The Impact of Key Environmental Factors on the Vital Rates of two Baltic Sea Copepods

Dissertation

zur Erlangung des Doktorgrades
des Fachbereichs Biologie
der Universität Hamburg

vorgelegt von

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September 2010

Genehmigt vom Department Biologie
der Fakultät für Mathematik, Informatik und Naturwissenschaften
an der Universität Hamburg
auf Antrag von Professor Dr. M. A. PECK
Weiterer Gutachter der Dissertation:
Herr Professor Dr. M. ST. JOHN
Tag der Disputation: 30. April 2009

Hamburg, den 16. April 2009

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SUMMARY

In this Ph.D. thesis, the impact of various environmental factors impacting on copepod vital rates was investigated with a main focus of examining reproductive success of two key Baltic copepod species (*Acartia tonsa* and *Temora longicornis*) that serve as major food source for larval and planktivorous fish. Therefore, gaining knowledge on how populations of these copepods are expected to respond to changing environmental factors is critical for projecting changes in marine system trophodynamic structure and function (*e.g.*, due to climate change). This thesis is structured into four chapters, containing two chapters built around five manuscripts. Four manuscripts are published in peer-reviewed journals while the fifth is prepared for submission. Those two chapters are preceded by a general introduction. A discussion and general conclusions, including future perspectives, are provided in the final portion of this thesis.

Within the first MANUSCRIPT "The effects of temperature and salinity on egg production and hatching success of Baltic *Acartia tonsa* (Copepoda: Calanoida): A laboratory investigation" the functional response of aspects of reproductive success of a southwestern Baltic population of *Acartia tonsa* was quantified using wide ranges in temperatures and salinities. Specifically, daily egg production was determined over a broad range of temperatures and the time course and success of hatching were evaluated. The effect of salinity on egg hatching success was also examined. As optimal temperature for this population of *A. tonsa*, 22 to 23°C for egg production as well as for hatching was determined. No hatching was observed for eggs incubated at low temperatures (≤ 12°C) that were produced by females acclimated to temperatures ≤ 10°C indicating a possible thermal threshold between 10.0 and 13.0 °C below which only the production of diapause (or low quality) eggs exists in this population. Salinities ≥ 17 psu seem to be optimal for hatching success at intermediate temperatures. The high reproductive success observed over wide ranges in temperatures and salinities in this Baltic population demonstrates one of the mechanisms responsible for the cosmopolitan distribution of this species within productive, estuarine and marine habitats.

In MANUSCRIPT 2: "The effects of temperature and salinity on reproductive success of *Temora longicornis* in the Baltic Sea: a copepod coping with a tough situation**"** the influence of temperature and salinity on aspects of reproductive success and naupliar survival of a southwestern Baltic population of *Temora longicornis* was characterized. The thermal reaction norm constructed from measurements of egg production rate over a broad range in temperatures suggested an optimal temperature of 17°C for this Baltic population. Reproductive success, including egg production and hatching success, was strongly impacted by salinity (rearing and/or incubation salinity). At salinities ≥14 psu, egg production rate was highest when tested at a cohort's rearing salinity and lower when tested at other salinities. For adults reared at 8 psu, a commonly encountered salinity in Baltic surface waters, egg production rate was relatively low at all tested salinities – a pattern indicative of osmotic stress. Hatching success increased asymptotically with increasing salinity and was maximal between 24 and 26 psu. However, hatching success did depend upon the adult acclimation salinity.

Finally, the 48-h survival of nauplii at one of six different temperatures was measured after exposure to a novel salinity (either 7 or 20 psu). Upon exposure to 7 psu, 48-h naupliar mortality increased with increasing temperature. In contrast, after exposure to 20 psu, mortality was relatively low at all temperatures. An intra-specific comparison of egg production for three different *T. longicornis* populations revealed markedly different temperature optima and clearly demonstrated the negative impact of brackish (Baltic) salinities. These results provide estimates of reproductive success and early survival of *T. longicornis* to the wide ranges of temperatures and salinities that will aid ongoing biophysical modeling examining climate impacts on this species within the Baltic Sea.

Within the third MANUSCRIPT: "Effects of salinity, photoperiod and adult stocking density on egg production and egg hatching success in *Acartia tonsa* (Calanoida: Copepoda): Optimizing intensive cultures", the main focus was to optimize the production of a calanoid copepod for use in marine fish aquaculture. It was examined how large ranges in each of three factors (salinity, photoperiod duration, and culture density) influenced egg production rate and 48-h egg hatching success of *Acartia tonsa*. The effect of anaerobic storage time (2 to 180 d) at 4°C on egg hatching success was also quantified. In this species, hatching success was more strongly impacted by differences in salinity and photoperiod than was egg production while the opposite was true for the impact of adult stocking density. In terms of salinity, the lowest and highest mean egg production rate was observed at 30 and 14 psu, respectively, and hatching success was estimated to be > 75% for all salinities > 13 psu. The photoperiod duration (used to rear copepods and incubate eggs) had little effect on egg production rate but significantly influenced hatching success. Adult stocking density had no effect on egg hatching but the relative number of eggs harvested (# female^{-1}) was highest at 50 ind l^{-1} and lowest at 400 ind l^{-1}. For maximum egg production rates and egg hatching success of *A. tonsa* in culture, results of this study suggest using salinities of 14 to 20 psu, photoperiods between 16 and 20 h, and low adult stocking densities (~50 ind l^{-1}).

In the fourth MANUSCRIPT: The "Impacts of light regime on egg harvests and 48-h egg hatching success of *Acartia tonsa* (Copepoda: Calanoida) within intensive culture" was examined on daily egg harvest (eggs tank^{-1} d^{-1}), and 48-h egg hatching success (%) by *Acartia tonsa* in intensive 130-L cultures. Since this copepod produces more eggs during darkness than in the light, it was tested whether egg harvests could be increased by utilizing unnatural light regimes. Egg harvests were between 0.85 to 1.20 million eggs culture^{-1} wk^{-1} and mean egg harvest was not significantly different among tanks maintained at 3 h:3 h, 4 h:4 h, 6 h:6 h and 12 h:12 h light:dark. Egg hatching was not significantly different for eggs produced in the different light regimes and incubated at 12 h:12 h. In a second experiment, cohorts were reared (from nauplii) in constant darkness (D) and constant light (L) and eggs produced in each cohort were incubated in darkness (D–D, L–D) or light (D–L, L–L). Egg hatching success was significantly different among the treatments and increased with increasing light exposure. These and published data were combined to generate an equation predicting 48-h hatching success for eggs produced and incubated at photoperiods between 0.5 and 24 h. Our experiments indicated that light can be an important factor affecting the success of intensive cultures of *A. tonsa* and that copepod culture protocols should include information on light regimes used during rearing and incubation of eggs.

In the fifth MANUSCRIPT: "Handling Copepods and Egg Production Rates: A Note of Caution", the impact of handling stress on egg production rates by *Acartia tonsa* was examined from the data reported in > 30 studies on this species. The data collected in six experiments that differed markedly in scale (250 ml to 100 L replicate containers) and in the environmental factors tested (temperature, salinity, photoperiod, light intensity or stocking density) were more closely examined. In nearly every replicate in every treatment in each of those six experiments, egg production rate increased during the first two or three days. Significant treatment effects were often found for copepods acclimated to different treatment levels prior to testing. However, in these experiments, significant treatment effects were never found when data from day 1 were compared. In the case of egg production rates by *A. tonsa*, significant differences among treatments appeared to be masked by a handling effect for up to two days. A review of the literature indicated that the majority of studies measuring copepod egg production acclimate copepods for < 2 days and do not include time for copepods to recover from handling stress. Some published manuals suggest that controlling for the effect of handling is unnecessary if copepods are carefully handled. However, findings of this study should urge researcher to test for handling effects as they develop egg production measurement protocols. Spurious measurements of egg production will seriously undermine attempts to understand the dynamics of copepod populations (and/or secondary production) in most marine systems.

OUTLINE OF PUPLICATIONS:

The following overview outlines the five publications included in this thesis and the contribution of the various co-authors to those manuscripts. The overall objectives of this study were derived from the RECONN (DFG "AQUASHIFT" Priority Program) science plan.

CHAPTER II: Reproductive success of two copepods in near shore environments of the Baltic Sea: *Acartia tonsa* and *Temora longicornis*

Ms 1) The effects of temperature and salinity on egg production and hatching success of Baltic *Acartia tonsa* (Copepoda: Calanoida): A laboratory investigation

Linda Holste* and Myron A. Peck

Linda Holste designed the experiments, analysed the data and wrote the manuscript. These activities were done in close collaboration with Prof. Myron Peck. The manuscript was published in *Marine Biology* (2006), a peer-reviewed journal.

Ms 2) The effects of temperature and salinity on reproductive success of *Temora longicornis* in the Baltic Sea: a copepod coping with a tough situation

Linda Holste*, Michael A. St. John and Myron A. Peck

Linda Holste designed the experiments, collected and analysed the data and wrote the manuscript. These activities were done in close collaboration with Prof. Myron Peck. Prof. Michael St. John was a co-PI on the research project that funded this work and provided detailed editorial comments on various drafts of the manuscript. This manuscript was published in *Marine Biology* (2008), a peer-reviewed journal.

CHAPTER III: *Acartia tonsa* as live feed for fish: Optimizing mass cultures for aquaculture

Ms 3) Effects of salinity, photoperiod and adult stocking density on egg production and egg hatching success in *Acartia tonsa* (Calanoida: Copepoda): Optimizing intensive cultures

Myron A. Peck* and Linda Holste

Myron Peck designed the experiments, collected and analysed the data and wrote the manuscript. Linda Holste collected data and assisted in the writing of the manuscript and presented the results at scientific meetings. This manuscript was published in *Aquaculture* (2006), a peer-reviewed journal.

Ms 4) Impacts of light regime on egg harvests and 48-h egg hatching success of *Acartia tonsa* (Copepoda: Calanoida) within intensive culture

Myron A. Peck, Bianca Ewest, Linda Holste, Philipp Kanstinger, Meike Martin

Myron Peck designed the experiments, analysed the data and wrote the manuscript. He also helped collect data along with all of the co-author. All co-authors provided comments on drafts of the manuscript. Additionally, Linda Holste helped analyze data and assisted in the writing of the manuscript. This manuscript was published in *Aquaculture* (2008), a peer-reviewed journal.

Ms 5) Handling Copepods and Egg Production Rates: A Note of Caution

Linda Holste*, Berenike Diekmann and Myron A. Peck

Linda Holste analyzed the data and wrote the manuscript. Myron Peck designed the study and helped with the writing. Berenike Diekmann provided data and editorial comments. The manuscript is planned for submission to *Limnology and Oceanography Methods*, a peer-reviewed journal.

CHAPTER I:

GENERAL INTRODUCTION:

Calanoid copepods play an important role in marine ecosystems because they form the largest trophodynamic link between primary (phytoplankton) and tertiary (zooplanktivorous fish and invertebrates) producers. Populations of calanoid copepods have been shown to impose such a high feeding pressure on phytoplankton communities that they can control the phytoplankton production (*e.g.,* Frost 1987). On the other hand, copepods also influence growth rates and distribution of their predators via bottom-up control (Springer and Rosenneau 1985). Due to this vast importance of copepods to the trophodynamic structure and functioning of estuarine and marine systems, a number of large-scale field programs have been funded to disentangle the various factors controlling changes in the copepod community biomass and production (i.e., IGBP – regional GLOBEC programs, Trans-Atlantic Study of *Calanus* (TASC)). Environmental factors such as for instance temperature, salinity, oxygen concentration, food availability and -quality and their interactions control copepod distribution and abundance. These factors affect copepod vital rates (*e.g.*, reproductive success and growth) in a species-specific manner at both the individual and population levels. Through either adaptation and/or acclimation, the range in environmental factors that a certain species can tolerate can be shifted or expanded. In some cases, species colonize areas having an environment that is substantially different from their original habitat. Species whose vital rates respond differently to environmental factors can often coexist, overlapping within habitats that may be sub-optimal. Within the Baltic Sea, brackish species share this habitat with marine species whose origin is from full strength seawater (North Sea, North Atlantic). Within this system, copepod species that have their centre of geographical distribution in different latitudinal zones often coexist. For example, there are Arctic species (*e.g., Acartia longiremis*, *Psuedocalanus acuspes*), boreal species (*e.g., Temora longicornis*, *Eurytemora affinis*) and even species originating from sub-tropical regions (*Acartia tonsa*). Depending on their ability to tolerate, and in some cases adapt or acclimate to, these Baltic Sea conditions, populations of these species will be more or less successful.

Copepod Vital Rates:

Rates of development, reproduction, feeding and metabolism by calanoid copepods have been previously reviewed by different authors (Hart 1990, Kiørboe and Sabatini 1995; Peterson 2001). In some cases, meta-analyses have been performed to generate predictive equations describing the effects of temperature and body size on vital rates such as weight-specific growth (Hirst and Lampitt 1998), metabolism (Ikeda *et al*. 2001) and reproduction (Bunker and Hirst 2004).

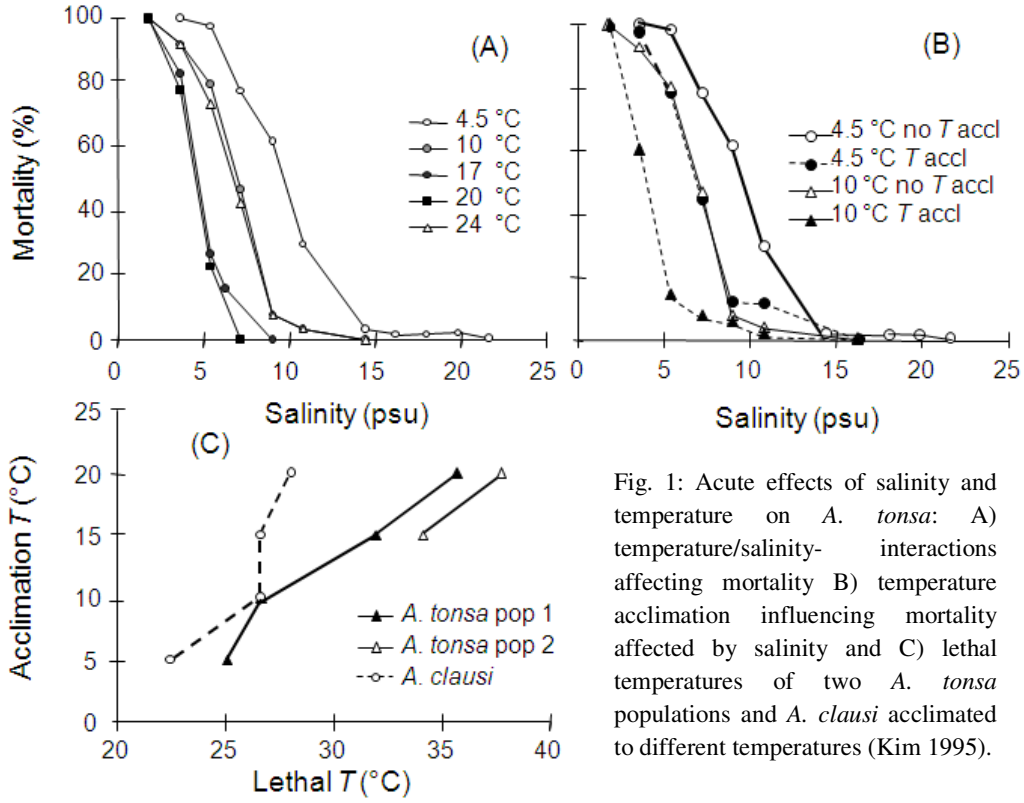

Fig. 1: Acute effects of salinity and temperature on *A. tonsa*: A) temperature/salinity- interactions affecting mortality B) temperature acclimation influencing mortality affected by salinity and C) lethal temperatures of two *A. tonsa* populations and *A. clausi* acclimated to different temperatures (Kim 1995).

These analyses are important first steps toward evaluating the role of copepods at the global scale (*e.g.*, for carbon cycling) but they ignore the species-specific manner in which organismal vital rates respond to changes in abiotic and biotic factors. These inter-specific differences may be particularly relevant for understanding trophodynamic processes at regional-scales where only a few species may exert considerable influence on food web structure and energy cycling. Furthermore, due to differences in long-term habitat characteristics, intra-specific (inter-population) differences are likely to develop that may influence responses of individuals to environmental factors.

Adaptation and Acclimation: Changes due to the environment

Results of research comparing different populations of copepod species underscore the important, intra-specific differences that can exist and the ability of copepod populations to adapt to local conditions (González 1974, Decker *et al*. 2001, Lee *et al.* 2003). Copepods with a wide distribution in temperate and/or sub-tropic marine and estuarine habitats, such as *A. tonsa* and *T. longicornis*, inhabit areas with markedly different ambient conditions in terms of the mean (and short-term and seasonal variance in) abiotic and biotic conditions. An example of adaptation by *A. tonsa* populations to different environments includes changes in the critical (lethal) temperatures. The critical thermal maximum was several degrees higher for *A. tonsa* from a population from Puerto Rico (18°N) compared to conspecifics from Mt. Hope Bay, Rhode Island, USA (41°N) (Figure 1a). This species also tolerates a wide range of salinities (Cervetto *et al*. 1999) and populations of *A. tonsa* exist in

habitats having vastly different mean salinities. Euryhaline copepods have been shown to exhibit shifts in salinity tolerance, in terms of both low and high critical thresholds, in habitats with different salinity characteristics. One of the best examples of this was the change in salinity tolerance of the copepod *Eurytemora affinis* documented during its freshwater invasion (Lee and Petersen 2002, Lee *et al*. 2003). Not surprisingly, evidence for population-specific responses to salinity also exists from a comparison of reproductive success of *A. tonsa* populations inhabiting the North Sea (32 to 35 psu), Danish Bight (10 to 32 psu) and southwestern Baltic Sea (12 to 16 psu) (Chinnery and Williams 2004). A recent study also suggests that population-specific differences in *A. tonsa* may include changes in copepod behaviour. Individuals of *A. tonsa* from a population originating from a region having seasonally low dissolved O_2 concentrations were able to avoid low O_2 in the laboratory whereas individuals from a region without seasonal hypoxia did not avoid lethal conditions (Decker *et al*. 2001). These specific findings show potential phenotypic responses that may have a genetic (inheritable) basis and have important implications for the applicability of the same model formulation across different regions and populations.

An important distinction should be made between population-specific responses (adaptation via selection on genetic variability) and acclimation effects (non-genetic basis) as discussed, for example, by Bradley and Ketzner (1982) for temperature tolerances for *E. affinis* (Poppe). Environmental acclimation (to one factor) or acclimatization (to two or more factors) can markedly shift vital rates at specific environmental conditions. As is the case with detecting population differences, a change in critical tolerance to an abiotic or biotic factor tends to be the most conspicuous way of detecting the acclimation effects. For example, the upper thermal maximum of *A. tonsa* was markedly influenced by acclimation temperature (Fig. 1a). Short-term acclimation to different temperatures and salinities can also change the temperature x salinity (*T***S*) tolerance of individuals (Figure 1b). In most eurythermal and euryhaline animals, acclimation to environmental conditions changes cellular machinery (*e.g.*, the number of ribosomes and mitochondria) and biochemical constituents (*e.g.*, allozymes) that influence organismal-level bioenergetic rates at specific environmental conditions. Indeed, several bioenergetic parameters (rates of oxygen consumption and ammonia excretion) were shown to be affected by acclimation temperature when measured after acute changes in temperature (Gaudy *et al*. 2000). In many key copepod species (*e.g.*, calanoids such as *A. tonsa* and *T. longicorus*) however, the biochemical / cellular changes accompanying acclimation have not yet been investigated.

Egg Production:

During the last 50 years, numerous field studies have been conducted to quantify the various environmental factors influencing calanoid copepod egg production (*EP*) and hatching success (*HS*). This is, in part, due to the technique of estimating secondary production in the field from weight-specific *EP* multiplied by copepod biomass from net hauls (*e.g.*, Poulet *et al*. 1995, Hansen *et al*. 2006). Temporal (seasonal) and spatial variability in reproductive success has commonly been observed and likely results from a number of factors, the effects of which are difficult to distinguish *in situ*. For example, the main factors correlated with *A. tonsa EP* in various studies were food quantity (biomass, *Chl-a* concentration), food quality (species, proteins, lipids), temperature and salinity (*e.g.*, Durbin and Durbin 1984, Poulet *et al*. 1995, Kleppel and Hazzard 2000). Field data collected for that species have been

subjected to multiple linear regression analyses to identify the environmental factors explaining the most variability in *EP* in this species, but often with mixed results. For example, in Chesapeake Bay, no correlation was found between *EP* and Chl-*a*, and most of the variability in *EP* was explained by temperature, protozoan micro-zooplankton biomass and the C:N ratio in suspended particulate matter (White and Roman 1992). In contrast, Ambler (1986) found significant correlations of *EP* with chl-*a* concentration in East Lagoon, Texas. Significant correlations were also found between *EP* and measures of food quality, specifically the content of 18:3ω-3 fatty acids in the seston (Hazzard and Kleppel 2003). Finally, diel variations of *A. tonsa EP* have been observed in the field (White and Roman 1992, Cervetto *et al*. 1993). This species tends to produces most of its eggs during the night or early morning hours due likely to diel behavioural differences that affect growth bioenergetics.

The effects of various abiotic and biotic factors on copepod *EP* have also been studied within controlled, laboratory conditions for many decades and results of these studies help disentangle the effects of various abiotic and biotic factors that operate simultaneously in the field to establish observed *EP*. For example, *A. tonsa EP* has been measured with respect to a range in temperatures at ad libitum feeding levels (Castro-Longoria 2003), and in feeding levels at one temperature (Kiørboe *et al*. 1985) and using a variety of foods having different nutritional qualities (Jonasdottir 1994, Broglio *et al*. 2003). *EP* by *A. tonsa* was also shown to be affected by difference between *in situ* and experimental temperature (Kim 1995) and female age (*e.g.* Parrish and Wilson 1978). Disease agents, such as viruses (Drake and Dagg 2005) have not been observed to affect *EP* nor *HS*. In Fig. 2, two main factors affecting *EP* (food quality and food quantity) are shown with reliable functions.

Even though reproduction has been well studied in both laboratory and field, gaps in knowledge still exist. For example, studies examining the impacts of temperature-salinity interactions, light intensity, water turbulence and intra-specific competition are generally lacking for most copepod species. For physiologically-based modelling of copepod population dynamics, understanding the influence of temperature-salinity interactions is critical within systems with seasonal and spatial variability in salinity (*e.g.*, coastal areas and brackish water systems like the Baltic Sea).

Egg Hatching:

Although *A. tonsa* and *T. longicornis* are among the most intensively studied species, parameterizing stage-based population models for these species is challenging due to gaps in knowledge concerning egg hatching success and resting life stages. In the laboratory as well as in the field, a large number of studies have quantified egg hatching success under various different environmental factors (*e.g.*, temperature, salinity, food quality). Nevertheless, in most cases realistic mathematical descriptions of hatching rates are still missing. Temperature has an impact on all physiological processes and, hence, has been examined more often than other factors. Still the range in temperatures tested is often not broad enough and therefore does not always allow reliable predictions. Naturally, not only abiotic factors, but also biotic factors such as food quality (Fig. 3) impact hatching success. Controlled experiments examining the interaction of various abiotic and biotic factors on egg hatching success are still lacking.

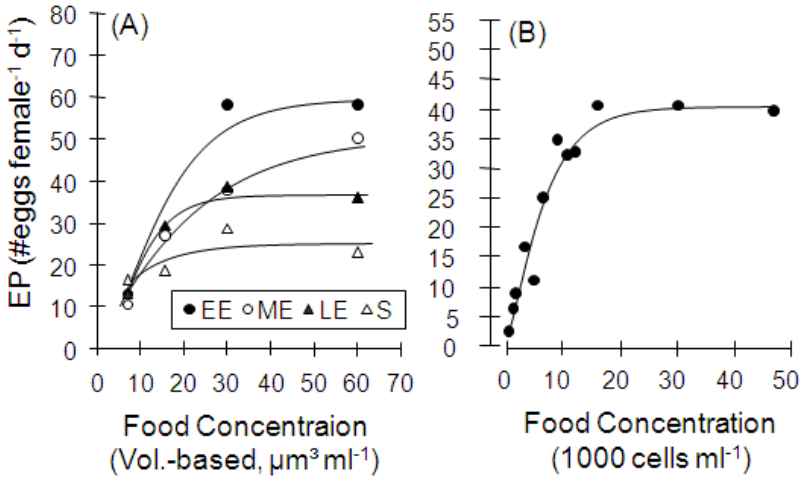

Fig. 2: A) The influence two different biotic factors on egg production rate (*EP*) by *A. tonsa* : Food quality (Jonasdottir, 1994) feeding *Thallasiosira weisflogi* in four different culture states: EE (early exponential) ME (mid exponential), LE (late exponential) and S (senecent), and B) food quantity (*Rhodomonas* sp.) (Kiørboe, 1985).

Diapause egg production has been known as one of the overwintering strategy for various copepod species for more than three decades (Zillioux and Gonzales 1972). The environmental triggers for the production and hatching of diapause eggs (and therefore also the period of time that these eggs can spend within diapause in the sediments) are unclear. Field and laboratory investigations have considered temperature, light and oxygen (*e.g.*, Landry 1975, Uye 1980, Uye and Flemminger 1976, Uye *et al*. 1979) to be the main abiotic factors controlling diapause egg production and hatching in *Acartia* congeners. Naturally, one of the biggest challenges to modelling the phenology of this species remains the overwintering strategy of populations of *A. tonsa* within temperate latitudes.

Due to uncertainties regarding the quantity of overwintering eggs within sediments and the environmental trigger(s) inducing their hatching, realistic modelling of this species with emergence of hatched nauplii from the sediment in spring remains problematic.

There is debate regarding the strategy of production of resting eggs in species such as *A. tonsa* (continuous, small amounts versus less frequent large quantities) which may have helped contribute to the lack of a consensus regarding the different types of eggs that can be produced by this species. The finding of morphological differences in eggs of *A. tonsa* and *T. longicornis* is still recognized as useful way of separating subitaneous from diapause eggs. While some authors report long spines and a slightly larger egg size as morphological criteria identifying diapause (resting) eggs (*e.g.*, Grice and Gibson 1981, Belmonte and Puce 1994), others found no difference in hatching of eggs either with or without spines (Drillet *et al*. 2007). In the following we will abide by the definition that diapause eggs are a genetically controlled resting stage with an arrested development, caused by the producing female.

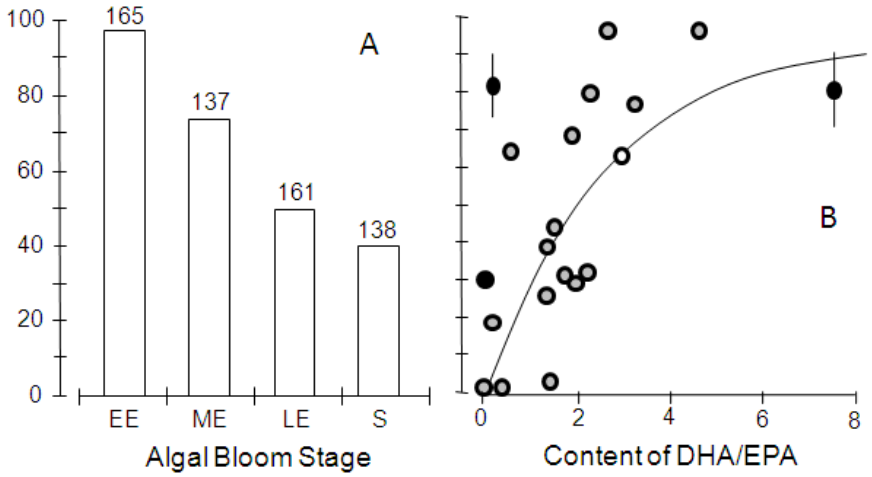

Fig. 3: Hatching success (*HS*) of *A. tonsa* influenced by food quality (Jonasdottir 1994; n=137 to 165, left panel) and *HS* of *T. longicornis* affected by DHA/EPS content (Ahrend 2005).

These eggs require a period of time during which they experience low temperatures before they can hatch (Marcus and Schmidt-Gegenbach 1986) which is described as refractory phase. After passing the refractory phase resting eggs can still spend years in the sediment without hatching if environmental conditions are unfavourable. Hence quiescent eggs are identical to subitaneous eggs (eggs that hatch directly if conditions are favourable) but remain within a state of retarded development caused by unfavourable environmental conditions. Controlled laboratory experiments that generate data on hatching success (*HS*) of eggs produced by adults maintained within different environmental conditions are the first necessary step in understanding the mechanisms responsible for the induction of diapause egg production. However, more specific experiments and field observations are required to answer questions regarding the exact mechanisms and dynamics that mark the production and hatching of overwintering eggs in the field.

Other processes such as egg development rate (*ED*) and naupliar survival are rarely studied in terms of environmental factors. For instance, *ED* is a process that is rarely examined in terms of the potential impacts of salinity and light (photoperiod as well as light intensity). These could be key factors impacting the development rate of eggs and could be the key to information on diapause egg dynamics. To the best knowledge, there have only been studies conducted on the effects of temperature on *ED* (*e.g.*, McLaren *et al.* 1969) that provide useful information for modelling. In terms of food quality, there have been studies finding arrested or disturbed (abnormal) development based on toxic diatoms (*e.g.,* Ianora *et al.* 2004) but no clear mathematical relationships have been formulated for modelling activities. The question of how phytoplankton bloom conditions impacting cohort development and reproduction is even of more importance to modellers as there is likely a succession of algal growth implemented in models than different diatom groups that even distinguish between toxic and non toxic diatoms. In laboratory experiments, *A. tonsa* copepodites developed normally from copepodite stage 1 to copepodite stage 6 when fed diatoms (*Thallassiosira weisfloggi*) in the exponential growth phase but ceased at the third copepodite stage when individuals from the same cohort were fed with senescent (non-exponential) phase diatoms (Fig.: 4 Diekmann *et al.* submitted). These results indicate the importance of knowledge of

the algal condition experienced by copepods for estimating growth and population dynamics.

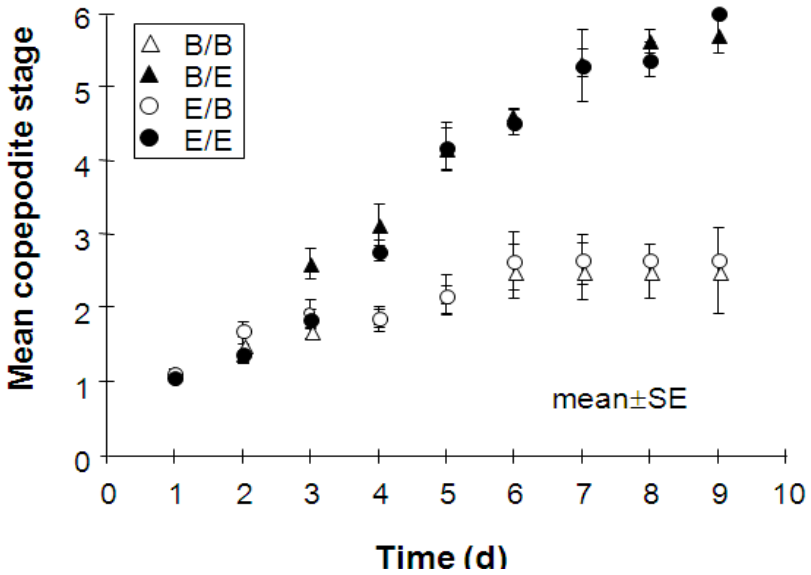

Fig. 4: Mean stage development of *Acartia tonsa* over time (d). Filled symbols: treatments fed *ad libidum* with *T. weisfloggi* in exponential phase, open symbols: treatments fed *ad libidum T. weisfloggi* under bloom condition B/B = offspring produced under bloom conditions and then fed algal bloom, B/E = offspring produced under bloom conditions and fed algae in the exponential phase, E/E = offspring produced under exponential growth conditions of algae and fed algae in the exponential phase, E/B = offspring produced under exponential growth conditions of algae and fed algal bloom.

