at that segment where temperature corresponded to the maintenance temperature. Data acquisition was started using an automated MATLAB routine described in Chapter I and the
animals were left undisturbed throughout the whole experiment. After 20h of exposure to the thermal gradient, temperature preference was analyzed during the following 3h period to determine gravitational thermal preference (see Chapter II) using a custom designed MATLAB routine described in Chapter I. To avoid pseudoreplication, the preferred temperature of brown shrimp within one run was calculated as the mean of the median selected temperatures of each shrimp in one experimental trial (Mathur & Silver 1980; Karlsson et al. 1984).

Following each experiment, brown shrimp were removed from the SC, placed in plastic bags filled with seawater and stored in -20°C. After complete freezing, samples were thawed and TL of each individual was determined to the nearest mm using an USB camera (uEye, UI-1485LE-C-HQ, IDS Imaging Development Systems GmbH, Obersulm, Germany) equipped with a Pentax lens (C1614-M, Pentax Ricoh Imaging Deutschland GmbH, Hamburg, Germany) by means of a custom made MATLAB program. TL was measured from the tip of the scaphocerite to the end of the telson. As gender determination of live animals, especially the small size groups used for the experiments can be error-prone, gender was again verified under a magnifier. If a misidentification of gender in one of the specimen was noted, the data derived by the respective experiment were not included in the data set. Following gender determination, brown shrimp were freeze-dried for 24h (Alpha 1-4 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and weighed to the nearest 0.1mg on an electronic balance (SE2 ultra micro balance, Sartorius AG, Göttingen, Germany). Dry weight (DW) and TL were used to calculate the mean dry weight condition index (DWCI) following Perger and Temming (2012). In egg-bearing females, the eggs were removed before freeze-drying. Eggs were differentiated for the presence of absence of eyespots and the percental amount of eggs featuring eyespot was calculated.

4.2.3 Data and statistical analysis
Statistical data analysis was performed using the R statistical program version 2.15.0 (R Development Core Team, 2011) including the mgcv (Wood, 2006, 2008) and the AED (Zuur et al., 2009) packages. Data exploration was conducted according to the protocol from Zuur et al. (2009, 2010). Data were checked for potential outliers using Cleveland dotplots. Multipanel scatterplots were used to investigate the relationship between variables. Colinearity between covariates was assessed by Pearson correlation and variance inflation factors (VIF).

We originally considered twelve covariates for our statistical analysis (Tab. 4.2). The number of the day an experiment was conducted was included as a categorical predictor. The month
the experiment was conducted was included as a less resolved categorical predictor of time. Month was incorporated in two different ways: (1) Month was treated as a covariate with 12 levels (month-ID12) conflating Jan2011/12 as well as Feb2011/12. (2) Month was treated as a covariate with 14 levels (month-ID14) stating with Jan2011 = 1 to Feb2012 = 14. The categorical predictor season was initially considered in two different ways: Season was (1) defined as a categorical predictor with 4 factor levels (winter-ID4), assigning Dec2011&12, Jan2011&12 and Feb2011&12 as “winter”, Mar2011-May2011 as “spring”, June2011-Aug2011 as “summer” and Sept2011-Nov2011 as “autumn”. In addition, season was (2) assigned as a categorical predictor with 5 factor levels, assigning Jan2011-Mar2011 as “winter1”, Apr2011-June2011 as “spring”, July2011-Sept2011 as “summer”, Oct2011-Dec2011 as “autumn” and Jan2012-Feb2012 as “winter2”. Day length for experiments was calculated. The point in the lunar cycle for each experiment was derived from a sinusoidal function oscillating between full and new moon. Body size of the shrimp was used as a categorical predictor, grouping the animals into 4 categories, namely shrimp of 3.5 and 4.0, 4.5 and 5.0, 5.5 and 6.0 and 6.5 and 7.0 cm TL. Based on the mean TL of shrimp used within one experiment at a given date, individuals were further assigned to a respective cohort identity. Cohort identity was determined based on a growth model for mean growth of brown shrimp following Hufnagl & Temming (2011b) with growth of the shrimp starting at 5 mm on each first day of a respective month. Brown shrimp were assumed to recruit at 15 mm TL (Temming & Damm, 2002). Based on these calculations, brown shrimp used for the experiments were assigned to 16 different cohort identities.

Our initial data analysis revealed colinearity among the covariates and thus six of the twelve covariates (Tab. 4.2) had to be excluded from further statistical analysis. Initial data analysis further revealed non-linear patterns in thermal preference. Therefore and as no a priori specification of the response and the explanatory variables is needed, we used general additive models (GAM) for our statistical analysis (Wood, 2006, 2008). Starting with a full model including all covariates and interactions (Zuur et al., 2009) we applied dynamic backward selection removing non-significant interactions and covariates in turn until no further model improvement could be attained (Zuur et al., 2009). For dynamic backward selection we used the Akaike’s information criterion (AIC) which is a measure of the goodness of fit of a model. Finally, model assumptions were checked according to Zuur et al. (2007, 2009, 2010). This finally resulted in a model, including the covariates

$$ T_{\text{pref},i} = \alpha + f(\text{Day number}_i) + \text{factor(Tcatch}_i) + \text{factor(size group}_i) + \text{factor(Tcatch}_i) : \text{factor(size group}_i) + \epsilon_i $$
where $T_{\text{prefi}}$ is the preferred temperature in the $i$th observation within the experimental series. $T_{\text{catchi}}$ is the temperature at catch and size group, of the $i$th observation. $\text{factor}(T_{\text{catchi}}) : \text{factor}($size group$)$ indicates an interaction and $\varepsilon_i$ denotes the independently, normally distributed noise with expectation 0 and variance $\sigma^2$.

**Table 4.2:** List of covariates for generalized additive model (GAM) analysis of preferred temperatures in brown shrimp.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Continuous/categorial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day number</td>
<td>Continuous (number of day each experiment was conducted: day15-day405)</td>
</tr>
<tr>
<td>Dry weight condition index</td>
<td>Continuous (dry weight condition index calculated after Perger and Temming (2012))</td>
</tr>
<tr>
<td>Body size (TL)</td>
<td>Categorial (4 levels: 3.5 &amp; 4.5 cm TL = 4; 4.5 &amp; 5.0 cm TL = 5; 5.5 &amp; 6.0 cm TL = 6; 6.5 &amp; 7.0 cm TL = 7)</td>
</tr>
<tr>
<td>Gender</td>
<td>Categorial (2 levels: female &amp; male)</td>
</tr>
<tr>
<td>Temperature at catch</td>
<td>Continuous (temperature [°C] at catch)</td>
</tr>
<tr>
<td>Depth at catch</td>
<td>Continuous (depth [m] brown shrimp were caught)</td>
</tr>
<tr>
<td>Day length*</td>
<td>Continuous (calculated according to the coordinates of the SWRS)</td>
</tr>
<tr>
<td>Cohort ID*</td>
<td>Continuous (based on mean growth model after Hufnagl and Temming (2011b))</td>
</tr>
<tr>
<td>Moonindex*</td>
<td>Continuous (sinusoidal function oscillating between full and new moon)</td>
</tr>
<tr>
<td>Location*</td>
<td>Categorial (3 levels: SWRS, RS, WS)</td>
</tr>
<tr>
<td>Month*</td>
<td>Categorial ((1) 12 levels: 1-12; (2) 14 levels: 1-14)</td>
</tr>
<tr>
<td>Season*</td>
<td>Categorial ((1) 4 levels: winter, spring, summer, autumn; (2) 5 levels: winter11, spring11, summer11, autumn11, winter12)</td>
</tr>
</tbody>
</table>

Covaraites denoted with an asterisk have not been incorporated into the final statistical model due to colinearity.

**4.3. Results**

During the 14 month time course of the experiments, we performed a total of 271 individual temperature preference experiments. In these experiments, brown shrimp selected a wide range of temperatures and did not show a single or a constant thermal preferendum (Fig. 4.2). Brown shrimp selected mean median preferred temperatures between 4.4-22.3°C, revealing a spread of the thermal preference zone of ~18°C throughout the season. Occasionally throughout this 14 month period, we observed great behavioral differences and brown shrimp selected a wide range of temperatures, even in the same experimental trial. This was especially true for the smallest size group of 4 cm TL from March to July.
By means of dynamic backward selection, most of the initially considered explanatory variables (Tab. 4.2) were removed. Gender, DWCI as well as depth did not improve the model and were removed during the dynamic backward selection process. The resultant model comprising day number as a smoothing function as well as the size of the shrimp and the temperature at catch as categorical predictors explained 65.1% of the variance in the data (Tab. 4.3).

![Figure 4.2](image)

**Figure 4.2:** Mean median preferred temperatures of brown shrimp of different size groups, during the 14 month experimental period. Thermal preferenda were determined in an annular chamber. Brown shrimp were sampled at stations WS1-WS4, RS1-RS5 and SWRS (see Tab. 4.1). The grey line corresponds to water temperature at the SWRS in Büsum. Each data point denotes an individual thermal preference experiment.

Thermal preference of brown shrimp differed significantly throughout the seasonal cycle (Tab. 4.3). Following the relatively cold winter 2010/2011, brown shrimp selected low temperatures with a mean ± SD of ~7.9 ± 1.8°C. During this phase, we observed the coldest record of preferred temperature, i.e. 4.4°C. With increasing water temperatures from March to July 2011, thermal preferenda increased and brown shrimp selected temperatures up to 17.5°C but hardly selected temperatures below 8°C. With already decreasing field water temperatures from August 2011 onwards, thermal preference increased further. From August to October just few brown shrimp selected temperatures below 12.5°C and preference temperatures reached up to 22.3°C, the highest recorded thermal preference in this study.
From November 2011 on, thermal preference decreased just slightly and remained on a relatively high level until February 2012. The course of the smoothing function indicated a massive shift in thermal preference around day 210-280 (Fig. 4.3). Following day 280, thermal preferenda were on average higher compared to the prior phase.

Figure 4.3: Smoother and 95% confidence bands obtained by generalized additive model (GAM) analysis, illustrating the relationship between mean median preferred temperatures of brown shrimp of different size, gender as well as origin and the co-variates day (day number is referring to the day number of the experiment), body size and catch temperature. Thermal preferenda were determined in an annular chamber system.

In addition to day number, thermal preference was significantly affected by the body size (Tab. 4.3). Compared to the 4 cm group, the three larger groups selected significantly colder temperatures (Tab. 4.3). However, we observed a significant interaction of body size and the temperature the brown shrimp were caught, indicating that the effect on thermal selection is not driven by size of the shrimp exclusively. The mere effect of the temperature at catch was not significant. The effect of size on thermal selection is shown in Fig. 4.4 illustrating the higher thermal preferenda of small shrimp during the whole experimental period and a decreasing thermal preference with increasing TL. However, in autumn 2011 and winter
2011/12, thermal preferenda of brown shrimp of the 7 cm group increased and they almost selected temperatures as high as the 4 cm TL group.

4.4. Discussion

4.4.1 Seasonal variability
Brown shrimp were found to be thermosensitive and perform behavioral thermoregulation confirming and supporting previous findings on crustacean species in general (Lagerspetz & Vainio, 2006) as well as on brown shrimp in particular (see Chapter II). Seasonal variability in preference temperature of brown shrimp was pronounced and covered a wide thermal range between 4.4-22.3°C. The overall range of ~18°C was far beyond the typical expansion of the thermal preference zone which is usually around 2-4°C (Magnuson et al., 1979), suggesting the existence of distinct seasonal thermal preference zones in brown shrimp.

