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Shell disease in *Crangon crangon* (Linnaeus, 1758): The interaction of temperature and stress response

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ABSTRACT

The prevalence of black spot shell disease is increasing among marine crustaceans worldwide. Rising seawater temperatures – often stressful for ectothermic species – are assumed to enhance the occurrence of shell disease. In the North Sea > 50% of local populations of the brown shrimp (*Crangon crangon*) are affected by the disease. While fisheries are suffering because diseased crustaceans are barely merchantable, the impact of shell disease on life history traits of crustaceans is little understood. To determine the role of temperature on the development of black spots and its implications for survival and physiology in the brown shrimp, a prolonged (3 months) thermal stress experiment was performed. We measured the increment of shell disease and the effect of molting in shrimps kept at control (15 °C = equivalent to the seafloor temperature in the North Sea during sampling) and increased temperature (20 °C = according to predictions for the end of the century). The resting metabolic rate was analyzed to determine the physiological state of diseased compared to non-diseased animals. In the present study, the warmer temperature in the range of 20 °C did not increase the spot size of shell disease and no differences were observed between the two temperatures. The process of molting thereby seemed to diminish and in most of the cases even completely remove the signs of shell disease. At 15 °C but not at 20 °C, metabolic rate was reduced in diseased in contrast to healthy individuals. This study showed that shell disease might lead to a higher mortality rate and an impairment of the physiological state in *C. crangon*.

1. Introduction

The predicted water temperature of the world oceans will increase by 2–4 °C by the end of the century (IPCC, 2013) and the North Sea is one of the fastest warming continental shelf seas (Burrow et al., 2011). This raises the question of how marine invertebrates such as crustaceans in the North Sea will be affected by these changes and if they will be more stressed and more prone to diseases. The brown shrimp *Crangon crangon* is an ecological important shrimp due to its central role in the food web and as a valuable fishery resource along the northern European Atlantic Coast with total landings of up to 37,500 t in e.g. 2014 (ICES, 2015; Revill and Holst, 2004). To date, in England already > 80% (Dyrynda, 1998) and in the German Bight up to 50% of brown shrimps (Knust, 1990) were found to be infected with shell disease.

Shell disease is a common term for many different melanization of necrotic lesions, erosions, and disintegrations in crustaceans such as classical or endemic shell disease (Smolowitz et al., 1992; Sindermann, 1979) and epizootic shell disease (Shields, 2013; Smolowitz et al., 2005). The proximate causes are thereby a dysbiosis of different chitinolytic and lipolytic bacteria such as *Plesiomonas*, *Vibrio* and *Aquimarina* (Cipriani et al., 1980; Feinmann et al., 2017; Getchell, 1989; Meres et al., 2012; Rosen, 1967). These pathogens are common in marine environments (Chistoserdov et al., 2005; Fisher et al., 1978; Hock, 1940; Malloy, 1978; Smolowitz et al., 1992) and are also present on the cuticle of healthy crustaceans (Rosen, 1967), in this case without any impact on the host. Under stressful environmental conditions (ultimate causes e.g. temperature increase, chemical pollution) or mechanical damage, they can degrade the cuticle and thereby facilitate the entrance of other pathogens (Baross et al., 1978; Cook and Lofton, 1973; Schlotfeldt, 1972; Shields, 2013; Vogan et al., 2001). Shell disease is therefore interpreted as a maladaptation to changing environmental conditions by decreasing the immunocompetence of the organisms (Chen et al., 1995; Dove et al., 2005) and reducing the defense mechanisms against pathogens (Sindermann, 1979; Tlusty et al., 2007). Due to the altered expression of different genes and hormones crustaceans can be energetically compromised or produce elevated levels of ecdysone, pointing towards changes in the molting behavior (Castro

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et al., 2006; Laufer et al., 2005; Tarrant et al., 2012). Shell disease can also be lethal due to secondary invading pathogens into the epidermis and underlying tissues causing sepsis of the host or impairments of locomotion and feeding functions (Dyrynda, 1998; Hoenig et al., 2017; Smolowitz et al., 1992). In all ectothermic organisms such as *C. crangon*, temperature determines and interacts with embryogenesis, growth, molting frequencies and also the reproduction and survival (Caudri, 1939; Hufnagl and Temming, 2011a; Lloyd and Yonge, 1947; Siegel et al., 2008; Tiews, 1954). Hence, increasing water temperatures can result in physiological stress and therefore decreased defense mechanisms, which in turn lead to more susceptibility to shell disease as has been shown in the American lobster (*Homarus americanus*) (Dove et al., 2005; Glenn and Pugh, 2006; Tlusty et al., 2007). Additionally, elevated water temperature enhances the amount of bacteria and thus the risk of an infection (Dove et al., 2005; Glenn and Pugh, 2006).

In the current study, a prolonged thermal stress experiment was performed to understand the effect of an elevated temperature on the onset and progression of shell disease in *C. crangon*, and to examine the effect of shell disease on the organisms' physiology.

2. Materials and methods

2.1. Habitat and sampling of brown shrimps

Crangon crangon is widely distributed in the North Sea and is also common in the Irish Sea, Baltic Sea, Mediterranean Sea, Black Sea and at the Atlantic coast. In the southeastern part of the North Sea the habitat of brown shrimps' is mostly characterized by sandy and muddy bottoms where they live buried in the sediment (Pinn and Ansell, 1993). The temperatures in the North Sea range from 0 °C in winter near the coast to 30 °C in summer in the shallow areas and can locally vary by 10 °C within a few hours.