Copepod Growth and Development:

Various studies have explored the effect of biotic and/or abiotic factors on development and growth of copepods. Data from intensively studied species like *Pseudocalanus elongatus* (*e.g.*, Klein-Breteler and Gonzales 1988, Klein-Breteler *et al*. 1995, Koski and Klein-Breteler 2003) or *Calanus finmachicus* (*e.g.*, Campbell *et al.* 2001, Hirche and Kosobokova 2003, Yebra *et al.* 2006) have been incorporated within various models (*e.g.*, *P. elongatus*: Stegert *et al*. 2007, *C. finmarchicus*: Carlotti and Wolf 1998). These data are a compilation of field, laboratory and mesocosm data, from which developmental time and somatic growth can be projected.

Different copepod species have different energy requirements for successful growth and development as well as different abilities and efficiencies of prey capture. Paffenhöfer and Stearns (1988) reported that *A. tonsa* was restricted to shallow waters such as estuaries and other near-shore environments and speculated that this distribution was a result of food limitation within deeper, offshore areas. The threshold particle concentration for filtration (< 0.25 $mm^3\ l^{-1}$ of particulate organic matter) is very high in *A. tonsa* compared to, for example, *P. elongatus* (0.05 $mm^3\ l^{-1}$). The former species does not accrue lipid reserves, rather, it invests most excess assimilated food energy into either somatic growth or egg production. Consequently, starvation tolerance is rather low in *A. tonsa* and 100% mortality occurs after six to 10 days of food deprivation of food deprivation (Dagg 1977) whereas *C. finmarchicus* can survive > 21 days without feeding (Dagg 1977).

Stage-based Copepod Modelling:

The identification of key zooplankton species is based on overall abundance, seasonal importance and their trophic interactions as prey and predator. Because of their outstanding importance as food for all marine fish larvae and planktivorous fish (*e.g.* Last 1980, Nielsen and Munk 1998, Möllmann *et al*. 2004) and their wide distribution, copepods form the most important zooplankton group within models.

Recent emphasis has been placed on generating stage-based (life-cycle) copepod models linked to circulation models to depict the spatio-temporal dynamics observed *in situ*. This coupled modelling approach has been applied to specific species within specific regions such as *P. elongatus* (likely *P. acuspus*) in the Baltic Sea (Fennel 2001, Fennel and Neumann 2003) and *Calanus finmarchicus* in shelf areas of the northwest Atlantic (Miller *et al*. 1998, Bruno *et al*. 2003, Li *et al*. submitted). Carlotti *et al*. (2000) provide a review of copepod modelling activities.

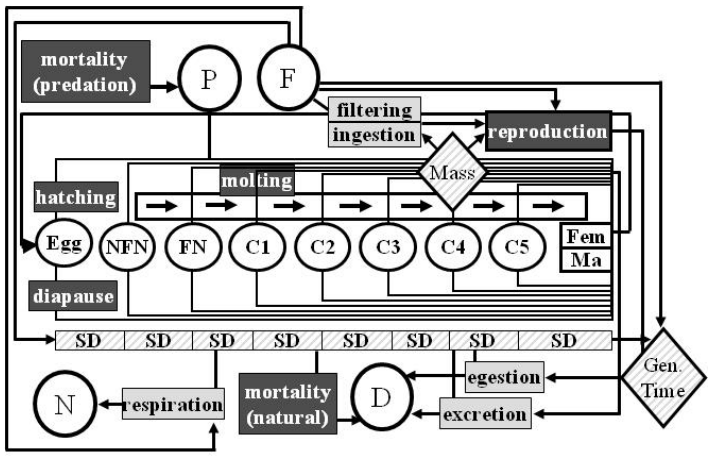

Figure 5: Schematic diagram of a stage-based (nine-stage) copepod model showing inter-relationships among stage six control processes (dark grey), five bioenergetic processes (light grey) and three stage characteristics (hatched). NFN = non-feeding nauplii, FN = feeding nauplii, C = copepodite, Fem = female, Ma = male, SD = stage duration. Inputs from, and feedbacks to, the environment are depicted in circles including food (F), detritus (D), nutrients (N) and predation (P).

The underpinning of such models is basic, quantitative knowledge on how various abiotic factors (*e.g.*, temperature, light, salinity, etc.) and biotic factors (*e.g.*, food quantity, food quality, predation, etc.) influence copepod life history traits and vital rates (i.e., rates of feeding, growth, reproduction and mortality).

To correctly depict the seasonal population dynamics within stage-based population models, the values of parameters representing changes in the critical molting mass of different stages may need to be a dynamic variable and not merely a static parameter. This may be particularly important for modeling populations within temperate areas that exhibit marked seasonality in prey availability and temperature. Clearly, laboratory studies examining the

relative contribution of changes in temperature, food quantity and food quality are needed to disentangle (and model) seasonal changes in body length (and carbon content). Finally, the causes and consequences of inter-individual variability in stage-specific length and mass of copepods within cohorts grown under different environmental conditions is an active area of ongoing laboratory research. Although, at the present time, variability in copepod size is not a feature of most stage-based models, it may, nonetheless, be correlated with potential differences in size that may occur due to environmental factors that vary seasonally.

Clearly, stage-based models attempting to simulate spatio-temporal dynamics of single species in specific areas would benefit most from parameter estimates derived from life-history traits measured on populations of the target species inhabiting the model domain. However, this may be unrealistic in some cases due to a lack of *in situ* and/or laboratory data. However, the question as to whether stage-based models should include acclimation effects (perhaps by keeping track of "environmental history") remains open since the process of acclimation can occur at time-scales that may not be relevant for biophysical modeling activities. For example, acclimation times of only 24 h to different (higher or lower) temperatures and salinities, shifted critical *T*S* tolerance in *A. tonsa* (Kim 1995). In our opinion, the inclusion of acclimation effects within models would be warranted if modeled copepods experience sporadic fluctuations in environmental factors at time scales shorter than those required for acclimation (24-48 h). This might be the case after an intense wind-driven mixing event that breaks down strong water column stratification.

Copepods and Aquaculture:

Due to worldwide overexploitation of fishing grounds, aquaculture has become a very important source of fish production within the last decades. The establishment of exclusive economic zones in the 1970s has played an important role for the development of marine aquaculture. For instance, Japan could no longer exploit the marine flora and fauna of coastal waters of many nations, so they decided to become independent in seafood production, resulting in a massive economic investment in aquaculture infrastructure and research. During the last years, the role of calanoid copepods as live prey within aquaculture became more and more important. Today 35% of the total fisheries production consumed by humans has its source from marine aquaculture. The necessity for aquaculturists to understand in detail the physiology and biochemistry of the organisms that they raise has contributed much to making marine aquaculture a sophisticated industry. Traditional live prey such as rotifers (*e.g. Brachionus plicatilis*) or brine shrimp (*Artemia*) are usually reared in mass culture for fish larvae or planktivorous fish (for review see Støttrup and McEvoy 2003). Both are usually fed artificial emulsions to enrich their nutrient composition to simulate the nutritive status of natural prey items (Lubzens and Zmora 2003, Dhont and von Stappen 2003). Larval fish require a diet high in DHA (Docosahexaenoic acid; 22:6n-3) to achieve better growth, stress resistance and a proper pigmentation (*e.g.* Watanabe *et al* 1983, Kraul *et al*. 1993, Copeman *et al.* 1999). Rotifers and brine shrimp *nauplii* have a very low DHA:EPA (Eicosapentaenoic acid 20:5n-3) ratio and are often artificially enriching directly prior to feeding larval fish. The disadvantage of this method is that DHA is rapidly lost or converted, so it is difficult to maintain high DHA values in mass cultures (Navarro *et al*. 1999).

Although more than 11,500 species of copepods have been classified (Humes 1994), the number of species that are cultured at larger scales relevant for rearing fish larvae are very few and fall within the orders of Calanoida, Harpacticoida and Cyclopoida. The calanoid species are most abundant in the pelagic environment in coastal waters and have therefore received the most attention by researchers (Mauchline 1998). In aquaculture, species belonging to the genera *Acartia*, *Centropages* and *Eurytemora* are in most widespread use in mono- or mixed cultures (Støttrup 2003). Among the primarily epibenthic harpacticoid copepods, species belonging to the genera *Euterpina*, *Tigriopus* and *Tisbe* have been among the preferred candidates for aquaculture (Støttrup 2003). Although they are easier to cultivate than calanoids (higher densities, faster reproductive success) the disadvantage is the epibenthic life-stages, a life history trait that reduces the range in prey sizes that can be utilised by pelagic fish larvae. Very few cyclopoid species have been reared in the laboratory. *Oithona* spp. and *Apocyclops* spp. appear to be the best candidates suitable for multi-generation cultures and ideal as food for marine fish larvae (Støttrup 2003). But the major disadvantage is the inability to harvest eggs as in calanoid species. More recently paracalanid copepods belonging to the genus *Parvocalanus* have been reported to be well suited for intensive culture as well as suitable live prey for marine fish larvae (McKinnon et al. 2003, Shields et al. 2005).

The use of calanoid copepods as live prey has several advantages: 1) they form the natural food source of all marine fish larvae, 2) the nauplii can be smaller than traditional live food which is particularly important for first-feeding larvae of warm-water fish species that have a relatively small mouth gape compared to the larvae of temperate fish species, and 3) adult calanoid copepods can be grown in cultures using natural microalgae with a high nutritional value (DHA:EPH ratio of 4:1 or higher) and this nutritional value is transferred to eggs and newly hatched nauplii (Shields *et al*. 1999) and so costly emulsions can be avoided. 4) *Acartia* species such as *A. tonsa*, and *A. clausi*, *Eurytemora affinis*, *Centropages hamatus* (Marcus 2005) and *Temora longicornis* (Næss 1996) all have diapause eggs. There are several advantages of diapause eggs: 1) Lavens & Sorgeloos (1996) suggested using copepod resting eggs as an inoculum to initiate copepod cultures. 2) their use for short- or more importantly long-term storage to ensure stable production of newly hatched nauplii for feeding marine fish larvae Marcus (2005) and 3) the reduction of contaminant risks due to the fact that resting eggs of several taxa were "resistant to surface disinfection agents commonly used in aquaculture".

The main obstacle to wide-scale use of copepods (or any live food) within aquaculture is defining protocols that optimize their efficient, productive mass culture. This implies finding the optimal environmental conditions required for rearing. The knowledge of environmental factors controlling copepod populations in the field and/or laboratory can be partly applied to aquaculture (see Støttrup 2003). Still, mass cultures have to be seen as exceptional circumstance since animals are grown at exceptionally high (unnatural) concentrations and, due to this, the effect of environmental factors could be enhanced or shifted. Additionally the practical handling and facilities are of great importance to aquaculture since the effort of growing live food for fish larvae (including food for the copepods) should be as minimized for cost effectiveness.

The ability to culture these organisms at a scale adequate for marine larviculture would present a major step forward for the production of many marine species that require a nutritionally better-suited diet than that provided by the traditional live prey.

Study Species:

Acartia tonsa:

Acartia tonsa belongs to the most intensively studied calanoid copepod species in the world with more than 400 citations (Maucheline unpubl data). Its native location is described as the Indo-Pacific. Presumably through ballast waters of ships, this species is nowadays distributed throughout the world's oceans within temperate to tropical marine and estuarine waters. In the year 1925 it has been described for the Baltic Sea for the first time (Elmgren 1984). *A. tonsa* is known as a euryhaline, eurythermic species (Lumberg 1976) that feeds omnivorously (Lonsdale *et al*. 1979). In the Baltic Sea, *A. tonsa* normally undergoes between eight to nine generations per year (Arndt and Schnese 1986). Within constant environmental conditions (*e.g*., temperature, food availability), the development of *Acartia* species has been described as isochronal, meaning that the duration of one stage is more or less equal to that of every other (Miller 1977). Under favourable conditions, an adult *Acartia tonsa* female can ingest up to 360% of her body weight d^{-1} (Roman 1977; Paffenhöfer and Stearns 1988) and can produce up to 78 eggs d^{-1} (Parrish and Wilson 1978). As a result of these high egg production rates, *Acartia tonsa* can have a high intrinsic rate of population increase and, during periods when it is abundant, may exert high grazing pressure on lower trophic levels (top-down control). Paffenhöfer and Stearns (1988) found this species restricted to shallow waters as estuaries and other near shore environments as a result of food limitation in deep offshore areas. The result of previous studies conducted in the Baltic Sea (Arndt and Schnese 1986, Madhupratap *et al.* 1996) and elsewhere (*e.g*., Sullivan and McManus 1986, Marcus 1996) indicate that *A. tonsa* along with other congeners produces resting eggs as an overwintering strategy. The environmental triggers for resting egg production are thought to be temperature, photoperiod and oxygen concentration (*e.g.*, Castro-Longoria and Williams 1999, Chinnery and Williams 2003, Katajisto 2004). Still the definition of resting egg characteristics is unclear. In the Baltic, highest abundances of this species are measured during summer months August/early September in near sore areas. During winter months *A. tonsa* is virtually absent from plankton samples due to low temperatures and insufficient feeding conditions.

Temora longicornis:

Similar to *A. tonsa, Temora longicornis* is a neritic and euryhaline copepod species inhabiting temperate estuarine and marine habitats. In contrast to *A. tonsa* it is not restricted to near shore environments but can also be found further off shore. Due to its wide distribution from the Portuguese coast (Halsband-Lenk *et al*. 2002) up to higher latitudinal habitats, *e.g.* the Barents (Klekowski and Weslawski 1990) and White Seas (Pertzova 1990 as cited by Lukashin *et al.* 2003, Chikin *et al*. 2003) *T. longicornis* is very well studied (500-600 citation (Maucheline unpubl data). Within the Baltic Sea it forms one of the key calanoid copepod species (Hernroth and Ackefors 1979) and therefore an important food item for larval and planktivorous fish. Its omnivorous feeding (Lebour 1922, Turner 1984) allows females and ingestion of 72 $10^6 \mu m^3$ of food (O`Connors *et al.* 1980). This species

undergoes 2-6 generations per year (Digby 1950, Petersen and Kimmerer 1992, Halsband-Lenk *et al.* 2004) and can avoid unfavorable environmental conditions by resting egg production (Castellani and Lucas 2003). But field data of *T. longicornis* hatching success give evidence that resting egg production is not essential and therefore avoided in the Baltic Sea (Madhupratap *et al.* 1996, Dutz *et al.* in prep). In the Baltic, maximal egg production appears in May (Hansen *et al.* 2006). In contrast to *A. tonsa*, *T. longicornis* is present in all stages all year long but reaches its maximal abundance in late spring.

Study Area - Baltic Sea:

The Baltic Sea is with an area of 370 000 km² one of the largest brackish water systems of the world. Through a narrow connection in the southwestern part to the North Sea, the Kattegat, saline water is able to penetrate into the Baltic Sea and forms oxygen rich and saline bottom water (Fig. 6). The great amount of fresh water surplus resulting from river runoff leads to a constant outflow of Baltic Sea water on the other hand. Therefore a steep gradient of salinity in the horizontal and vertical with a permanent halocline is characteristic for this system. Because of its special bathymetry, where very shallow sills (mean depth is 56 m) alternate with basins that are down to > 450m deep (Gotland Basin), the flow of saline and oxygen rich water is strongly impeded. Major inflow events as a result of strong winds renew the bottom water within the basins. These strong wind events are coupled to the North Atlantic Oscillation (NAO) (Matthäus and Schinke 1994, Schinke and Matthäus 1998). While before the 1980s, these inflow events occurred every two to four years, in the last two decades only two major inflows took place (Schinke and Matthäus 1998).

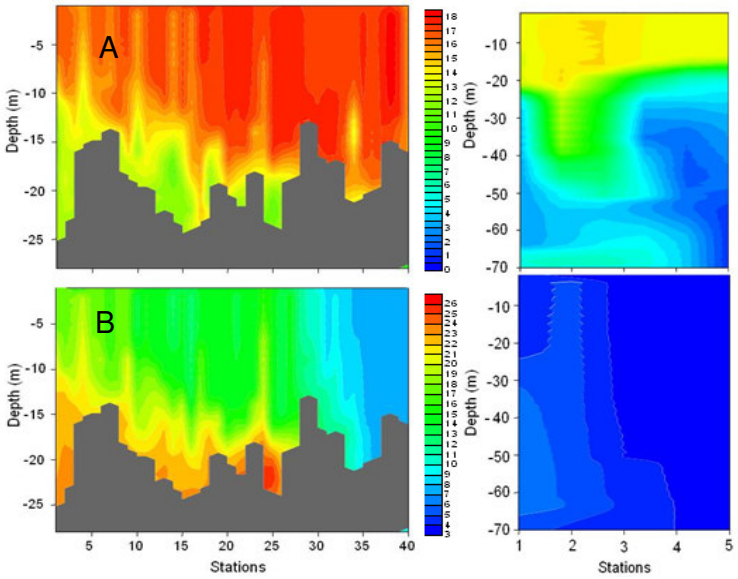

Fig. 6: A) Mean Summer temperature (°C) and B) mean Salinity (psu) in 1992/93 in the southern Baltic Sea (40 stations, left panels) and the Gulf of Finland (5 stations, right panels) ICES Data Base.

In the case of long stagnation periods, oxygen depletion due to break-down of organic matter leads to anoxic conditions in the deep basins The system offers remarkable conditions for ecological research due to: 1) the wide ranges in temperatures and salinities from the southwester to the northeast and 2) the mixture of marine and brackish water (estuarine) spieces inhabiting the ecosystem (Leppokoski *et al.* 2002) and 3) a relatively simple trophic structure (*e.g.*, Möllmann *et al.* 2000). Cod (*Gadus morhua*) forms with sea birds and few mammals the upper trophic level. It preys on sprat (*Sprattus sprattus*) and herring (*Clupea harengus*), the two most abundant zooplanktivorous fish in this system. Four major copepod species dominate the zooplankton in the central Baltic Sea: *Pseaudocalanus acuspes*, *Temora longicornis*, *Acartia longiremis* and *Acartia bifilosa*. Within the southwestern part of the Baltic the near shore waters form also a habitat for *Acartia tonsa*.

OBJECTIVES:

1) Reproductive success of two copepods

Ongoing research focuses on the impact of climate change on trophodynamic structure and function within estuarine and marine ecosystems. Abrupt changes in species dominance termed "regime shifts" have been reported for several ecosystems including the North Sea, North Atlantic (*e.g.*, Beaugrand and Reid 2003, Beaugrand and Ibanez 2004) and the Baltic Sea (Möllmann *et al.* 2000, Alheit *et al.* 2005). Within the Baltic Sea, a decrease in *Pseudocalanus acuspes* abundances was significantly correlated to a decrease in salinity within the last two decades, whereas an increase in the abundance of *Acartia spp*. was correlated to an increase in spring temperature within the upper 50 m (Möllmann *et al*. 2000). Annual indices of the abundance of *T. longicornis*, although more stable, were also positively correlated with temperature in the uppermost portion of the water column. To understand the mechanisms behind this shift in structure, essential information on vital rates, the potential of acclimation and the ecological consequences are needed. Therefore the first objective of the present work was to help eliminate gaps in knowledge the currently exist on how vital rates and life history traits of Baltic *A. tonsa* and *T. longicornis* respond to extrinsic (environmental) factors (Chapter II, *manuscripts 1* and *2*).

2) Optimizing mass cultures for aquaculture

Aquaculture forms one of the fastest-growing economic sectors of the animal production in the world (FAO 2006). Due to ocean-wide overexploitation of fishing grounds, the world wide need for fish farming increases continuously and new techniques, facilities and feeding procedures have to be developed. Traditional live prey used for larval rearing, such as *Artemia salinia* and *Brachionus plicatilis* have limitations due to their low nutritive value and relatively high economic expenses (*e.g.*, Støttrup and McEvoy 2003, Wilcox *et al.* 2006). Therefore rearing calanoid copepods in large cultures seems to be a realistic alternative. To optimize large-scale, intensive cultures, the factors controlling vital rates have to be explored on a population level. Optimal environmental conditions (*e.g.*, water temperature, light intensity, photoperiod, and water salinity) have to be defined and protocols developed that utilize these conditions. Thus the second goal of this thesis was to provide thorough knowledge on factors controlling copepod production within intensive cultures and to explore practical techniques that potentially could optimize those cultures (Chapter III, *manuscripts 3, 4* and *5*).

2) Parameters for stage based copepod models

The third objective in this thesis was to close some gaps in knowledge of how intrinsic responses (vital rates and life history traits) of copepods respond to extrinsic (environmental) factors and to predict those responses using robust mathematic functions. Processes that are important controls on copepod production and biomass in some species which, to date have not been included within model applications are discussed. Since that species is particularly well-studied, gaps in knowledge for this species likely exist for other species as well (*all chapters including appendix*).

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CHAPTER II: Reproductive success of two copepods in near shore environments of the Baltic Sea: *Acartia tonsa* and *Temora longicornis*

Ms 1) The effects of temperature and salinity on egg production and hatching success of Baltic *Acartia tonsa* (Copepoda: Calanoida): A laboratory investigation

Linda Holste* and Myron A. Peck

Ms 2) The effects of temperature and salinity on reproductive success of *Temora longicornis* in the Baltic Sea: a copepod coping with a tough situation

Linda Holste*, Michael A. St. John and Myron A. Peck

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The effects of temperature and salinity on egg production and hatching success of Baltic *Acartia tonsa* (Copepoda: Calanoida): A laboratory investigation

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ABSTRACT

The functional response of aspects of reproductive success of a southwestern Baltic population of *Acartia tonsa* (Copepoda: Calanoida) was quantified in the laboratory using wide ranges in temperatures and salinities. Specifically, daily egg production (*EP,* # female^{-1} d^{-1}) was determined for four or five days at 18 different temperatures between 5 and 34°C and the time course and success of hatching were evaluated at ten different temperatures between 5 and 23°C. The effect of salinity (0 to 34 psu) on egg hatching success was also examined. The highest mean rates of *EP* were observed between 22°C and 23°C (46.8 to 50.9 eggs female^{-1} d^{-1}). When studied at 18 psu, hatching success of eggs increased with increasing temperature and was highest (92.2 %) at 23°C. No hatching was observed for eggs incubated at low temperatures (≤ 12°C) that were produced by females acclimated to temperatures ≤ 10°C indicating a possible thermal threshold between 10.0 and 13.0°C below which only the production of diapause (or low quality) eggs exists in this population. When tested at 18°C, the hatching success of eggs incubated at 15 different salinities increased asymptotically with increasing salinity and was maximal (81.4 to 84.5%) between 17 and 25 psu. The high reproductive success observed over wide ranges in temperatures and salinities in this Baltic population demonstrates one of the mechanisms responsible for the cosmopolitan distribution of this species within productive, estuarine and marine habitats.

Keywords

Acartia tonsa, temperature, salinity, egg production, egg hatching

INTRODUCTION:

Calanoid copepods play a key role in the cycling of nutrients and energy in marine ecosystems by forming a trophodynamic link between primary (phytoplankton) and tertiary (e.g. planktivorous fish) production (DeYoung 2004). The widespread distribution and abundance of members of this family results, in part, from adaptation of life history traits to match specific environmental (physical and chemical) conditions and/or constraints. For example, diapause eggs have been developed by some calanoid species inhabiting relatively shallow temperate habitats (e.g. Marcus 1984; Lindley 1990; Viitasalo and Katajisto 1994) to cope with intolerable annual ranges in biotic (e.g., seasonal primary production) and/or abiotic (e.g., temperature) factors within these areas.

Within the Baltic Sea, hydrographic changes in recent decades have been correlated with trophodynamic changes in terms of zooplankton and fish (e.g., Möllmann et al. 2000). Specifically, Möllmann et al. (2000) suggested that decreasing salinity was one of the causal mechanism behind a regime shift in the dominant calanoid copepod species in the Baltic from *Pseudocalanus elongatus* and *P. acuspes* to *Acartia spp*. (mostly *A. longiremis* and *A. bifilosa*) since the former species may require higher salinities for high reproductive success than the latter ones (Mauchline 1998). These changes in species abundance help demonstrate that the wide ranges in salinities and temperatures of the Baltic Sea often exceed those of the preferred niche of the calanoid species found there. Unfortunately, the functional response of reproductive success (i.e., egg production and hatching) to salinity and/or temperature in many calanoid species is not well known, having been studied in only a handful of species such as *Eurytemora affinis* (e.g., Gonzalez and Bradley 1994) and a number of *Acartia* congeners (e.g., Tester and Turner 1991; Chinnery and Williams 2004). Moreover, within estuaries and brackish enclosed waters, the considerable temporal and spatial variation in abundance and distribution of calanoid copepods has not been explained merely by variations in abiotic factors such as salinity and temperature (Bradley 1991; Wellershaus and Soltanpour-Gargari 1991) but also by the dynamics of biotic variables such as food concentration and predation pressure (Paffenhöfer and Stearns 1988).

Acartia tonsa (Dana) is easily maintained in laboratory culture (Støttrup 2000) and hence is among the most intensively studied calanoid species (Mauchline 1998). Previous studies have quantified the effect of temperature and/or feeding on *A. tonsa* vital rates including growth and egg production (e.g., Heinle 1969; Miller et al. 1977; Klein Breteler and Gonzales 1986; White and Roman 1992; Broglio et al. 2003). However, relatively little attention has been paid to the effect of salinity on vital rates (Heinle 1981; Cervetto et al. 1999; Gaudy et al. 2000). Chinnery and Williams (2004) found a significant effect of both temperature and salinity on egg hatching success in four *Acartia* species including *A. tonsa*. However, it is clear that studies on this (and other) calanoid species often have not covered sufficiently wide ranges in temperatures and or salinities to develop complete functional responses of vital rates to these factors. For this reason, attempts to understand and model the life history dynamics of *A. tonsa* within the Baltic Sea (and other calanoid species in other systems, i.e., Norberg and DeAngelis 1997; DeYoung 2004) may be met with limited success.

The present study examined the effects of temperature (5 to 34°C) and salinity (0 to 34 psu) on aspects of the reproductive success of a southwestern Baltic population of *A. tonsa.* Specifically, the effect of temperature on egg production, hatching success and the time course of

hatching and the influence of salinity on hatching success were examined. These experiments, conducted at unlimited feeding levels, were designed to generate more complete functional responses of *A. tonsa* reproductive success to these environmental factors.

MATERIALS and METHODS:

Acartia tonsa used in this study were the progeny of adults collected from two WP2 seawater samples (12m to surface, 55 µm mesh size) taken in August 2003 (S = 14 psu, T = 18°C) in Kiel Bight in the southwestern Baltic Sea (54°N; 10°E). In the laboratory, zooplankton samples were acclimated to S = 18 psu and T = 18°C over the course of four days after which *A. tonsa* was isolated from each field sample in a ratio of 3:1 (females: males) and placed into each of two cylindrical 8 L tanks (density ~ 23 ind L^{-1}; 180 ind $tank^{-1}$). Cultures were provided daily rations of a cryptophyte (*Rhodomonas sp.*) at concentrations (> 50 000 cells mL^{-1}) providing unlimited growth and egg production in *A. tonsa* (Kiørboe et al. 1985; Støttrup and Jensen 1990). Cultures were maintained on a 13L:11D light regime and received gentle aeration for mixing. Eggs were collected every two days by removing the aeration, letting the eggs settle and siphoning the bottom of the tank. Collected eggs were stored at 4°C and hatched later within six, 350 L "starter culture" tanks. Cohorts of *A. tonsa* were maintained at 30 to 50 ind L^{-1} in these tanks and fed *Rhodomonas sp.* at ≥ 50 000 cells mL^{-1} each day. The copepods used in experiments described in later sections were the progeny of the aforementioned starter cultures that were maintained for approximately eight generations in the laboratory at 18 psu and 18 to 20°C. Three different experiments were conducted in this study within a controlled-environment room having a 12L:12D light regime with a daytime water surface light intensity of 1 to 5 µE (µmol m^{-2} s^{-1}).

Exp 1: Temperature and Egg Production

The effect of temperature on egg production (*EP*, # $female^{-1}$ d^{-1}) was quantified at ten temperatures between 5 and 23°C (trial 1) and between 21 and 34 °C (trial 2) with two common temperatures (21 and 23°C) used in each trial. Copepods were acclimated to different temperatures prior to the trials due to the influence of temperature history on temperature tolerance in this species (González 1974). A total of ~40 ind L^{-1} (nauplii to adults) was loaded into each of five 8-L (trial 1) and three 250-L acclimation tanks (trial 2) containing filtered (1 µm) seawater and acclimated at a rate of ~0.6°C d^{-1} to one of seven different temperatures (6, 9, 13, 17 and 22°C in trial 1; 22, 24, and 28°C in trial 2). *Rhodomonas sp.* was also acclimated to and grown at three different temperatures (6, 12 and 20°C).

Both *EP* trials were conducted using a thermal gradient table (Thomas et al. 1963), an aluminium block that was heated and cooled by pumping temperature-controlled water through holes drilled in both ends. Copepod *EP* was measured in three replicate 250 mL glass beakers at each temperature. Thermal stratification within beakers was avoided by conducting trials at relatively cold air temperatures (6°C in trial 1 and 18°C in trial 2). Temperatures were maintained within ±0.15°C (at low temperatures) to ±0.7°C (at two highest temperatures).

To avoid egg cannibalism, five females and one male were held within mesh-bottom sieves (8.4 cm height, 4.5 cm diameter, 130 µm mesh size) suspended in each container. Adults used in the trials had been previously acclimated to within 1 to 2 °C of the test temperature for at least two days. Since developmental rates are temperature dependent, C_5 stage *A. tonsa* were

initially loaded into containers at temperatures ≥18 °C to minimize differences in the amounts of temperature-time (i.e., degree-days) individuals were within the adult stage at the different test temperatures. Every 24 h, the adults were placed into a new container by carefully transferring the sieve. The new container had filtered seawater (same temperature) containing > 50,000 cells mL^{-1} *Rhodomonas* sp. The contents of the old container were collected (35 µm sieve), rinsed into a Bogorov dish, and the number of eggs counted under a Leica MZ 9_5 dissecting scope. Using these methods, data were collected from each of the 30 replicate containers each day for four (trial 2) or five (trial 1) days. Containers were checked daily for mortalities and any dead individuals were replaced with individuals acclimated to a similar (± 1 to 2 °C) temperature. At the end of the trials, adults were videotaped and prosome lengths measured using computer image analysis (Optimas 6.51).

Exp 2: Temperature and Egg Hatching

Eggs were collected from the adult cultures acclimated and maintained at either 6, 9, 13, 17 or 22°C (±0.2°C) and then loaded (n = 30) into each of three replicate 150 mL containers at each of 10 different temperatures (Table 1) within the thermal gradient table (conditions were the same as in Exp 1, trial 1). The number of unhatched eggs was counted periodically until no further hatching was noted over a two-day period (total time course of experiment was 168 h). The frequency of observations depended upon the temperature. During the first 48 h, containers incubating eggs at 14 to 23°C were checked every hour and the number of unhatched eggs was recorded. Containers between 8 and 12°C were checked every four h while those at 5, 7 and 8°C were examined every 12 h. Additionally, the prosome length of 20 adults within each of the five acclimation temperatures was measured. The cumulative egg hatch (H_{CUM}) versus time (h) and the total hatch success (HS_T, %) of eggs were calculated.