Table 4.3: Results of the generalized additive model (GAM) analysis for covariates affecting thermal preference in brown shrimp.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate ± SE</th>
<th>t value</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>14.466 ± 1.160</td>
<td>12.468</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tcatch</td>
<td>-0.023 ± 0.113</td>
<td>-0.200</td>
<td>0.841</td>
</tr>
<tr>
<td>factor(SIZE_GROUP)5</td>
<td>-2.996 ± 0.924</td>
<td>-3.244</td>
<td>0.001</td>
</tr>
<tr>
<td>factor(SIZE_GROUP)6</td>
<td>-5.832 ± 1.076</td>
<td>-5.422</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>factor(SIZE_GROUP)7</td>
<td>-5.015 ± 1.211</td>
<td>-4.140</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tcatch : factor(SIZE_GROUP)5</td>
<td>0.196 ± 0.076</td>
<td>2.576</td>
<td>0.011</td>
</tr>
<tr>
<td>Tcatch : factor(SIZE_GROUP)6</td>
<td>0.350 ± 0.082</td>
<td>4.250</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Tcatch : factor(SIZE_GROUP)7</td>
<td>0.299 ± 0.109</td>
<td>2.732</td>
<td>0.007</td>
</tr>
<tr>
<td>smooth term</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day number</td>
<td>7.032</td>
<td>7.032</td>
<td>33.97</td>
</tr>
</tbody>
</table>

The factors causing this seasonal change in thermal preference are mostly unknown. In fish, seasonal variability in thermal preferenda is often linked to seasonal migratory behavior. In non-migratory fish species, thermal preferenda did not show much variation during the seasonal cycle (deVlaming, 1971; Roed, 1979) whereas migratory fish species showed a seasonal pattern in their preference temperatures (Zahn 1964; Hesthagen, 1979; Clark & Green, 1991; Magnuson et al., 2007). Consequently, the extended seasonal migrations in brown shrimp throughout the year as well as during their developmental cycle (Havinga 1930; Boddeke, 1976) might explain seasonal and size specific differences in temperature preference observed in the present study.
4.4.2 Photoperiod and reproductive state

Besides migratory behavior, photoperiod, effects of reproductive state or other endogenous physiological and endocrinological cycles and rhythms have been shown to affect and modulate preference temperatures as well (Richards et al., 1977; Ihnat & Bulkley, 1984; Roscoe et al., 2010). Photoperiod was therefore kept constant during the whole 14 month period to avoid any confounding effect in our study. However, as brown shrimp were sampled throughout the season they might still have been affected by and adapted to the photoperiod they experienced in the field before sampling. As brown shrimp for our experiments were used up to two weeks post sampling, we cannot exclude that this adaptation to the natural photoperiod expired. We intended to account for this by incorporating photoperiod, represented by the length of the day as a continuous predictor in our statistical analysis. However, due to high collinearity we had to finally exclude this identifier. Although the dates when the experiments were conducted are linked to day length, we cannot conclude on an effect of the photoperiod on preferred temperature based on the here presented results. This has to be evaluated in separate experiments where the photoperiod is modulated during animal maintenance accordingly.

Figure 4.4: Seasonal mean median preferred temperatures of brown shrimp of different size groups determined in an annular chamber. Boxes include 1st and 3rd quartiles. Whiskers denote 95% of the data range.
Potential effects of the maturation state on thermal preference, however, were partially covered by our data. We determined whether female brown shrimp carried eggs and whether eggs were with or without eye spots. The presence of eye-spots corresponds to embryos that are further developed compared to eggs without eye-spots (Havinga, 1930; Wehrmann & Kattner, 1998). We observed a clear pattern in the proportion of eggs with eye-spots (Fig. 4.5) coinciding with previous reports (Havinga, 1930). Eggs were less developed in winter 2010/11 and the proportion of eggs with eye-spots increased in spring 2011. During summer 2011, most eggs had eye-spots before mainly fleshly laid eggs were observed during autumn 2011. Impacts of the reproductive cycle may explain the high thermal preferenda of large females during autumn 2011. To our surprise the thermal preferenda of large (≥6 cm TL) females in autumn were just slightly lower compared to temperatures preferred by the 4 cm group (Fig. 4.3) (day 270-330). This coincided with the period where females had either no or mainly freshly laid eggs without eye-spots (Fig. 4.5). High thermal preferenda of large, non-ovigerous female brown shrimp could in fact indicate an effect of the reproductive state on thermal preference. Potentially, preference towards higher temperatures promotes ovarian development and boosts egg production by increased metabolic performance due to increased temperature. Unfortunately, the number of experiments investigating egg-bearing females was low throughout the whole study (32 independent experiments), especially during summer (no eggs: n=8; eggs: n=7) and autumn (no eggs: n=14; eggs: n=3) 2011, as egg-bearing females were scarce in the shallow field stations (Siegel et al., 2008). Therefore, we were not able to test for the effects of egg-bearing and egg development state in our statistical analysis.

4.4.3 Body size and gender

Body size of brown shrimp was identified as an additional determinant affecting thermal preference (Tab. 4.3; Fig. 4.4). Smaller individuals were found to select significantly higher temperatures compared to brown shrimp >4 cm TL. This finding coincides with previous results obtained by numerous field studies (reviewed in Campos & van der Veer, 2008). Brown shrimp recruit in the shallow coastal areas at a size of 10-20 mm TL (Kuipers and Dapper, 1984; Beukema, 1992) and benefit from high temperature in those regions allowing for rapid growth (Havinga, 1930; Lloyd and Yonge, 1947) until becoming sexually mature at 35-40 mm TL (Meredith, 1952). Our study provided experimental evidence that the aggregation of small animals in more shallow regions as well as occurrence of larger specimens in deeper water might be related to distinct thermal preferenda. However, we observed a significant interaction of body size and catch temperature indicating that the
effect on thermal selection is not driven by the size of the shrimp exclusively. Due to this interaction, we cannot clarify the mere effect of body size even though the effect of catch temperature alone was not significant.

In addition to size and ontogeny, previous field studies reported that male and female brown shrimp hold distinct thermal requirements as depth distribution throughout the year differs among both sexes (Havinga, 1930; Tiews, 1970). In general, female brown shrimp seem to hold higher thermal requirements compared to males as males are less abundant in the shallow regions during summer (Havinga, 1930). However, the results of the present study did not reveal any differences in thermal preferenda among female and male brown shrimp. Gender specific differences of vertical distribution in the field might thus be caused by other factors than temperature.

![Graph showing seasonal variability in the percental amount of eggs with eye spots in egg-bearing female brown shrimp.](image)

**Figure 4.5:** Seasonal variability in the percental amount of eggs with eye spots in egg-bearing female brown shrimp. Boxes include 1st and 3rd quartiles. Whiskers denote 95% of the data range.

**4.4.4 Cohort identity**

The pattern of the smoother in our statistical analysis revealed an additional factor potentially influencing thermal preference in the common brown shrimp as well (Fig. 4.3). From the start of our experiments until mid-July (day 200), thermal preferenda showed just a slight variability. Following day 200, however, we observed a steep increase in preference temperature until end of October (day 300) that was accompanied by considerably less
variability in the data compared to the period before and thereafter. This steep increase in preference temperature coincided with the recruitment of the new cohort that usually starts in April and peaks between May and June (Temming & Damm, 2002). Assuming growth rates as determined by Hufnagl & Temming (2011b), shrimp recruiting on the mudflats at a TL of 1.5-2.0 cm in April-June would achieve 3.5 cm TL, i.e. the smallest size of shrimp we used for our experiments, around June-August. The respective cohorts (cohort ID 9-16) were used for our experiments from ~day 200 onwards (Fig. 4.6). However, due to the great variability in growth of brown shrimp, we cannot exclude that new recruits were already present in cohorts 7 and 8. Although the occurrence of new brown shrimp recruits coincides with the increase of thermal preference, the effect of cohort identity in thermal preference remains speculative. Cohort identity was highly colinear with the continuous predictor “day number” and had therefore to be excluded from our final model.

![Figure 4.6: Cohort identity of brown shrimp used for thermal preference experiments. Cohort identity was calculated on the growth model for mean growth by Hufnagl & Temming (2011b) using body length (TL) of brown shrimp as determined after each experiment.](image)

Although we observed a seasonal pattern in preference temperatures, thermal selection of brown shrimp coincided only weakly with the corresponding water temperatures in the field
During winter and late autumn, thermal preferenda exceeded field temperatures revealing that brown shrimp have to cope with suboptimal thermal conditions during that time of the year. In the field, brown shrimp can partially counteract suboptimal thermal conditions as they conduct extended seasonal migrations starting during late summer and proceeding until early winter before the mudflats get covered with ice (Boddeke, 1976). This winter migration leads the shrimp off the coast avoiding low temperatures in the littoral zone. In severe winters, brown shrimp migrate further offshore to deep and less cold bottom waters (Boddeke, 1963; Boddeke, 1976). However, thermal preferenda determined during winter, especially during 2012, were still above thermal conditions usually experienced by brown shrimp during that period even when migrating far offshore.

In contrast to the preferenda in winter, brown shrimp selected considerably lower temperatures when compared to field temperatures during spring and summer. During that time, thermal preferenda hardly exceeded field temperatures and were considerably lower compared to the optimal temperature range of brown shrimp reported by Campos & van der Veer (2008) and Freitas et al. (2007, 2010). Campos and van der Veer (2008) defined an optimal temperature range of 5-25°C for juveniles and 5-20°C for adults and Freitas et al. (2007, 2010) defined 23°C as the thermal optimum for brown shrimp based on their results of a dynamic energy budget model. In contrast, Hufnagl and Temming (2011b) comparing 25 studies on brown shrimp growth rates, revealed highest growth rates in 20-30 mm juveniles at 23-27°C. Shrimp of 40-60 mm, however, grew best at 18-22°C. Our results obtained during the winter season coincide with the lower temperature range specified by Campos and van der Veer (2008) as brown shrimp were found to actively select temperatures as low as 4.4°C. In contrast, the upper range of preferred temperatures in the present study was lower as the optimum temperature range for juveniles and adults as specified by Campos and van der Veer (2008) as well Freitas et al. (2007, 2010). Brown shrimp barely selected temperatures >20°C although we provided the shrimp with a temperature gradient up to 25°C allowing for such high preferenda. However, our findings agree with the results obtained by Hufnagl and Temming (2011b) for shrimp of 40-60 mm as well as the field observations by Henderson et al. (2006) who observed low brown shrimp abundance in the field when water temperatures exceeded 22°C. Henderson et al. (2006) ascribed this to a thermal avoidance response of the shrimp. Indeed, brown shrimp in the present study did not select temperatures above 22.3°C providing experimental evidence for the assumption made by Henderson et al. (2006). Still, the highest observed preferendum of 22.3°C was lower than the optimum temperature determined by Freitas et al. (2007, 2010). However, it has been previously reported that thermal preferenda will hardly exceed physiological thermal optima.
(Martin and Huey, 2008). This has been related to ectotherms being not perfect thermoregulators and that temperatures slightly above the optimum will depress fitness considerably more than temperatures slightly below. This is also considered as the reason why thermal reaction norms are usually skewed (Martin and Huey, 2008). Indeed, since we calculated thermal preferenda as the mean median preferred temperatures over all animals within one experimental trial (Mathur & Silver 1980; Karlsson et al. 1984) the upper thermal preference range might be underestimated in our study. Apart from that, the lower range of preferred temperatures was less variable compared to the upper (Fig. 4.1, day 80-day 280 and day 280-day 370) indicating a considerable amount of variability in thermal selection among brown shrimp. This as well as utilization of different food resources (Hufnagl & Temming, 2011a) might further explain the great differences in growth trajectories observed in brown shrimp (Hufnagl & Temming, 2011b).

4.5 Conclusion
The common brown shrimp was found to be thermosensitive and perform behavioral thermoregulation. Moreover, this study shows that behavioral thermoregulation of brown shrimp did not result in a species-specific preference temperature but that brown shrimp selected a wide range of temperatures during the seasonal cycle. We also found that body size affected thermal selection, providing evidence for an ontogenetic effect on thermal preference in brown shrimp as shown for vertebrate ectotherms before (McCauley & Huggins, 1979; Lafrance et al., 2005). The data of the present study therefore suggest that brown shrimp do not hold a species-specific preference temperature, but that the FTP in brown shrimp is affected at least by season and differs between size classes. These results coincide with previous findings on vertebrate aquatic ectothermic species (Zahn, 1964; Hesthagen, 1979; McCauley and Huggins, 1979; Clark & Green, 1991; Lafrance et al., 2005; Mortensen et al., 2007) and represent the first evidence for a marine, invertebrate ectotherm.

4.6 Acknowledgements
The authors thank Thomas Neudecker and the crew from the research vessel FFS Solea as well as Norbert Temming for providing life brown shrimp for this study. Annika Mues and Zaki Sharawy are thanked for their help in sampling brown shrimp in Büsum. Carmen Czerwinski and Wiebke Bretting are thanked for data validation and laboratory assistance. Sven Lütke and Sven Stäcker are acknowledged for their help in animal husbandry. We also thank the “Landesbetrieb für Küstenschutz, Nationalpark und Meeresschutz Schleswig-Holstein” (LKN) (http://www.schleswig-holstein.de/LKN/EN/LKN_node.html) for providing daily temperature
data for SWRS (Fig. 4.2). This study was partly funded by the Cluster of Excellence “Integrated Climate System Analysis and Prediction” (CliSAP) of the University of Hamburg.