The experimental period lasted three months (sampling, acclimation and recording experiment), from July 2015 until October 2015. Shrimps were caught with a beam trawl by the research vessel FK *Uthörn* in the waters of the German Bight, North Sea southwest of the Island of Helgoland (54°11′ N, 7°53′ E) and transported to the Biological Station Helgoland (BAH) of the Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research. After collection, male and non-gravid females of a mean body length of 48 (± 6) mm were kept in holding tanks at 15 °C according to temperatures in the field. Shrimps were allowed to acclimate to the holding tanks and the surrounding conditions for a minimum period of three days. The water temperature in these holding tanks was then gradually heated to the required study temperature over 5 days (1 °C per day). Individuals, which were not immediately used in experiments, remained in these tanks under ad libitum conditions (see below) to replace dead animals during the experiment. *Crangon crangon* were fed every second day at dusk with live plankton collected locally or with frozen mysis (*Mysis relicta*). The remaining food was removed the next day. The entire aquarium was cleaned once a week to minimize possible accumulation of bacteria and organic waste.

2.2. Experimental design

Experiments were conducted in temperature-controlled rooms at low light conditions. The control group was held at 15 °C and the warm treatment group at 20 °C (representative of the water temperature in the North Sea in summer plus 2–4 °C of the predicted ocean warming until the end of the century (IPCC, 2013)). The experimental setup of either temperature treatment consisted of five flow-through aquaria (40 × 60 × 20 cm; 33 L) with an insert of 12 compartments each (Fig. 1A). Aquaria were supplied with filtered seawater directly pumped from the waters surrounding Helgoland with 2 L/min^{-1} through perforated pipes located above the five boxes (Fig. 1) to provide fresh seawater supply to each compartment. The bottom of each compartment was covered with a mesh of 300 μm^2 so that the water could run through (Fig. 1B). Randomly chosen and apparently healthy brown shrimps were set into the aquaria (one animal per compartment) to prevent injuries and to allow for individual measurements of the size of black spots.

The water temperature was checked daily throughout the experiment using a digital thermometer and if necessary adjusted by heating devices. Shrimps were examined twice daily and all cases of molting or mortality were recorded. All individuals were photographed at the beginning of the experiment and after molting or death with a Nikon P7000 using a scale bar. Images were subsequently analyzed for body length and size of black spots using ImageJ software (http://rsb.info.nih.gov/ij/). The size of the spots was normalized by the body length of the individual and divided by the days of observation to receive the relative increment of black spots per day using the longest time span between two measuring points (start, molting or death of the organism).

2.3. Resting metabolic rate

The resting metabolic rate of shrimps was determined in closed chamber respiration and low light conditions. Animals were used, which had been in the experiment for a minimum of three weeks. A single shrimp was incubated in a 3.5 L sealed glass respiration chamber filled with filtered seawater (0.2 micron pore) (modified from Teschke et al., 2011). The animal (healthy or diseased) was randomly chosen from either of the two temperature treatments, and was placed on a mesh bottom separating the animal from a stir bar, which was driven by an electromagnetic stirrer. This setup was chosen to guarantee a constant mixing of the water. Measuring temperature corresponded to the temperature the animals had experienced in the two treatments. Oxygen consumption of the shrimps in the respiration chambers was measured with an Oxy-4mini 4 channel fiber optic oxygen transmitter (PreSens, Regensburg, Germany), allowing four parallel recordings. The oxygen concentration was recorded every 5 min for 24 h or until the O_2 concentration reached 70%. Oxygen consumption in chambers without shrimps provided information on potential metabolic activity of microorganisms (e.g. plankton and bacteria) in the filtered seawater. At the end of each incubation run, shrimps were removed from the chambers and photographed to calculate its body length and, if existing, the size of the shell disease spots.

It was determined that the shallowest respiration rates occurred between the hours of 0200 and 0700 pm. The decrease in oxygen concentration during this period was then used to calculate the resting metabolic rate in mg O_2 g^{-1} h^{-1}, accounting for the different solubility of O_2 in seawater at the different temperatures, corrected for oxygen saturation changes inside control chambers, and subsequently standardized for the body weight of the organisms according to Begum et al. (2009) and Teschke et al. (2011).

2.4. Statistical analysis

Statistical analyses were conducted with SPSS 24 (SPSS, Chicago, IL) and the significance threshold was set to $\alpha = 0.05$ throughout the study. The data were tested for normality and homogeneity of variance using the Shapiro-Wilk and Levene's tests before further statistical analysis.

Kaplan-Meier analysis (Kaplan and Meier, 1958) was performed to test the effect of temperature and shell disease infection on mortality rates in the control and the warm temperature shrimps as well as in diseased and healthy ones. This procedure is a descriptive method, allowing an estimation of survival over time by using the log-rank method (Mantel, 1966), which gives equal weight to all time points. Spearman correlation was used to test the effect of the increase of shell disease per day on the survival time of the shrimp. Differences in the increase of black spots per day between the two temperature treatments