Exp 3: Salinity and Hatching Success

Egg hatching success (HS_S, %) was quantified at 15 different salinities from 0 to 34 psu by conducting four separate trials. In each trial, due to technical limitations, seven or eight different salinities were tested (Table 1). Egg hatching among the four trials was compared at three common salinities (6, 17 and 25 psu).

In each trial, a known number of eggs (59 –65) was loaded into a 250 mL culture flask containing 200 mL of gently aerated, 1 µm filtered seawater. Three replicate flasks were used at each salinity. All flasks were incubated for 48 h within a controlled-environment room at 18°C (range ±0.5°C). After 48 h, the contents of the flasks were gently poured through a 35 µm sieve and rinsed into a Bogorov dish. Unhatched eggs were counted with the aid of a Leica MZ 9_5 dissecting scope. A duration of 48 h was based upon the time course of hatching in previous salinity hatching trials conducted at the same temperature (Peck & Holste Submitted) and results of Exp 1.

Statistics:

Data collected in this study were analysed by linear and non-linear regression analysis. Predictive regressions were used and parameter estimates were obtained by the least-squares method. The functional form of regressions was chosen based upon several statistical criteria (significance level, coefficient of determination (r^2), sum of squared errors (SSE) and residual

trend analysis). A one-way ANOVA tested for differences in adult size (prosome length) among the different acclimation temperatures used in Exp 1 and Exp 2. A two-way ANOVA was used in Exp 1 (*EP*, trial x temperature) and Exp 3 (arcsin transformed percent hatch [arcsin*$(\%/100)^{0.5}$], trial x salinity). Q_{10} values were calculated for data collected in this (Exp 1) and other studies from a linear regression of ln*EP* versus *T* (ln*EP* = lna +b*T*, where $Q_{10} = e^{b*10}$). *EP* data from other studies were taken directly from published text, tables, or figures. Data from figures were collected after digitization of the images (MATLAB 5.3, Mathworks-Inc, Natick, MA, USA; DIGIREAD shareware). All statistical tests were performed using SAS software (SAS 1989) and were considered significant at $p \leq 0.05$.

RESULTS:

Exp.1: Egg production and temperature

An increasing trend in the egg production rate (*EP*) observed during the first two (trial 2) or three (trial 1) days was considered to denote an acclimation period to the test chambers. Only *EP* data collected after this period were averaged (n = 2 days) and used in subsequent analyses. Mean(±SE) *EP* at common temperatures (*EP* at 21 and 23°C, trial 1= 29.7(±7.7) and 32.7(±8.7), trial 2 = 29.6(±9.8) and 32.9(±5.6), respectively) was not significantly different between trials and data from the two trials were combined and analysed together.

Under unlimited feeding conditions, mean *EP* increased with increasing temperature (*T*) from 0 (zero) at 5.2°C to a maximum (EP_{MAX}) of 50.9 eggs female $^{-1}$ day $^{-1}$ at 22.9°C and declined at higher temperatures (Fig. 1A). Between temperatures of 5.2 and 22.9 °C, mean *EP* was related to *T* based upon:

1) $$LnEP = 0.28(\pm 0.02) * T - 2.38(\pm 0.26) \qquad r^2 = 0.90,\ n = 33$$

where mean(±SE) parameter estimates are provided ($p < 0.001$). The slope estimate in Eq. 1 (0.28±0.02) corresponds to a Q_{10} value of 16.6(±2.8). At the warmest water temperature used in the present study (34°C), 100% mortality occurred. This temperature was considered the thermal maximum (T_{MAX}) for this population. Observed values for EP_{MAX} and T_{MAX} and the estimated Q_{10} value were used within a slightly modified version of an equation developed by O'Neill (1968) to estimate the functional relationship of mean *EP* versus *T* and the optimal temperature (T_{OPT}):

2) $$EP = EP_{MAX} * \left(\frac{T_{MAX} - T}{T_{MAX} - T_{OPT}} \right)^{x} * e^{\left[\frac{x*(T - T_{OPT})}{(T_{MAX} - T_{OPT})} \right]}$$

where *x* is equal to:

3) $$x = \frac{W^2 * \left[1 + \sqrt{1 + \frac{40}{W}} \right]^2}{1000}$$

and W is a function of the Q_{10}:

4) $$W = (Q_{10} * \alpha - 1) * (T_{MAX} - T_{OPT})$$ $r^2 = 0.82$, n = 60.

T_{OPT} and α were estimated parameters equal to (mean(±SE)) 24.78(±0.28) and 0.216(±0.010), respectively ($p < 0.01$). When plotted against T, no trend in the residuals of Eq. 2 was noted.

Exp. 2: Egg hatching and temperature

No hatching was observed over the course of 168 h for eggs produced by females acclimated to the two lowest temperatures (6 and 9°C) and incubated at temperatures between 5 and 10.5°C (5.4, 6.9, 8.7 and 10.4°C). However, for eggs produced at temperatures ≥ 13°C and incubated at 12.4, 14.0, 15.9, 18.0, 20.4 and 22.4°C, hatching was observed within one hour of the start of observations and the total hatch success (HS_T, %) increased in a linear fashion with increasing T:

5) $$HS_T = 3.80(\pm 0.44) * T + 5.73(\pm 7.68)$$ $r^2 = 0.81$, n = 18

where mean(±SE) parameter estimates are provided ($P < 0.0001$) (Fig. 1B). Hatching was completed within 24 h at the highest temperature tested (22.4°C), within 40 h at an intermediate temperature (18.0°C) but occurred over a time course of 120 h for eggs incubated at 12.3°C. At time = 0, the average age of eggs was approximately 6 h.

Between 12.3 and 22.4°C, the cumulative percent hatch (H_{CUM}) versus time was best described by a non-linear function:

6) $$H_{CUM} = A - B * (e^{(-C*t)})$$

where H_{CUM} was expressed in percent (%), t = time (h) and A, B, and C were estimated parameters. Two of the parameters in Eq. 6 were significantly influenced by T according to:

7) $$A = A_0 + A_1 * T$$

8) $$C = C_0 + C_1 * T .$$

Thus, the effect of T on the cumulative time-course of hatching (H_{CUM+T}) was:

9) $$H_{CUM+T} = A_0 + A_1 * T - B * (e^{(t*(C_0 + C_1 * T))}) .$$

Parameter estimates for A_0, A_1, B, C_0, and C_1 were 10.16(± 4.04), 3.66(± 0.25), 62.61(± 1.49), -0.0537(± 0.0247) and -0.0023(± 0.0015), respectively, $r^2 = 0.87$, n = 385, $p < 0.01$ (Fig. 2). According to Eq. 9, the time to 50% hatch was 29.5, 19.8, 13.6, 9.2, 5.8 and 3.8 h at 12.4, 14.0, 15.9, 18.0, 20.4 and 22.4°C, respectively. Variability existed in the time course of hatching

among replicates at some temperatures, particularly at the intermediate temperatures (15.9°C and 18.0°C). Interestingly, the replicate with the most rapid increase in cumulative hatch (%) was usually, but not always, the replicate with the highest total hatch at each temperature.

No significant differences were found in the mean length of females acclimated to the different temperatures (p = 0.2). The mean(±SE) prosome length of females used in Exp 1 and Exp 2 acclimated to 6, 9, 13, 17, 22 (trial 1), 22 (trial 2), 24 and 28°C was 0.82(±0.03), 0.86(±0.03), 0.85(±0.01), 0.87(±0.04), 0.86(±0.02), 0.83(±0.04), 0.83(±0.01) and 0.84(±0.01) mm, respectively.

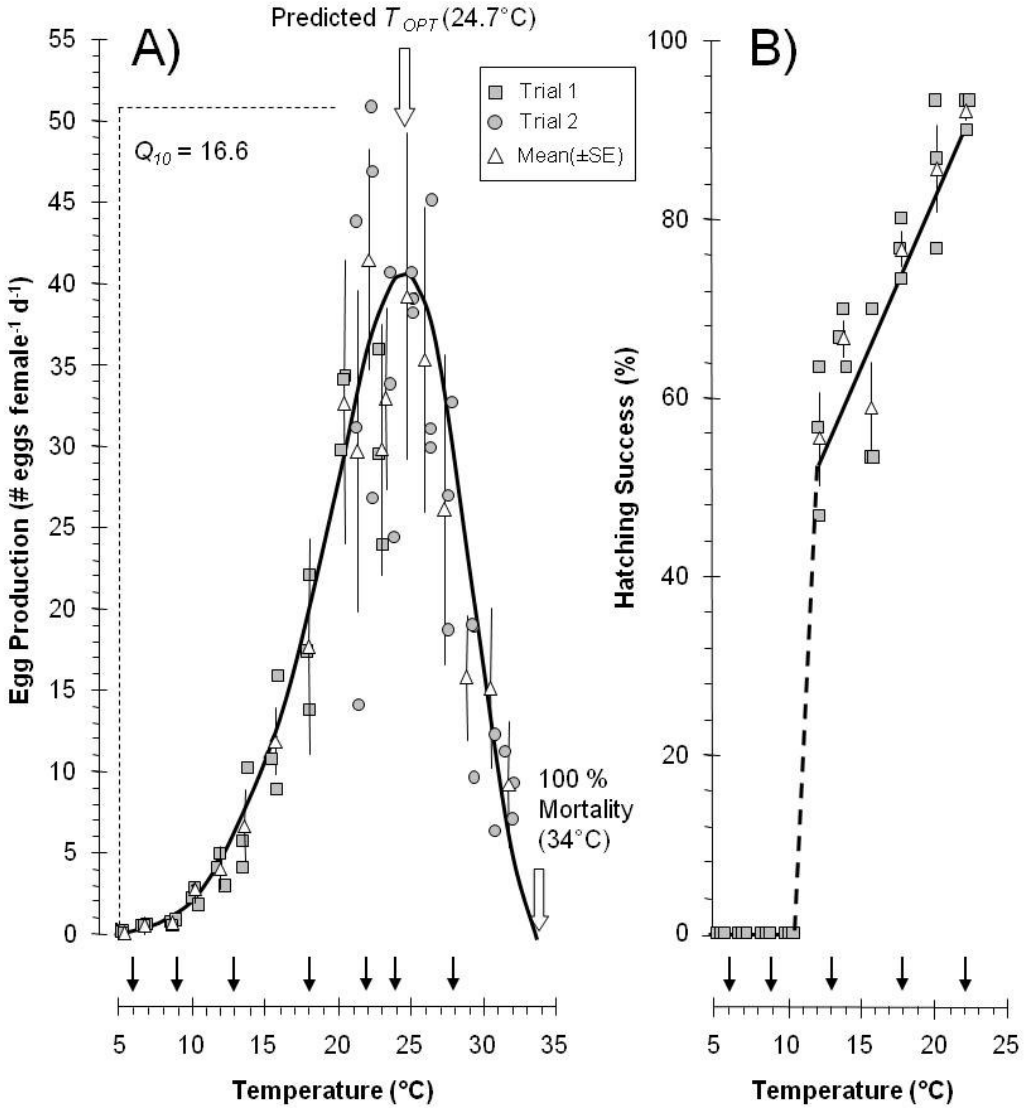

Fig. 1: *A. tonsa* egg production rate (*EP*, Panel A) and hatching success (*HS*, Panel B) as affected by temperature (trial 1, squares; trial 2, circles). In Panel A, the observed mean value for each replicate (n = 2 days) is provided. In Panel B, each datum represents the percentage hatch of 30 eggs. The mean(±SE) *EP* and *HS* at each temperature in each trial (n = 3) is also given (triangles). Arrows indicate female acclimation temperatures. Total mortality of adults was observed at the highest test temperature (34°C). Parameter estimates for predicted O`Neill function (Panel A) and linear regression (Panel B) are indicated within the text.

Exp. 3: Hatching Success and Salinity

The percent (%) hatch of *A. tonsa* eggs was lowest at 0 psu (11.4 %), increased asymptotically with increasing salinity and was highest (84.5 %) at 25 psu (Fig. 3). A modified logistic equation best described the effect of salinity (*S*) on the percent hatch (HS_S):

$$HS_S = \frac{62.74(\pm 7.20)}{1 + e^{-0.44(\pm 0.12)*(S - 6.63(\pm 0.73))}} + 15.15(\pm 6.44) \qquad n = 30,\ r^2 = 0.86 \tag{10}$$

where mean(±SE) parameter estimates are provided ($p < 0.001$). There were no significant differences in HS_S among the four trials for the HS_S at each of the common salinities (6, 17 and 25 psu, $p = 0.91$).

At low salinities, results of a previous study (Holste 2004) indicated that *A. tonsa* eggs ruptured or burst (hypo-osmotic effect) within 48 h and that burst eggs were identified as hatched eggs in trials. Based upon those results, a correction value (*CR*) was calculated and used to modify all observed hatch values in this study at $S \leq 10$ psu ($CR = 0.3192 + 0.0608*S$). The data used to parameterise Eq. 10 were corrected values.

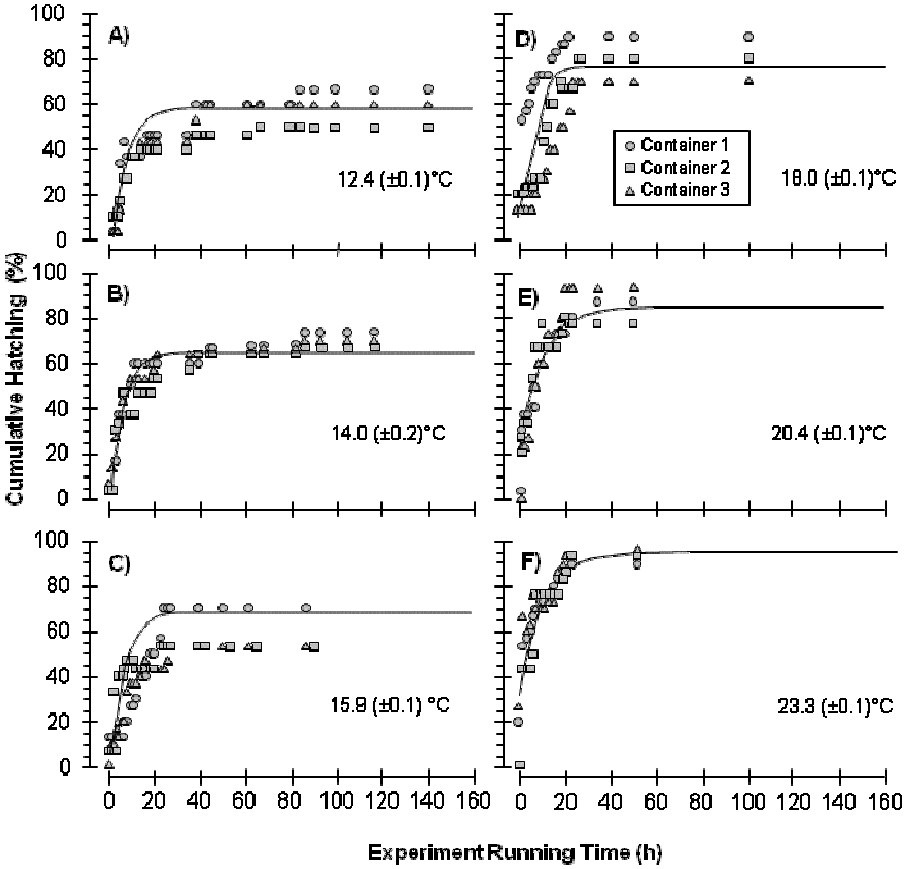

Fig. 2: Cumulative hatching percent (%) versus time (h) for eggs within three replicate containers (triangles, squares, circles) at each of six different temperatures (Panels A-F). Within each panel, the regression line denotes predicted H_{CUM} based upon Eq. 9 in the text.

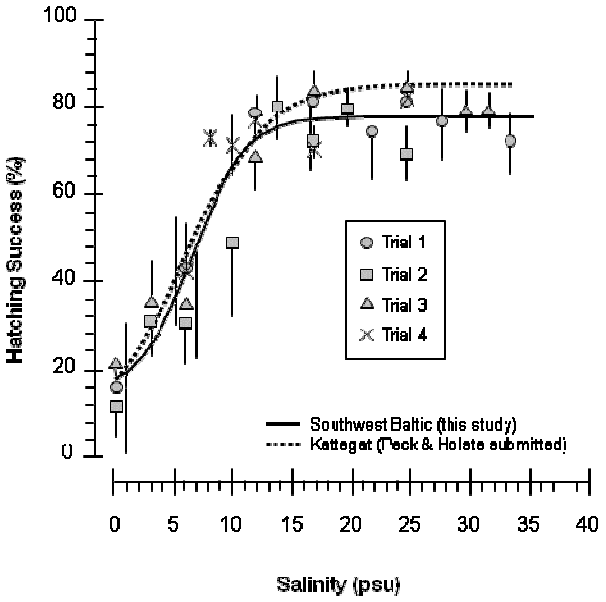

Fig. 3: The mean(±SE) hatching success (HS_S, %) of *A. tonsa* eggs incubated for 48 h at 14 different salinities. For visual clarity, each datum represents the mean of three replicates. Different symbols denote different trials. The equation and parameter estimates for the regression are indicated in the text. For comparison, the predicted hatching success of a Kattegat population of *A. tonsa* (reared at 25 to 30 psu) tested over the same range in salinities is shown (dashed line) (Peck & Holste Submitted).

DISCUSSION:

Temperature and egg production:

Acartia tonsa is considered a typical warm water copepod species and is often most abundant during the summer months in temperate coastal environments (Arndt and Heidecke 1973; Hirche 1974; Behrends and Schneider 1995). Therefore, finding an observed EP_{MAX} and a predicted T_{OPT} for *EP* at 22.9 and 24.8 °C, respectively, was not unexpected since these temperatures would be commonly encountered during summer months in shallow coastal estuaries. Interestingly, although *EP* rapidly decreased at temperatures ≥ 25°C, it was still relatively high at 32°C (11.2 eggs female^{-1} d^{-1}), a temperature that is likely rarely experienced in nature by this Baltic population and is close to its upper lethal temperature (González 1974, this study). *A. tonsa* is one of the most cosmopolitan calanoid copepod species and its widespread distribution in low to mid latitude waters from the Indo-Pacific to northern Atlantic is likely due, in part, to the capacity of this species to successfully reproduce over large ranges in temperatures as indicated in the present study.

A large range in temperature-specific values of *EP* has been reported for *A. tonsa* in previous studies which is not unexpected since *EP* in this (and other) species results from not only the effect of temperature (Castro-Longoria 2003; this study) but from the interplay of a number of different factors including the difference between *in situ* and experimental temperature (Kim 1995), salinity (Peck and Holste Submitted, this study), female age (e.g. Parrish and Wilson 1978) and food concentration and quality (e.g. Kiørboe et al. 1985; Broglio et al. 2003). Due to differences in one or more of these factors, *EP* values in different studies are often difficult to

compare. For example, at a common temperature of 18°C and using the same algal species and concentration, Kiørboe et al. (1985) observed an *EP* of 48 eggs female^{-1} d^{-1} in a Kattegat population cultured at 27 psu, a rate that is twice that (24 eggs female^{-1} d^{-1}) measured in the present study using a Baltic population of *A. tonsa* maintained at 18 psu. In this case, differences in female ages, water salinities, as well as inter-population differences may have contributed to the different results. Our *EP* results between 17.6 and 20.1°C (17.7 to 32.7 eggs female^{-1} d^{-1}, respectively) agree well with those observed by Broglio et al. (2003) at 17 and 20°C (25 to 27 eggs female^{-1} d^{-1}) using slightly lower concentrations of *Rhodomonas sp*.

EP is often used as a growth proxy for adults and populations (e.g. Kiørboe et al. 1985) since it represents the difference between energy inputs and metabolic costs. Differences in reproductive modes and body sizes preclude direct comparison of *EP* among different copepod species. However, an interspecific comparison of Q_{10} values for *EP* provides one method of identifying species-specific patterns in how the balance between metabolic costs and energy gains changes with increasing temperature (Table II). Depending upon the species, *EP* can respond weakly to increasing temperature (e.g., Q_{10} = 1.8 for *A. bifilosa* between 4 and 24°C, Koski and Kuosa 1999) or strongly (Q_{10} = 4.6 for *Calanus finmarchicus* between -2 to 8°C, Hirche et al. 1997; Q_{10} =5.8 for *Temora longicornis* between 2 and 10°C, Maps et al. 2005; Q_{10} = 11.1 for *A. margalefi* between 5 and 20°C, Castro-Longoria 2003). Not only species- but also population-specific differences in *EP* Q_{10} values and temperature optima likely exist due to adaptations to local conditions. These differences between species- and populations may contribute to the consistently low Q_{10} values for *EP* (i.e., 1.33 to 1.93) derived from data sets containing mixtures of copepod species (Ikeda 1985; Hirst and Bunker 2003) that have different (and perhaps contrasting). The strategy of analysing mixtures of species is useful when community-level effects of temperature are desired, but should not be applied to single species. This discussion on calanoid *EP* and Q_{10} appears to be especially germane for copepod modeling efforts since 1) thermal effects on growth are often depicted using a Q_{10} parameter, and 2) two-fold differences can exist in the Q_{10} parameter applied within models constructed for the same copepod species (e.g. *C. finmarchicus*: Carlotti and Slagstad 1997; Carlotti and Wolf 1998; Hansen et al. 2003).

Due to the positive relationship between body size and *EP* (Mauchline 1998), the suggestion was previously made to normalize *EP* data from field and laboratory studies to female length (McLaren and Leonard 1995). In the present study, the mean prosome length of females acclimated to and tested at the different temperatures was not significantly different. However, if not taken into account, body size may be a confounding variable in field studies examining the effect of temperature on *EP* since, in many temperate calanoid species, adult body size is smallest during the warmest months (Viitasalo et al. 1995). Naturally, *in situ EP* can also be influenced by a variety of other, uncontrolled factors such as food quality that may vary seasonally, explaining, in part, the higher Q_{10} estimates obtained in this study (with controlled conditions and ad libitum feeding) compared to most field-based estimates.

Temperature and egg hatching:

Temperature not only affects *EP* but also hatching success (HS_T) (Chinnery and Williams 2003; this study). The present study quantified the hatching of eggs produced by adults acclimated to five temperatures (6, 9, 13, 17, and 22°C) and incubated within 1 to 2 °C of acclimation *T*. The most conspicuous result of Exp 2 was a lack of egg hatching at relatively cold

temperatures. Eggs produced by adults acclimated to $T \leq 9.0$°C did not hatch within 168 h when incubated at $T \leq 10.5$°C, whereas eggs produced by adults acclimated to T ≥ 13°C initiated hatching within 1 h of the start of the experiment and finished hatching 115 h later when incubated at 12.3°C. Tester and Turner (1991) observed poor hatching when incubating *A. tonsa* subitaneous eggs at temperatures below 10°C. Furthermore, no hatching was found by Castro-Longoria (2003) when eggs of *A. tonsa* and three other *Acartia* congeners were incubated at 5 and 10°C. The values of HS_T at warmer temperatures in this study agree with those in other laboratory studies. For example, the high HS_T observed at 20°C in the present study (~92%) agrees well with that (85.4 %) obtained by Chinnery and Williams (2004) for *A. tonsa* and other congeners at the same temperature. Moreover, commencement of hatching was similar between the two studies (i.e., in both studies hatching was observed within one hour of the start of observations at all incubation temperatures). Field data for several *Acartia* congeners collected in the Bornholm Basin, Baltic Sea suggested that egg hatching success was low in cold months (January to April) but increased rapidly and was highest (80%) in warm months (May to August) (Dutz et al. 2004).

The results of previous studies conducted in the Baltic Sea *(*Arndt and Schnese 1986; Madhupratap et al. 1996) and elsewhere (e.g. Sullivan and McManus 1986; Marcus 1996) indicated that *A. tonsa* produces normal eggs, subitaneous eggs and resting eggs. Normal eggs hatch rapidly within the water column. Subitaneous eggs have been described as "quiescent eggs" that may forego hatching in unfavourable conditions but can hatch as soon as improved environmental conditions are experienced. Resting eggs or "diapause eggs" have an obligatory refractory phase that may span several years (Watson and Smallman 1971; Grice and Marcus 1981; Marcus et al. 1994). For members of the *Acartia* genus, temperature, photoperiod and oxygen concentration seem to be the major environmental cues influencing the production of eggs that are considered to be diapause eggs (e.g. Castro–Longoria and Williams 1999; Chinnery and Williams 2003; Katajisto 2004). In recent laboratory trials conducted at 17°C (Peck and Holste Submitted), the 48 h percent hatch of eggs produced by *A. tonsa* reared from nauplii to adults at 8 h, 12 h, 16 h and 20 h photoperiods was 25 %, 55%, 85% and 78% respectively, indicating a strong influence of photoperiod on 48-h hatching success.

Depending upon the species, diapause eggs can be morphologically distinct from subitaneous eggs. Although no differences in egg morphology of hatched and unhatched eggs were noted in the present study (magnification 96 x), a recent study on a congener suggested that differences between the two egg types could be difficult to recognize without scanning electron microscopy (SEM) (Castellani and Lucas 2003). The strategy of diapause egg production may be more strongly influenced by abiotic factors such as *T*, light and oxygen concentration when feeding levels are high. But poor food quality and quantity may override these abiotic effects.

Temperature-specific diapause egg production would explain the lack of hatching observed at cold temperatures in the present study, although other interpretations are also possible (i.e., temperature effect on egg quality). The relationships observed between temperature, *EP* and HS_T in the present experiments suggest that temperature affected the proportion of either diapause eggs, poor quality eggs (that do not hatch and die), or both that was produced each day (i.e., 100% diapause eggs at $T \leq 10$, decreasing proportions of diapause eggs with increasing $T \geq$ 13°C). The results of the present study offer no direct evidence for the presence of diapause egg

production. Future hatching trials conducted after long-term storage of eggs produced at different temperature combined with SEM should help resolve whether the results of the present study can be explained solely by differences in diapause egg production.

Salinity effect on egg hatching:

Few studies have examined the effect of salinity on hatching success of calanoid copepod eggs, which is surprising given the abundance of many members of this family within estuarine and brackish waters. The present study using a Baltic population demonstrated that hatching success (HS_S) increased with increasing salinity and was maximal at 25 psu. The non-linear relationship suggested that HS_S markedly declined with decreasing salinity after a threshold of ~17 psu. For a North Sea population of *A. tonsa*, salinity had an even stronger impact on hatching (Chinnery and Williams 2004). In that study, only 55 % of eggs hatched at a salinity of 15 psu, whereas in this study hatching success of eggs from the Baltic population at 14 psu was 1.5 times greater (78 %). Recent egg hatching trials performed on a Kattegat (27 psu) population using similar methods and salinity ranges as the present study indicated nearly the same non-linear response of HS_S to salinity except that the Kattegat population had a higher HS_S at salinities ≥ 15 psu (Peck and Holste Submitted). Not only hatching success but also *EP* can be affected by salinity and a recent study using the same Baltic population as the present study indicated significantly higher *EP* at 14 psu compared to 30 psu (Peck and Holste Submitted). These studies and field observations indicating population persistence during warm periods at very low salinities (i.e., 4 psu, northeastern Baltic) suggest a high degree of phenotypic plasticity in the response to salinity among populations of *A. tonsa* in the North and Baltic Seas.

The reproductive characteristics of *A. tonsa* examined in the present study showed clear functional responses to temperature and salinity when these abiotic factors were studied separately. However, the interaction between temperature and salinity (*T*x*S*) was not examined, a limitation of the present research. The *T*x*S* interaction can be important especially with regard to physiological tolerances affecting vital rates. For example, pelagic invertebrates often have higher tolerances to lower salinities at higher temperatures (Kinne 1970), a finding inferred for *A. tonsa* from seasonal field distributions (Jeffries 1962). Moreover, the effect of the *T*x*S* interaction on vital rates can be species-specific in copepods. For example, when the effect of temperature on rates of energy loss (respiration, *R*, and excretion, *E*) was compared at different salinities, Gaudy et al. (2000) observed no significant differences for *A. clausi,* whereas the Q_{10} (10 to 20°C) at 15 psu for both *R* and *E* in *A. tonsa* was significantly lower (1.5 and 1.21) compared to 35 psu (4.79 and 2.2). Interestingly, these results (direct measurements of energy loss) agree well with the finding at 18°C of higher *EP* (proxy for surplus energy) in *A. tonsa* at an intermediate salinity (14 psu) compared to a higher salinity (30 psu) more characteristic of coastal marine habitats (Peck and Holste Submitted).

CONCLUSION:

Within the Baltic Sea, seasonal temperature differences spanning 15 to 20°C are often observed in waters having surface salinities of ~4 psu (northeast) to 22 psu (southwest). Populations of *A. tonsa* normally exist within shallow, coastal areas of the Baltic Sea, areas likely to experience larger seasonal (and daily) ranges in temperatures compared to the deeper basins. In this regard, laboratory experiments were conducted using a southwestern Baltic population to

evaluate the functional response of factors associated with reproductive success (egg production and hatching) to wide ranges in temperatures (5 to 34°C) and salinities (0 to 34 psu). The results of this and other studies suggest several reasons for the numerical abundance and cosmopolitan distribution of this species in productive near-shore estuarine and marine environments including: 1) an increase in egg production rate with increasing temperature that was far stronger than that estimated from studies of other calanoid copepod species, 2) a considerable phenotypic plasticity in the effect of salinity on egg hatching success, and 3) the development of a diapause life strategy that may be triggered in response to either (or both) abiotic (decreasing temperatures and photoperiods) and biotic (feeding resources) factors.

Acknowledgements

We are grateful for the help of Philipp Kanstinger, Bianca Ewest, Meike Martin and Gudrun Bening with laboratory rearing and data collection. We would also like to thank Drs. Mike A. St.John and Axel Temming and two anonymous reviewers for helpful comments and suggestions on earlier drafts of this manuscript. This research was funded by the Global Ocean Ecosystem Dynamics (GLOBEC Germany) program by the German Federal Ministry for Education and Research (BMBF 03F0320E) and the German Science Foundation (DFG) AQUASHIFT program cluster Resolving Trophodynamic Consequences of Climate Change ("RECONN", DFG # JO556/1-1).

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Ms 2) The effects of temperature and salinity on reproductive success of *Temora longicornis* in the Baltic Sea: a copepod coping with a tough situation

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The effects of temperature and salinity on reproductive success of *Temora longicornis* in the Baltic Sea: a copepod coping with a tough situation

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ABSTRACT:

At specific locations within the Baltic Sea, thermoclines and haloclines can create rapid spatial and temporal changes in temperature (*T*) and salinity *(S*) exceeding 10 °C and 9 psu with seasonal ranges in temperature exceeding 20°C. These wide ranges in abiotic factors affect the distribution and abundance of Baltic Sea copepods via species-specific, physiological-based impacts on vital rates. In this laboratory study, we characterized the influence of *T* and *S* on aspects of reproductive success and naupliar survival of a southwestern Baltic population of *Temora longicornis* (Copepoda: Calanoida). First, using ad libitum feeding conditions, we measured egg production (*EP,* # eggs female^{-1} d^{-1}) at 12 different temperatures between 2.5 and 24°C, observing the highest mean *EP* at 16.9°C (12 eggs female^{-1} d^{-1}). Next, the effect of *S* on *EP* and hatching success (*HS, %)* was quantified at 12°C for cohorts that had been acclimated to either 8, 14, 20 or 26 psu and tested at each of five salinities (8, 14, 20, 26 and 32 psu). The mean *EP* was highest for (and maximam *EP* similar among) 14, 20 and 26 psu cohorts when tested at their acclimation salinity whereas *EP* was lower at other salinities. For adults reared at 8 psu, a commonly encountered salinity in Baltic surface waters, *EP* was relatively low at all test salinities– a pattern indicative of osmotic stress. When incubated at 12°C and 15 different salinities between 0 to 34 psu, *HS* increased asymptotically with increasing *S* and was maximal (82.6 to 84.3%) between 24 and 26 psu. However, *HS* did depend upon the adult acclimation salinity. Finally, the 48-h survival of nauplii hatched and reared at 14 psu at one of six different temperatures (10, 12, 14, 16, 18, and 20°C) was measured after exposure to a novel salinity (either 7 or 20 psu). Upon exposure to 7 psu, 48-h naupliar mortality increased with increasing temperature, ranging from 26.7% at 10°C to 63.2% at 20°C. In contrast, after exposure to 20 psu, mortality was relatively low at all temperatures (1.7% at 10°C and ≤ 26.7% for all other temperatures). An intra-specific comparison of *EP* for three different *T. longicornis* populations revealed markedly different temperature optima and clearly demonstrated the negative impact of brackish (Baltic) salinities. Our results provide estimates of reproductive success and early survival of *T. longicornis* to the wide ranges of temperatures and salinities that will aid ongoing biophysical modeling examining climate impacts on this species within the Baltic Sea.