4.7 References


SEASONAL THERMAL PREFERENCE OF BROWN SHRIMP


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CHAPTER IV

5. Lower thermal capacity limits of the common brown shrimp
(Crangon crangon, L.)

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Abstract

Brown shrimp (Crangon crangon, L.) are subjected to a huge annual temperature range and certain thermal conditions during winter have been identified to affect the overall brown shrimp population. Despite that, little is known about the thermal biology of brown shrimp as well its thermal capacity towards low temperatures. In the present study, we determined the critical thermal minima (CTₘᵋᵣₗ) and the critical lethal minima (CLₘᵋᵣₗ) of male and female brown shrimp of different body sizes and maturation states in laboratory based experiments. For the CTₘᵋᵣᵣₗ trials, brown shrimp were acclimated to 4.0, 9.0 and 14.0°C for two weeks and exposed to a cooling rate of -0.2°C min⁻¹ afterwards. In the CLₘᵋᵣᵣₗ trials, brown shrimp were exposed to a cooling rate of -1.0°C day⁻¹ without prior thermal acclimation. Acclimation temperature significantly affected temperature tolerance of brown shrimp in the CTₘᵋᵣᵣₗ trials, however, CTₘᵋᵣᵣₗ among the experimental groups just varied slightly and no clear effect of gender or body size was observed. Depending on the acclimation temperature, CTₘᵋᵣᵣₗ in brown shrimp ranged around -1.4°C and 2.6°C. In the CLₘᵋᵣᵣₗ trials, brown shrimp even tolerated the coldest temperature of -1.7°C that could be established in the experimental setup. Thus, CLₘᵋᵣᵣₗ values for brown shrimp could not be determined, however, lower critical thermal limits can be considered to be well below -1.7°C. Indeed, we observed a negative relationship of temperature and reactivity within the range of 7.0°C and 1.0°C in the CLₘᵋᵣᵣₗ trials that was determined by means of the flicking response following a single electrical pulse. This relationship suddenly broke between 1.0°C and 0.0°C where an abrupt drop in reactivity of the shrimp became apparent. Therefore, although the respective criteria for CLₘᵋᵣᵣₗ testing were not met, temperatures below 1.0°C can be supposed critical under prolonged exposure even when giving brown shrimp the opportunity for acclimation. The results this study reveal,
that brown shrimp hold a wider thermal range as originally reported and that brown shrimp can cope with subzero temperatures. Implications of low temperatures tolerance are discussed in the context of the brown shrimp’s ecology as well as stock assessment.

Keywords: brown shrimp, beam trawl, critical temperature, CLmin, CTmin, pulse fishing, temperature limits, temperature tolerance, Wadden Sea
5.1 Introduction
The common brown shrimp (*Crangon crangon*, L.) is a decapod crustacean species inhabiting eu- and sublittoral habitats. Brown shrimp play a pivotal role in coastal ecosystems, being an important prey for crustacean species, fish and birds (Pihl, 1985; van der Veer and Bergmann, 1987) as well as an epibenthic predator of epi- and infaunal species (del Norte-Campos and Temming, 1994; Pihl and Rosenberg, 1984; Oh et al., 2001). Within the Wadden Sea, which is considered as its main area of distribution, brown shrimp are exceptionally numerous and support a commercially important fishery with annual landings around 30000 t (ICES, 2011).

The North Sea brown shrimp stock is subjected to a considerable degree of year-to-year variability (Temming and Damm, 2002; Siegel et al., 2005; Campos et al., 2010). Several biotic as well as abiotic factors have been identified to drive these fluctuations. Besides river in-flow, winter North Atlantic Oscillation Index (NAOI) and predator abundance, winter water temperature has been identified as a main determinant for the subsequent spring as well as the autumn shrimp stock (Oh et. al, 1999; Siegel et al., 2005; Campos et al., 2010). The strong effect of winter water temperature on the brown shrimp population in the North Sea can mainly be related to the brown shrimp's life cycle. Following Hufnagl and Temming (2011), the brown shrimp population is driven by an annual population cycle. Brown shrimp carry eggs almost throughout the whole year, however, brown shrimp recruitment in spring and peak abundances in autumn are mainly derived from winter-eggs spawned from October to March (Havinga, 1930; Boddeke, 1982; Temming & Damm, 2002; Hufnagl & Temming, 2011). Conditions affecting the winter population will therefore heavily influence the overall shrimp stock in the next year.

Winter water temperature affects spring and autumn abundance in different manner. Spring abundance of brown shrimp is markedly reduced following cold winters and recruitment of juveniles on the shallow mudflats is delayed (Boddeke, 1976; Beukema, 1992). During cold winters, brown shrimp migrate far offshore increasing the distance the shrimp as well as drifting larvae have to cover in the following spring thus postponing their arrival (Boddeke, 1976). Additionally, low temperatures slow down egg development resulting in later hatch compared to mild winters (Temming and Damm, 2002). Beukema (1991), Siegel et al. (2005) as well as Campos et al. (2010) also discussed direct consequences of low temperatures that might increase winter mortality resulting in decreased abundances. Following Campos et al. (2010) the effects of low winter water temperatures are especially notable when coinciding with low mean sunshine duration. Here, net productivity is reduced, causing starvation (Hufnagl et al., 2010) and increased overwintering mortality. In contrast to spring abundance...
and within a certain range of temperatures, cold winters have been shown to promote shrimp abundance in autumn (Siegel et al., 2005; Campos et al., 2010). This is potentially due to temporal shifts in predator-prey overlap, thus releasing larval and juvenile shrimp from predation pressure. Moreover, recruitment is more pronounced following cold winters potentially causing an over-saturation of predators and therefore increasing the probability of survival (Ziegelmeier, 1970; Beukema, 1991; Temming & Damm, 2002). Additionally, higher susceptibility of adult shrimp towards deep water temperatures compared to juvenile shrimp (Campos and van der Veer, 2008) might release small individuals from cannibalism of adult shrimp in the next season.

Despite this sweeping effect of temperature on the North Sea brown shrimp population, only little is known about the brown shrimp’s thermal capacity and the direct consequences of exposure towards low temperatures. Across its habitat, brown shrimp are confronted with a high degree of seasonal temperature variability as well as regular exposure towards temperatures below 0°C during the winter season. Campos and van der Veer (2008), actualizing and extending the synopsis on brown shrimp by Tiews (1970) reported brown shrimp to be able to survive at temperatures as low as 6°C. Freitas and colleagues (2007, 2010), using a dynamic energy budget model to estimate thermal sensitivity based on rates of oxygen consumption, stated 0°C as the lower temperature limit brown shrimp can sustain. Following the severe winter 1962/1963 with subzero seawater temperatures, Boddeke (1963) reported brown shrimp to still occur in the landlocked Veerse Meer and concluded that brown shrimp can sustain seawater temperatures as low as -1.8°C.

However, critical lower temperature limits for brown shrimp have not been determined yet, impeding assessments on the effect of low winter temperatures on brown shrimp. Critical temperature limits are typically determined by means of laboratory based experiments under controlled conditions. Several methods for critical temperature limit testing have been used so far, resulting in different types of measures (Lutterschmidt and Hutchison, 1997a, 1997b; Beiting et al., 2000; Dallas and Ketley, 2011). Besides the incipient lethal temperature (ILT) and chronical lethal method (CLM), the critical thermal method (CTM) has frequently been used to determine upper and lower critical thermal limits (Shafland & Pestrak, 1982; Cuculescu et al., 1998; Hoang et al. 2002; Madeira et al., 2012). In contrast to ILT and CLM, the CTM uses a pre-defined sub-lethal endpoint rather than death as in the aforementioned methodologies to ascertain the critical temperature threshold (Lutterschmidt and Hutchison, 1997a, 1997b; Beiting et al., 2000). The onset of disorganized locomotory movements, the onset of spasms, a loss of the righting reflex or the loss of equilibrium (LOE) is generally applied as endpoints in the CTM. These endpoints all represent a state where the tested
organism loses its ability to escape from conditions which ultimately may lead to its death (Cowles and Bogert, 1944; Lutterschmidt and Hutchison, 1997b; Beitinger et al., 2000). For critical thermal maxima (CT$\text{max}$), a heating rate of 0.3°C min$^{-1}$ has been suggested, allowing the body temperature to track the temperature in the surrounding and to avoid reacclimation (Lutterschmidt and Hutchison, 1997b; Beitinger et al., 2000). In contrast, cooling rates of 0.1 -0.15°C min$^{-1}$ have been applied in most studies on critical thermal minima (CT$\text{min}$), mainly due to technical reasons (Beitinger et al., 2000). In CL$\text{min}$ trials, cooling rates of 1.0-2.0°C day$^{-1}$ are being used resulting in longer exposure times and allowing for reacclimation of the test organisms during the trial (Beitinger et al., 2000).

The aim of the present study was to evaluate the thermal capacity of the common brown shrimp towards low seawater temperatures and thus to obtain a better understanding of its thermal biology and temperature tolerance limits. We tested whether body size, gender or maturation state affects tolerance towards low temperature. For this purpose, two types of approaches were conducted. Acute CT$\text{min}$ were determined at a cooling rate of -0.2°C min$^{-1}$ to test for the effect of previous acclimation on thermal tolerance of brown shrimp. In chronic CL$\text{min}$ trials, we exposed brown shrimp to gradually decreasing temperatures at a cooling rate of -1.0°C day$^{-1}$.

5.2 Materials and Methods

5.2.1 Brown shrimp sampling and maintenance

Acute CT$\text{min}$ and chronic CL$\text{min}$ experiments were conducted according to the flow chart in Figure 5.1. Brown shrimp were caught by the research vessel FFS Solea in January and February 2012 at 53°56’N, 007°51’E and 53°57’N, 007°54’E, respectively. On board the vessel, brown shrimp were kept in an aerated tank with surface water flow-through until arrival in Cuxhaven, approximately 5h post catch. Shrimp were transferred to tanks with continuous aeration and transported to the laboratory facilities of the Institute of Hydrobiology and Fisheries Science, University of Hamburg, Germany. Here, animals were maintained in 1 m$^3$ circular tanks with aerated artificial seawater of 30 PSU at 8 ± 0.5°C connected to an in-house temperature controlled recirculating water system with a foam fractionator and a trickling biofilter. Shrimp were fed dry feed (Marico Advance, Coppens International), live Artemia nauplii (SEPArt, Inve Aquaculture) and chopped herring and sprat pieces to apparent satiation twice per day. Following two days of acclimation to husbandry conditions, brown shrimp were sorted to the nearest 5 mm total length (TL) and sex was determined based on the appendices of the first and second endopodite (Tiews, 1954).
Figure 5.1: Schematic illustration of the work flow for acute $CT_{\text{min}}$ and chronic $CL_{\text{min}}$ experiments. After sampling, brown shrimp were maintained until sorting and sex determination. For acute $CT_{\text{min}}$ experiments, brown shrimp were acclimated 4, 9 or 14°C for 2 weeks. Post acclimation, $CT_{\text{min}}$ experiments were conducted. For chronic $CL_{\text{min}}$ experiments, brown shrimp were transferred to the experimental chambers right after sorting and determination of sex. Starting at 7.0°C, temperature was decreased in 1.0°C intervals until -2.0°C were reached.

5.2.2 Experimental apparatus
Short- and long-term experiments were conducted in six poly-ethylene (PE) boxes of 103.5 x 61.0 x 20.0 cm (length x width x height) (Fig. 5.2). The PE-boxes were subdivided into an inflow compartment on both long sides as well as a central outflow compartment in the middle of each box. Between in- and outflow compartments, perforated PE plates constituted 24 separate 2.4 l experimental chambers (12 on each side of the outflow compartment) of 20.0 x 8.0 x 15.0 cm each. Water from the inflow compartment passed into the chambers through the perforated plates via underflow and was drained via an overflow into the central outflow compartment. Within the inflow compartment, the water was vigorously aerated. The six PE boxes were mounted on an aluminium frame and isolated with polystyrene plates. A reservoir tank of 150 l was arranged below the frame, collecting the discharged water from the outflow compartments of the PE boxes. Water was redistributed to the boxes by means of two pumps (OR 6500, Aqua Medic GmbH), each pump charging either the left or the right strand of inflow compartments.