Key words: *Temora longicornis*, Copepods, Baltic Sea, Reproduction, Salinity, Temperature, Mortality

INTRODUCTION:

The Baltic Sea is one of the largest brackish water bodies in the world. Its hydrographic conditions are periodically altered due to low frequency, major inflows of North Sea water (Matthäus & Franck 1992). These large inflow events can result in widespread changes in salinity (*S*) and temperature (*T*) conditions. Inflow events, seasonal changes in river runoff, and other factors affect the strength of haloclines and thermoclines often creating depth differences in *S* and *T* exceeding 9 psu and 10 °C (Segerstråle 1957). Therefore the Baltic Sea forms a challenging habitat for organisms. Within its rather simple food web, the zooplankton community is dominated by a few calanoid copepods: *Pseudocalanus acuspes*, *Acartia spp* and *Temora longicornis* which serve as the main prey items for clupeid fishes such as sprat (*Sprattus sprattus*) and herring (*Clupea harengus*) (e.g. Sandström 1980, Flinkman et al.1998, Möllmann et al. 2000)).

Regime shifts have been documented in many large marine ecosystems and a synchronous change occurred in both the North Sea (e.g., Beaugrand & Reid 2003, Beaugrand & Ibanez 2004) and the Baltic Sea (Möllmann et al. 2000, Alheit et al. 2005) in the late 1980ies. Within the Baltic Sea, a decrease in *Pseudocalanus acuspes* abundances was significantly correlated to a decrease in salinity within the last two decades, whereas an increase in the abundance of *Acartia spp*. was correlated to an increase in spring temperature within the upper 50 m (Möllmann et al. 2000). Annual indices of the abundance of *T. longicornis*, although more stable, were also positively correlated with temperature in the uppermost portion of the water column. The strong response of both of these copepods to temperature is well demonstrated (e.g., for *Temora*: Mauchline 1998, Halsband-Lenk et al. 2002; for *Acartia*: Holste & Peck 2006) but the effect of salinity has only been studied on *Acartia sp* (e.g., Peck & Holste 2006).

Based upon its field distribution *T. longicornis* has been classified as a temperate, neritic and euryhaline species (Krause et al. 1995 and references therein) but has a wide latitudinal range inhabiting waters from the Portuguese Coast (Halsband-Lenk et al. 2002 and references therein) north to the Arctic Ocean (Klekowski & Weslavski 1990, Lukashin et al. 2003, Chikin et al. 2003). Marine and coastal euryhaline species such as *T. longicornis* are osmoconformers (see Mauchline 1998 and references therein), regulating ionic composition on a cellular level to match the osmolarity of the external environment. In the Baltic Sea, late copepodite stages and adults of *T. longicornis* are known to vertically migrate across the permanent halocline in summer (Schmidt 2006) and therefore individuals have to cope with large differences in salinity (> 9 psu) each day. The costs associated with exposure to wide fluctuations in salinity likely reduce the energy available for other processes such as growth and reproduction but it is unknown to what extent adaptation to lower salinities might compensate for these costs. Furthermore, patterns of field abundance (Jeffries 1962) and direct experiments (Kinne 1971) suggest that copepods and a variety of other pelagic invertebrates tend to have higher tolerances to lower salinities at higher temperatures. Therefore, the magnitude of the impact of daily migrations through thermoclines and haloclines on rates of survival and population growth of copepods is currently unknown and the possible consequences of increased warming due to climate change are difficult to explore. Furthermore, time series from the Baltic Sea show a decrease in *T. longicornis* biomass in summer (Möllmann et al. 2003) since 1990. A multiple linear regression indicated a positive correlation with salinity that was significant for early copepodite stages (and almost for nauplii) and adults. Hence the authors suggested that when temperature in summer is sufficiently high, *T. longicornis* suffers due to the low salinities encountered in waters above the

halocline. The combination of low salinity and high temperature would negatively impacts reproductive success of the population through a reduction in the numbers of adults available to produce eggs.

In the present study, we quantified the reproductive success of *T. longicornis* over wide ranges in temperatures and salinities and the interaction between these abiotic factors (*T*x*S*). Specifically, we quantified egg production rate (*EP*) at 12 different temperatures (Exp 1), and measured *EP* (Exp 2) and egg hatching success (*HS)* (Exp 3) at a variety of different acclimation salinities and after changes in salinity. We also examined the influence of *T*x*S* on naupliar mortality using two different salinities and six different temperatures. Our experiments were designed to provide estimates of environments promoting optimal survival of *T. longicornis* early life stages and yield ecologically relevant information on how abiotic factors shape life history strategies of Baltic *T. longicornis*. These data are needed to advance parameterizations of coupled biophysical stage-based copepod models (e.g., Fennel & Neumann 2003, Neumann & Fennel 2006) developed to explore how climatic processes act to influence variability in secondary production of this and other key copepod species in the Baltic Sea.

MATERIAL and METHODS:

Temora longicornis used in this study were the progeny of adults collected from two WP2 seawater samples (12m to surface, 55 µm mesh size) taken in May 2005 (S = 14 psu, T = 10°C) in Kiel Bight in the southwestern Baltic Sea (54°N; 10°E). In the laboratory, zooplankton samples were acclimated to S = 14 psu and T = 12°C in order to enhance production over the course of four days after which *T. longicornis* was isolated from each field sample in a ratio of 3:1 (females: males) and placed into each of two cylindrical 8 L tanks (density ~ 25 ind L^{-1}). Cultures were provided daily rations of a cryptophyte (*Rhodomonas sp.*) at concentrations (> 50 000 cells mL^{-1} equal to ≥400µg C L^{-1}) providing unlimited growth and egg production for *T. longicornis* (Dutz et al. 2008). Cultures were maintained on a 13L:11D light regime and received gentle aeration for mixing.

After several generations, four different salinity cohorts were established (8, 14, 20 and 26 psu) which are hereafter referred to experimental cultures (EC_8, EC_{14}, EC_{20} and EC_{26}, respectively). Each *EC* was reared for at least two generations before the start of experiments (for details see Table I). Four different experiments were conducted in this study within a controlled-environment room having a 12L:12D light regime with a daytime water surface light intensity of 1 to 5 µE (µmol m^{-2} s^{-1}) (Biospherical Instruments Inc. QSL 100). In all experiments, temperature (±0.1°C) was monitored using data loggers (Onset computer corporation Box Car Probe and salinity (±0.1 psu) was measured daily (WTW Tetra Con® Probe).

Exp 1: Temperature and Egg Production

The effect of temperature on egg production rate (*EP*, # $female^{-1}$ d^{-1}) was quantified at nine temperatures between 2.6 and 24°C. This experiment was conducted at an intermediate salinity (14 psu) using two trials. Prior to experimental trials, adults were acclimated to the different test temperatures within five days. After being acclimated, copepods were reared at the test temperature for two days prior to the start of the experiment.

Five *T. longicornis* females and two males were carefully pipetted into each of three replicate 250 mL glass beakers at each test temperature. Beakers were held within a thermal gradient table (Thomas et

al. 1963), an aluminium block that could be heated and/or cooled by pumping temperature-controlled water through holes drilled in both ends. Thermal stratification within beakers was avoided by conducting experiments at a relatively cold air temperature (10 °C). Within replicate glass beakers, water temperatures were maintained within ±0.15°C (at low temperatures) and ±0.5 to 0.7°C (at the two highest temperatures).

To avoid egg cannibalism, individuals were held within mesh-bottom sieves (8.4 cm height, 4.5 cm diameter, 130 µm mesh size) suspended in each beaker. Every 24 h, the adults were placed into a new container by carefully transferring the sieve. The new container had filtered seawater (at the appropriate test temperature) containing > 50,000 cells mL^{-1} *Rhodomonas* sp. The contents of the old beakers were collected (35 µm sieve), rinsed into a Bogorov dish, and the number of eggs counted under a Leica MZ 9_5 dissecting scope. Using these methods, data were collected from each replicate beaker each day for 72h. The first 24 hours was considered an acclimation period for copepods to become accustomed to the beakers. Beakers were checked daily for mortalities and any dead individuals were replaced with individuals acclimated to a similar temperature. Any dead female was assumed to have died after 12 h and had produced eggs until death (i.e., 6 females incubated, one found dead the next day, total eggs produced were divided by 5.5). At the end of the trials, adults were videotaped and prosome lengths measured using computer image analysis (Optimas 6.51).

Exp 2: Salinity and Egg Production

The effect of salinity on egg production (*EP*, # eggs $female^{-1}$ d^{-1}) was quantified at each of five test salinities of 8, 14, 20, 26 and 32 psu with individuals from each of the four salinity cohorts (EC_8, EC_{14}, EC_{20} and EC_{26}). Five *T. longicornis* females and two males were carefully pipetted into each of three replicate 250 mL glass beakers at each test salinity. Data were collected over the course of three days (see Table I) and the daily methods were the same as those used in Exp 1. Experiments took place at an intermediate temperature (14±1°C).

Exp 3: Salinity and Egg Hatching

Eggs used in this experiment were produced by EC_8 to EC_{26}. Copepods that produced eggs had been in the adult stage for approximately 5 to 7 days prior to the start of measurements. Egg hatching success (*HS*, %) was quantified at 15 different salinities from 2 to 30 psu by conducting five separate trials. In each trial, due to technical limitations only seven or eight different salinities could be simultaneously tested (Table I). Egg hatching was compared at four common salinities (8, 12, 20 and 26 psu) among the four trials.

In each trial, a known number of eggs (48 –59), that were produced during the previous 24 hours, was loaded into a 250 mL culture flask containing 200 mL of gently aerated, 1 µm filtered seawater. Three replicate flasks were used at each of the test salinities. All flasks were incubated for 48 h within a controlled-environment room at 14°C (range ±0.5°C). After 48 h, the contents of the flasks were gently poured through a 35 µm sieve and rinsed into a Bogorov dish. Unhatched eggs were counted with the aid of a dissecting scope.

Exp 4: Temperature/Salinity interaction and Naupliar Mortality

For this experiment, eggs were collected from the EC_{14} at 14°C (to enhance production) by letting eggs settle and siphoning the bottom of the tank. Eggs were incubated at 14°C and 14 psu. Freshly-hatched nauplii were stepwise acclimated from 14°C to six different temperatures (9.5, 11.6,

13.9, 15.7, 18.2 and 20.4 °C) and fed *Rhodomonas* sp. until 90% reached the N4 stage. Both, acclimation and experiment were conducted using the thermal-gradient table mentioned above. A total of 20 to 25 N4 nauplii was then placed into 50 mL wells containing 35 mL of SW having either 7 or 20 psu at the appropriate acclimation temperature (three replicates each). Every 12 hours for a total of 48 h, replicates were checked for mortalities by gently rinsing them into a petri dish and examining each individual under a dissecting scope.

Literature comparison:

In order to compare our data and those from various literature sources on the effect of temperature on *EP*, data from published figures were digitized with the help of MATLAB 7.0.4 (2005). Field data (*EP,* # eggs female^{-1} d^{-1} and water temperature) were calculated from monthly mean values.

Statistics:

Data collected in this study were analyzed by linear and non-linear regression analysis. Predictive regressions were used and parameter estimates were obtained by the least-squares method. The functional form of regressions was chosen based upon several statistical criteria (significance level, coefficient of determination (r^2), sum of squared errors (SSE) and residual trend analysis). A one-way ANOVA tested for differences in adult size (prosome length) among the different acclimation temperatures and salinities used in Exp 1 and 2. A two-way ANOVA was used in Exp 1 (*EP*, trial x T), in Exp 2 (*EP,* acclimation *S* x test *S*) and in Exp 3 (*HS, S* x trial). Additionally an ANCOVA was performed to test the interactive effect of rearing salinity and incubation salinity in Exp 3. All values of *HS* (%) were arcsin transformed [arcsin*(%/100)$^{0.5}$]). An ANCOVA was also used for testing the significance of *T* & *S* interactions in experiment 4. All statistical tests were performed using SPSS (SPSS 1990) and were considered significant at $p \leq 0.05$.

RESULTS:

Exp 1: Temperature and Egg Production:

Egg production rate (*EP*, # eggs female^{-1} d^{-1}) was measured at nine different temperatures within two trials: trial 1: 2.6 to 16.5°C, trial 2: 12.1 to 24°C. Mean (±SE) *EP* of the two measurement days at 12, 14 and 16.5 °C in trial 1 (3.6±0.4, 6.7±0.3 and 7.09±0.8, respectively) was not significantly different ($p = 0.551$) than that measured at the same temperatures in trial 2 (3.5(±0.3), 4.8(±1.1) and 8.8(±1.5), respectively). Therefore, the data collected in the two trials were pooled and analyzed together.

Under unlimited algal concentrations, mean (±SE) *EP* increased with increasing *T* from 1.5 (±0.4) to 12 (±2.1) eggs female^{-1} d^{-1} from 2.6 to 16.6°C, respectively, and declined at higher temperatures (Fig. 1). At 24°C, 100% mortality occurred and this was defined as the lethal temperature (T_{LETH}). The simplest functional describing the effect of temperature on *EP* form was a three parameter Gaussian function:

$$EP = a * e^{\left[-0.5\frac{(T-T_{OPT})^2}{b}\right]}$$

where mean (±SE) parameter estimates for a, b and T_{OPT} were 6.40 (±0.54), 5.98 (±0.80) and 16.58 (±0.77), respectively (r^2= 0.83, n=34, p≤0.0001).

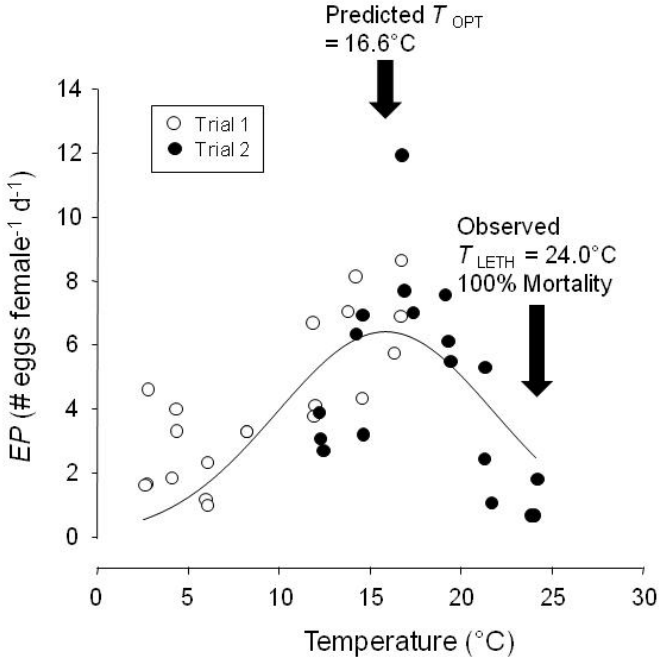

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Fig. 1: *Temora longicornis* egg production rate (*EP*, # eggs female^{-1} d^{-1}) versus acclimation temperature (°C). Mean values for each of the three values are shown. The observed *EP* for each of the three replicates and 48 h is provided. Total mortality of adults was observed at the highest test temperature (24°C). Filled circles = trial 1, unfilled circles = trial 2.The non-linear regression is provided within Table II.

Exp 2: Salinity and Egg Production:

For copepod cohorts reared at the four different acclimation salinities, clear influences of incubation salinity on *EP* were observed. Females that were reared at 8 psu exhibited generally low *EP* (1.8 to 11 eggs female^{-1}d^{-1}) and *EP* changed little after short-term incubation in the different salinities (Fig. 2A). Animals cultured at 14 psu produced more eggs (2.7 to 16.9 eggs female^{-1}d^{-1}) with a maximal production at 14 psu and a decline in *EP* with further increase in *S* (Fig. 2B). This pattern was also observed in the other treatments, where the highest *EP* occurred close to the rearing salinity (20 psu treatment the highest *EP*: 18.5 eggs female^{-1}d^{-1}; 26 psu treatment highest *EP*: 14.1 eggs female^{-1}d^{-1}; compare Fig. 2C&D). *EP* in each of the three treatments could be described well by a dome-shaped function (Gaussian, 3 parameters) in which parameters were in all cases highly significant (P ≤ 0.0001). *EP* was significantly different for all acclimation salinities when tested via a two-way ANOVA (F = 7.32, p = 0.0005).

Mortality occurred with time in all treatments during the experiment (Fig. 2E–H)). Copepods at 8 psu could not withstand salinities higher than 20 psu for longer than one day and, after 100% mortality, dead copepods were not replaced with alive ones in those replicates (Fig. 2E). Females reared at 14 psu survived well at 8 and 14 psu but above 14 psu mortality increased with increasing salinity and was 100% at 32 psu Fig. 2F). A different pattern emerged at 20 psu where survival was high at 8, 14, 20 and 26 psu (Fig. 2G) on day 1. On the second day, mean mortality was 55.6% at 8 psu up to 55.6% and at 32 psu was mortality higher than at the intermediate salinities (49.2%). In adults reared at 26 psu, relatively high mortality (50.0%) only occurred at the lowest test salinity (8 psu) (Fig. 2H).

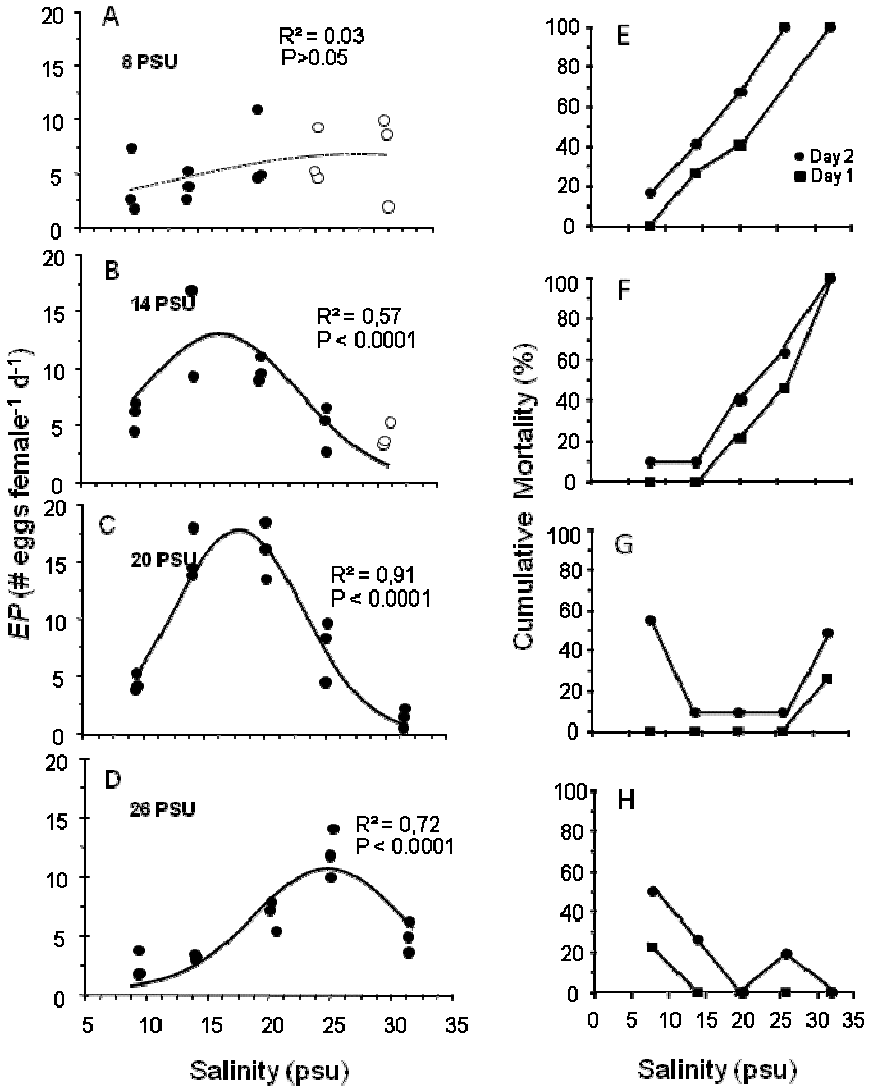

Fig. 2: *Temora longicornis* egg production rate (*EP*, # eggs female^{-1} d^{-1}) and cumulative mortality (%) for adults acclimated to one of four salinities (8, 14, 20 and 28 psu) and tested at 8, 14, 20, 26 and 32 psu (Panel A to D). Values of egg production are means (n = 2 days) for each of the three replicates. Unfilled circles = data for 24 h only due to 100% mortality occurring on second day. Non-linear regression parameter estimates are provided in Table II. Panel E to H: Cumulative mortality (%) during experiment.

Exp. 3: Salinity and Egg Hatching Success:

Within the four trials, hatching success (*HS*) of eggs produced at 8, 14, 20 and 26 psu was measured at a total of 15 different salinities. No significant differences were detected between trials (Two-way ANOVA, F = 1.623, p = 0.412) and for rearing salinity (ANCOVA, F = 0.194, p = 0.899) and, therefore, data from all performed trials were combined for subsequent analyses. Hatching success increased with increasing incubation salinity (Fig. 3) and changes in *HS* (%) with salinity were best described by a three parameter sigmoidal function (Table II). Hatching success was not predicted

to increase at salinities > 23 psu. The highest mean±SE hatching success (91.2±1.1%) occurred when eggs produced at 26 psu were incubated at the same salinity. The lowest *HS* (25.6±3.7%) was measured when eggs produced at 20 psu were incubated at 2 psu.

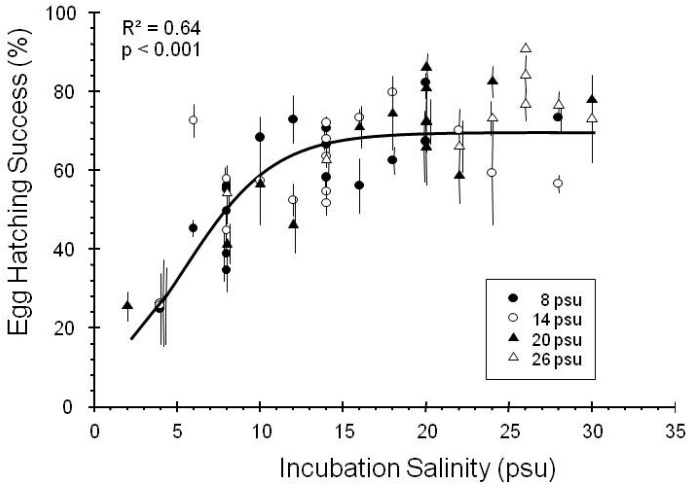

Fig. 3: Egg hatching success (*HS*, %) of *Temora longicornis* versus salinity. Eggs were incubated for 48 h at 15 different salinities. For visual clarity, each datum represents the mean of three replicates and standard error bars were offset slightly. Different symbols denote cohorts reared at different salinities (EC_8, EC_{14}, EC_{20} and EC_{26}). Regression for predicted hatch is provided in Table II.

The pattern in the change in *HS* vs. the change in incubation *S* (ΔHS vs. ΔS) for each cohort that produced eggs (Fig. 4) depended upon the rearing salinity of the cohort (EC_S). Eggs produced at 8 psu and incubated at a higher (lower) *S* had generally higher (lower) *HS* than those at 8 psu. Eggs produced at 14 psu had relatively high values of *HS* (10 incubations of 13 in total) when incubated at either lower or higher S. When eggs produced at 20 psu were incubated at higher *S*, *HS* was only slightly (~10%) higher than at the rearing salinity but was much lower (up to 40% decrease) when those eggs were incubated at lower salinities. Only three out of 12 batches incubated at a salinity ≥ 20 psu exhibited a positive difference in *HS*, while seven out of 12 incubations showed a lower *HS*, mostly at lower salinities. Eggs produced from cohorts reared at 26 psu exhibited a decrease in *HS* when incubated at either lower or higher salinity. It should be noted that, in that case, only a few trials were conducted at higher incubation *S*, since 30 psu was the highest test salinity.

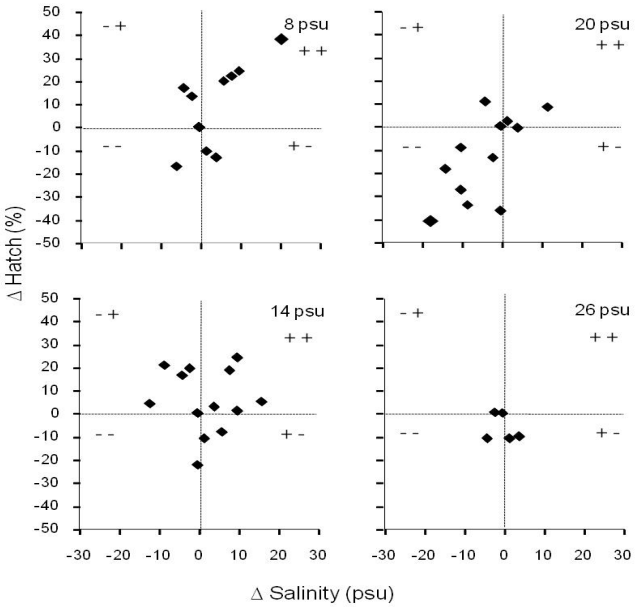

Fig. 4: Absolute change in the percent hatch of *Temora longicornis* eggs versus the absolute change in salinity experienced during incubation of eggs produced by four different salinity cohorts (EC_8, EC_{14}, EC_{20} and EC_{26}). Dashed lines denote references between positive and negative effects of salinity on hatching.

Exp. 4: Temperature /Salinity interaction and Naupliar Mortality:

There was a significant interaction between *T* and *S* on cumulative naupliar mortality (*CNM*) (ANCOVA, p = 0.03). During the 48-h test, mean±SE *CNM* at 20 psu was relatively low at low temperatures (10 and 12°C, CNM = 1.6±1.6, and 8.2±1.7%, respectively), highest at 14°C (26.6±8.8%) and did not increase at higher temperatures (Fig. 5). 2 In contrast, *CNM* was higher at 7 psu, especially at relatively low temperatures (e.g., 10 and 12°C, *CNM* = 26.70±6.00; 26.7±7.3%, respectively). For nauplii acclimated to higher temperatures (14 to 20°C), mean±SE *CNM* markedly increased to a maximum of 63.2±4.6% at 18°C and 62.5±4.6 at 20°C. Hence *CNM* was up to a threefold higher in the 7 psu treatments compared to the 20 psu treatments at each of the different temperatures tested.

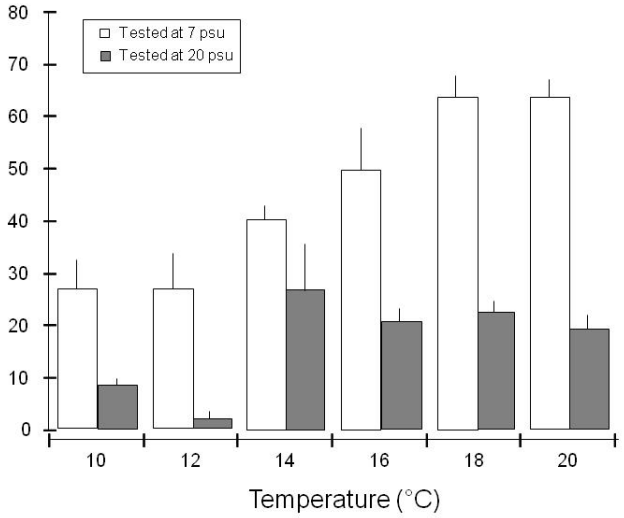

Fig. 5: *Temora longicornis* 48-h cumulative naupliar mortality for nauplii that had been acclimated to 14 psu at six different temperatures and then exposed to either a lower (7 psu) or higher (20 psu) salinity. Bars (white = 7 psu; grey = 20 psu) represent means (+SE) of three replicates.

DISCUSSION:

Copepods form the bulk of secondary production in marine environments being the most important food item for all marine fish larvae and zooplanktivorous adult fish. Resolving environmental (climate) impacts on the trophodynamics of marine ecosystems demands a thorough knowledge of changes occurring in the copepod community. The Baltic Sea is characterized by steep horizontal and vertical gradients of salinity and Baltic populations of *T. longicornis* can be exposed to quite different salinity conditions both daily (via vertical migration) and/or seasonally depending upon their geographic location. Laboratory experiments conducted in this study investigated two reproductive processes: egg production as influenced by temperature and acclimation salinity and 48h-hatching success of eggs as affected by acclimation salinity. The interactive effect of temperature and salinity on *CNM* was also investigated in an effort to provide estimates about possible survival conditions of *T. longicornis* offspring in the wild.

Egg Production and Temperature:

Temora longicornis is considered to be a typical temperate calanoid copepod species and occurs within the Baltic Sea throughout the whole year (e.g. Viitasalo et al. 1995a & b, Vuorinen et al. 1998, Möllmann et al. 2000, Hansen et al. 2006). Therefore, the finding of an intermediate temperature optimum (16.6 °C) for *EP* was not surprising. Values of *EP* reported in different studies

are often difficult to compare due to differences in one or more factors. Within the literature a wide range in values of *EP* have been reported including 3 to 50 eggs female^{-1} d^{-1} at 7 and 8°C in November 1997 in the Irish Sea (Castellani & Altunbaş 2006) to 10 to 50 eggs female^{-1} d^{-1} at 18 and 10°C, respectively, in the North Sea (data collected in May 2004 by Wesche et al. 2007) and 43.5 eggs female^{-1} d^{-1} at 15°C in Long Island Sound (Peterson & Kimmerer 1994) with variability in temperature-specific rates depending upon the time of the year, feeding conditions and prosome length of females (e.g., Klein Breteler & Gonzales 1988, Klein Breteler et al. 1990). These reported literature values of *EP* were always for incubations performed at salinities ≥ 30 psu. One has to note that our experiments were conducted with individuals experiencing a much lower salinity of 14 psu. In the Baltic Sea, maximal *EP* coincided with the spring phytoplankton bloom occuring between March and May at 4 to 8 °C at salinities around 7 psu. A second peak in egg production has been measured in September/October at surface water temperatures of 17 to 20 °C. Values of egg production obtained in our laboratory experiments with a maximum of 12 eggs female^{-1} d^{-1} were much lower compared to rates measured in other areas. In Exp 2, egg production was higher when females were acclimated to 14 or 20 psu. Females reared at 20 psu produced 18.5 eggs female^{-1} d^{-1}. Recent studies in the Bornholm Basin (Baltic Sea) reported values of *EP* between 3 to 14 eggs female^{-1} d^{-1} (Peters 2006) at salinities of approximately 7 to 9 psu and temperatures between 17 and 20°C. Those findings agree closely with values of *EP* found in the present study. In comparison, when normalized to a temperature of 10°C (using a Q_{10} of 3, after Kiørboe & Sabatini 1995), maximum values of *EP* reported for *T. longicornis* in the North Sea were much higher (28.4 to 35.0 eggs female^{-1} d^{-1}) (Peters et al. 2007). Therefore our findings suggest that, in the case of the Baltic Sea, salinity is a masking factor. Comparing values from the literature (Fig. 6 & table III) one can find differences in both *EP* (eggs female^{-1} d^{-1}) and temperature optima for *EP* in different systems. These differences appear to depend: 1) strongly on the food quantity and quality, 2) on adaptations to the prevailing temperatures of the habitat, and 3) on adaptations to different salinities. For instance: Field data (Fig.: 6A) on *T. longicornis* in the North Sea indicate a production of almost 60 eggs female^{-1} d^{-1} at temperatures around 10°C (Halsband & Hirche 2001). The same species within the Baltic Sea has its temperature optimum at 3°C with a much lower total *EP* (Peters 2006). Differences in female size can partly explain the difference between Baltic and North Sea. These size differences of animals between the two ecosystems are well known to be salinity dependent. Hence in this case salinity is explaining differences in total *EP* (eggs female^{-1} d^{-1}). Looking at specific *EP*, Peters (2006) found a specific *EP* of 0.19 (µgC female^{-1}) in the Baltic, which is similar to that of Halsband-Lenk in the North Sea (0.25). The differences in carbon-specific *EP* between the habitats might then be explained by salinity, directly on the effect of osmotic stress and indirectly by size differences.