Water in the reservoir tank was cooled by a recirculating chiller (ProfiCool Genius 41.02-NEB, National Lab GmbH) charging a titanium heat exchanger (VT04 CD16, GEA Ecoflex). Outflow temperature of the heat exchanger was controlled by a Pt100 thermistor (Pt100 Class B sensor, RS Components GmbH) connected to a PID process controller (4100+, West Control Solutions). The PID controller regulated a three-way control valve (type 323,
Belimo Automation AG) via a modulating rotary actuator (LR24A-SR, Belimo Automation AG) keeping the temperature at the respective set value ± 0.1 °C. Inside each box, the water temperature was monitored by three equally spaced temperature sensors (DS1820-LC, B+B Thermo-Technik GmbH), connected to a digital USB-thermometer (TLOG64-USB, B+B Thermo-Technik GmbH). Temperature inside the boxes was recorded every minute and visualized in real-time using the PC-Datalogger Software (PC-Datalogger, B+B Thermo-Technik GmbH). Additionally, water temperature in all experimental compartments was recorded at least four times a day using a handheld digital thermometer (Technoterm 9500, Testoterm KG).

The whole setup was connected to the in-house temperature controlled recirculating water system. Inflow from the in-house water system to the experimental setup was regulated via a magnetic valve (type D132V9-Z114A, Zimmer Automation GmbH) controlled by a level sensor (Type LS803-51, Gentech International LTD) that was interconnected to an additional pump (OR 1250, Aqua Medic GmbH) placed in the reservoir tank. The pump was regulated by the magnetic valve and gradually lowered the gauge in the reservoir until the level sensor dropped, thus opening the magnetic valve until the original water level was reattained. By this, a 100% water exchange could be accomplished within 24 h, without disturbing the adjusted temperature in the experimental setup.

5.2.3 Critical thermal minima (CTmin) trials

Upon sorting and sexing, six groups of brown shrimp, male shrimp of 4.5 and 5.5 cm TL as well as females of 4.5 cm, spent females of 5.5 cm TL and egg-bearing individuals of 5.5 and 6.5 cm TL were transferred to three separate circular holding units for acclimation (Fig. 5.1). Animals <4 cm TL could not be tested due to size selectivity of the gear type used for sampling. The holding units were provided with continuous aeration and connected to three temperature controlled reservoirs tanks. Water in the reservoir tank was tempered by means of the central in-house cooling system, charging a titanium heat exchanger (VT04 CD16, GEA Ecoflex, Sarstedt, Germany) as well as an electrical water chiller (Titan 1500, Aqua Medic GmbH) and titanium heating rods (600 W, Schego). The titanium heat exchangers were controlled as described above, whereas the titanium heaters were regulated by temperature sensors (Pt100 RTD temperature probe, JUMO GmbH & Co. KG, Fulda, Germany) connected to an electronic microstat (Jumo eTRON M, JUMO GmbH und Co KG, Fulda, Germany) keeping temperatures at the respective set value ± 0.2°C. After brown shrimp were transferred to each of the three temperature controlled reservoirs tanks, temperature was adjusted gradually during three successive days to the respective
acclimation temperatures of 4.0, 9.0 and 14.0°C. Hereafter, brown shrimp were acclimated for two weeks. During acclimation, brown shrimp were fed as described above.

**Figure 5.2:** Experimental setup used to evaluate critical thermal minima (CT<sub>min</sub>) and critical lethal minima (CL<sub>min</sub>) of the common brown shrimp. (1) Test chamber (for illustration purposes this chamber is flipped by 45°). The test chamber was subdivided into an inflow compartment (2) that was separated by perforated PVC plates from the (3) experimental chamber for brown shrimp. One test chamber contained 24 individual experimental chambers. Water from the experimental chamber was drained by the (4) outflow compartment. (5) Valve, (6) experimental chamber, (7) water inlet pipe from in-house RAS, (8) water outlet pipe to in-house RAS, (9) experimental chamber inlet pipe, (10) experimental chamber outlet pipe, (11) magnetic valve controlling inflow from in-house RAS, (12) 150 l reservoir tank, (13) level sensor, (14) set-reset flip-flop circuit connected to a (15) pump to reservoir tank water outlet, (16) pumps charging experimental chambers, (17) pump charging heat exchanger water inlet, (18) PT100 thermistor, (19) heat exchanger water inlet, (20) heat exchanger water outlet, (21) titanium heat exchanger, (22) three-way control valve and modulating rotary actuator. (23) PID process controller controlling (22) via temperature values from (18). (24) recirculating chiller. For reasons of simplification, temperature sensors placed in the experimental chambers were omitted.

The experiment for determining CT<sub>min</sub> had a factorial design with three acclimation temperatures and six groups of brown shrimp. Each combination of acclimation temperature and brown shrimp group was replicated three times, resulting in 54 individual trials. For each individual trial, three brown shrimp were used and transferred to three separate experimental
chambers of the setup (Fig. 5.2) adjusted to the respective acclimation temperature. Subsequently, the water in the setup was cooled at -0.2°C min⁻¹. During cooling, water temperature was continuously controlled by a hand held thermometer and water temperature was noted every 30 sec to check for consistency in cooling rates between the different trials and to tune the cooling process when necessary. Periodically during cooling, the righting reflex was tested by prodding the shrimp with a glass rod and turning them over on their back. The critical temperature limit was determined as that temperature, where the shrimp lost its righting response (Lutterschmidt and Hutchison, 1997b; Beitinger et al., 2000). The mean was calculated for the three shrimp from one respective trial and this temperature was depicted to represent the critical temperature.

5.2.4 Critical lethal minima (CLₘᵢₙ) trials

In contrast to the acute CTₘᵢₙ trials, chronic CLₘᵢₙ trials were conducted at a considerably lower cooling rate of -1.0°C day⁻¹. Death of the experimental animals was set as the endpoint in CLₘᵢₙ testing. During subsequent cooling, we also tested whether cooling affected the reactivity of brown shrimp. Instead of prodding with a glass rod, we used an electrical pulse generator to check for brown shrimp reactivity during the CLₘᵢₙ trials. By this, we intended to test two different types of responses. First, the single electrical pulse was used to stimulate the shrimp in a standardised and repeatable manner (Onnen and Zebe, 1983). The pulse should provoke an escape reaction and the intensity of this escape was quantified by means of the number of successive flicks until the shrimp settled on the bottom again. This escape reaction should disclose the temperature at which shrimp become hyperexcitable or when muscular spasms occurred (Friedlander et al., 1976; Lutterschmidt and Hutchison, 1997a, 1997b; Beitinger et al., 2000). Second, once the shrimp stopped their escape reaction, we checked whether the animals lost their equilibrium as they descended to the bottom (Lutterschmidt and Hutchison, 1997a, 1997b; Beitinger et al., 2000). Here, the loss of equilibrium (LOE) corresponds to the loss of the righting reflex in the acute CTₘᵢₙ trials.

The pulse generator for this experiment consisted of a power supply (0-32 V, 3.2 A, Statron Gerätetechnik GmbH) that was connected to two 25 cm stainless steel electrodes, insulated with rubber tubes except for the lower one cm of the material. A MOSFET relay (10 A, 100 V, Custom Sensors and Technology) was interconnected between electrodes and the power supply and coupled to a control button that, when activated, provoked a single electric pulse of 7 ms by means of a set-reset flip-flop circuit. The electrodes were inserted in each of the experimental compartment at 20 cm distance, resulting in a field strength of 20 V or 1.0 V cm⁻¹, respectively. In an initial pretrial, survival of the pulse treated specimen did not differ
significantly compared to a control of 72 brown shrimp confirming the results of Polet et al. (2005) using 65 V or 1.3 V cm⁻¹, respectively.

Upon sorting and sexing, six groups of brown shrimp with equal body size and gender were transferred to the experimental boxes. Two chronic CLₘᵢₙ trails were conducted. The first trial was performed using male shrimp of 4.0 and 5.0 cm, females of 5.0 and 6.0 cm as well as egg-bearing females of 6.0 and 7.0 cm. In the second trial, male shrimp of 4.5 and 5.5 cm, females of 4.5 and 5.5 cm as well as egg-bearing females of 5.5 and 6.5 cm were tested. 24 shrimp of each group were placed in one of the six PE boxes, each shrimp in a separate experimental compartment and were remained undisturbed for 2 days at 7°C water temperature. Hereafter, excitability and reactivity of the brown shrimp were checked by means of a single electrical pulse as described above. Each shrimp was tested once per day. Testing was performed between 10-11am. Upon testing, temperature was reduced by -1.0°C for testing at the following day until the lowest achievable temperature of -1.7°C was reached. The whole trial, including the two days of acclimation, lasted for 12 days. In parallel to the temperated trials, 24 brown shrimp of different gender and size were used as a control group. These shrimp were maintained at 10 ± 0.6°C during the whole experiment. Controls were pulse tested right after the temperated treatments to test for a potential effect of repeated pulsing on reactivity and flicking intensity.

5.2.5 Statistical analysis

All statistical analyses were conducted with the R statistical software (R Development Core Team, 2011) using the car package (Fox and Weisberg, 2011). Data of short-term tests were checked for normality and variance homogeneity by means of a Shapiro-Wilks W test and Levene’s test, respectively. The effect of acclimation temperature on critical temperature limits for each experimental group was tested by means of one-way ANOVAs. A linear model was used to test whether acclimation temperature, gender, body size as well as presence and absence of eggs in female brown shrimp affected the CTₘᵢₙ.

Reactivity data from chronic CLₘᵢₙ trails, determined as the number of flicks, were checked for normality and variance homogeneity as described above. The interrelation of temperature and reactivity was analysed by means of a segmented regression using the segmented package (Muggeo, 2003, 2008). Segmented regressions for each group were conducted to identify potential breakpoints in the temperature-reactivity relationship. The difference in the slope before and after the breakpoint was analysed by means of the Davies-Test as incorporated in the segmented package (Muggeo, 2008). The temperature below this breakpoint was considered as the lower critical temperature. As for the temperated trial, the
segmented regression analysis was conducted for the control group. Here time and reactivity were used as explanatory and response variables, respectively.

5.3 Results
Acclimation temperature significantly affected acute CT_mn values in brown shrimp (Fig. 5.3). Within the range of the acclimation temperatures of 4.0, 9.0 and 14.0°C, acute CT_mn decreased with decreasing acclimation temperature. The effect of acclimation temperature in acute CT_mn was highly significant within all the experimental groups (p<0.001). Female brown shrimp of 4.5 cm and egg-bearing females of 5.5 cm reached the lowest CT_mn of -1.40 ± 0.18 and -1.41 ± 0.46 when acclimated to 4°C, respectively. Male shrimp (-0.66 ± 0.26) and freshly spent females (-0.68 ± 0.29), both of 5.5 cm TL had the highest CT_mn values and were least tolerant to low temperatures when acclimated to 4°C. Data for the effect of acclimation on CT_mn of shrimp are given in Table 5.1. Cooling rates did not differ among individual CT_mn trails (p>0.05). In a linear model on the effect of acclimation temperature, gender and size on CT_mn over all size groups, acclimation temperature was the dominant factor affecting acute CT_mn (p<0.001). Gender was identified to be of borderline significance (p<0.05) whereas size did not contribute significantly to the overall model (p>0.05). Additionally, a borderline significant interaction of body size and gender was observed (p<0.05). Mortality during acclimation to 4.0, 9.0 and 14.0°C for the CT_mn trials varied considerably among the different experimental groups and was highest in shrimp acclimated to 14.0°C. In 4.5 cm female brown shrimp, all animals acclimated to 14.0°C died during the acclimation period and the respective CT_mn could not be determined. Female brown shrimp of 4.5 cm TL were thus excluded from the statistical analyses.

During the 12 day long-term chronic CL_mn trial, mortality was considerably low in all experimental groups. Brown shrimp even survived the coldest temperature of -1.7°C that could be established in the experimental setup. Besides in spent females and males of 5.5 cm where 10% mortality were observed, mortality in the remaining groups ranged around 5%. Thus, CL_mn of brown shrimp could not be determined. Regular temperature measurements confirmed the stable temperature conditions between the gradual temperature decreases throughout the trial. Following the daily temperature decrease of -1.0°C day⁻¹, the new set temperature was achieved within 3-4 hours.
Table 5.1: Acute critical thermal minima (CT\text{min}) of brown shrimp (Crangon crangon) acclimated to 4.0, 9.0 and 14°C. Shrimp were acclimated for two weeks. CT\text{min} for male and female brown shrimp were determined at a cooling rate of -0.2°C min\(^{-1}\). CT\text{min} for female brown shrimp of 4.5 cm TL at 14°C acclimation could not be determined due to high mortality during acclimation. Data represent mean values ± SD.

<table>
<thead>
<tr>
<th>Acclimation temperature</th>
<th>4.5 cm</th>
<th>5.5 cm, spent</th>
<th>5.5 cm, egg-bearing</th>
<th>6.5 cm, egg-bearing</th>
</tr>
</thead>
<tbody>
<tr>
<td>female</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-1.40 ± 0.18</td>
<td>4</td>
<td>-0.68 ± 0.29</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>1.00 ± 0.25</td>
<td>5</td>
<td>0.83 ± 0.37</td>
<td>6</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>-</td>
<td>2.49 ± 0.48</td>
<td>7</td>
</tr>
</tbody>
</table>

| male                    |        |               |                     |                     |
| 4                       | -1.00 ± 0.42 | 9              | -0.66 ± 0.26       | 9                   |
| 9                       | 0.83 ± 0.23  | 9              | 1.23 ± 0.30        | 8                   |
| 14                      | 2.04 ± 0.38  | 9              | 2.58 ± 0.56        | 4                   |

Brown shrimp remained at the bottom and did not show any sign of disturbance when the electrodes were inserted into the experimental compartments. Following a single pulse, the tail muscle contracted vigorously followed by several successive flicks. As temperature decreased, the reactivity of brown shrimp following the pulse was found to be markedly altered (Fig. 5.4). First, the number of successive flicks increased as temperature decreased until ~1.0°C. At temperature below 0°C, however, the number of flicks suddenly dropped. At -1.0°C, the flick generated by the pulse was just occasionally followed by one or two more successive flicks. Below -1.0°C, almost all animals just responded by one flick and hardly any flicks were observed afterwards. However, almost no shrimp lost equilibrium after the flicking phase and most shrimp came down horizontally. No sign of muscular spasms was detected.

The segmented regression analysis revealed a discontinuous temperature-reactivity relationship in the CL\text{min} trials and a single breakpoint was identified. Individual experimental groups just differed slightly with regard to individual breakpoints. Therefore, all groups were aggregated for further analysis. Pooled for all groups, the breakpoint ± SE was determined as 1.04°C ± 0.16 °C. The breakpoint was verified as the slopes on both sides of the breakpoint differed significantly (Davies-Test, p<0.001).
Figure 5.3: Linear relationship between acute critical thermal minima (CT$_{\text{min}}$) and acclimation temperature of brown shrimp (Crangon crangon) acclimated to 4.0, 9.0 and 14.0°C. Acclimation lasted two weeks. Acute CT$_{\text{min}}$ experiments were conducted at a cooling rate of -0.2°C min$^{-1}$. Regression parameters for each experimental group are given in the respective panel. (a) 4.5 cm males, (b) 5.5 cm males, (c) 5.5 cm spent females, (d) 5.5 cm egg-bearing females, (e) 6.5 cm egg-bearing females.

In contrast to the tempered trials, reactivity in the controls did not change after repeated pulsing throughout the experiment (Fig. 5.5). Using time vs. reactivity as explanatory and
response variables, the segmented regression did not identify a single breakpoint and no significant difference in slope of the relationship was detected (Davies-Test, p>0.05).

**Figure 5.4:** Reactivity of brown shrimp (*Crangon crangon*) during gradual cooling at a cooling rate of -1.0°C day⁻¹ from 7.0°C to -2.0°C in the chronic CLₘᵦ trials. Reactivity of brown shrimp was determined as the number of flicks following a single electrical pulse. Filled circles denote temperatures above the breakpoint. Open circles represent temperatures below the breakpoint. The breakpoint was determined by means of a segmented regression. For further details, see text.

### 5.4 Discussion

#### 5.4.1 Critical thermal minima (CTₘᵦ)

By means of controlled laboratory experiments, this study revealed a typical acclimation temperature and CTₘᵦ relationship for the common brown shrimp as thermal tolerance determined as the CTₘᵦ decreased with decreasing acclimation temperature (Fig. 5.3). Over the test range of 4.0-14°C, brown shrimp were found to tolerate temperatures between -1.4°C and 2.6°C. Lowest CTₘᵦ of -0.7 to -1.4°C were found in brown shrimp acclimated to 4.0°C. Brown shrimp acclimated to 14.0°C were less tolerant. Here, CTₘᵦ between 1.7-2.6°C were observed. The strong effect of acclimation temperature on thermal tolerance as observed in the present study has also been reported for other crustacean species before (Diaz et al., 1998, 2002; Hoang et al., 2002; Kir and Kumlu, 2008). Still, brown shrimp tolerated considerably lower temperatures compared to previously investigated subtropical and tropical crustacean species like *Penaeus merguiensis* (Hoang et al., 2002), *P.*
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*semisulcatus* (Kir and Kumlu, 2008), *Macrobrachium rosenbergii* and *M. acanthurus* (Diaz et al., 1998, 2002).

**Figure 5.5:** Reactivity of brown shrimp (*Crangon crangon*) following a daily repeated, single electrical pulse. Reactivity of brown shrimp was determined as the number of flicks. Brown shrimp kept at constant temperature served as a control for the temperate pulse treatment in the CL<sub>min</sub> trials (Fig. 4). The absence of any pattern in brown shrimp reactivity as well as the lack of a breakpoint at constant temperature confirmed increased flicking as well as the breakpoint in temperate trial to be related to a temperature effect and was not due to repeated pulsing. Boxes include the 25% and 75% quartiles. Error bars denote 90% of the data range. Black dots denote the 5<sup>th</sup> and 95<sup>th</sup> percentile range. Open circles represent the water temperature at each respective day.

Compared to acclimation temperature, gender and body size of the brown shrimp affected CT<sub>min</sub> only to a minor degree. Thermal tolerance among the different experimental groups was similar (Tab. 5.1, Fig. 5.2), however, the effect of gender on CT<sub>min</sub> was marginally significant. Female brown shrimp were found to be most tolerant to low temperatures. When compared to males this difference was just marginal (Tab. 5.1, Fig. 5.2). In contrast to gender, body size did not contribute to explaining the overall variance of the CT<sub>min</sub> data. However, as we observed a marginally significant interaction of gender and body size in our analysis, the detected gender specific differences could also be ascribed to a combined effect of gender and body size as well. Thus, although we observed a minor effect of gender on thermal tolerance, the results of the present study confirmed the findings from field studies by Boddeke (1963, 1975) who reported brown shrimp of all live stages to be equally tolerant to low temperatures.
Based on the results of the acute CT$_{\text{min}}$ trials, brown shrimp can tolerate lower temperatures as reported in previous field and modelling studies (Freitas et al., 2007, 2010; Campos and van der Veer, 2008). Campos and van der Veer (2008), extending the synopsis on brown shrimp by Tiews (1970) and reviewing numerous field studies, reported that brown shrimp can sustain temperatures as low as 6°C. Freitas et al. (2007, 2010) using a dynamic energy budget model on rates of oxygen consumption of brown shrimp, calculated a lower thermal tolerance limit of 0°C. Based on our findings, however, the lower temperature tolerance limit of the common brown shrimp was determined as -1.4°C. Indeed, the lowest tolerable temperature of -1.4°C observed in the present was slightly higher compared to the critical temperature threshold hypothesized by Boddeke (1963). Following the severe winter 1962/1963 with subzero seawater temperatures, Boddeke (1963) reported brown shrimp to still occur in the landlocked Veerse Meer and concluded that brown shrimp can sustain seawater temperatures as low as -1.8°C. However, in the study of Boddeke (1963), temperature measurements did not cover the whole area of the Veerse Meer and brown shrimp might have sought less cold refuges for overwintering. Indeed, the results of the present study confirm that brown shrimp can survive temperatures close to the freezing point of natural seawater.

The CT$_{\text{min}}$ in the present study were based on the loss of the righting response set as the end point in the CT$_{\text{min}}$ trials. So far, various behavioural endpoints have been used to specify the onset of critical temperature effects (Lutterschmidt and Hutchison, 1997a, 1997b; Beitinger et al., 2000). When approaching suboptimal temperatures, aquatic ectotherms usually respond by increased activity as a first sign of stress (Lutterschmidt and Hutchison, 1997a, 1997b; Hoang et al., 2002; Kir and Kumlu, 2008). When temperature decreases further, this is followed by jerky motions and uncoordinated motor activity as observed in crustaceans as well as fish (Beitinger et al., 2000; Hoang et al., 2002). If conditions still exacerbate, a loss of the righting response might occur followed by decreased and shallowed respiratory movements before a comatose state arises (Prosser and Nelson, 1981). Muscular spasms usually occur before a loss of the righting response (Prosser and Nelson, 1981; Lutterschmidt and Hutchison, 1997a, 1997b; Beitinger et al., 2000). Lutterschmidt and Hutchison (1997a, 1997b) suggested the onset of muscular spasm as the definite end point to be a more precise and conservative measure rather than a loss of the righting response. However, during the CT$_{\text{min}}$ trials of the present study, we did not observe any sign of muscular spasm when brown shrimp approached critically low temperatures. In contrast to the chronic CL$_{\text{min}}$ trials, just a slight increase in activity was observed in the CT$_{\text{min}}$ trials,
leaving the loss of the righting response as the only applicable endpoint for $CT_{\text{min}}$ testing in the present study.

5.4.2 Critical lethal minima ($CL_{\text{min}}$)

Apart from the strong effect of previous acclimation demonstrated in the acute $CT_{\text{min}}$ trials, this study also showed that the rate of cooling strongly influences the lower temperature tolerance of brown shrimp as has been shown for other crustaceans before (Diaz et al., 1998, 2002; Hoang et al., 2002; Kir and Kumlu, 2008). In contrast to acute $CT_{\text{min}}$, chronic $CL_{\text{min}}$ do not only account for the effect of temperature but also include the effect of time the animals are exposed towards low temperatures (Lutterschmidt and Hutchison, 1997b; Beitinger et al., 2000). However, chronic $CL_{\text{min}}$ also account for the capacity of reacclimation as well as cold-hardening during the experimental trial (Beitinger et al., 2000). Even though the time given the shrimp for acclimation during the successive steps of cooling is limited in $CL_{\text{min}}$ experiments, chronic $CL_{\text{min}}$ trials represent natural conditions during winter cooling more realistically than do acute $CT_{\text{min}}$ experiments. In the present study, the lower cooling rate in the $CL_{\text{min}}$ trials revealed the great potential for reacclimation and cold-hardening of brown shrimp as brown shrimp even sustained the lowest temperature of $-1.7^\circ\text{C}$ that could be achieved in the experimental setup. Death used as the endpoint in chronic $CL_{\text{min}}$ testing (Lutterschmidt and Hutchison, 1997b; Beitinger et al., 2000) did not occur and thus chronic $CL_{\text{min}}$ of brown shrimp could not be exactly determined in the present study. As with the results of the $CT_{\text{min}}$ trials, the results of the $CL_{\text{min}}$ trials further confirm the field observations by Boddeke (1963, 1975) on low temperature tolerance of brown shrimp.

Even if brown shrimp can survive temperatures as low as $-1.7^\circ\text{C}$, we observed that brown shrimp are adversely affected by temperatures approaching $0^\circ\text{C}$. When subjected to decreasing temperatures between $7^\circ\text{C}$ to $-2^\circ\text{C}$ and excited with a single electrical pulse, reactivity of brown shrimp initially increased in a linear manner as temperature decreased. This increase in reactivity might represent a first sign of stress due to low temperature (Prosser and Nelson, 1981) and indicate that the shrimp were trying to escape from critical thermal conditions (Neil and Ansell, 1995). A rapid increase in reactivity, i.e. hyperexcitability, was not detected at any of the respective temperatures in the chronic $CL_{\text{min}}$ trials. However, at temperatures below $1.0^\circ\text{C}$ we observed a sharp decrease in the flicking response, indicating the onset of suboptimal thermal conditions with brown shrimp possibly entering a torporous state. At temperatures below $1.0^\circ\text{C}$, most shrimp just responded by two flicks, whereof one was initiated directly by the action potential induced by the pulse. As in the
acute CT\textsubscript{min} trials, all size classes were found to be equally susceptible to low temperature when cooled at -1.0°C day\textsuperscript{-1}.

The breakpoint specified in the chronic CL\textsubscript{min} trials by means of the segmented regression was determined at a temperature where considerable flicks were still detectable. Therefore, adverse thermal effects may start below this breakpoint. In contrast to the CL\textsubscript{min} trials, reactivity in the controls did not change after repeated pulsing throughout the experiment (Fig. 5.5) and the segmented regression did not identify a single breakpoint. Therefore, the controls revealed that reactivity in the temperate trials changed due to temperature rather than repeated application of the electrical pulse.