Comparing rates of egg production by *T. longicornis* measured in laboratory studies (Fig. 6 B) conducted with populations in the Gulf of St Lawrence (Maps et al. 2005) and the North Sea (Halsband-Lenk et al. 2002) with those in our study, there are two findings besides a salinity effect: 1) the total *EP* differs due to food quality and quantity and 2) the temperature optimum is shifted toward the acclimation temperature. Obvious differences in *EP* can be noted among *T. longicornis* fed a relatively large heterotrophic dinoflagellate (*Oxyrrhis marina*, Maps et al. 2005) a somewhat smaller cryptophyte, (*Rhodomonas*,this study) and a smaller nanoflagellate (*Hymenomonas elongate*, Halsband-Lenk et al. 2002). In these cases, temperature–specific *EP* tends to decline with decreasing prey size. A second explanation for differences in *EP* observed among different populations seems to be differences in water temperatures experienced during the growing season. For example, the Gulf of St. Lawrence is the coldest habitat in this comparison, and therefore a rather low T_{OPT} is expected. The Baltic Sea is a habitat with intermediate summer temperatures while the shallow North Sea region

Helgoland Roads is a relatively warm habitat. These findings suggest that there is not only a direct effect of temperature on *EP* but population specific shifts in temperature optima based on differences in temperatures prevalent during spring phytoplankton bloom.

Egg Production and Hatching and Salinity:

In the present study, we found significant differences in the reproductive success (*EP* and *HS*) when cohorts acclimated to one of four different salinities were tested at five different salinities. While *EP* was always highest at the acclimation salinity of cohorts tested at 14, 20 and 26 psu, the 8 psu individuals exhibited no differences in *EP* when tested at the various salinities. Generally, *EP* by females acclimated to 8 psu followed no trend with salinity and was very low, especially when individuals were tested at higher salinities. This is obviously a result of the high mortality that occurred within treatments exposed to salinities above 20 psu. Our results provide evidence for a low salinity threshold for egg production in the Baltic Sea of ~ 8 psu. As previously stated, the numbers of eggs produced in this experiment generally agreed well with the findings of field studies conducted in the Bornholm Basin (Peters 2006). A second indication for an influence of acclimation salinity on vital rates was the differences in cumulative mortality: Adults from EC_8 and EC_{14} did not survive in 32 psu water while EC_{26} had a higher mortality at 8 psu. When all hatching success data were combined, HS_S followed a three parameter sigmoidal function with an increase in hatching success with increasing incubation salinity. Percent hatching success within this study was similar to values reported in studies conducted at the same temperature (14°C) in other regions. For example, in the North Sea (≥ 30 psu) hatching success for this species has been reported to be between 78 to 90% (Peters et al. 2007) and 67% (Koski et al. 2006) while a slightly lower value (42.8%) was reported for the Gulf of St. Lawrence within salinities between 26 to 30 psu (Maps et al. 2005). Looking at the ΔHS vs. ΔS, we found that eggs produced at 14 psu seemed to be most robust when incubated at lower or higher salinities. For example, 13 different incubations were conducted with eggs produced at 14 psu and 10 batches of these, exhibited higher hatching success at salinities lower or higher than 14 psu. The results of both experiments suggest that reproductive success of this species would be markedly different depending upon subtle differences in vertical position of animals with respect to the halocline or differences in the horizontal location of this species along salinity gradients within estuarine regions.

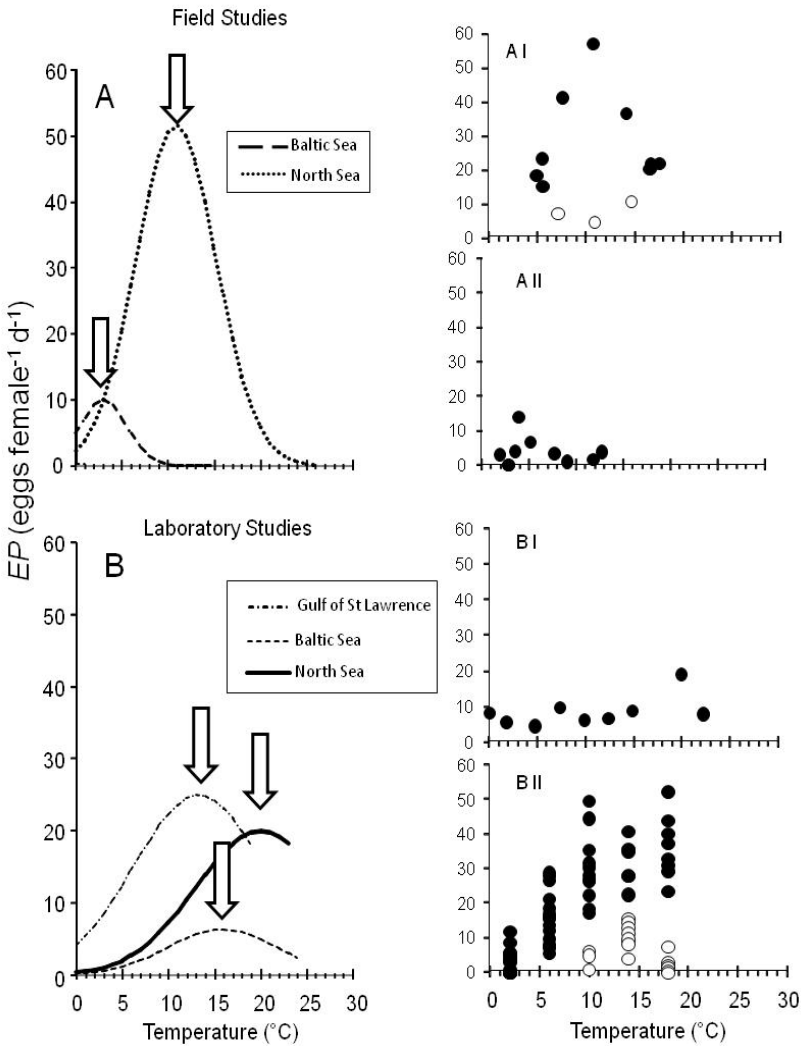

Fig. 6: Summary of laboratory and field data collected on egg production rates (# eggs female^{-1} d^{-1}) of different *Temora longicornis* populations. Panel A) Predicted seasonal egg production rate versus temperature based data collected in different field studies: Baltic Sea (dashed line, Peters (2006)), North Sea (dotted line, Halsband & Hirche 2001). Panel B) Predicted egg production rate of three different *Temora longicornis* populations versus temperature based upon data collected in the laboratory: Gulf of St Lawrence (dash-dotted line, Maps et al. 2005), Baltic Sea (dashed line, this study) and North Sea, Helgoland Road (solid line, Halsband-Lenk et al. 2002). AI, AII, BI and BII: data used for prediction of panel A and B. Compare also Table III. Unfilled circles = data not used for prediction because of obvious food limitation as stated by the authors Halsband & Hirche 2001, and Maps et al. 2005.

Naupliar Mortality and Temperature/Salinity interaction:

Euryhaline copepod species such as *T. longicornis* are thought to perform ionic regulation at a cellular level, but mechanisms of osmoregulation in copepods are still not well understood. Within our study, 48-h cumulative naupliar mortality (*CNM*) increased with increasing temperature within the 7 psu treatment and was threefold higher compared to *CNM* within the 20 psu treatment. There have been only a few studies conducted examining the interaction between temperature and salinity (*T***S*) on the survival of copepods, with most studies examining adults. For example, Damgaard and Davenport (1994) found a higher salinity tolerance at lower compared to higher temperatures looking at adult survival of a harpacticoid copepod (*Tigriopus brevicornis*). A similar finding was reported for adults of the calanoid copepod *Eurytemora velox* by Nagaraj (1988). Nauplii often form the most fragile and vulnerable copepod life stages in terms of salinity tolerance. For example, Lee and Petersen (2002) observed that nauplii of *Eurytemora affinis* were less tolerant to low salinities compared to copepodite and adult (CVI) stages. An important study on the impact of salinity on the survival of nauplii of Acartia congeners was conducted by Chinnery and Williams (2004). Those authors found that, for all congeners examined, nauplii survived better (up to 86.3%) at full strength

seawater (33.3 psu) compared to nauplii incubated at lower salinities. Since animals used in the present study were acclimated to 14 psu, a rather favorable salinity for egg production and egg hatching success for this southwestern Baltic population and were examined over a wide range in temperatures, we suggest that osmotic stress was the main factor driving mortality when individuals were exposed to a lower salinity (7 psu). As metabolism is higher at higher temperatures (Ikeda 1970; Marshall 1973), it is not unexpected that animals` condition seems to suffer from the energy spent on regulating the ion content of body fluid. The additional energy costs associated with ionic regulation of body fluids at high temperatures had marked consequences in this study. Cellular-level and bioenergetics measurements such as measuring enzyme activities and the level of specific dynamic action (e.g., see work on *E. affinis* (Kimmel & Bradley 2001) and on *A. tonsa* and *Calanus finmarchicus* (Thor 2000)) in response to salinity changes should help identify the physiological mechanisms acting to cause the patterns in mortality noted at different *T*S* combinations in the present study.

Ecological consequences:

Our laboratory experiments were designed to examine the reproductive success of *T. longicornis* over wide ranges in temperatures and salinities that encompass those experienced by this species in different regions of the Baltic Sea and allow us to interpret the life history strategy of this calanoid copepod in this system. The vertical distribution of *T. longicornis* in the Bornholm Basin in July and August is stage-specific (Schmidt 2006). Younger stages (nauplii, C1 and C2) mostly dwell in the upper water column (10 to 30 m) and do not perform any noticeable vertical migration. The intermediate copepodite stage (C3) is also found in deeper waters down to 50 m. The late copepodites and adults, however, occur during the daytime at depths of ~80 m (below the permanent halocline situated at ~50 m) at 10 to 15 psu. At night, the abundance of late copepodites and adults is often highest in the uppermost 20 m of the water column (Schmidt 2006). Hence these animals experience a salinity difference of up to 9 psu within a relatively short period of time (hrs). For *Acartia spp.* and *Centropages spp.* it is known that *EP* is highest during the night until the early morning and takes place within the upper water column (Checkley et al. 1992). Assuming the same temporal dynamics for *T. longicornis*, egg production by individuals ascending from the deeper, more saline waters would seem to be a sub-optimal strategy based upon the results of the present study. The increase in temperature during their migration into the upper 20 m should enhance *EP* but the decrease in salinity experienced by adults would limit the production of eggs to approximately a third of the potential temperature-specific egg output for that population. Moreover, eggs produced at lower salinity in the upper water column would have a relatively low probability of hatching (see Fig. 4) compared to eggs produced at higher salinities.

The abundance of *T. longicornis* nauplii is highest between April and August when upper water layers reach temperatures of approximately 6 to 8 and 18 to 20°C, respectively (Hansen et al. 2006). Nauplii dwell in the upper 40 m and reach their maximum seasonal abundance in July at a depth of 30 m at temperatures of 5 to 16°C and salinities of 7 to 9 psu (Fig.7). Looking at the nauplii abundance in the Bornholm Basin (Central Baltic Sea) in different months, the highest abundance of nauplii is found at low salinities (in the upper water column) in May. As surface water temperatures increase during early summer up to 16°C (July), most nauplii still reside in these low salinity conditions. As soon as the surface water temperature increases to ≥ 20°C), the majority of nauplii are found in deeper water layers where temperatures are between 10 and 16 °C suggesting that nauplii avoid high surface temperatures by migrating to the region of the thermocline. These findings

correspond well with our results concerning cumulative mortality in which survival was found to be highest at low temperatures when exposed to low salinities. In terms of organismal metabolism, temperature and salinity were classified by Fry (1971) as controlling and masking factors, respectively. Fry writes (1971 pg 17): "A masking factor is an identity which modifies the operation of a second identity on the organism". In the case of *T. longicornis* in the Baltic Sea, temperature-specific rates of egg production are lower than would be expected at higher salinities. The masking effect of salinity is clearly evident when specific egg production rates by this species are compared in the North Sea and in the Bornholm Basin of the Baltic Sea. The relatively low *EP* in the Baltic supports our data that suggest a strong influence of salinity on early life stage survival.

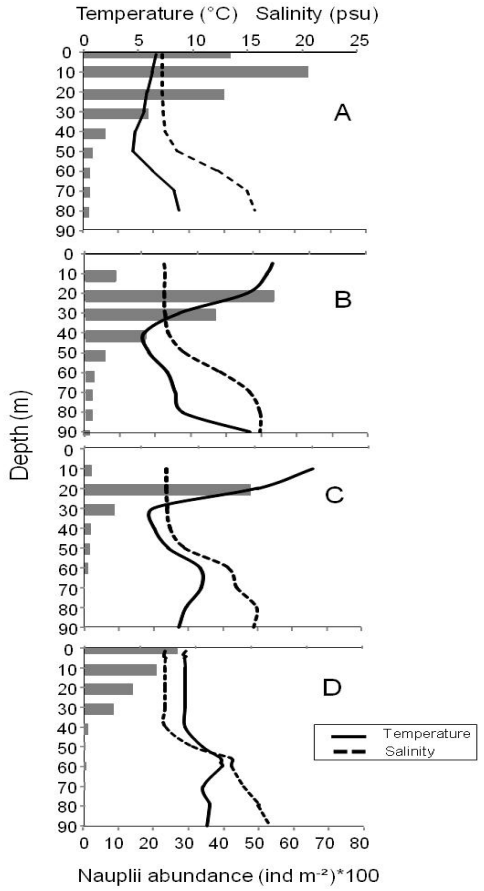

Fig. 7: Depth profiles of the mean abundance (ind m^{-2}) of *Temora longicornis* nauplii (bars), salinity (psu, dashed lines) and temperature (°C, solid lines) within the Bornholm Basin (Central Baltic Sea) in each of four different months: May (Panel A), July (Panel B), September (Panel C) and November (Panel D).

Within the Baltic Sea, *T. longicornis* is widely distributed during the summer months (July to September in offshore regions of the Gotland Deep (Johansson et al. 2004) and dominates the open sea off the Gulf of Finland (Viitasalo 1992). Going further to the North, the biomass of this copepod significantly decreases likely as a result of the increased freshwater run-off and decreased salinity in those habitats (Vuorinen et al. 1998). It has been stated that, after a decline of abundance for over two decades, "*Temora* had virtually disappeared from the plankton samples within the 1990s" (Vuorinen et al. 1998, pg 769). A negative correlation between *T. longicornis* abundance and salinity was previously reported based upon time series data collected off the southwest coast of Finland, where this species was classified as halophilic, preferring low stability of the water column when salinity was highest (Viitasalo et al. 1995b).

Our study indicated that *T. longicornis* can persist in many areas in the Baltic Sea due to its tolerance of low salinities but that the costs and tradeoffs of utilizing low salinity waters include severe reductions in reproductive potential. The hydrographical conditions, often characterized by sharp vertical and horizontal gradients in abiotic factors, are a main environmental challenge faced by animals inhabiting the Baltic Sea. Temperature as well as salinity conditions strongly impact the spatial distribution of *T. longicornis* both horizontally and vertically. Therefore, strong changes in climate conditions in the Baltic Sea coupled with the potential for severe top–down control of zooplankton resources by zooplanktivorous fish are expected to have a substantial impact on this species. A general circulation model predicted a 3 to 5°C increase in the temperature of the upper water layer of the Baltic Sea during the 21st century (see HELCOM Thematic Assessment 2006). Our results suggest that predicted temperature increases will make some areas of the Baltic Sea uninhabitable for *T. longicornis* due to physiological limitations imposed by the interactions of temperature and salinity. Based on our laboratory data, scenarios of the possible influences of climate variability will be easier to explore using coupled biophysical models (e.g., Fennel & Neumann 2003, Neumann & Fennel 2006) developed for *T. longicornis* and other key copepod species in the Baltic Sea.

Acknowledgements:

We are grateful for the help of Philipp Kanstinger, Bianca Ewest, Meike Martin and Gudrun Bening with laboratory rearing and data collection and would like to thank Christian Möllmann and Janna Peters for helpful discussions and comments on this work. This research was supported by the German Science Foundation (DFG) AQUASHIFT program cluster project Resolving Trophodynamic Consequences of Climate Change (RECONN, # JO556/1-2).

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CHAPTER III: *Acartia tonsa* as live feed for fish: Optimizing mass cultures for aquaculture

Ms 3) Effects of salinity, photoperiod and adult stocking density on egg production and egg hatching success in *Acartia tonsa* (Calanoida: Copepoda): Optimizing intensive cultures

Myron A. Peck* and Linda Holste

Ms 4) Impacts of light regime on egg harvests and 48-h egg hatching success of *Acartia tonsa* (Copepoda: Calanoida) within intensive culture

Myron A. Peck, Bianca Ewest, Linda Holste, Philipp Kanstinger, Meike Martin

Ms 5) Handling Copepods and Egg Production Rates: A Note of Caution

Linda Holste*, Berenike Diekmann and Myron A. Peck

Ms 3) Effects of salinity, photoperiod and adult stocking density on egg production and egg hatching success in *Acartia tonsa* (Calanoida: Copepoda): Optimizing intensive cultures

Myron A. Peck* and Linda Holste

Effects of salinity, photoperiod and adult stocking density on egg production and egg hatching success in *Acartia tonsa* (Calanoida : Copepoda): Optimizing intensive cultures

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ABSTRACT:

The interest in large-scale culturing of copepods for marine fish aquaculture is growing, however studies quantifying the optimal conditions for intensive copepod production are generally lacking for most species. In the present study, we examined how large ranges in each of three factors (salinity, photoperiod duration, and culture density) influenced the egg production (*EP*) and 48-h egg hatching success (*HS*) of *Acartia tonsa* Dana (Copepoda : Calanoida). The effect of anaerobic storage time (2 to 185 d) at 4°C on *HS* of eggs was also quantified. In this species, *HS* was more strongly impacted by differences in salinity and photoperiod than was *EP* while the opposite was true for the impact of adult stocking density. In terms of salinity, the lowest and highest mean *EP* (17 and 40 eggs female^{-1} d^{-1}) was observed at 30 and 14 psu, respectively, and *HS* was estimated to be > 75% for all salinities > 13 psu. The photoperiod duration (used to rear copepods and incubate eggs) had little effect on total daily *EP* but significantly influenced *HS* which was 27, 55, 85 and 78 % at photoperiods of 8, 12, 16, and 20 h, respectively. Adult stocking density had no effect on *HS* but the relative number of eggs harvested (# female^{-1}) was highest at 65 ind l^{-1} and lowest at 425 ind l^{-1}. For eggs produced using a 12 h photoperiod, *HS* (%) decreased linearly by 4% every 20 days (i.e., the *HS* of eggs incubated at 20 psu was predicted to be ~82% and 47% after one week and six months of storage, respectively). For maximum egg production and 48-h egg hatching success of A. tonsa cultures, results of this study suggest using salinities of 14 to 20 psu, photoperiods between 16 and 20 h, and low (~50 ind l^{-1}) adult stocking densities.

Key words

Acartia tonsa, intensive culture, salinity, photoperiod, stocking density, egg production, egg hatching

INTRODUCTION:

Copepods are the natural prey items for most marine fish larvae and have been successfully cultured for this purpose in both extensive outdoor (e.g., Svåsand et al., 1998; Toledo et al., 1999) and intensive indoor systems (for review see Støttrup, 2003). When grown on easily cultured phytoplankton (e.g., *Rhodomonas sp*. (Cryptophycea)), copepods are often highly nutritive, especially in regard to essential fatty acids such as docosahexaenoic acid (DHA) and other polyunsaturated fatty acids important for marine fish early growth, their development and survival (Sargent and Falk-Petersen, 1988; McEvoy et al., 1998). Moreover, due to mouth gape limitations, newly-hatched larvae of some warm-water marine fish species have difficulty ingesting rotifers (i.e., *Brachionus* sp.) and brine shrimp (*Artemia* sp.) nauplii, but are able to feed upon copepod nauplii (Støttrup, 2003). Due to these and other attributes, the interest in large-scale culturing of copepods is growing and recent reviews (Støttrup, 2003; Lee et al., 2005) discuss culturing techniques and the application of copepods as live prey in marine fish aquaculture.

Acartia tonsa (Dana) is a free-spawning, calanoid copepod species with a cosmopolitan distribution, being the dominant copepod in many subtropical and temperate coastal marine and estuarine areas (Mauchline, 1998). It is easily maintained in culture (Støttrup, 2000) and hence is among the most intensively studied copepod species. To address ecological questions, previous laboratory and field studies have evaluated some of the major factors influencing *A. tonsa* growth and egg production including the effect of temperature (e.g., Miller et al., 1977; Holste and Peck, in press) feeding level and/or food quality (e.g., Kiørboe et al., 1985; Støttrup and Jensen, 1990; Libourel Houde and Roman, 1987; Broglio et al., 2003) and the interaction of temperature and feeding (e.g., Klein Breteler and Gonzales, 1986; White and Roman, 1992). Other factors possibly affecting vital rates are less well studied including the effect of salinity on egg production and hatching success (Cervetto et al., 1999; Holste and Peck, in press). Although protocols for batch culture of *A. tonsa* have been previously described (Støttrup et al., 1986), published accounts of controlled experiments attempting to optimise cultures techniques are rare.

In an effort to optimize intensive culture of *A. tonsa*, we conducted four experiments to evaluate the effects of various abiotic variables and culturing conditions on aspects of the reproduction of this species. Specifically, *A. tonsa* egg production and egg hatching success were quantified: 1) at different salinities, 2) under different day length durations (photoperiods), and 3) at different adult stocking densities in cultures. We also evaluated the effect of the duration of anaerobic storage time at 4°C on the hatching success of *A. tonsa* eggs.

MATERIALS and METHODS:

Acartia tonsa cultures:

The present experiments used *A. tonsa* from two populations. First, a Danish Sound population was obtained from eggs produced at the Danish Institute for Fisheries and Marine Research (Charlottenlund, Denmark). This Danish Sound population had been previously grown for > 70 generations within laboratory cultures. Second, a southwestern Baltic Sea population was obtained from plankton samples (Kiel Bay, Germany) and maintained for >4 generations at our Elbe Aquarium laboratory facility (IHF, Hamburg, Germany) prior to experiments. The two populations were maintained separately and were cultured in ~220 l round plastic tanks. All copepod cultures were provided daily rations of *Rhodomonas* sp., an algal diet normally high in eicosapentaenoic acid (EPA) (DHA:EPA = 0.6) (Støttrup et al., 1986). Specifically, *Rhodomonas* sp. was maintained at 20-22°C in semi-continuous (0.5 replacements d^{-1}, for two to three weeks) 30 to 60 l cultures at 1.5-2.2 x 10^6 cells ml^{-1} and grown using 1 µm cartridge-filtered seawater with Guilliard's F/2 nutrient solution added

under continuous (24 h) full spectrum (Osram "Fluora", L 36W/77) surface light intensities of ~50 µE s^{-1} m^{-2}. Algae were fed to copepods at ≥ 50 000 cells ml^{-1}, a concentration of *Rhodomonas* sp. that does not limit feeding or growth of *A. tonsa* (Kiørboe et al., 1985; Støttrup and Jensen, 1990).

Copepod culture water temperature was between 18 and 23°C, salinity ranged from 25 to 30 psu (Danish Sound population) or 18 to 20 psu (Baltic Sea) and a 13 h photoperiod was used with a light intensity of ~2 to 6 µE s^{-1} m^{-2}. Cultures were gently aerated and eggs were collected every one or two days by removing aeration, allowing time for the eggs to settle (0.5 h) and siphoning the bottom of the tank. Collected eggs were rinsed onto a 35 µm sieve and stored in capped vials (20 to 60 ml anoxic seawater, ~10,000 to 50,000 eggs ml^{-1}) at 4°C until the start of the experiments.

Experiments

All of the experiments in this study were conducted within a controlled-environment room using the same water temperature (18°C, range ± 0.5°C). Water salinity was 18 ±0.5 psu (except in Exp 1 and Exp 2), the light regime was 12L:12D (except in Exp 3) and daytime surface light intensities were 1 to 4 µE s^{-1} m^{-2}. During experiments, measurements of temperature (± 0.1°C) and salinity (± 0.1 psu) were made daily (WTW Microprocessor Conductivity Meter LF 196, TetraCon 96-1.5 probe). All counts of *A. tonsa* adults, eggs and nauplii were made in Bogorov dishes with the aid of a dissecting microscope (Leica MZ 9_5). *Rhodomonas* sp. was used as food and fed at > 50,000 cells ml^{-1}.

Exp 1: salinity and egg production

The daily rate of egg production (*EP*, # eggs $female^{-1}$ d^{-1}) by Baltic *A. tonsa* was quantified at five different salinities (6, 10, 14, 20, and 30 psu) over the course of five days. Prior to the start of the experiment, one mixed-stage *A. tonsa* culture at 18 psu was randomly split into five separate cultures. Each of these five cultures was acclimated (1.3 psu d^{-1}) to one of the different test salinities and maintained at a constant salinity for at least four days prior to the start of measurements. At the start of measurements, ten females and two males were loaded into each of three 880-ml cylinders (holding tubes) at each salinity. A previous study using a 5:1 sex ratio for *A. tonsa* yielded temperature-specific *EP* values that were among the highest reported for this species (Holste and Peck, in press). To avoid egg cannibalism and facilitate egg collection, each holding tube had a 100 µm mesh bottom and was suspended in a 1-l cylinder containing a mixture of seawater and algae at the test salinity. Aeration was not used so that eggs produced would sink through the mesh.

At the same time every day, the adults were transferred to a new cylinder by gently removing the holding tube and placing it in a new cylinder containing filtered (1 µm) seawater and *Rhodomonas* (>50,000 cells ml^{-1}). The contents of the old cylinder were collected (35 µm sieve) and the number of eggs and nauplii counted. Holding tubes were checked daily for mortalities. Dead individuals (immobile when gently prodded, ~5% mortality during experiment) were removed from tubes and replaced with new individuals of the same sex that were acclimated to the test salinity.

Exp 2: salinity, storage time and egg hatching success

The 48-h hatching success (*HS*, % hatch) of *A. tonsa* eggs (Danish Sound population) was quantified at 19 different salinities from 0 to 34 psu by conducting six separate trials. In each trial, due to technical reasons, a maximum of eight different salinities (three replicates each) was tested. To evaluate the effect of storage time at 4°C on egg hatching success, eggs chosen for trials had been stored for 2, 14, 19, 90 and 185 days prior to testing. Eggs used in trials were produced by three different cohorts (A, B and C). Eggs produced by cohort A were tested after 2 and 185 days of storage, those produced by cohort B were tested at 14 and 19 days and those from cohort C were tested after 90 days. All cohorts were tested at a

common salinity (16 psu).

In each trial, a known number of eggs (93 –110) was loaded into a 250 ml culture flask containing 200 ml of gently aerated, filtered (1 µm) seawater. Based upon the time course of hatching presented elsewhere (Peck & Holste, in press), all flasks were incubated for 48 h at 18 ± 0.5°C after which the contents of the flasks were collected onto a 35 µm sieve and unhatched eggs were counted. A 48-h egg incubation period was used based upon hatching times at 18°C (Holste and Peck, in press) and the duration of the first two non-feeding naupliar stages (Peck and Holste, unpublished data).

When incubated for 48 hours at low salinities (0 to 6 psu), results of a previous study indicated that some *A. tonsa* eggs burst and can be incorrectly identified as hatched eggs (Holste, 2004). We corrected for this in the present study based upon the incidence of bursting observed at different salinities. Specifically, 196 eggs produced at 18 psu were individually incubated in 2.5 ml of water within Microtiter ® plate wells at four salinities (0, 3, 6 and 18 psu, n = 37 to 57 eggs at each *S*). A dissecting scope and digital camera (Leica MZ 16, (11.5x); Leica DC 300) was used to take pictures of each egg at 12 h intervals for 48 h. Ruptured or burst eggs were easily distinguished from hatched and unhatched eggs (Holste, 2004). The percentage of burst eggs decreased linearly with increasing salinity and was equal to 67.6, 50.9 and 31.1% at 0, 3 and 6 psu. No burst eggs were observed at 18 psu. Based upon regression analysis of these data, a correction value (*CR*) was calculated ($CR = 0.3192 + 0.0608 \cdot S$) and multiplied against the hatch values observed in the present study at incubation salinities ≤ 10 psu.

Exp 3: photoperiod, egg production and egg hatching success

Baltic *A. tonsa* eggs were hatched and, after two days, the resulting cohort of nauplii was equally divided into four separate, 100-l tanks. Each cohort was reared using a different photoperiod (8, 12, 16, or 20 h). After the cohorts reached the adult stage (development rate was same for each cohort), a known number (146 to 151) copepods from each cohort were randomly placed within each of three chambers (total n = 12). Similar to Exp 1, chambers consisted of two cylindrical tubes, an inner holding tube was 5.5 l with a 100 µm mesh bottom that was suspended within an 8 l outer tank. After every phase of the light-regime (10 to 15 minutes after the lights turned on or off) over the course of five days, adults were transferred to a new tank by gently removing the holding tube and placing it within a new outer tank containing filtered (1 µm) seawater and algae (*Rhodomonas*, > 50,000 cells ml^{-1}). The eggs and nauplii within the old tank were collected (35 µm sieve) and counted. In some cases, eggs and nauplii were concentrated and stored within a seawater formalin solution and counted later.

On day 2 of *EP* measurements, the 48-h hatching success *(HS)* of eggs was quantified. Methods used were the same as those in Exp 2 except that, in this case, four replicate flasks were used from each treatment group (eggs from the three replicate tanks at each photoperiod were mixed before loading the hatching flasks) and fewer eggs (46 to 54 eggs) were loaded into flasks. Salinity and temperature (18 ± 0.5°C and 18± 0.2 psu) were the same as those used in photoperiod *EP* measurements.

Exp 4: adult stocking density, egg production and hatching success

Baltic adult copepods (14 to18 days old) were transferred to each of nine, 100-l tanks at initial stocking densities of 50, 200, and 400 adults l^{-1} in each of three tanks. Tanks were maintained using standard culture techniques (1 µm filtered, 18 psu seawater, gentle aeration, feeding 2x d^{-1} at > 50,000 cells l^{-1} *Rhodomonas sp.*). To maintain ad libitum feeding concentrations, volumes of algal culture were provided during each feeding period at a ratio of 8:4:1 to tanks with 400, 200 and 50 ind l^{-1}, respectively. On each of eight consecutive

days, eggs produced in each tank were collected using standard culture procedures. Eggs were vacuumed off the bottom of each tank using a siphon after removing the aeration for 0.5 h. A total of 20 l of water was siphoned from each tank in the same manner (a second siphoning normally collects < 10% of the number of eggs collected during the first siphoning). The adults collected in the siphoned water were returned to the tanks and eggs were concentrated onto a 35 µm sieve and re-suspended in 500 ml of seawater. The number of eggs (and nauplii) in the 500 ml sample was estimated from the mean number in nine, 1-ml sub-samples. For the calculation of relative egg production (# female^{-1}), the female:male ratio was assumed to be 1:1 in the population.

Hatching success was quantified for eggs produced on day 7 using the methods outlined in Exp 2. Eggs collected from the three tanks at each of the three initial stocking densities were mixed and a known number (92 to 107) was incubated in each of four replicate flasks (total n = 12) for 48 h.

Statistics

Statistical analyses were carried out using SAS software (SAS, 1989) and the significance level was set at a = 0.05. Treatment effects in Exp 1, 3 and 4 were analyzed using oneway ANOVAs or GLM (if the design was unbalanced). Percentage data (% hatch) were arcsine transformed [arcsine $(\% / 100)^{0.5}$] prior to testing. When significant differences were found, a Tukey-Kramer post-hoc test (Sokal and Rohlf, 1995) was used for pair-wise comparisons of treatment means. The data collected in Exp 2 were analyzed via non-linear regression analyses and parameter estimates were obtained via the least squares method.

RESULTS:

Exp 1: salinity and egg production

The rate of egg production (*EP*) within each tank was lowest on the first day, increased on the second day and remained relatively constant thereafter. The first day of the experiment was considered an acclimation period to the tanks and these data were excluded from statistical analyses. After day 1, the mean(± standard error, SE) *EP* by Baltic *A. tonsa* was 21.9(0.1), 25.6(7.5), 41.8(4.9), 26.9(0.6) and 17.4(1.2) eggs female^{-1} d^{-1} for copepods acclimated to 6, 10, 14, 20, and 30 psu, respectively (Figure 1). Salinity significantly affected *EP* (ANOVA, df = 4,10; F = 4.60; p = 0.023) with the daily mean *EP* at 14 psu being significantly greater than that at 30 psu (Tukey-Kramer, $p < 0.05$) (Figure 1).

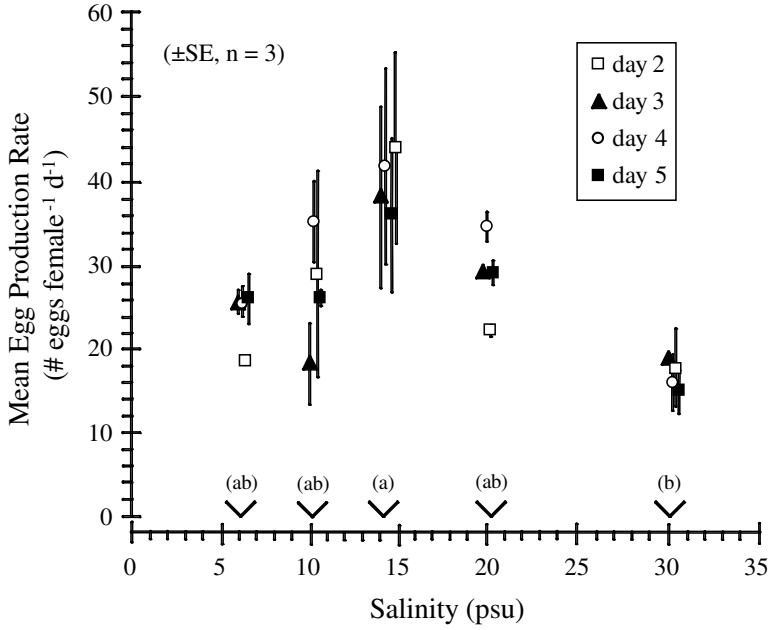

Figure 1: Egg production rate (*EP*, # eggs female^{-1} d^{-1}) on each of four days for *A. tonsa* acclimated to and tested at five different salinities (6, 10, 14, 20 and 30 psu). Each datum represents the mean (± SE) *EP* of three replicate tanks. Dissimilar letters along abscissa denote significant differences in *EP* (grand mean, averaged across days) among the salinities.

Exp 2: salinity, storage time and egg hatching success

The hatching success (*HS*, %) of *A. tonsa* eggs (Danish Sound) produced at 25 to 30 psu was influenced by both salinity and the duration of storage time at 4°C (Figure 2). The mean(± SE) *HS* was highest (88.8(3.3)%, n =3) at 20 psu for eggs that had been stored for two days. At a salinity of 8 psu, eggs that were stored for only two days in trial 1 and trial 2 (both from cohort A) had a mean(± SE) *HS* of 63.2(7.0) and 57.8(11.2), respectively. The mean(± SE) 48-h *HS* of eggs incubated at 16 psu that had been stored for 2, 14, 19, 90, and 185 days (all cohorts) was 84.1(1.2), 77.9(5.4), 73.3(5.6), 66.2(1.1) and 40.7(5.6) %, respectively. The effect of both salinity (*S*, 0 to 34 psu) and storage time (*t*, 2 to 185 days) on hatch success ($HS_{S,t}$) was described well by a modified logistic equation:

$$1) \qquad HS_{S,t} = \frac{a}{1 + e^{b\cdot(S-c)}} - d \cdot t$$

where *e* is the natural logarithm base, *a* is the predicted maximum *HS*, *b* represents the predicted rate of change in *HS* due to *S*, *c* is an offset adjustment and *d* is the predicted rate of change in *HS* due to storage time. Parameters *a*, *b*, *c* and *d* were estimated to be equal to (mean (± SE)) 85.50(1.53), -0.26(0.03), 5.40(0.38) and
0.20(0.01), respectively ($p < 0.0001$, $r^2 = 0.949$). The storage slope value (*d*) n Eq. 1 indicated that *HS* (%) was predicted to decrease linearly by 4% every 20 days.

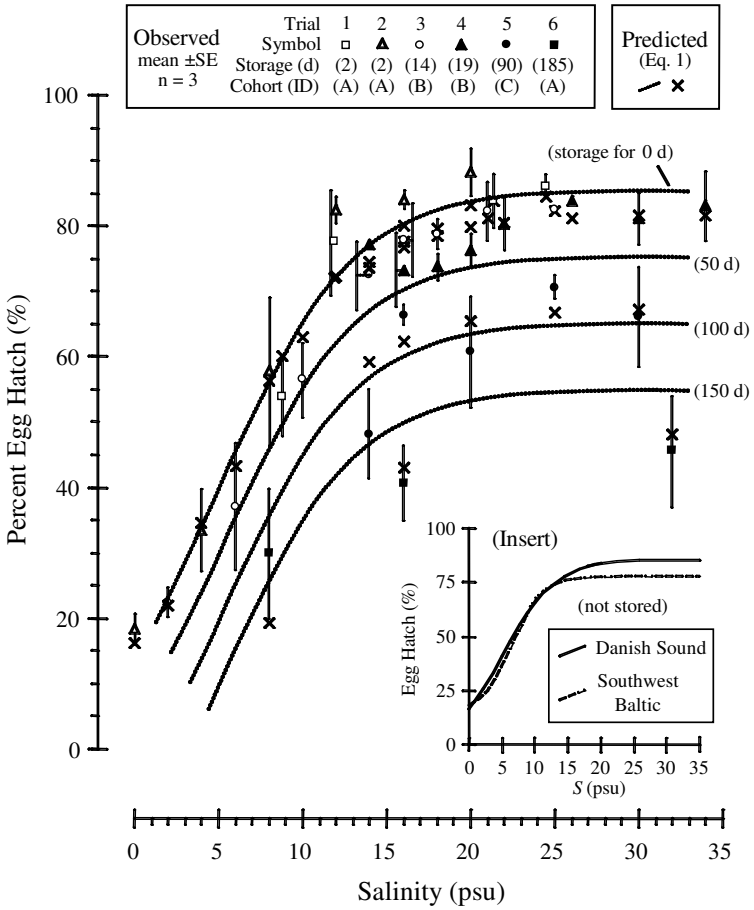

Figure 2: The 48-h percent hatch (%) of *A. tonsa* eggs versus incubation salinity (0 to 34 psu) and storage time at 4°C (2 to 185 days). The observed hatch in each of the six separate trials and predicted hatch (lines and points calculated using Eq. 1 in the text) are provided. All eggs used in trials were produced by adults within three cohorts, each in a 12L:12D light regime at 25-30 psu. Each observed datum is the mean (± SE) value for three replicates. Insert: Comparison of the predicted 48-h percent hatch at different salinities for eggs produced by a Danish Sound population (this study) and eggs produced by a southwestern Baltic population (Holste and Peck, in press).

Exp 3: photoperiod, egg production and egg hatching success

Total egg production decreased with time due to mortality during the experiment. Since the number of females within each tank at any time was unknown, *EP* was not calculated on a per female basis. The mean(± SE) total number of eggs produced in tanks maintained at 8, 12, 16 and 20 h photoperiod was equal to 4217(658), 2817(427), 3510(317) and 4361(658), respectively, and was not significantly different (ANOVA, df = 3, 8; F = 1.99; p = 0.19).

To compare differences in egg production during night and day among the photoperiod treatments, hourly egg production rates were calculated for each phase of the light regime on each day. Trends in differences between hourly rates of production during dark (*D*) and light (*L*) periods among the four photoperiods were not consistent but hourly production in darkness tended to increase with increasing light period (photoperiod) duration. At the three longest photoperiods, the ratio of hourly egg production during darkness to that during light periods (*D* / *L*) was positive and, on average, ≥ 2.0. In tanks at the shortest photoperiod (8 h), the hourly egg production during darkness was either the same as or slightly lower than that calculated for light periods (Figure 3A-D). Within one tank containing adults at a 20 h photoperiod, hourly egg production during the 4-h dark period was

nearly 10 fold higher than that during the 20-h light period. The diel production ratio (*D* / *L*) was unrelated to the total number of eggs produced in a tank during the experiment.

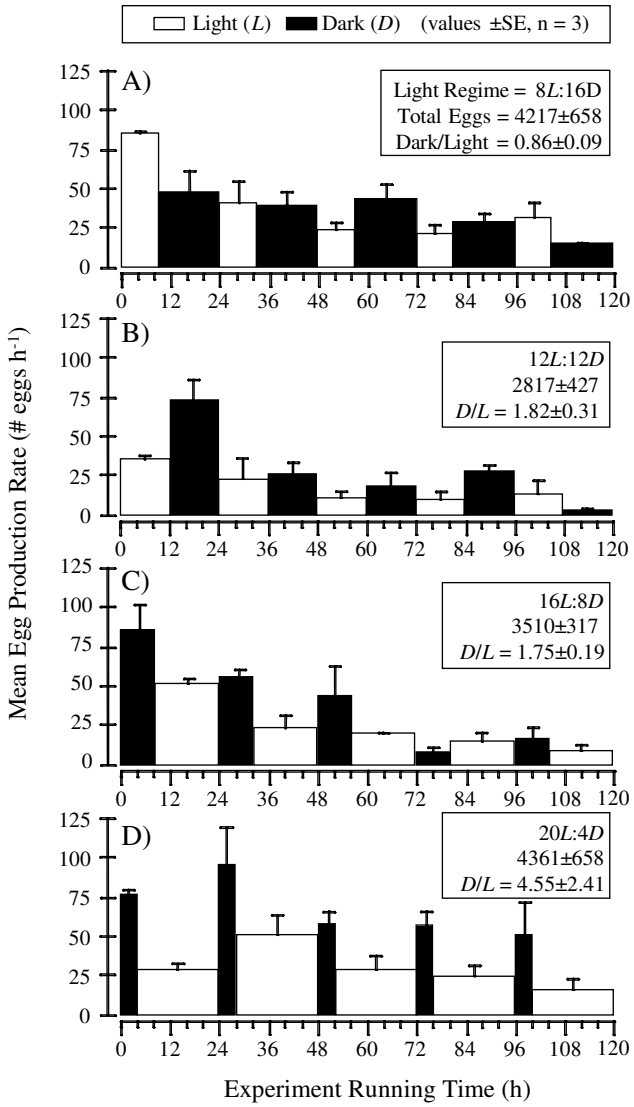

Figure 3: Egg production rate (# h^{-1}) during periods of darkness (*D*, filled bars) and light (*L*, unfilled bars) by *A. tonsa* adults reared (from nauplii) and tested at each of four different light regimes having photoperiod durations of 8 h (Panel A), 12 h (panel B), 16 h (panel C), and 20 h (panel D). The mean(+ SE) total number of eggs produced in each treatment (n = 3 tanks) and the ratio of hourly egg production in darkness and light (*D*/*L*) are also provided in each panel. The width of the bars corresponds to the number of hours. Also note that egg production is plotted versus experiment running time (*t*, hours) and that the light regime started (at $t = 0$) on the dark phase and ended on the light phase in the 16L:8D (panel C) and 20L:4D (panel D) treatments.

Although total egg production was unaffected by photoperiod, the 48-h hatching success (*HS*) significantly increased with increasing photoperiod (ANOVA, df = 5, 22; F = 21.42, $p < 0.001$) (Figure 4A). The 48-h *HS* of eggs obtained from adults reared at the eight hour photoperiod was < 30% whereas *HS* was 55% for eggs obtained from adults at 12 h and between 78 to 85% for eggs obtained at photoperiods ≥ 16 h.

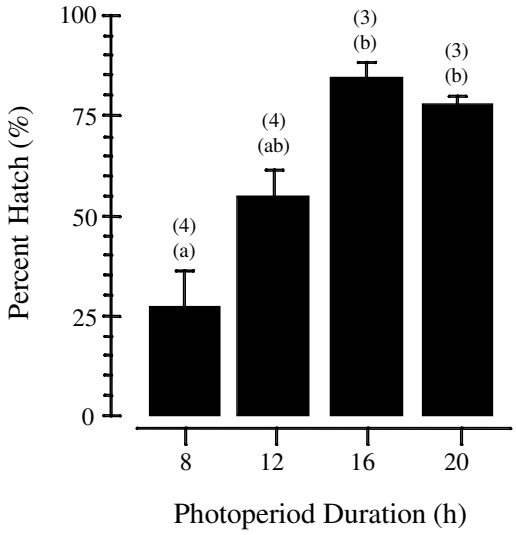

Figure 4: The effect of photoperiod on the 48-h percent hatch (%) of newly harvested *A. tonsa* eggs. Copepod cohorts were reared (late stage nauplii to adults) and eggs were produced and incubated at each of four different photoperiods. The number of replicates in each treatment is shown above bars. Significant differences in the % hatch (arcsine transformed) are denoted by dissimilar letters above bars.

Exp 4: adult stocking density, egg production and hatching success

Based upon daily estimates of adult density in the each tank (adults in subsamples of water from each tank on each day were counted), the mean(±SE) density of adults within the 50, 200 and 400 ind l^{-1} (nominal) treatment groups was 65.0(9.5), 166.3(24.5) and 424.7(10.3) ind l^{-1}, respectively. The number of eggs harvested from all tanks was initially low (Day 1 & 2) and reached peak levels at different times (Figure 5A). The number of eggs harvested increased rapidly in high-density tanks then declined at the end of the eight-day period, whereas the number harvested from low-density tanks generally increased with time and was equal to that in high-density tanks at the end of the eight-day period. The mean egg harvest from the final two days of the experiment was 50%, 61% and 76% of the maximum harvest obtained for the high-, medium- and low-density treatment groups, respectively.

Not unexpectedly, the total (8-d) number of eggs harvested from tanks with different adult densities was significantly different ($df = 2$, $F = 24$, $p < 0.002$) with the highest mean number of eggs (1.76 million) produced in the treatment having the highest mean stocking density (Figure 5B). However, the mean relative egg production (# $female^{-1}$) over the 8-d period was significantly higher in tanks with the lowest mean stocking density (65 ind l^{-1}) compared to that in tanks with higher adult densities (Tukey-Kramer, $df = 5$, crit value 5.218, $p < 0.05$ (Figure 5). The latter result assumed the same (1:1) female:male ratio in all tanks. The hatching success of eggs produced by copepods at the three culture densities was not significantly different (ANOVA, $df = 2,8$; $F = 0.22$; $P = 0.80$). The mean(±SE) 48-h *HS* of eggs was equal to 48.4(8.0), 36.7(16.8) and 38.1(15.9) % for eggs produced by adults maintained at mean stocking densities of 65, 166 and 425 adults l^{-1} at a 12L:12D light regime.

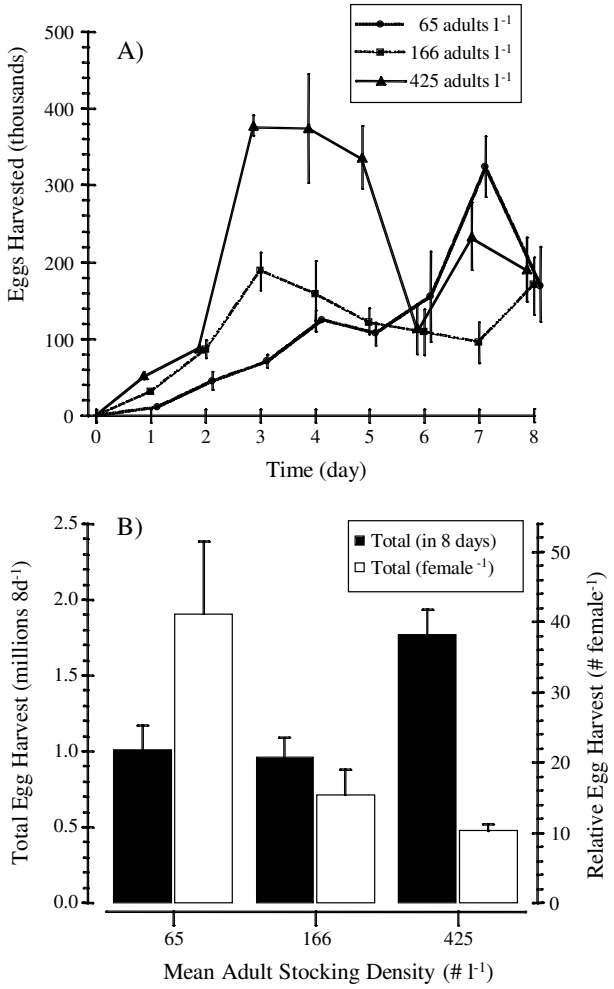

Figure 5: Panel A) The number of eggs (thousands) harvested versus time (days) for tanks having mean adult stocking densities of 65, 166 or 425 adults l^{-1}. Values are mean±SE (n = 3). Panel B) The mean(+SE) number of eggs harvested from tanks during an eight-day period versus adult stocking density. Egg harvest was expressed in both absolute units (total number, filled bars) and relative units (number $female^{-1}$, open bars). Relative harvest assumed the same (1:1) female:male ratio in each tank. Statistical differences are presented within the text.

DISCUSSION:

Copepod nauplii are being produced as a live feed for first-feeding larvae of a number of warm-water marine fish species including red snapper (*Lutjanus campechanus*), mangrove jack (*Lutjanus argentimaculatus*), striped trumpeter (*Latris lineata*) and grouper (*Epinephelus coioides*) (Schipp et al., 1999; Lee et al., 2005 and references therein). Cost-effective culturing of copepods within intensive systems for this purpose will rely on maximizing both the efficiency of egg production and the success of egg hatching. The present research focused on optimizing culture conditions for maximum daily egg production and 48-h hatching success of *A. tonsa*, a common calanoid species used in aquaculture. Our results suggested that 48-h hatching success (*HS*) was more strongly impacted by differences in

photoperiod and salinity than was the rate of egg production (*EP*) while the opposite was suggested for the impact of adult stocking density, which impacted *EP* but not *HS*.

Salinity

The effects of salinity on copepod egg production (*EP*) has not been frequently examined which is surprising given the strong gradients in salinity often experienced by numerically abundant genera (e.g., *Temora* spp., *Acartia* spp.) inhabiting coastal regions (Mauchline 1998). The species examined in the present study is euyhaline; populations of *A. tonsa* persist in both coastal marine waters as well as within estuaries having low salinity (e.g., 4 psu, Gulf of Finland, Baltic Sea, Katajisto et al., 1998). In the present study, *A. tonsa EP* was highest at intermediate salinities (14 and 20 psu) and reduced at lower and higher salinities (6, 10 and 30 psu). The relationship between *EP* and salinity observed in the present study may stem from differences in the costs of osmoregulation (e.g., costs associated with the regulation of free amino acid pools, Farmer and Reeve, 1978) at different salinities. When fed high concentrations of algae, the energy savings afforded to *A. tonsa* in water of nearly isotonic salinity will likely be reflected in increased *EP* relative to adults maintained in hyper- or hypotonic water salinities. However, it should be noted that the 2.4-fold difference in *EP* observed among the salinities examined in the present study (17 to 40 eggs female^{-1} d^{-1}, at 30 and 14 psu) is relatively small compared to the ten-fold difference in *EP* due to temperature (e.g., 3 to 30 eggs female^{-1} d^{-1} at 10 and 20 °C) (Holste and Peck, in press).

The present results indicated that salinity had a marked impact on the 48-h hatching success (*HS*) of *A. tonsa* eggs. For eggs harvested from adults that were maintained at 18°C within 25 to 30 psu water the *HS* (%) was predicted to be relatively high (> 70%) when eggs were incubated at salinities > 12 psu. These results are somewhat contradictory to those of Chinnery and Williams (2004) in which only 55% of North Sea *A. tonsa* eggs hatched at 15 psu. Interestingly, hatching success of eggs harvested from a south-western Baltic population (18 °C, 18 psu) was 78% at 14 psu (Holste and Peck, in press) which is the same as that (77%) predicted at 0 days storage for the Danish Sound population used in the present study (Figure 2, Insert). This suggests that, at least in terms of the effects of salinity on *HS*, a high degree of phenotypic plasticity may exist among populations of this copepod residing in different salinity conditions.

Increasing storage time at 4 °C decreased the 48-h *HS* of *A. tonsa* eggs in the present study, an effect that was described well by a linear decrease in *HS* with time (parameter *d*, in Eq 1). Based upon Eq. 1, at an incubation salinity of 20 psu the 48-h *HS* would be 84, 78, 72 and 61% after storage times of 0, 4, 8 and 16 weeks, respectively. It should be noted that the eggs used in salinity/storage hatching trials originated from three cohorts and it is possible that using eggs produced from different cohorts (or even from different days from the same cohort) could have contributed to variability in egg hatch success. However, intra- and inter-cohort variability in 48-h *HS* appears to have been low in the present study since 1) *HS* was similar between eggs collected on two different days from the same cohort, and 2) the trend in the decrease in *HS* with storage time was similar among different cohorts tested at the same salinity.

Photoperiod

In the present study, differences in photoperiod duration did not influence the total number of eggs produced, but did influence diel differences in *EP*. Stearns et al. (1989) previously reported diel differences in *EP* for *A. tonsa*. In their study, females collected from estuaries in Georgia and North Carolina, USA had hourly egg production rates during nighttime that were, on average, 2.8 times greater than those during daylight. These results agree with the present study in which the hourly rate of egg production in darkness (D) tended to be more than twice the hourly rate during light periods (L) when cultures were exposed to photoperiods ≥ 12 h. A novel finding of the present study was that the ratio of eggs produced

during darkness to that during light (D/L) increased with increasing photoperiod duration. Given these results, it would be interesting to study the influence of un-natural light regimes (e.g., alternating 4 h pulses of darkness and light) as a method to maximize *EP* in intensive cultures.

Although photoperiod had no effect on total *EP*, it had a marked effect on the hatching success of the produced eggs. Results of this study indicated that 48-h *HS* decreased markedly with decreasing photoperiod experienced by adults such that half and three quarters of the egg produced at 12 h and 8 h photoperiods did not hatch within 48 h. These findings are interesting and we speculate, based upon other factors being equal among treatments (i.e., high feeding levels, temperatures, daily egg production rates), that they possibly result from differences in the proportions of different types of eggs produced among the photoperiod treatments. Previous studies conducted in the Baltic Sea (Arndt and Schnese 1986; Madhupratap et al. 1996) and elsewhere (e.g. Sullivan and McManus 1986; Marcus 1996) indicated that *A. tonsa* produces normal eggs, subitaneous eggs and resting eggs. Normal and subitaneous eggs hatch rapidly in favourable environmental conditions whereas resting eggs have an obligatory refractory phase that may span several years (Watson and Smallman 1971; Grice and Marcus 1981; Marcus, 1996).

For other members of the *Acartia* genus, photoperiod, temperature and O_2 concentration seem to be the major environmental cues affecting resting egg production (Katajisto et al., 1998; Castro–Longoria and Williams, 1999; Chinnery and Williams, 2003). The 48-h egg incubation period used in the present study would not be sufficient for resting eggs (Marcus, 1996; Marcus and Murray, 2001). Depending upon the species, resting eggs can be morphologically distinct from normal and subitaneous eggs. Although no differences in egg morphology of hatched and unhatched eggs have been noted at our facility (magnification 96 x, M. Peck, unpublished data), a recent study on a congener suggested that differences between the two egg types could be difficult to recognize without scanning electron microscopy (SEM) (Castellani and Lucas, 2003).

Manipulating culture conditions to increase the production of resting eggs would benefit efforts to stockpile and hatch eggs after long-term storage. Unfortunately, the results of the present study offer no direct evidence for the presence of resting egg production. Future hatching trials conducted after long-term storage of eggs produced at different photoperiods combined with SEM should help resolve whether the decrease in 48-h *HS* observed with decreasing photoperiod in the present study was due to 1) increasing proportions of resting eggs that were produced, 2) increasing proportions of non-viable eggs that were produced, or 3) some combination of the former and latter.

Adult stocking density

If space is not limited within the production facility, results of this study indicated that using low adult copepod stocking densities increased the efficiency of egg production. Culturing at 425 adults l^{-1} was possible (a total of 5.3 million eggs was harvested from three 100-l cultures in 8 days, or ~20 eggs female^{-1} 8d^{-1}) but was less efficient than culturing at a lower stocking density of 65 ind l^{-1} (~40 eggs female^{-1}). Moreover, eight-times more algae was used at the higher stocking density.

The method of intensive culturing and egg collection used in the present study is simple and inexpensive (Støttrup et al., 1986; Støttrup, 2003) but also poses potential problems due to egg cannibalism and poor water quality. Differences in both of these factors likely contributed to the finding in this study of the highest relative *EP* (eggs female^{-1}) at the lowest stocking density. The increased algal requirements of tanks with high adult densities may decrease the time between batches (complete water changes) due to the increased algal grazing, fecal production and amount of bacterial substrate for ciliates. Poor, short-term egg hatching may also result from lower water quality in high-density tanks since some copepod species produce resting eggs in response to high concentrations of their own metabolites (e.g.,

Ban and Minoda, 1994). Water quality parameters were not measured in the present study. Although, no differences in 48-h egg hatching success were noted among the density treatments, egg harvests declined in high-density tanks toward the end of the eight-day experiment. This indicates that the high-density tanks likely had inadequate water quality. Production tanks designed with conical-shaped bottoms with stopcocks would likely make egg collection (and tank cleaning) more rapid and efficient.

CONCLUSION:

Based upon the results of this and other studies (Kiørboe et al., 1985; Støttrup et al., 1986; Holste and Peck, in press) the following can be recommended to optimize the intensive, batch culturing of *A. tonsa*. To achieve relatively high rates of production of eggs that can be immediately hatched (48 h) and fed to fish, cultures should be maintained at 22 to 24 °C, between 14 and 20 psu, and at photoperiods of 16 to 20 h using high concentrations of algae (i.e., > 50, 000 cells ml^{-1} *Rhodomonas* sp.). Using the culture methods outlined in the present study, the 48-h egg hatch success (%) declined linearly by 4% for every 20 d of storage at 4 °C.

Acknowledgements

The help of Philipp Kanstinger, Meike Martin and Gudrun Bening with laboratory rearing and data collection is greatly appreciated. Bianca Ewest provided valuable laboratory support and comments on this manuscript. We would also like to thank Jens-Peter Herrmann for technical assistance with laboratory equipment and three anonymous reviewers for their helpful comments. This research was supported by the Global Ocean Ecosystem Dynamics (GLOBEC Germany) program funded through the German Federal Ministry for Education and Research (BMBF 03F0320E) and the German Science Foundation (DFG) AQUASHIFT program cluster Resolving the Trophodynamic Consequences of Climate Change ("RECONN", DFG # JO556/1-1).

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Ms 4) Impacts of light regime on egg harvests and 48-h egg hatching success of *Acartia tonsa* (Copepoda: Calanoida) within intensive culture

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Impacts of light regime on egg harvests and 48-h egg hatching success of *Acartia tonsa* (Copepoda: Calanoida) within intensive culture

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ABSTRACT:

We examined the effect of light regime on daily egg harvest (*EH*, eggs $tank^{-1}$ d^{-1}), and 48-h egg hatching success (*HS*, %) by *Acartia tonsa* (Copepoda : Calanoida) in intensive 125-l cultures. Since this copepod produces more eggs during darkness than in the light, we tested whether *EH* could be increased by utilizing unnatural light regimes. Egg harvests were between 0.85 to 1.20 million eggs $culture^{-1}$ wk^{-1} and mean *EH* was not significantly different among tanks maintained at 3h:3h, 4h:4h, 6h:6h and 12h:12h light:dark. *HS* was not significantly different for eggs produced in the different light regimes and incubated at 12h:12h. In a second experiment, cohorts were reared (from nauplii) in constant darkness (*D*) and constant light (*L*) and eggs produced in each cohort were incubated in darkness (*D-D*, *L-D*) or light (*D-L*, *L-L*). Mean(±SE) *HS* was significantly different among the treatments, increased with increasing light exposure, and equal to 3.7(1.1), 32.2(15.1), 38.3(0.8) and 52.2(16.5)% for *D-D*, *L-D*, *D-L* and *L-L* treatments, respectively. These and published data were combined to generate an equation predicting 48-h *HS* for eggs produced and incubated at photoperiods between 0.5 and 24 h. Our experiments indicated that light can be an important factor affecting the success of intensive cultures of *A. tonsa* and that copepod culture protocols should include information on light regimes used during rearing and incubation of eggs.

INTRODUCTION:

In general, copepods constitute a large percentage of the diet of marine fish larvae in nature (Munk and Nielsen, 1994; Pepin and Penney, 1997) and, when used in aquaculture, often enhance larval survival, growth and the percentage of normally pigmented individuals compared to traditional live feeds such as rotifers (e.g., *Brachionus* spp.) and brine shrimp (*Artemia* spp.) nauplii (McEvoy et al., 1998; Nanton and Castell, 1999). Thus, the ability to rear copepods at large scales would present a major advancement in the larviculture of many marine fish species. Although copepods within three families (Calanoida, Harpacticoida and Cyclopoida) are currently cultured at scales relevant for rearing fish larvae, the calanoid species are most abundant in pelagic coastal waters and form the bulk larval fish gut contents in these regions. Subsequently, calanoids have been studied most intensively in both the laboratory and field (Mauchline, 1998).