Apart from the present study, electrical pulsing has already been used for experiments on brown shrimp successfully (Onnen & Zebe, 1983; Polet et al., 2005). Onnen & Zebe (1983) used repeated electrical pulses to provoke tail flicks of different intensity, investigating energy metabolism of brown shrimp during muscle activity and subsequent recovery. Polet et al. (2005) investigated the tolerance of brown shrimp to electrical pulses while evaluating an electrical beam trawl for commercial brown shrimp fishery. In contrast to the present study, Polet et al. (2005) used continued pulsing to carry the shrimp off the ground. The authors reported a strong flicking response of brown shrimp when stimulated with electrical pulses of 40-110 V or 0.8-2.2 V cm\textsuperscript{-1}, respectively. In their experiments, shrimp where shown to stand pulsing well and pulse related mortality was low. In contrast to these previous studies, we just used a single electrical pulse to frighten the shrimp and trigger one single flick. For short term testing, pulsing was assumed to be inappropriate as the high cooling rate would necessitate repeated pulsing within a short period. Repeated pulsing in CT\textsubscript{min} testing might bring the shrimp to exhaustion (Onnen and Zebe, 1983) and corrupt the temperature effect.

5.4.3 Critical temperature limits of the common brown shrimp: Ecological significance, implications for fisheries and field surveys

The critical thermal limits obtained by the acute CT\textsubscript{min} trial and the thermal threshold determined at a cooling rate of 1.0°C day\textsuperscript{-1} are hard to compare. Different endpoints were used to determine the threshold where brown shrimp reach their thermal capacity limits. In general, both types of experiments revealed the high capacity of brown shrimp towards low temperatures and even subzero temperatures. In the acute CT\textsubscript{min} trials it became apparent that brown shrimp can tolerate temperatures from 2.6°C to -1.4°C when acclimated between 4.0 and 14.0°C. In contrast, the threshold determined by electrical pulsing at a cooling rate of 1.0°C day\textsuperscript{-1} reveals a slightly higher critical temperature limit of 0°C. Still, shrimp exposed to -1.7°C in the chronic CL\textsubscript{min} trials did not show indications of a loss of the righting reflex used
as endpoint criterion in the acute $CT_{\text{min}}$ trials. We therefore assume that brown shrimp can survive temperatures even below the threshold stated in the $CT_{\text{min}}$ when they are given the chance for acclimation and cold-hardening. The sudden drop of reactivity without a clear sign of hyperexcitability in the $CL_{\text{min}}$ might indicate that brown shrimp lapse into torpor, starting at temperatures below 1.0°C. Burying in the sediment as reported for brown shrimp (Tiews, 1970) might be used to sustain the torpor and endure critically low temperatures until thermal conditions improve and energy reserves (Kattner et al., 1994) might help the brown shrimp to sustain this critical phase with low food availability (Hufnagl et al., 2010).

With regard to the life cycle of brown shrimp, the results of both trials are highly relevant. This especially applies to egg-bearing females. Following Hufnagl and Temming (2011), the brown shrimp population mainly derives from eggs spawned in winter, whereas eggs spawned during summer are of less importance for autumnal peak abundance in the Wadden Sea. Adaptability of egg-bearing females towards low temperatures is thus of crucial importance for the overall brown shrimp population. Egg-bearing individuals were found to be as tolerant as the remaining experimental groups in the present study. Higher thermal sensitivity in specimens at advanced maturation state as well as in egg-bearing females as previously reported (Boddeke, 1976; Campos and van der Veer, 2008) may therefore be exclusively related to the onset of the autumnal migration but not to temperature tolerance. However, it has yet to be investigated whether eggs and developing larvae can tolerate such low temperatures as well.

Apart from this ecological context, the findings from this study are also highly relevant for scientific surveys assessing total abundance and biomass of the brown shrimp stock. In these surveys, gear types are being used that rely on an active flight response of the shrimp (Berghahn et al., 1995). When startled by the gear, usually by bobbins, tickler chains as well as the rapidly rising water currents and pressure waves induced by the gear, the shrimp emerge from the ground and are being directed towards the mouth of the net (Berghahn et al., 1995). As some of these surveys are being conducted during the winter season covering shallow coastal areas, critical temperatures as determined in the present study might occur, affecting catchability of brown shrimp (ICES, 2011). Jeffery and Revill (2002) already showed that the escape reaction of brown shrimp is affected by water temperature resulting in reduced catchability at lower temperatures. Based on the findings of the chronic $CL_{\text{min}}$ trials, however, brown shrimp might not respond to the approaching gear at all. Low catches at subzero water temperatures (ICES, 2011) might therefore be due to a reduced or even lacking escape reaction instead of absence in a given swept area and potentially affect and even distort abundance estimates. This should be considered and evaluated in respective
field trials at low seawater temperatures, comparing the commonly used gear types as well as fishing gear that does not rely on an active escape response of the shrimp.

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5.6 References


6. GENERAL DISCUSSION

The aim of this thesis was to contribute to a better understanding of the brown shrimp’s thermal biology. On the one hand, this thesis investigated the directive role of temperature by means of experiments on the thermal preference behavior, which has so far not been considered for the common brown shrimp. On the other hand, the lower critical thermal limits were determined to reveal the thermal capacity of brown shrimp towards low temperatures and thus to complete the current knowledge on the brown shrimp’s thermal niche. Moreover, the present thesis contributes to recently introduced methodological approach for thermal preference determination, i.e. the annular chamber system, and presents a computer based routine for automated data analysis of thermal preference experiments in this type of setup.

6.1. Experimental setup for thermal preference determination

Selecting an appropriate experimental system for the thermal preference experiments comprised a crucial first step in this thesis. As a wide range of experimental setups has been used to determine thermal preferenda in laboratory based experiments so far (McCauley, 1977; Myrick et al., 2004) several methodological approaches were under consideration. From the various types of setups (McCauley, 1977), linear gradient tanks, shuttleboxes and annular chamber systems were of particular interest as this thesis started.

Linear gradient tanks of rectangular shape were taken into consideration as this type of setup has been used in numerous thermal preference studies before (e.g., Mathur et al., 1982; Kivivuori and Lagerspetz, 1990; Chen and Chen, 1991; Lafrance et al., 2005; Bates et al., 2010). Thus, a vast amount of information concerning construction, handling as well as strengths, weaknesses and potential pitfalls of this type of setup was available. In addition, rectangular gradient tanks are easy to construct and would allow for a contemporary start of data collection. However, the use of linear gradient tanks becomes problematic when thermal gradients of a wide range are being established as intended in this thesis. The length of the trough has to be adapted accordingly and as the length of the test apparatus increases, automated recording and object detection in automated analysis becomes problematic, especially when small individuals shall be investigated in the setup as well. Still, the biggest drawback of rectangular setups is the various points of thigmotactic cues that are associated with the different temperature levels inside the system. The presence of corners or the proximity to corners meaning potential cover might bias thermal selection as the test organisms orientate towards both ends of the setup (Badenhuizen, 1967; Bevelhimer 1996; Dillon et al. 2009).
In contrast to linear gradient tanks, shuttleboxes use a temporal rather than a spatial temperature gradient allowing for a wide thermal range (Neill et al. 1972; Reynolds & Casterlin 1979a; Staaks et al. 1999; Mortensen et al. 2007). Shuttleboxes further offer the advantage of thermal preference experiments being recorded and analysed automatically (Neill et al., 1972; McCauley, 1977). However, only one specimen can be tested at a time (McCauley, 1977) unless the animals show synchronous swarming behavior (Ohlberger et al., 2008) which does not apply to brown shrimp (Tiews, 1970). Non synchronous movements of numerous brown shrimp within the shuttlebox would thus prevent a temporal thermal gradient from being established in the system. It is further unknown whether brown shrimp are able to learn how to control the shuttlebox and thus their body temperature. As this thesis started, there were just few examples of thermal preference experiments with crustaceans in shuttleboxes (Casterlin and Reynolds, 1977; Reynolds and Casterlin, 1979a; Tattersall et al., 2012). These studies, however, investigated solitary members of the Reptantia which usually walk rather than swim. Brown shrimp appear in groups and might perform differently as it belongs to the Natantia which predominantly move by swimming. The study by Tattersall et al. (2012) moreover showed that the thigmotactic cues in shuttleboxes affect the distribution within the chambers and might thus affect thermal preferenda of brown shrimp.

As both types of systems seemed to be unsuitable to determine thermal preference in the common brown shrimp, an annular chamber system (Myrick et al., 2004) was used for the experiments in the present thesis. Annular chambers counteract most of the problems in the aforementioned systems and are considered advantageous compared to more classical setups (Myrick et al., 2004). Annular chambers have been successfully employed in several thermal preference studies on fishes already (Myrick et al., 2004; Chen et al., 2008; McMahon et al., 2008; Gräns et al., 2010, 2012; Klimley et al., 2011; Behrens et al., 2012; Schram et al., 2013). However, the suitability of the advantageous design for experiments on the common brown shrimp had to be evaluated in the present thesis. Still, in addition to extensive evaluation and construction works that are associated with the establishment of a relatively new and technically complex experimental setup, an automation procedure for data recording and data analysis was not available at the beginning of this thesis. Myrick et al. (2004) as well as Chen et al. (2008) used an observer to record position and temperature data, making the experiments with annular chambers laborious and time consuming. Indeed, permanent observation does not allow for highly resolved preference data over a prolonged experimental period. McMahon et al. (2008) therefore used a video camera for observation and recording of the animal’s positions within the annular chamber. Temperatures were
assigned subsequently based on temperature measurements that have been conducted prior and after each experiment. Gräs et al. (2010), Klimley et al. (2011) and Schram et al. (2013) extended this approach and provided the annular chamber with thermistors for automated temperature measurements throughout the swimming channel. Still, assignment of position and temperature data had to be conducted manually. Behrens et al. (2012), in a very recent study, used a custom made combination between the National Instrument Vision Builder and the LabView software. In their approach, a single experimental animal per run was tracked over the whole experimental period resulting in highly resolved thermal preference data. The MATLAB routine developed for the present thesis (Chapter I, supplementary information) avoids the time consuming manual assignment of temperature data as this procedure is conducted automatically. By means of this program, multiple animals got detected in the setup and the respective temperatures were assigned accordingly. Extensive evaluations have been performed to assure proper functioning and correct assignment by the routine. This revealed a high precision and low error rate. Supposing some slight modifications and adjustments, the here presented program can be easily transferred to other annular chamber systems. Thus, the automated routine presented in this thesis will facilitate future thermal preference experiments in annular chambers considerably allowing for highly resolved preference data, even in prolonged trials. Besides this, the general principle underlying the routine can also be applied to other types of experimental systems where position data have to be assigned to any spatially resolved factor. Preliminary tests of the annular chamber that were conducted without a thermal gradient in the swimming channel, revealed the suitability of the system for experiments on the common brown shrimp (Chapter I). However, a slight preference of the brown shrimp towards the outer and the inner walls of the swimming channel was observed. This confirmed the current setup as a prerequisite to obtain unbiased preferenda as the circular shape counteracts previously observed site-specific bias and preference towards the end of rectangular setups (Badenhuizen, 1967; Bevelhimer 1996; Dillon et al. 2009). Apart from the numerous advantages of the annular chamber system (Myrick et al., 2004), this thesis also identified some drawbacks in this type of setup. As for any spatial, either horizontal or vertical thermal gradient, the test organisms can easily get access a relatively wide range of temperatures within a limited distance. Brown shrimp could thus shuttle within a temperature range that is considerably wider compared to natural conditions and the huge thermal range at narrow space might prompt the shrimp to explore extreme temperatures more frequently as discussed by Behrens et al. (2012) already. This experimental constrain, however, is inherent to almost all choice and preference experiments using setups holding a
gradient. However, the present thesis obtained thermal preferenda by means of the gravitational approach (Chapter II). In contrast to preferenda determined by the acute approach, gravitational preferenda are retrieved once shuttling stopped, meaning that brown shrimp select a relatively narrow temperature range. Spatial proximity of a wide range of temperatures might therefore be of less concern for the results of this thesis.

Another drawback of the system has already been identified in the original setup by Myrick et al. (2004). As in the original setup, the space occupied by the different temperatures was not equal in the system used in this thesis. Due to the annular design, the thermal gradient consisted of two semicircles of equal temperatures (Chapter I). The warmest and coldest temperatures were therefore less available compared to intermediate temperatures as these temperatures were available on both sides of the circular gradient. McMahon et al. (2008) increased the warmest and coldest section of the swimming channel accordingly counterbalancing this discrepancy. Indeed, McMahon et al. (2008) as well as Myrick et al. (2004) investigated considerably larger animals in their annular chamber systems. In contrast, the annular chamber used of the present study was, first, considerably larger compared to the two aforementioned systems and, second, the experimental animals were much smaller. Thus, all temperatures should be provided in a sufficient spatial amount. Still, as preference temperature was calculated as the median preferred temperature among all individuals within one experimental trial (Mathur & Silver 1980; Karlsson et al. 1984) the upper thermal preference range might potentially be slightly underestimated.