Acartia tonsa (Dana) is the dominant calanoid copepod in many low to mid-latitude coastal marine and estuarine areas, is easily maintained in culture (Støttrup et al., 1986) and hence, has been very well-studied. Previous investigations have examined some of the major factors influencing *A. tonsa* growth and egg production including temperature (e.g., Miller et al., 1977; Holste and Peck, 2006), feeding level and/or food quality (e.g., Kiørboe et al., 1985; Støttrup and Jensen, 1990; Broglio et al., 2003), the interaction of temperature and feeding (e.g., Klein Breteler and Gonzales, 1986; White and Roman, 1992), as well as the effect of salinity on egg production and hatching success (Cervetto et al., 1999; Holste and Peck, 2006). However, the influence of light, both in terms of light intensity and duration of the daily photoperiod, has received relatively little attention compared to research on the effects of other environmental factors affecting copepod vital rates.

The results of a number of previous studies suggest that light regime can markedly influence diel rates of egg production in this species (Stearns et al., 1989; Cervetto et al., 1993; Peck and Holste, 2006). In one study, *A. tonsa* females collected from mid-Atlantic coast estuaries of the USA had hourly rates of egg production during nighttime that were, on average, 2.8 times greater than those during daylight (Stearns et al., 1989). Similarly, Peck and Holste (2006) observed hourly rates of egg production during darkness (D) that were > twice those during light periods (L) when cultures were exposed to photoperiods ≥12 h. Furthermore, the ratio of L/D eggs increased with increasing photoperiod duration and was > 8 in one tank maintained at a photoperiod of 20 h (Holste and Peck, 2006). These findings suggested that it would be worthwhile to explore whether egg harvests in intensive cultures of *A. tonsa* could be increased by using unnatural light regimes. Only one study has examined the effect of different light regimes on egg hatching (e.g., Peck and Holste, 2006) and, in that study, no photoperiods < 8 h or > 20 h were examined.

In an effort to increase the productivity of intensive (130-l, ~150 to 250 adults / l) cultures of *A. tonsa*, we examined the effect of unnatural light regimes on egg harvests and 48-h egg hatching success (*HS*, %). We also examined the presence or absence of light on *HS* and developed a functional relationship between photoperiod (0 to 24 h) and *HS* in this species at a temperature (20°C) commonly used to rear intensive cultures.

MATERIAL and METHODS:

Acartia tonsa cultures

Individuals from a southwestern Baltic Sea population of *A. tonsa* were obtained from plankton samples (Kiel Bay, Germany) and maintained for >10 generations at our Elbe Aquarium laboratory facility (IHF, Hamburg, Germany) prior to experiments. Copepods were

cultured in ~220 l round plastic tanks at 18 to 23°C in 18 to 20 psu seawater under a 13h photoperiod (13:11 light:dark regime) and provided daily rations of *Rhodomonas* sp., an algal diet normally high in eicosapentaenoic acid (EPA) compared to docosahexaenoic acid (DHA:EPA = 0.6) (Støttrup et al., 1986). Specifically, *Rhodomonas* sp. was maintained at 20-22°C in semi-continuous (50% water replacement d^{-1}, for two to three weeks) 30 to 60 l cultures at 1.0-2.0 x 10^6 cells ml^{-1} and grown using 1 µm filtered (Whatman) seawater with Guilliard's F/2 nutrient solution added under continuous (24 h) full spectrum (Osram "Fluora", L 36W/77) using outside surface (inside culture) light intensities of ~50 (~15) µE s^{-1} m^{-2}. Algae were fed to copepods at ≥ 25 000 cells ml^{-1}, a concentration of *Rhodomonas* sp. that does not limit egg production by *A. tonsa* (Kiørboe et al., 1985; Støttrup and Jensen, 1990).

Experiments

All experiments in this study were conducted within two controlled-environment rooms using a mean(± range) water temperature of 20.5(0.7)°C and a water salinity of 18.5(0.5) psu using *A tonsa* from a southwestern Baltic Sea population (collected near Kiel, Germany). Measurements of temperature (± 0.1°C) and salinity (± 0.1 psu) were made daily (WTW Microprocessor Conductivity Meter LF 196, TetraCon 96-1.5 probe) on each tank. *Rhodomonas* sp. was used as food and fed at > 25,000 cells ml^{-1}. The amount of algae fed to tanks was determined from cell counts (Coulter Counter Multisizer TM) made on two, well-mixed water samples taken from each tank each morning. Tanks were maintained using standard culture techniques (1 µm filtered seawater, gentle aeration, feeding algae 1x d^{-1}) using a 14L:10D light regime (except Exp 1) with a light intensities of ~3.5 µE s^{-1} m^{-2} during the photoperiod. On each day, eggs produced in each tank were collected using standard culture procedures. The aeration was removed from tanks for 0.5 h and eggs were vacuumed off the bottom. A total of 10 l of water was siphoned from each tank in the same manner (a second siphoning normally collects < 10% of the number of eggs collected during the first siphoning). The copepodites in the siphoned water were collected by pouring the water through a 200µm sieve and returned to the tank. Eggs were concentrated onto a 35 µm sieve, re-suspended and well mixed within a known volume of seawater (between 300 and 500 ml) and the total number of eggs (and nauplii) was estimated from counts made on six, 50-µl sub-samples. The number of eggs counted in each sub-sample was normally between 25 and 50. The egg harvest (*EH*, no. eggs $tank^{-1}$ d^{-1}) was calculated using the mean(±SE) concentration (eggs ml^{-1}) obtained from those six counts multiplied by the volume (ml) of the seawater sample. *EH* was based only on the number of eggs harvested from tanks, nauplii collected were enumerated but not included in the estimates.

The 48-h hatch success (*HS*, %) of eggs was measured by incubating a known number of eggs (97 –104) within a 250 ml culture flask containing 200 ml of gently aerated, filtered (1 µm) seawater and ~20,000 cells ml^{-1} of *Rhodomonas* spp. The eggs collected from tanks at each treatment level were mixed prior to loading the flasks. Eggs from each treatment were loaded into three replicate flasks. All flasks were incubated for 48 h at 20.5±0.5°C, 18.5±0.2 psu, after which the contents of the flasks were collected onto a 35µm sieve and unhatched eggs were counted. A 48-h egg incubation period was used based upon hatching times at 20°C for *A. tonsa* (Holste and Peck, 2006) and the duration of the first two non-feeding naupliar stages (Peck, unpublished data).

Table I: Initial and final stage distribution (frequency, %) and the concentration (n l^{-1}) of all stages combined and adults within tanks in an experiment examining unnatural photoperiods. The light regime (hours light : hours dark) is indicated. Cop = copepodite.

Stage	*Acartia tonsa* Stage Frequency (%, mean(SE))															
	3L:3D (tank a)		3L:3D (tank b)		4L:4D (tank a)		4L:4D (tank b)		6L:6D (tank a)		6L:6D (tank b)		12L:12D (tank a)		12L:12D (tank b)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Nauplii	4 (4)	72 (2)	10 (2)	77 (5)	9 (4)	65 (4)	23 (8)	72 (8)	14 (5)	81 (2)	26 (8)	72 (1)	12 (2)	84 (3)	9 (2)	71 (3)
Cop. I-III	43 (7)	20 (1)	33 (8)	15 (5)	11 (5)	19 (2)	19 (4)	20 (5)	24 (3)	11 (2)	10 (5)	17 (4)	21 (3)	7 (4)	17 (2)	21 (1)
Cop. IV-V	33 (12)	3 (1)	20 (2)	3 (1)	25 (5)	8 (0.4)	17 (6)	3 (1)	22 (4)	4 (0.3)	19 (4)	7 (4)	20 (3)	4 (0.3)	24 (3)	5 (3)
Adults	21 (3)	5 (0.2)	37 (10)	6 (1)	55 (5)	8 (2)	41 (1)	6 (1)	40 (2)	4 (0.3)	46 (9)	5 (2)	48 (1)	5 (0.1)	51 (3)	4 (0.04)
	Acartia tonsa Concentration (number l^{-1}, mean(SE))															
All stages	80 (6)	1547 (97)	145 (18)	1099 (55)	98 (12)	1927 (419)	82 (17)	1396 (62)	113 (17)	1189 (51)	97 (4)	1139 (137)	83 (12)	1121 (45)	78 (6)	1450 (178)
Adults	16 (4)	78 (8)	54 (23)	63 (9)	54 (12)	158 (72)	34 (8)	77 (23)	45 (9)	48 (6)	44 (10)	51 (25)	40 (7)	61 (4)	40 (5)	54 (8)

Exp 1: Light Regime

This experiment evaluated the effect of four different light regimes (3h:3h = 3 h light:3 h dark, 4h:4h , 6h:6h and 12h:12h) on *EH* and *HS*. Eight, 120-l (65 cm diameter) tanks were used, two at each of the four treatment levels. Copepods were three to four weeks of age (adults were one to two weeks old). Copepods were staged into four categories (nauplii, copepodite stages I-III, copepodite stages IV-V, adults) and counted at the start and end of the experiment. All counts of adults, eggs, nauplii and copepodites were made on two replicate samples using Bogorov dishes with the aid of a dissecting microscope (Leica MZ 9_5). Between 50 and 150 adults were counted within 1-ml sub-samples of a concentrated solution of known volume of each replicate sample. An initial concentration of ~40 adults l^{-1} was used in this experiment (Table I). *HS* was assessed for eggs collected on day six. Abiotic and biotic conditions within each egg flask were similar during incubation. Therefore, any differences in *HS* would reflect differences in eggs related to treatment effects experienced during egg production and not during egg incubation. In this experiment, eggs were incubated at a 12h:12h, the light regime most closely matching that routinely used at our facility.

Exp 2: Photoperiod and Egg Hatching

Two copepod cohorts were reared (from the late naupliar stage), one in constant darkness (*D*) and one in continuous (24-h) light (*L*). Rearing tanks (130-L), water temperature and salinity, and culture methods were the same as those previously described. Eggs were produced by both cultures for ~ one week at which point *HS* was measured for eggs collected on the same day from each cohort (development rate of copepod was the same in the two cultures). On the day of *HS* measurements, eggs were collected two times over the course of three hours and the second egg collection was used for the experiment. Eggs collected from the *D* cohort were incubated in constant darkness (*D-D*) and constant light (*D-L*) and eggs collected from cohort *L* were also incubated using constant light (*L-L*) or constant darkness (*L-D*). A known number of eggs (52 to 58) were incubated within four replicates at each of the four treatment levels. The incubation flasks, water temperature, water salinity and methods employed were identical to those previously described.

Statistics

One-way ANOVAs were used to assess the effect of light regime (Exp 1) on *EH* and *HS* as well as the effect of long-term photoperiod (Exp 2) on *HS*. When statistically significant treatment effects were observed, a Tukey post-hoc test was used to identify significant differences among treatments. Non-linear and linear regression analyses were also performed

with parameter estimates fit using the least squares method. Percentage data were arcsine transformed [arcsine*(%/100)$^{0.5}$] prior to testing for statistical differences. Statistical analyses were carried out using SAS software (SAS, 1989) and the significance level was set at $\alpha = 0.05$.

RESULTS:

Exp 1: Light Regime

During the first two days of the experiment, daily egg harvests (*EH*) in five of the eight tanks was relatively low (Fig. 1) and these days were considered an acclimation period (data not used in analyses). From day three to day seven, the mean(±SE) *EH* within tanks (n = 2) maintained at 3L:3D, 4L:4D, 6L:6D, and 12L:12D was 166000(3500), 153300(6200), 167600 (23800), and 190000(7200) eggs tank^{-1} d^{-1}, respectively (Fig. 1). There were no significant differences in *EH* among the treatments (ANOVA, df = 7, F = 1.40, p = 0.37).

The mean number of nauplii harvested from each tank on each day was between 10.9 and 19.3 % of the mean number of eggs harvested from each tank each day. There was no effect of light regime on the mean number of nauplii female^{-1} d^{-1} collected from the tanks (ANOVA, *df*=7,*F* = 0.5, *p*=0.70). At the end of one week, the mean(±range) number of nauplii within each of the 120 l tanks was 120,000(±31,000), the concentration of adults increased two-fold, and copepod densities were between 1100 and 1900 individuals l^{-1} (Table I). The relative abundance of nauplii : copepodite stages I-III, copepodite stages IV-V, and adults at the end of the experiment were similar among tanks and equal to ~75:15:4:5, respectively.

The mean(±SE) egg *HS* from the 3L:3D, 4L:4D, 6L:6D and 12L:12D light regimes was 82.7(5.1), 77.3(3.4), 79.6(1.6) and 68.6(3.1)%, respectively (Fig. 2a). Although there was a tendency for *HS* to decrease with increasing photoperiod duration, differences among the treatments were not significant at the $p \leq 0.05$ level (df = 11, F = 2.82, p = 0.1067).

Exp 2: Photoperiod and Egg Hatching

In this hatching experiment, it was not possible to set up (count eggs for) the dark incubation without exposing eggs to light. Furthermore, eggs from the *L* cohort could have received (at most) 3.5 hours of light prior to being incubated in darkness (the *L-D* treatment). We estimated that eggs in the *D-D* and *L-D* treatments were exposed to light for ~0.5 and 2.5 h, respectively, prior to 48-h incubation in darkness. Significant differences existed in the *HS* of eggs among the different treatments (ANOVA, *df*=11, *F*=4.63, *p*=0.037). The mean(±SE) *HS* of eggs in the *D-D*, *L-D*, *D-L* and *L-L* treatment groups was 3.7(1.1), 32.2(15.1), 38.3(0.8) and 52.2(16.5)%, respectively (Fig 2c). Mean *HS* was significantly lower in the *D-D* treatment compared to that in other treatment groups.

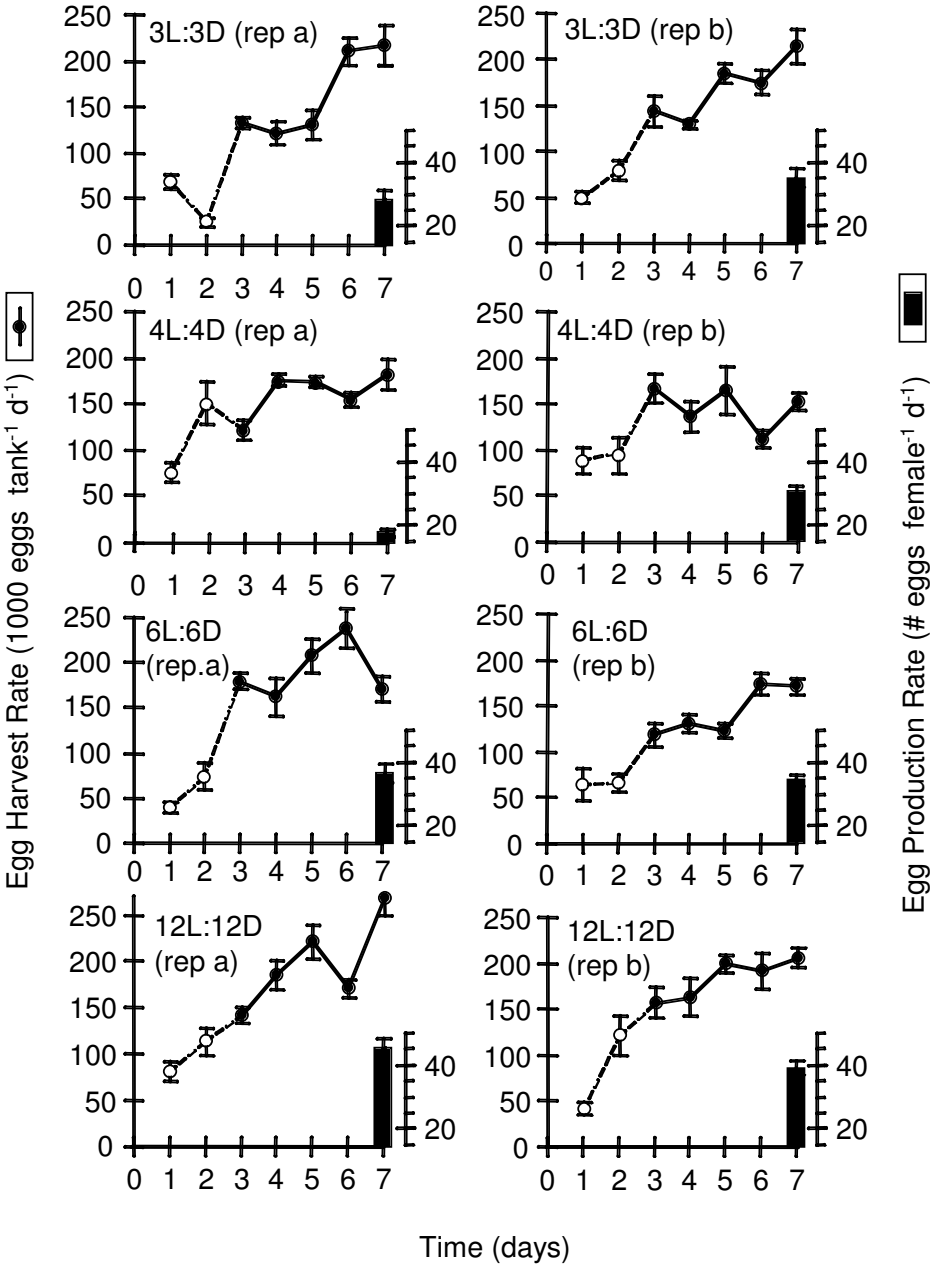

Fig. 1. Acartia tonsa daily egg harvest (number of eggs tank− 1) versus time (days) in each of eight, 130-l culture tanks exposed to light regimes of either 3 h:3 h, 4 h:4 h, 6 h:6 h or 12 h:12 h light:dark. The first two days (open symbols) were considered acclimation days. The total number of eggs female− 1 (mean ± SE) collected during the final day of the experiment (when counts were made of adults in tanks) is provided in each panel.

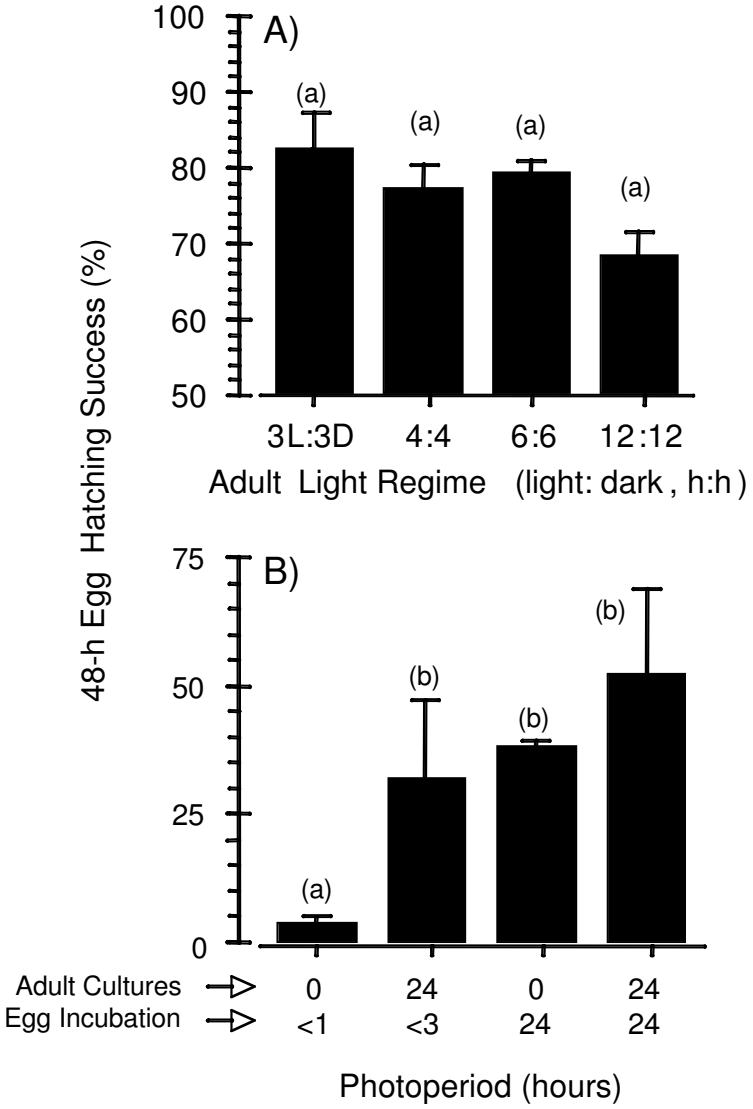

Fig. 2. The 48-h percent hatch (%) of Acartia tonsa eggs versus the long-term light regime experienced by adults during rearing and short-term light regime experienced by eggs during incubation. Panel A) adults experienced different light regimes and eggs were incubated at 12 h:12 h (L:D). Panel B) copepod cohorts were reared (from nauplii) in darkness or 24-h light and adults from those cultures produced eggs that were incubated for 48 h in either darkness or light. Within each panel, bars with different letters were significantly different.

DISCUSSION:

Light and Egg Production

The attempt to increase daily egg harvests by exposing *A. tonsa* cultures to unnatural light regimes was not successful. The pattern of daily egg harvest with time was similar among most of the tanks and maximum *EH* (~200,000 eggs d^{-1}) was reached after about four to five days. Egg harvests on the last day of the experiment and the adult counts made on that day can be combined to estimate egg production rates (*EP*, no eggs $female^{-1}$ d^{-1}) (filled bars in Fig. 1). In most tanks, *EP* was relatively high (between 30 and 45 eggs $female^{-1}$ d^{-1}) on the last day of the experiment. These rates were underestimates since a large number of nauplii was present within tanks on the final day of both experiments. The number of nauplii in tanks would add an

additional ~10% to our estimates of *EP* (33 to 50 eggs female^{-1} d^{-1}). Under optimal conditions in small-scale (controlled) experiments, maximum *EP* by *A. tonsa* has been reported to be 44 to 55 eggs female^{-1} d^{-1} between 18 and 25°C (e.g., Durbin et al., 1983; Schmidt and Jónasdóttir, 1997; Holste and Peck, 2006; Peck and Holste, 2006). Naturally, these final day estimates of *EP* in the present study were not as precise as those from smaller-scale, controlled laboratory experiments. Nevertheless, it appears that *EP* was relatively high and close to the physiological limit of energy allocation to reproduction (growth of gonadal tissue) in this species. Since there was little physiological "room for improvement", the lack of a positive treatment effect was not unexpected and our results merely suggest that *EH* was not negatively impacted by exposure to the unnatural light regimes used in this study.

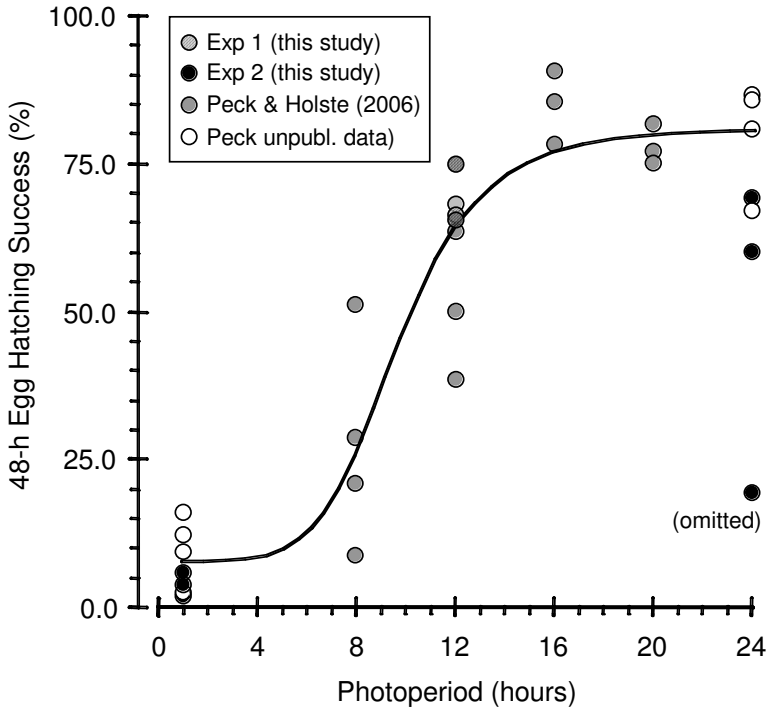

Fig. 3. The effect of photoperiod duration (h) on 48-h hatching success of *Acartia tonsa* eggs. The data from Exp 1 (12:12 light:dark) and Exp 2 (1:23, 24:0 light:dark) in the present study, those from Peck and Holste (2006) as well as unpublished measurements were combined for the analysis. All incubations were performed at 20 to 22 °C. The regression equation and parameter estimates are provided in the text. Copepods used in these trials were from a southwestern Baltic Sea population (collected in the harbor of Kiel, Germany).

Light and Egg Hatching Success

Results of the present experiment indicated that *HS* was significantly impacted by the photoperiod experienced by adults (when the eggs were produced) and by the eggs (during their incubation). Our results agree with those of a previous study (Peck and Holste, 2006) indicating that long-term differences in photoperiods experienced by developing cohorts, adults and eggs markedly influence 48-h *HS*. After combining the data from this study and those of Peck and Holste (2006), the relationship between photoperiod (*PH*, hours) and 48-h hatching success (*HS*, %) could be described by a four-parameter logistic function:

1) $$HS = HS_{\min} + \frac{HS_{\max} - HS_{\min}}{1 + \left(\frac{PH}{PH_{HS\,50\%}} \right)^{SL}}$$ n = 30, r^2 adj = 0.875, $p < 0.001$.

The mean(±SE) parameter estimates for HS_{min}, HS_{max}, $PH_{HS50\%}$ and SL (slope) were 7.7(2.7), 81.0(3.9), 9.7(0.5) and -5.62(1.21), respectively. All parameter estimates were significant at the $p < 0.01$ level. The relationship indicated that a 10 h photoperiod would be required to obtain 50% hatching and that hatching was high and changed little at photoperiods between 16 and 20 h (Fig. 3). Hatching was often high but most variable for eggs produced and incubated in constant light (24 h). It should be stressed that Eq. 1 is only appropriate when photoperiods are experienced by both the adult cultures (during rearing) and by the eggs (during incubation) since results of our crossing experiment (Fig. 2b) indicated that changes in photoperiods experienced by eggs modified 48-h hatching success.

Evidence suggests that normal and/or resting eggs of a variety of invertebrates (e.g., crustaceans, insects, rotifers) can exhibit both photoreception (Hagiwara and Hino, 1989; Itoh and Sumi, 2000; Blackmer et al., 2002) and chemoreception (Hagiwara et al., 1995; Lass et al., 2005). For example, eggs of the silverleaf whitefly (*Bemisia argentifolii*) that were produced at 14h:10h L:D or at high light intensity had higher hatch rates than eggs oviposited at 10:14 L:D or low light intensity (Blackmer et al., 2002). Eggs of the cricket (*Gryllus bimaculatus*) could be entrained to different diel periodicities in hatching when exposed to new photoperiods, but only when eggs were exposed to new photoperiods midway through embryogenesis (Itoh and Sumi, 2000). In resting eggs of the rotifer (*Brachionis plicatilis*), the time to hatch and the synchrony of hatching were influenced by environmental conditions (e.g., temperatures, salinities, photoperiods) experienced by both the adults and by the eggs during incubation (Hagiwara and Hino, 1989). Moreover, the results of Hagiwara et al. (1995) indicated that hatching of rotifer resting eggs was affected by both light intensity, spectral composition and, more importantly, that hatching could be induced in darkness with the addition of prostaglandins E_1, E_2 and F_2. Those authors speculated that the production of peroxide in seawater caused by light and the oxidation of fatty acid to prostaglandins inside the embryo were the mechanisms triggering resting eggs to hatch (Hagiwara et al., 1995). Whether the hatching of copepod resting eggs could be triggered by similar chemical cues is unknown and an interesting avenue for future research.

Working with *Eurytemora affinis*, Ban and Minoda (1994) observed that females produced higher percentages of diapause (resting) eggs in crowded cultures and when maintained at low densities in water from crowded cultures. Those authors concluded that the buildup of metabolites decreased the percentage of rapidly hatching eggs that were produced. In Exp1 of the present study, the number of copepods l^{-1} increased ~ 12-fold in seven days and reached values of 1000 to 2000 individuals l^{-1}, 65 to 85% of which were naupliar stages. The 48-h *HS* was high (between ~70 and 85%) for eggs collected from tanks on day 6 of the experiment suggesting that intensive cultures of *A. tonsa* could use higher concentrations of individuals to increase the number of eggs harvested each day without a concomitant loss of the production of eggs that can be rapidly hatched.

The results of the present experiments underscore the need for descriptions of the light environment to be included within protocols describing intensive culture methodology used for

calanoid copepods (e.g., Støttrup et al., 1986; Lee et al., 2005). Information on the light environment will be especially important to include for species that produce resting eggs such as *A. tonsa*.

Acknowledgements
This research was supported by the Global Ocean Ecosystem Dynamics (GLOBEC Germany) program funded through the German Federal Ministry for Education and Research (BMBF 03F0320E) and the German Science Foundation (DFG) AQUASHIFT program cluster Resolving the Trophodynamic Consequences of Climate Change ("RECONN," DFG # JO556/1-1) awarded to M.A. Peck. We would like to thank Gudrun Bening for her help with maintaining long-term copepod cultures.

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Ms 5) Handling Copepods and Egg Production Rates: A Note of Caution

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Handling Copepods and Egg Production Rates: A Note of Caution

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ABSTRACT:

Protocols used to obtain egg production rates (*EP*) in marine copepods may be incorrect if they do not account for the potential impact of handling stress on adults. In this study, we found that handling effects significantly impacted *EP* (# eggs female^{-1} d^{-1}) by *Acartia tonsa* (Calanoida) in each of six laboratory experiments that differed markedly in scale (250 ml to 100 L replicate containers) and in the environmental factors tested (temperature, salinity, photoperiod, light intensity or adult stocking density). In nearly every replicate in every treatment in every experiment, *EP* increased during the first two or three days. Significant treatment effects on *EP* were often found in those experiments. but never when only the data from day 1 were compared. In the case of *A. tonsa*, significant differences among treatments appeared to be masked by a handling effect for up to two days. The effect of increasing female age could be discounted. A review of the literature indicated that, in the majority of studies measuring *EP* , copepods were acclimated to novel environmental conditions for < 2 days and the vast majority did not include any additional time (after the start of EP measurements) for copepods to recover from handling stress. Some published manuals suggest that controlling for the effect of handling is unnecessary if copepods are carefully handled. We disagree and urge researcher to test for handling effects as they develop *EP* measurement protocols. Any impact on *EP* from handling will undoubtedly be species-specific. Spurulous measurements of *EP* will seriously undermine attempts to understand the dynamics of copepod populations (and/or secondary production) in most marine systems.

INTRODUCTION:

Due to their abundance and trophodynamic importance of copepods in marine systems, "copepod production" is often synonymous with "secondary production". Understanding the dynamics of the latter demands thorough investigations of how various abiotic and biotic factors influence the former. Copepod egg production rates (*EP*, # eggs female^{-1} d^{-1}) are commonly measured and can provide estimates of the relative condition or growth "status" of individuals and populations of copepods (e.g. Omori and Ikeda 1984, Poulet et al. 1995). Furthermore, controlled measurement of the impacts of different environmental factors on copepod *EP* allow factors responsible for changes in, the phenology in abundance and productivity to be identified.

When copepod *EP* is measured, the common practice is to: 1) collect copepods from the field or from cultures grown in the laboratory, 2) quickly identify and sort (females and males), 3) thoroughly acclimate those animals to test conditions (if different than *in situ*/rearing condition), 4) carefully load animals into measurement chambers, and 5) make measurements (in some cases, step 3 comes before step 2, for detailed review of common methods, see Runge and Roff 2000). Although the duration of time allowed/needed for each step is study-specific, a recovery period between step 4 and step 5 is often not used. The general point of view is that handling stress is avoided (or minimal) when animals are carefully treated, a conclusion that stems from discussions started nearly 50 years ago.