Running an annular chamber turned out to be quite delicate and labour intensive. The complex setup with numerous water inlets to heating and cooling reservoirs as well as water lines into the annular chamber needed to be adjusted, controlled and readjusted on a regular basis. Especially when operated in seawater, fouling is of concern and the setup has to be cleaned thoroughly to avoid the formation of biofilms. Biofilms will especially arise in the warm sections of the annular chamber and might attract the test organisms and thus bias thermal preference. Fouling is also a great problem regarding the pipes and pumps for water supply as well as the perforated walls of the annular chamber as flow rate and thus the discharged water volume might be affected. Additionally, debris from the pipes might get detached and enter the swimming channel, potentially interfering with automated object detection. Thus, one day per week was exclusively provided for cleaning the setup and readjusting the installation to assure proper functioning throughout this thesis. Some of these issues, however, might be related to the present system being incorporated in the in-house recirculating water system. In a second annular chamber system constructed during this thesis (Mues, 2012), the chamber was provided with a separate water circulation making the
overall system more stable and independent from potential effects that may derive from the main recirculating system. In this closed setup, fouling was less of a problem and maintenance was by far less labour intensive, potentially as the reduced amount of nutrients delayed microbial growth.

6.2. Acute and gravitational thermal preference

Using the annular chamber, thermal preference could be determined in two different ways. These two methodologies, i.e. the acute and the gravitational approach, were derived from the bipartite definition of the final thermal preferendum, originally postulated by Fry (1947). In Chapter II of this thesis, both methodologies were applied and the respective outcomes compared, first, to evaluate the effect of previous thermal acclimation on thermal selection and, second, to determine whether gravitational preference was unaffected by prior acclimation. The results obtained by these approaches should reveal the most suitable methodology for determining thermal preference throughout the seasonal cycle in Chapter III.

By means of the acute temperature preference tests in Chapter II, a positive temperature-preference relationship in brown shrimp was identified. Positive temperature-preference relationships are typical for eurythermic species like the common brown shrimp experiencing a wide temperature range throughout the seasonal cycle (Johnson and Kelsch, 1998). Additionally, this positive relationship revealed acute preference to be highly affected by prior thermal acclimation. Brown shrimp were also shown to be thermosensitive and perform behavioral thermoregulation confirming previous results on other crustacean species (Lagerspetz and Vainio, 2006). In contrast to the acute approach, gravitational thermal preferenda were unaffected by prior acclimation. This finding can indeed be considered as a prerequisite for conducting the long-term experimental approach in Chapter III, where thermal preferenda were determined throughout the seasonal cycle. For the experiments in Chapter III, it was essential to exclude any effect of the prior thermal history on thermal preference revealing whether brown shrimp share a common final thermal preferendum (Fry, 1947; Reynolds and Casterlin, 1979b). The results in Chapter II thus revealed thermal preferenda to be unaffected by the temperature the animals experienced in the field as well as during husbandry when being exposed to the gradient for a sufficient amount of time. In contrast to previous studies on fish suggesting 24-96 h to obtain gravitational preference (Richards et al., 1977; Reynolds and Casterlin, 1979b), this thesis determined a period of (at least) 20h as sufficient to determine gravitational thermal preference in the common brown shrimp.
In contrast to the definition by Fry (1947), the acute and gravitational preferenda determined in Chapter II differed, as prolonged exposure was associated with lower preferred temperatures. The acute thermal preference experiments revealed a final thermal preferendum of 15.9°C. In the gravitational thermal preference experiments, however, brown shrimp preferred 13.5-15.0°C after 24 h and 12.0-14.9°C after 48 h of gradient exposure. Thus, both methodologies did not result in an identical preference temperature, i.e. the final thermal preferendum (Fry, 1947; Reynolds and Casterlin, 1979b) as stated in previous studies (Diaz et al., 1993; Hernandez et al., 1995; Perez et al., 2003; Diaz et al., 2007). Other studies (Badenhuizen, 1967; Reynolds and Thomsen 1974; deVLaming 1975; Richards et al., 1977; Reynolds, 1978) observed a similar discrepancy between both methodologies as did the present thesis. It has been speculated that the observed differences between acute and gravitational thermal preference might be related to nutritional correlates or starvation that arises during prolonged gravitational preference trials (Richards et al., 1977). Previous studies showed that starvation and poor food quality were associated with lower thermal preference compared to well fed specimens (Morgan, 1993; Magee et al., 1999; Pulgar et al., 1999; van Dijk et al., 2002; Pulgar et al., 2003). Apart from that, acute preferenda might be affected by increased stress levels immediately after the animals are introduced in the experimental system (Reynolds, 1977). Reynolds (1977) hypothesized that increased stress levels prompt the specimen to select higher temperatures. In contrast, following habituation to the system, the specimens might return to temperature preference values that are unaffected by stress and select lower temperatures in prolonged, gravitational trials. Indeed, the mismatch between acute and gravitational preferenda observed in Chapter II might be a first indication that brown shrimp do not hold a species specific FTP which has been further revealed in Chapter III.

6.3. Brown shrimp and the final thermal preferendum

To investigate whether thermal preference in the common brown shrimp coincides with the final thermal preferendum paradigm (FTP) (Fry, 1947), chapter III determined the thermal preferenda of male and female brown shrimp of different body size throughout the seasonal cycle. According to Fry’s bipartite definition of the final thermal preferendum (FTP) (Fry, 1947), gravitational thermal preference experiments should result in a species specific preference temperature being unaffected by the prior thermal history or any other non-thermal factor.

Although the long-standing paradigm on the FTP gains support by numerous authors (e.g. Richards et al., 1977; Reynolds and Casterlin, 1979b; Jobling, 1981) the FTPP has been
challenged by several recent studies, indicating that thermal preference is more flexible than initially expected and influenced by numerous factors. Several studies showed thermal selection and preferred temperatures to be affected by the nutritional state as well as starvation (Morgan, 1993; Magee et al., 1999; Pulgar et al., 1999; Despatie et al., 2001; van Dijk et al., 2002; Pulgar et al., 2003). Here, specimens that were fed a reduced ration or starved selected lower temperatures compared to well fed ones (Morgan, 1993; Magee et al., 1999; Pulgar et al., 1999; Despatie et al., 2001; van Dijk et al., 2002). Lower food quality also resulted in lower preferred temperatures compared to fish fed diets of high nutritional value (Pulgar et al., 2003). Ontogeny was observed to influence thermal preference as well, with juveniles selecting higher temperatures compared to adults (e.g. McCauley & Huggins, 1979; Lafrance et al., 2005). Thermal preference was also found to differ in genetically polymorphic cod (Petersen & Steffensen, 2003; Behrens et al., 2012) and was affected by the reproductive state in mountain whitefish (Ihnat & Bulkley, 1984) and salmon during their spawning migration (Roscoe et al., 2010). Recently, Bertolo et al. (2011) identified that brook trout perform distinct behavioral thermoregulatory tactics resulting in spatio-temporal segregation among individuals with distinct tactics. Above all, seasonality was identified to massively affect thermal preference behavior in several fish species, with lower preferred temperatures in winter compared to summer (e.g. Zahn, 1964; Hesthagen, 1979; Clark & Green, 1991; Tapaninen et al., 1998; Mortensen et al., 2007).

The results of Chapter III suggest that factors like season, ontogeny and maturity not only influence thermal preference in vertebrate ectotherms like fish, but do also affect thermal selection in invertebrate ectotherms like the common brown shrimp. Brown shrimp displayed a high individual variability in preferred temperatures. Thermal selection of brown shrimp was also found to be rather variable throughout the seasonal cycle and did not result in a common, species specific FTP. The overall range of selected temperatures, varying around $\Delta 18^\circ C$ was far beyond the typical expansion of the thermal preference zone which is usually 2-4$^\circ C$ wide (Magnuson et al., 1979), suggesting the existence of distinct seasonal thermal preference zones in brown shrimp. The statistical analysis from chapter III revealed that seasonality can be considered as a main factor influencing thermal selection and thermal preference in brown shrimp as has been observed in fishes before (e.g. Zahn, 1964; Hesthagen, 1979; Clark & Green, 1991; Tapaninen et al., 1998; Mortensen et al., 2007).

Apart from seasonality, body size affected thermal selection of brown shrimp as well, as small brown shrimp preferred warmer temperatures compared to large ones. As in fish (e.g. McCauley & Huggins, 1979; Lafrance et al., 2005) ontogeny therefore modulates thermal preference in the common brown shrimp. Chapter III also identified that large brown shrimp
may prefer high temperatures under certain circumstances. Large females favored relatively high temperatures during ovarian development in late summer, suggesting that maturation state affects thermal selection as previously observed in fish (Ihnat & Bulkley, 1984; Roscoe et al., 2010).

The results of Chapter III further suggest that thermal preference might be related to the seasonal origin of a cohort. However, this predictor could not be included into the statistical analysis due to high colinearity with the number of the day the experiments were conducted which was used as a continuous predictor of seasonality. The effect of cohort became apparent since thermal preferenda showed a steep increase as soon as the experimental animals originated from recruits of the present year. Here, the upper as well as the lower range of preferred temperatures increased notably. Moreover, thermal preferenda of brown shrimp belonging to late cohorts remained on a high level during the following winter and were considerably higher compared to the winter in the previous year. This might be related to distinct behavioral thermoregulatory tactics as observed in fish (Bertolo et al., 2011) or distinct preferenda among cohorts within and among successive years, arising from the thermal conditions experienced during recruitment and developmental acclimation (Angilletta, 2009).

Apart from the previous findings on fish, the results of Chapter III therefore further challenge the validity of the FTPP. The lack of a common FTP as well as the high amount of individual variability in thermal selection suggests a huge amount of phenotypic plasticity in thermal preference behavior of brown shrimp. This high individual variability might also account for the great potential for adaptation towards a wide range of environmental temperatures in the overall brown shrimp population and explain for the great expansion in the species’ distribution range.

### 6.4. Temperature and the annual life cycle of brown shrimp

The complex migration and distribution patterns of the common brown shrimp have been investigated in several field studies so far (Ehrenbaum, 1890; Havinga, 1930; Hartsuyker, 1966; Al-Adhub and Naylor, 1975; Boddeke, 1976; Janssen and Kuipers, 1980). In these studies, temperature was identified as one of the dominant factors influencing the regional distribution of this species. The results of the present thesis confirm these previous findings and suggest the regional distribution patterns to be driven via thermal preference behavior, differing seasonally as well as during the brown shrimp’s life cycle.

Chapter III showed that thermal preference of brown shrimp varied considerably throughout the annual cycle. The overall range of ~18°C was far beyond the typical expansion of the
thermal preference zone which is usually around 2-4°C (Magnuson et al., 1979), suggesting the existence of distinct seasonal thermal preference zones in brown shrimp. Thermal preference was lowest during winter, however, thermal preferenda were higher compared to the natural conditions usually discovered in the field during this season. During the cold winter 2010/11, brown shrimp selected mean temperatures around 8°C whereas a mean of ~14°C was selected during the relatively mild winter 2011/12. In general, preference towards higher temperatures during winter indicates that brown shrimp have to cope with suboptimal thermal conditions in the field. Additionally, preference for higher water temperatures might also guide brown shrimp towards the highest available temperatures in the field helping to avoid adverse temperature effects. Differences in preferred temperatures among years suggests a lack of a common “winter preferendum” reflecting a high plasticity in thermal preference behavior and might be related to an adaptation towards the current thermal conditions. This might also affect the extent of the brown shrimp’s winter migration (Boddeke, 1976). In severe winters, like that in 1962/63, brown shrimp were reported to migrate far offshore. Higher preferenda during mild winters might thus result in shorter winter migrations (Boddeke, 1976) or prompt brown shrimp to even overwinter inshore as reported by Boddeke (1976).

Although brown shrimp were found to prefer relatively high water temperatures during winter, chapter IV revealed that brown shrimp possess the capacity to even cope with extreme thermal conditions during this period. By means of acute CT$_{\text{min}}$ and chronic CL$_{\text{min}}$ experiments, brown shrimp of all investigated size classes (4.5-6.5 cm TL) were found to be equally resistant towards low temperatures confirming previous field observations by Boddeke (1963, 1976). In acute CT$_{\text{min}}$ experiments, brown shrimp tolerated temperatures around -1.4°C and 2.6°C, depending on the prior acclimation temperature. Due to the brown shrimp’s high tolerance, the chronic CL$_{\text{min}}$ could not be determined but critical lethal temperatures can be assumed to be close to or even below the freezing point of natural seawater. Still, the results of the CL$_{\text{min}}$ experiments suggested temperatures close to 0°C to indeed affect brown shrimp. At temperatures around 0°C, brown shrimp were observed to just weakly respond to external stimuli and seemed to enter a torporous state. Before this torpor arises, brown shrimp might burry themselves into the substratum sustaining low temperature conditions. It is unknown how long brown shrimp can sustain torpor during winter. However, Boddeke (1963) reported that brown shrimp were able to sustain low temperatures as low as -1.8°C for several weeks in the landlocked Verse Meer demonstrating that brown shrimp are adapted to even extreme temperature events in the habitat.
GENERAL DISCUSSION

Following low preferenda in winter, thermal preference just increased slightly during the following months. Preferenda during spring corresponded to temperatures measured in the shallow coastal areas indicating thermal preference behavior to play an important role in the onset of the spring migration as brown shrimp prefer temperatures common in shallow coastal areas. Still, other factors like increasing day length, light intensity or alterations in hormone levels like that of melatonin (Hickman et al., 2007; Aguzzi and Sarda, 2008) or a combination of these factors might be of equal importance as temperature. From spring onwards, thermal preference continued to increase slowly during the following months and rarely exceeded field water temperatures until late summer. Most notably, thermal preferenda were found to be considerably variable during this phase potentially reflecting different adaption or thermoregulatory strategies as previously reported in fish (Bertolo et al., 2011). Thermal preferenda being lower than field temperatures during April-August also suggest that temperature cannot be considered as the ultimate factor causing the high aggregations in the warm and shallow coastal areas. As temperature is more or less favorable throughout the habitat during this phase of the year, thermal selection might be dominated by avoidance towards critically high temperatures rather than preference behavior. In addition, brown shrimp might use other preference cues than temperature to select its whereabouts during summer. Here, food availability as previously suggested (Havinga, 1930; Lloyd and Yonge, 1947) as well as food quality might be of particular importance during this phase keeping the brown shrimp in the highly productive areas. High food supply and favorable thermal conditions then promote rapid growth and development of brown shrimp (Boddeke et al., 1986; Hufnagl and Temming, 2011a; Hufnagl and Temming, 2011b).

Thermal preferenda showed a marked increase as field water temperatures decreased during autumn. The seasonal origin of a cohort is suggested to be of particular importance for this observation, potentially resulting from developmental acclimation during the brown shrimp’s ontogeny (Angilletta, 2009). Still, preference towards the highest available temperature in the field might also serve to guide the shrimp towards deeper waters during winter migration.

Besides the general variability as well as the high amount of individual plasticity, the temporal pattern of thermal preference observed in this thesis suggests that thermoregulatory behavior in brown shrimp is adjusted to the annular temperature cycle in its natural habitat guiding the shrimp to the most favorable thermal conditions. Thermal preference behavior, however, is suggested to be one of many potential factors and food availability and food quality might be as important as temperature during certain phases of the year. Especially
during summer, thermal selection seems to be dominated by avoidance rather than preference behavior as preference was found to be highly variable during this period. Besides this behavioral adaptation towards temperature, this thesis further revealed that brown shrimp still possess the capacity to even cope with extreme temperature events.

6.5 Past and future implications of climatic variability on the common brown shrimp

During the last few years, landings of the brown shrimp fishing fleet revealed marked changes in brown shrimp geographic distribution throughout the North Sea (ICES, 2005, 2008). Landings in the southern part of the North Sea off France and western Belgium decreased whereas increased catches were reported from Danish waters (ICES, 2005). High densities of brown shrimp at the coast off Denmark were also observed during scientific surveys (ICES, 2005, 2007). In addition, analysis of time series on shrimp densities in the Dutch and German North Sea revealed a decreasing trend in inshore waters. In contrast, densities in offshore waters increased (ICES, 2005).

It has been hypothesized that the observed changes in abundance and distribution might be related to climatic related impacts with brown shrimp avoiding increasing maximum temperatures in summer and autumn, thus migrating to more northerly and deeper waters as has been previously reported for fish (Perry et al., 2005, Dulvy et al., 2008). However, the trend in northern waters seemed to be of temporary nature as landings did not increase any further. In contrast, landings even showed a slight decrease in more recent years (ICES, 2011, 2012a) which might be related to an again slightly decreasing trend in North Sea water temperatures from 2003 onwards (ICES, 2012b). Apart from temperature, high abundances in northern areas might also have resulted from altered drifting patterns of larval shrimp causing juveniles to recruit in more northerly areas. Moreover, as juvenile and even adult brown shrimp are known to perform extended migrations using tidal streams and currents (Hartsuyker, 1966; Al-Adhub and Naylor, 1975; Janssen and Kuipers, 1980), favorable currents might have occasionally transported the shrimp to northern realms.

In contrast to the situation in Danish waters, brown shrimp catches in the southern part of the North Sea continued to remain on a low level for several years already (ICES, 2012a). This, as well as the shift of the overall brown shrimp population to deeper waters (ICES, 2005) therefore suggests that regional distribution patterns of brown shrimp changed persistently. With regard to previous studies on the thermal biology of brown shrimp (reviewed in Campos and van der Veer, 2008; Freitas et al., 2010; Hufnagl and Temming, 2011a, 2011b) and based on the results of this thesis, temperature and thermal preference behavior are considered as important factors for these changes. Considering the prospected increases in
mean global temperatures of 2-4°C by the end of this century (IPCC, 2007), the observed changes might even become more pronounced in the future. However, as this thesis revealed a huge amount of individual variability as well as brown shrimp did not share a common FTP, it is rather unlikely that the overall population will be affected and respond to future changes in a uniform manner. The results therefore imply that climate driven effects on the common brown shrimp differ among life stages as well as during the seasonal cycle.

As shown for a 25-year time series in the Bristol Channel (Henderson et al. 2006), brown shrimp might benefit from increasing winter water temperatures as these conditions improve survival of larval shrimp. Recruitment, however, might be negatively affected by high winter water temperatures as observed by Siegel et al. (2005) as well as Campos et al. (2010) potentially due to increased mortality by predation during predation (Temming and Damm, 2002) as well as density-dependent control mechanisms (Henderson et al., 2006). As the results of Chapter IV suggest that brown shrimp are able to sustain extremely low temperatures by entering a torporous state, higher winter temperatures might increase energy demands of brown shrimp resulting in higher mortalities due to starvation (Hufnagl et al., 2010).

Juvenile and small (≤4cm TL) adult brown shrimp preferred temperatures with up to 22.4°C being considerably higher when compared to larger shrimp. Juveniles of 20-30 mm were also reported to have higher optimum temperatures for growth than larger specimens. Following Hufnagl and Temming (2011b), highest growth rates for brown shrimp of 20-30 mm were observed at 25°C being slightly higher compared to optimum temperatures of 23°C calculated by Freitas et al. (2007, 2010) by means of a dynamic energy budget model. Based on current summer water temperatures in the German Bight ranging around 17-18°C, temperature increases might thus result in better thermal conditions for juvenile brown shrimp suggesting that small brown shrimp might even benefit from future temperature increases. Still, it can be assumed that temperature extremes will have a negative impact on small shrimp, as juveniles were reported to avoid water temperatures approaching 27°C (Berghahn, 1983). Local temperature extremes might even have deleterious consequences when approaching 33.8°C as this threshold was reported to be the critical thermal maximum for brown shrimp of ~3.5 cm TL (Madeira et al., 2012).

In contrast to juvenile and small adult specimens, brown shrimp >4cm TL hold lower thermal requirements (Campos and van der Veer, 2008). Hufnagl and Temming (2011b) reported that brown shrimp of 40-60 mm showed highest growth rates at temperatures around 18-22°C, being considerably lower compared to smaller specimens. Following Henderson et al. (2006) temperatures above 22°C are being avoided, which is confirmed by the results of the
present thesis. Here, adults preferred significantly lower temperatures compared to juveniles and thermal preference in shrimp >4cm TL hardly exceeded 20°C. Other than in juvenile brown shrimp, climatic driven impacts causing North Sea water temperatures to rise are suggested to adversely affect adult brown shrimp starting at temperatures between 20-22°C.

### 6.6 Conclusions and outlook

The present thesis revealed annular chamber systems to be a powerful tool for determining thermal preferenda, not only in fish but also in invertebrate aquatic ectotherms like the common brown shrimp. By means of automated data recording and analysis, time and labor for manual data recording and processing can be significantly reduced while increasing the amount, resolution and quality of the data at the same time.

The experiments conducted in the frame of this thesis revealed temperature as an environmental factor of particular importance for brown shrimp. Brown shrimp were found to behaviorally respond to a thermal gradient, selecting certain temperatures over others. However, thermal preference behavior did not coincide with the final thermal preferendum paradigm as brown shrimp did not hold a common, species-specific preference temperature, as it differed by season as well as body size. This implies that the observed seasonal as well as ontogenetic patterns of geographic distribution in the field are caused by differences in thermal selection. Besides seasonal and ontogenetic differences, this thesis identified a high amount of individual variability suggesting a huge amount of phenotypic plasticity with regard to thermal preference. However, variability towards the upper range of preferred temperatures was more pronounced than to the lower potentially implying that the lower range of thermal preference is more determinate and genetically conserved in this species. This might emerge as brown shrimp are regularly exposed to low temperatures during ontogenetic development and as the lower limits is predetermined by the freezing point of natural seawater whereas the upper thermal range is more variable and also differs considerably among years. The higher variability in preference towards the upper thermal range might also determine whether brown shrimp can take advantage of high temperatures supporting high growth, potentially also explaining the highly variable growth rates observed in previous studies.

A projection of the effects of future climatic driven changes on the overall brown shrimp population is difficult. As thermal responses in brown shrimp were found to be highly variable, it is unlikely that future changes will affect the whole population in an equal manner. The prospected changes might favor certain ecotypes whereas others get adversely affected. Other factors than temperature, like food availability and quality or secondary effects arising from temperature increases might be as important as temperature, especially
as temperature increase is just one aspect of global change. Increases in mean North Sea water temperatures might be beneficial for juveniles as temperature conditions approach physiological optimum temperatures for maximal growth. In contrast, adults might be adversely affected and escape to deeper or northerly realms with unknown consequences for stock productivity. Still, local temperature extremes might bring brown shrimp to their thermal limits resulting in increased mortalities. Across the wide distributional range, thermal responses might differ among local populations especially at the northern and southern edges of the species’ distribution. However, the wide geographic distribution of brown shrimp inhabiting different habitats as well as the highly variability in thermal responses also implies a considerably capacity for adaptation in this species helping to cope with or even benefit from increasing water temperatures.

6.7 References


Individual contribution to chapters with multiple authors

CHAPTER I
Automation and critical evaluation of an annular chamber for aquatic ectotherm temperature preference experiments
Stefan Reiser, Axel Temming, André Eckhardt and Jens-Peter Herrmann
SR, AT and JPH designed the study. SR, AE and JPH designed and constructed the annular chamber system. SR and AE programmed the MATLAB code. SR performed the experiments, analysed the data and wrote the manuscript. AT critically commented and improved the manuscript.

CHAPTER II
Acute and gravitational thermal preference of the common brown shrimp (Crangon crangon, L.)
Stefan Reiser, Jens-Peter Herrmann and Axel Temming
SR and JPH designed the experiments. SR performed the experiments, conducted data analysis and wrote the manuscript. JPH and AT critically commented and improved the manuscript.

CHAPTER III
Seasonal variability in preferred temperature reveals invalidity of the “final thermal preferendum” for a marine, invertebrate ectotherm
Stefan Reiser, Axel Temming, Marc Hufnagl and Jens-Peter Herrmann
SR, AT and JPH designed the study. SR performed the experiments. SR and MH analysed the data. SR wrote the manuscript. AT, JPH and MH provided critical comments and improved the manuscript.

CHAPTER IV
5. Lower thermal capacity limits of the common brown shrimp (Crangon crangon, L.)
Stefan Reiser, Jens-Peter Herrmann, Thomas Neudecker, Axel Temming
SR, JPH and TN designed the study. SR and JPH set up the experimental apparatus. SR performed the experiments, analysed the data and wrote the manuscript. AT and JPH provided critical comments and helped to improve the manuscript.
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Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Dissertationsschrift selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe.

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Hamburg, Juni 2013                      Stefan Reiser