In the present study, we tested whether *EP* by *Acartia tonsa* (Dana), a copepod that is known to respond rapidly (<24 h) to changes in environmental conditions (e.g., Dagg 1977; Kiørboe et al. 1985), was influenced by handling. In this case, a "handling effect" was defined as (identified by) any significant increase in *EP* with time (day) within replicates and among treatments. We also reviewed the literature to examine common methods employed to measure copepod *EP* in this and other copepod species. In general, we posed the following questions: 1) could handling effects on copepod *EP* be identified? If so, did this handling effect 2) impair our ability to detect significant effects of environmental factors, and 3) depend greatly upon the protocol employed?

METHODS and PROCEDURE:

The data from six experiments on *A. tonsa EP* conducted at either small (250 ml, few individuals, pipetted) or large (~100 L few hundreds of individuals, 1-L beaker transfer) scales within different studies were combined for the analysis. Small-scale *EP* experiments tested the effects of different temperatures (EXP1) and salinities (EXP2) (see Holste and Peck 2006, Peck and Holste 2007, Diekmann et al. submitted) and large-scale *EP* experiments tested the effects of light regimes (EXP3), light intensities (EXP4), stocking densities (EXP 5) and salinities (EXP6) (Peck and Holste 2007; Peck et al. 2008; Peck unpubl. data).

Table I: Overview on acclimation time, - steps and - duration of experiments including experimental conditions. Na=not applicable

Experiment		Duration	Acclimation			Replicate Containers			Temperature	Salinity	*A. tonsa*
ID	Factor	total (d)	acclimation time steps	level	time (d) at exp. condition prior to exp	treatment^{-1} (n)	total (N)	Volume (L)	mean (range) (°C)	(psu)	replicate^{-1} (n)
Exp 1	Temperature	5	0.6 °C d^{-1}	6, 9, 13, 17, 22	3	3	30	0.25	5.5 to 23.4	18.0 (±0.5)	4 ♀ and 1 ♂
Exp 2	Salinity 1	15	2 psu d^{-1}	8, 18	3	12	24	0.25	15 (±0.15)	8.0 and 18(±0.5)	4 ♀ and1 ♂
Exp 3	Light Intensity	7	0	0.03, 0.49, 3.78, 13.81 µE	3	2	8	220	18 (±0.5)	18 (±0.5)	30 ind L^{-1}
Exp 4	Light Duration	7	0	3L:3D, 4L:4D 6L:6D, 12L:12D	3	2	8	220	10 to 20°C	7 and 20	27 ind L^{-1}
Exp 5	Stocking Density	8	na	*no acclimation*	na	3	9	100	18 (±0.5)	18.0 (±0.5)	54 to 440 ind L^{-1}
Exp 6	Salinity 2	5	1.3 psu d^{-1}	6, 10, 14, 20, 30	4	3	15	0.25	18 (±0.5)	6 to 30	4 ♀ and 1 ♂

Table II: Summary of statistics performed on reanalyzed data. Na=not applicable

	Temperature			Salinity 1			Light Duration			Light intensity			Stocking Density			Salinity 2		
	Time	Statistik		Time	Statistik		Time	Statistik		Time	Statistik		Time	Statistik		Time	Statistik	
	d	p	F	d	p	F	d	p	F	d	p	F	d	p	F	d	p	F
Handling ANOVA *EP* vs time	total	<0.0001	19.91	total	<0.0001	161.43	total	0.008	4.12	total	<0.0001	106.09	total	<0.0001	31.10	total	0.03	2.84
POST Hoc	1≠ all	≤0.009		1≠ all	<0.0001		1≠ 5	0.047		1=2	1		1	0.005		1≠ 3	0.02	
	2=3	1		2=3	0.185					1&2≠all	≤0.027		2	0.002				
	4≠all	≤ 0.025		4&5≠all	<0.0001								3	<0.0001				
	5≠all	<0.0001		4=5	0.817								4,5	<0.0001				
Significance of env. Factor	4	<0.0001	39.09	2	0.003	11.59	not signif.	na	na	not signif.	na	na	1	<0.0001	44.79	not signif.	na	na

In each case, laboratory-reared *A. tonsa* were used, an acclimation period was provided (step 3 above), females were tested at 30 individuals L^{-1}, different levels of treatments ($N \geq 3$) were replicated ($n \geq 3$), and copepod *EP* was measured on ≥ five subsequent days (Table I). These *EP* data were analyzed in three steps:

1. Data in each experiment were pooled and normalized to the highest *EP* measured during that experiment and the effect of time (day) was assessed (oneway ANOVA). A post hoc test (Tukey HSD) identified significant differences among days.
2. In Exps where a significant impact of the environmental factor on *EP* was originally reported (EXP 1, 2 and 5), a Bonferroni analysis determined the first day when *EP* among treatments was significantly different.
3. Finally, we examined the magnitude of the handling effect within small-scale versus large-scale experiments.

All statistical tests were performed using SPSS (SPSS 1990) and were considered significant at $p \leq 0.05$. For analyses of literature data, measurements of *EP* were taken directly from published tables and from figures. The latter were collected via digitizing (Matlab, dgtlgrph m-file) scanned images.

ASSESSMENT:

Re-analysis: Is there a handling effect?

There was a significant effect of time ($p<0.01$ to 0.001) on *EP* in the six experiments: normalized *EP* often increased until day 3 and was similar on subsequent days (Table II, Fig. 1). For example, in Exp 1 evaluating temperature, normalized *EP* significantly increased with Day1 < Days2&3 < Day4 <Day5 (Fig. 1A). In Exp 2 (evaluating salinity), significant differences included Day 1 > days 2&3 > days 4&5 (Fig. 1B). In Exp 6 (salinity, large-scale), normalized EP on day 1 was significantly less than that on Day 3 (ANOVA stats).

In Exps 1 (temperature), 2 (salinity) and 5 (stocking density), significant effects of environmental factors were first present, but often not on the first day of these experiments. In Exp 1, the greatest effect of temperature was noticeable on day 4 and 5. On these days, *EP* within at least two (and at most eight) of the 10 different temperature treatments were significantly different. Comparisons of *EP* among different temperatures yielded different patterns of significance with time with many more significant differences during progress of the experiment (Fig. 3 A insert). On day 3, *EP* was only significantly different between copepods in the lowest three (5 to 8°C) and highest two (21 and 23°C) temperatures. In Exp 2, the effect of salinity (8 and 18psu) on *EP* was significant on day 2 ($p=0.003$, $F = 11.59$) but not when compared on day 1 ($p=0.161$, $F=2.12$) (Fig 3 A insert). In contrast, the effect of stocking density (Exp 5) was significant at the start (Day 1) of the experiment ($p<0.0001$, $F=44.79$).

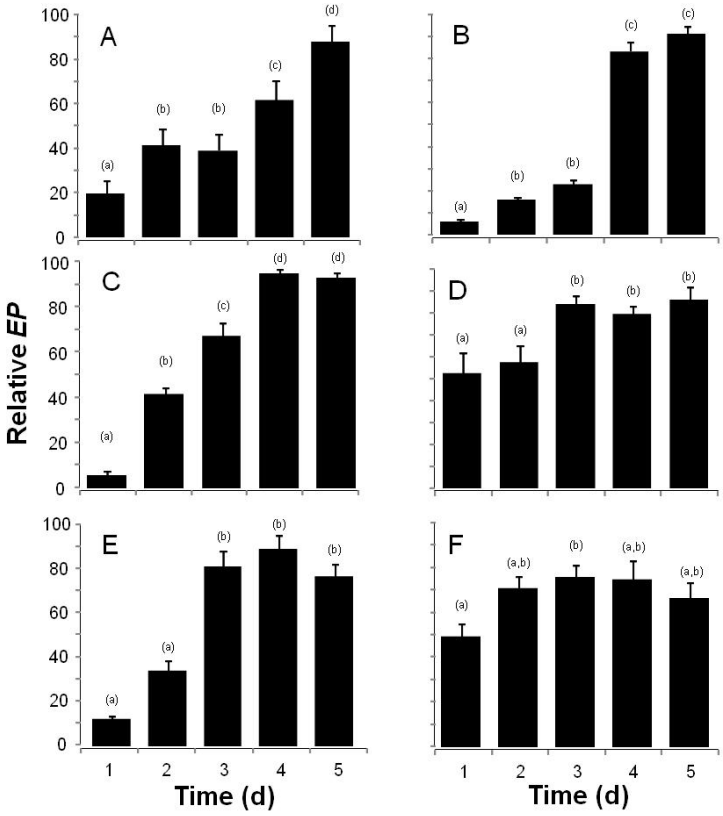

Fig. 1) Normalized mean (+SE) egg production rate (*EP*) versus time for *A. tonsa* within six different laboratory experiments quantifying the effects of temperature (panel A), salinity in small cultures (B), light intensity (C), light duration (D), stocking density (E) and salinitiy in large cultures (F). Egg production was normalized to maximum values obtained by treatment group and experiment to eliminate potential treatment effects and highlight temporal changes in (and possible impact of) initial handing on *EP*. Within each panel, days with different letters had significantly different normalized *EP*.

Finally, the effect of handling on *EP* was not significantly different (p=0.362, F=0.859) between small- and large-scale protocols.

Calculating the Q_{10} values for each of the experimental days of Exp 1 there is found a threefold increase in Q_{10} comparing Day 1 with Day 5. When the Q_{10} values were calculated for five different studies (Fig.3 panel B) using the best fit of an exponential function, there is found no coherence of Q_{10} value with acclimation procedure. While Kim (1995) with a very high Q_{10} did not use any acclimation time, Castro-Longoria (2003) who had acclimated test copepods to experimental conditions achieved only a relatively low Q_{10} of 2.9.

Protocols used to Measure EP in Acartia tonsa.

Acartia tonsa is among the most well-studied copepod species and methods employed to measure *EP* in this species are often similar to those employed to measure *EP* in other species. A brief review of 40 published studies examining *EP* by *A. tonsa* (Table III) indicated that 22.5 % of those studies measured *EP* in field-caught animals while the

remainder examined *EP* in laboratory-reared individuals. In total, 65% of all studies included an acclimation period (Step 3 in our scheme). However, only five of those 40 studies provided additional time (between step 4 and step 5) for the copepods to recover from any potential handling stress.

Mean(±SE) time of acclimation adds up to 58.4 h (±9.1) (equals 2.4 days) in total. Scientists that avoid handling after acclimation use a shorter mean acclimation time (47.3±6.4h) (meaning less than two days of acclimation) than scientists that handle their test copepods afterwards (51.4±9.2) (References in Table III). Scientists that allowed copepods to recover from handling stress (*RHS*) utilized a mean time of 51.6(±14.6) h. Unfortunately this is only the case in 5 of the 40 studies, so this *RHS* time has to be interpreted with caution. Within the selected field studies that quantify egg production only two studies take an acclimation time into account (24 and 72 h).

Similar to the results of our reanalysis, in the few studies that measured (and displayed) *A. tonsa EP* on consecutive days (versus time), *EP* tended to increasing with increasing time. For example, Støttrup and Jensen (1990) reported a two-fold increase in *EP* by *A. tonsa* during the first three days of measurements. Castro-Longoria (2003) examined *EP* by *A. tonsa* at different temperatures and observed increasing *EP* during the first three days at 15 and 20°C but stable *EP* at low temperatures (5 and 10°C). These temperature-specific findings correspond well to the results obtained from re-analysis of our EXP 3 data. Under optimal conditions (i.e., temperatures of 21 to 23 °C for *A. tonsa*) the effect of handling can have a greater impact on *EP* than under sub-optimal conditions (low temperatures).

Among these different studies, temperature-specific *EP* by *A. tonsa* was markedly different (Fig 1b). No matter whether measurements were made in the laboratory or in the field, minimal and maximal *EP* at the same temperature often differed by more than two-fold. In general, *EP* was higher in experiments that employed an acclimation period compared to those that did not. For example, the highest *EP* for *A. tonsa* was reported by Parrish and Wilson (1978) who conducted measurements for 8 days, more than enough time (e.g., > 5 days) for their copepods to recover from handling stress.

DISCUSSION:

The necessity of sufficient acclimation time to new environmental conditions prior to testing (Step 3 in our scheme) has been thoroughly discussed by other authors (e.g., see Tiselius et al. 1995). Clearly, sufficient time should be provided and the amount of time required will change depending upon both intrinsic (species, life stage and/or sex) and extrinsic factors (environmental variable tested and the level of that factor) (e.g., see work on temperature tolerance in *E. affinis* by Bradley (1978)). Our re-analysis and literature review highlights the potential importance of handling stress and also suggested that “handling effects” were present after copepods were transferred using either more or less gentle techniques (large beakers versus small pipettes). Possible explanations for handling stress include exposure to relatively high light intensities or high concentrations (crowding) during transfer of copepods to test chambers. Both factors are known to stress copepods (Marshall and Orr 1955; Hargrave and Geen 1968).

In terms of handling effects, our reanalysis of the data from each of six experiments indicated that *EP* significantly increased during the first days and that significant differences in *EP* due to some environmental factors (e.g., temperature) were absent on day 1. We interpret significant differences in *EP* with time to indicate a "handling effect" since, 1) copepods were acclimated to test conditions for a reasonable amount of time prior to experiments, 2) *A. tonsa EP* is known to respond rapidly to changes in environmental conditions (e.g., Dagg 1977; Kiørboe et al. 1985), and females employed at the start of measurements were of the age (3 to 8 days within C6 stage) where *EP* is maximal in this species (Parrish and Wilson 1978) However, our review of other studies performed on *A. tonsa* did not reveal consistent patterns of influence on *EP* related to differences in protocols utilized. Unfortunately, it was not possible to account for the impact of differences in acclimation (step 3 and handling recovery time (time between step 4 and 5) and possibly establish correction factors. In the 40 experiments that we reviewed, too many differences (beyond purely methodological differences) existed among those studies.

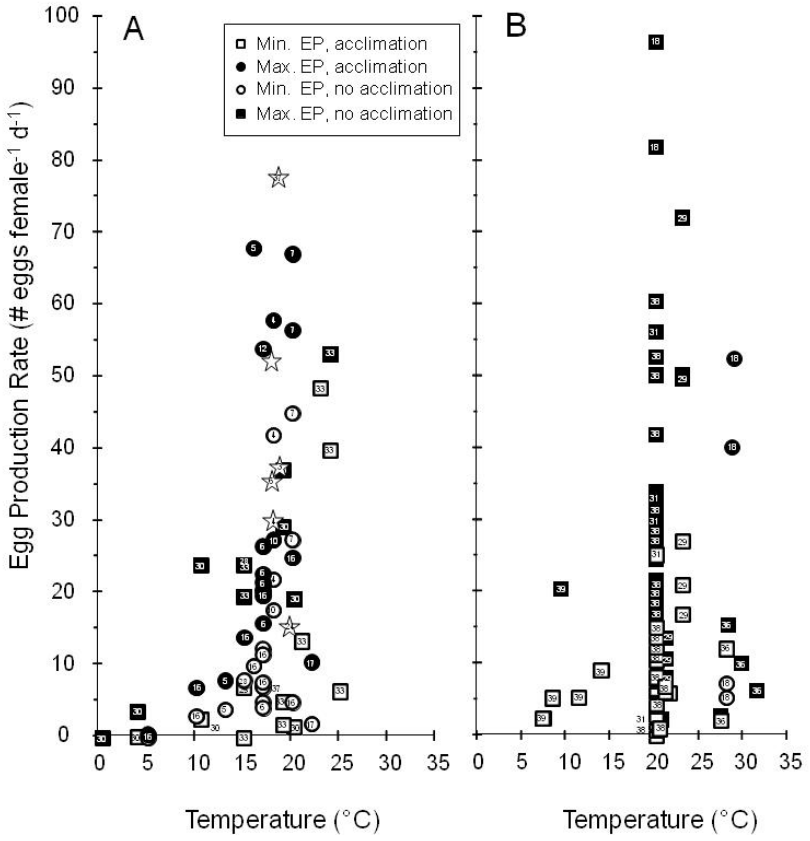

Fig. 2) Summary of egg production rates collected at different temperatures from different laboratory (Panel A) and field (Panel B) studies. Numbers within / next to data points represent study IDs listed in Table 3. Asterixes display data from experiments that had allowed copepods to recover from handling stress.

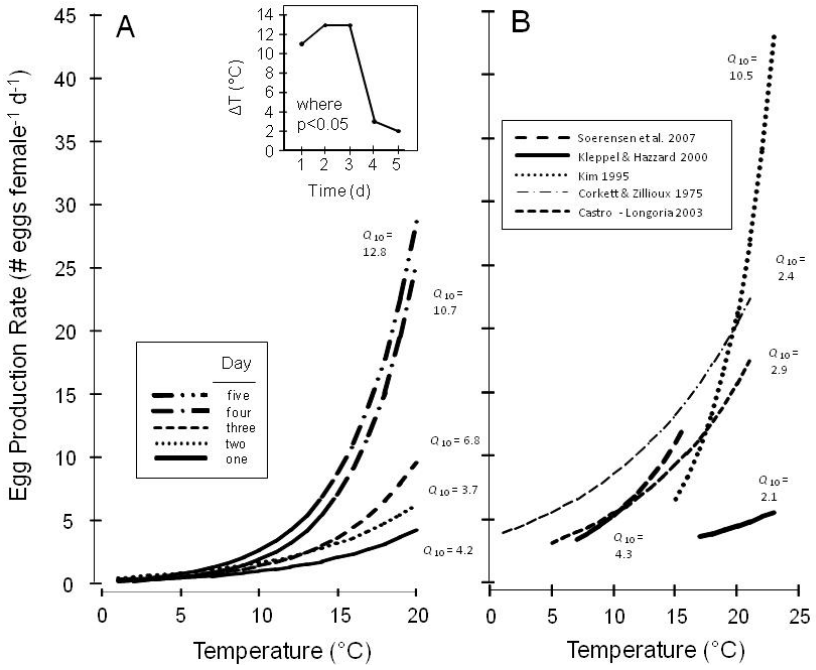

Fig. 3) Best fit, exponential regression lines describing egg production rate (EP) versus temperature (T) ($EP = a*e^{bT}$). Panel EP vs T on each of five subsequent days of an experiment conducted by Holste and Peck (2005). Insert: Minimal difference in temperature (DT) required for significantly different EP among temperatures verus time. Panel B) EP versus T for five different studies. In both panels, Q_{10} values are shown for the full temperature range ($Q_{10} = e^{b*10}$).

It should be noted that impacts of handling stress are not confined to copepods within the *Acartia* genus. Dutz et al. (2008) observed an increase in *Temora longicornis EP* during the first three to four days in four of their six treatment groups. In that study, *EP* increased ~80% within the first three days for copepods fed high-quality food (*Thalassionsira weisflogii*) but this temporal trend was absent in copepods fed poor-quality food (*Leptocylindricus danicus* and *Skeletonema costatum*) since those copepods produced very few eggs. Within both these studies, not only handling stress but also a diet shift may have contributed to temporal trends in EP. In both studies, the potential effect of copepod age can be discounted since females were an optimal age (~5 days in adult stage) for reproduction (Parrish and Willson 1978).

Marshall and Orr (1955) assumed that copepod *EP* measured within 24-h incubations would not reflect *in situ EP* due to stress associated with handling (and, in that case, exposure to light). In the subsequent decades many researchers have expressed the opposite opinion (e.g., Runge 1985, Stearns et al. 1989, Plourde and Runge 1993, Niehoff and Hirche 1996; Runge and Roff, 2000). These authors (and others) argue that: 1) although eggs could be released more rapidly due to handling stress, these eggs would have been released during the course of the incubation and the estimate of *EP* would nevertheless be robust, and 2) maximum *EP* in field incubations and in the laboratory are consistent. However, some studies suggest that

allowing time for copepods to recover after handling may be necessary to gain robust estimates of *EP*. For example, Poulet et al. (1995 and references therein) found no significant differences in *Calanus finmarchicus EP* at various temperatures between 5 and 24°C within the first 24 h but detected significant temperature effects when incubations were longer than 24 h. However, species within the *Calanus* genus are known to require long acclimation times as underscored by Halcrow's (1963) examination of O_2 consumption rate by *C. finmarchicus* acclimated to several temperatures; variability in rates depended on the thermal history, acclimation time and the season of field collection. Naturally, handling may not impact measurements of vital rates made on species of copepods that, unlike *Calanus* congeners, tend to respond relatively rapidly to changes in environmental conditions.

Protocols developed for *in situ* measurements of *EP* (used to estimate secondary production) strive to avoid the need for environmental acclimation by strictly conducting measurements only at in situ conditions (e.g. Poulet et al, 1995; Saiz et al., 1997 and references therein). Unfortunately, many protocols that were designed for field or laboratory measurements of copepod *EP* do not control for potential handling effects. However, handling effects have been demonstrated to impact *EP* in different species (e.g., Tiselius et al., 1995; Castro-Longoria 2003; the present study) and *T. longicornis* (Dutz et al., 2008), both relatively small, pelagic, broadcast-spawning calanoid copepod species that do not accumulate large amounts of lipid as energy stores.

COMMENTS & RECOMMENDATIONS:

Measurement protocols developed for copepod *EP* that have not accounted for possible handling impacts may yield erroneous data. Moreover, these data may lead to spurulous conclusions regarding the significance of the effects of various environmental factors on copepod reproduction. We demonstrated that, when testing a variety of different factors, significant treatment effects on *EP* were often masked (not observed) for 24 to 48 h due to a handling effect. This underscores the need for experimental designs to include sufficient time not only to completely acclimate copepods to changed environmental conditions but also to control for the impact of handling. The impacts of handling will be depend upon many factors and can be determined using pilot studies examining the time course of changes in *EP* (or any other vital rate) after transfer of adult copepods to test chambers.

Acknowledgements:
We would like to thank Philipp Kanstinger, Gudrun Behning, Meike Martin and Bianca Ewest for their help with laboratory cultures and experiments. This research was supported by the German Science Foundation (DFG) AQUASHIFT program cluster project Resolving Trophodynamic Consequences of Climate Change (RECONN, # JO556/1-2).

Table 3: Overview of literature reviewed on acclimation time and time copepods given to recover from handling stress before experiment testing environmental factors.

Study ID	Lab/ Field	Acclimation Y/N	Acclimation Time (h)	Handling Recovering time (h)	total Exp. Duration (h)	Environmental Factor tested	Reference
1	L	Y	36 to 72	24	24	*T* & *S*	Calliari et al. 2006
2	L	Y	168	72	8	*T* & *S*	Uye and Flemminger 1976
3	L	N	0	0	768	Age	Corkett and Zillioux 1975
4	L	Y	120	0	168	F_{QUAL}	Schmidt and Jonasdottir 1997
5	L	Y	24	0	72	Cadmium conc	Toudal and Riisgard 1987
6	L	Y	24	0	24	*S*	Miller and Marcus 1994
7	L	N	0	0	up to 1560	Age	Parrish and Wilson 1978
8	L	Y	48	0	24	F_{QUAL}	Broglio et al. 2003
9	L	Y	24	0	144	*T* & *S*	Castro-Longoria 2003
10	L	Y	10	0	144	*T* & *S*	Castro-Longoria 2003
11	F	Y	24	0	24	F_{QUAN}	Durbin et al. 1983
12	L	Y	168	0	0.75	F_{QUAN}	Houde and Roman 1987
13	L	Y	24	0	24	F_{QUAN}	Tiselius et al. 1995
14	F	Y	72	0	24	F_{QUAN}	Tiselius et al. 1995
15	L	Y	48	0	48	F_{QUAL}	Bellas and Thor 2007
16	L	Y	96	96	96	*StD*	Jepsen et al 2007
17	L	Y	18	18	120	F_{QUAL}	Price et al. 2006
18	F	N	0	0	24 to 48	*in situ*	Putland and Iverson 2007
19	L	Y	120	48	168	F_{QUAN}	Kiorboe 1989
20	L	N	0	0	48 to 72	F_{QUAN}	Kiorboe et al 1985
21	F	N	0	0	24	F_{QUAL}	Hazzard and Kleppel 2003
22	F	N	0	0	24	*T*	Kleppel 1992
23	L	Y	24	0	24	F_{QUAL}	Kleppel and Burkart 1995
24	F	N	0	0	24	F_{QUAL}	Kleppel and Hazzard 2000
25	L	N	0	0	24	*T*	Kim 1995
26	L	Y	2	0	96	F_{QUAL}	Ederington et al. 1995
27	F	N	0	0	24	F_{QUAL} and F_{QUAN}	Ambler 1986
28	F	N	0	0	24	*in situ*	Soerensen et al. 2007
29	L	N	0	0	48	F_{QUAL}	Augustin and Boersma 2006
30	L	Y	48 to 96	0	24	*S*	Calliari et al. 2006
31	L	Y	96	0	48	F_{QUAL}	Hassett 2004
32	L	Y	48	0	24	F_{QUAL}	Colin and Dam 2002
33	L	N	0	0	336	F_{QUAL}	Jones et al. 2002
34	L	Y	48	0	24	F_{QUAN}	Dam and Colin 2005
35	L	Y	96	0	24	*DO*	Sedlacez and Marcus 2005
36	L	Y	24	0	24	F_{QUAL}	Tang and Dam 2001
37	L	Y	48	0	24	F_{QUAL}	Jonasdottir 1994
38	F	N	0	0	24	Dial variation	Cervetto et al. 1993
39	L	Y	24	0	96	F_{QUAN}	Stoettrup and Jensen 1990
40	MC	N	0	0	16	*T*	Sullivan and McMarcus 1986

T = Temperature *S* = Salinity
F_{QUAL} = Food quality F_{QUAN} = Food quantity
DO = Dissolved oxygen *StD* = Stocking density
L = Lab F = Field MC = Mesocosm

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CHAPTER IV:

DISCUSSION AND FUTURE PERSPECTIVES:

Copepods in the Baltic Sea, Comparison of Life History Strategies:

Acartia tonsa versus Temora longicornis

In geological terms, the Baltic Sea is a relatively young habitat and, due to its evolutionary forming process, latitude and climate, the majority of Baltic Sea flora and fauna is composed of mixtures of species originating from widely different latitudes (*e.g.,* from subarctic and boreal to subtropical habitats). *Temora longicornis* and *Acartia tonsa* are not endemic to the Baltic Sea. The difference in origin becomes obvious when comparing their respective optimal temperatures for *EP* (MANUSCRIPT 1 and 2): *A. tonsa* is a subtropical species that expanded into boreal habitats and has an optimal temperature for *EP* of 23 to 24°C; *T. longicornis* is distributed in habitats from boreal to subarctic areas and has a lower optimal temperature for *EP* around 16°C. Although both species appear to have a high capacity to adapt to prevalent environmental conditions such as those encountered in the Baltic Sea. However, the distribution and productivity of these species in the Baltic Sea reveal limitations imposed by this environment. While *A. tonsa* is restricted to the highly productive and warmer near-shore areas where higher growth rates can be achieved, *T. longicornis* is more widely distributed over the entire basins. Its reproductive success is relatively low in the Baltic compared to marine populations of this species.

The studied population of *A. tonsa* depends strongly on relatively high temperatures for optimal reproductive success that can only be achieved in near shore environments in the Baltic Sea (MANUSCRIPT 1). Additionally, the low threshold for starvation makes it difficult for this species to achieve high abundances within off-shore, deeper Baltic Sea basins. A recent experiment investigating growth variability within *A. tonsa* cohorts at different feeding levels (see Appendix) suggested that the size of adults that were grown within *ad libitum* feeding conditions (50 000 cells ml^{-1} of *Rhodomonas* spp.) was greater than those grown under lower food concentrations (12 500 cells ml^{-1}) (Fig.2, Appendix). Furthermore, comparing the size frequencies of fast- versus slow-growing copepods within cohorts reared at each of three feeding levels (low, intermediate and high), fast-growing copepods, independent of feeding history, are smaller than the slow-growing individuals (Fig.3, Appendix). It is well known, that smaller females have lower rates of per capita egg production, thus these results suggest two, potential (albeit different) life strategies: 1) fast-growers with lower generation times can produce more cohorts per growth season but each cohort results from smaller adults producing fewer eggs, and 2) slow-growers with higher generation times can produce fewer generations per year and each cohort results from larger adults producing a greater (relative) number of eggs. Both strategies can be adaptive depending upon the environmental situation experienced by a developing cohort. Fast-growers likely have higher metabolic rates and higher prey requirements that slow growers (although increased growth efficiency is also a potential physiological mechanism increasing growth rates) and would likely do poorly when feeding conditions were sub-

optimal. On the other hand, slow growers may have a higher tolerance to sub-optimal feeding periods. One could speculate that the proportion of conspecifics that exhibit these different growth strategies within a population would change depending upon the amount of seasonal (or spatial) variability in prey encountered within that habitat (*e.g.,* subtropical areas that do not have a pronounced phytoplankton bloom versus temperate areas where phytoplankton production is dominated by one or two blooms).

The effect of salinity on *HS* was much stronger than on *EP* (MANUSCRIPT 1 and 3) and considering the strong horizontal salinity gradient (from higher values in the southwest to lower values in the northeast Baltic), salinity likely strongly dampens the productivity of *A. tonsa* in northeast areas (*e.g.,* this species is found in very low abundance in the Gulf of Finland (Viitasalo *et al.* 1995)). However, a comparison of the results in this thesis regarding temperature and salinity tolerance, this Kiel Bight population is presumably different than, for example, populations inhabiting the Limfjord (Denmark) where this species has been reported to withstands very low salinities (~4 psu) and relatively low temperatures about 5°C or even lower (Sørensen *et al.* 2007). This is not true for the southwestern Baltic population used in this study. In a comparison of different populations of *A. tonsa*, Drillet *et al.* (2008a) found significant, genetic and/or phenotypic differences in four tested populations. The two populations from Europe (southwestern Baltic Sea) did not differ in rRNA but differed significantly in hatching behaviour, indicating that, over time, acclimation to local temperatures and salinities changed the reproductive potential of those populations. The Kiel Bight population exhibited a significant difference in hatching pattern compared to all of the other populations (strains). Monitoring hatching success (*HS*) over a course of 150 days, *HS* initially declined during the first 30 days but then continuously increased until day 150. This indicates that the Kiel Bight population produced a high proportion of resting eggs. The abundance of *A. tonsa* in the Kiel Bight is relatively low compared to other calanoid copepods such as *Pseudocalanus elongatus* and has its peak abundance in August responding to the high surface temperatures of around 20°C or higher (Stransky 2007, Peschutta 2008). Salinities are usually relatively low (10 to 15 psu) at that time due to stratification and river runoff. Hence this population has adapted to summer conditions in this area and field measurements and the laboratory data collected in this thesis correspond well to one another. Resting egg production serves as strategy to avoid unfavourable conditions such as low winter temperatures, short photoperiods (MANUSCRIPT 1, 3 and 4) and perhaps low food quantity and/or quality. In subtropical habitats where seasonal variability in phytoplankton production is low, *A. tonsa* does not produce resting eggs, but only subitaneous eggs in which hatching is delayed (Chen and Marcus 1997).

Although *T. longicornis* is widely distributed from coastal areas to the basins, from south to the north, there productive success of this species (including *EP* and naupliar survival) appears most limited due to the low salinities prevalent in the Central Baltic (MANUSCRIPT 2). Within the Kiel Bight, *T. longicornis* can be collected all year. During the winter months, the standing stock consists of late copepodites and adults. Highest abundances are found in late spring/early summer (April and May) when temperatures are between 10 and 17°C. At this time salinities range from ~13 to 17 psu (Stransky 2007, Peschutta 2008). Considering the data collected on the impacts of salinity on *EP* (MANUSCRIPT 2) salinities of 13 to 17 psu are not critically low (*e.g.,* 7 psu) and are not expected to severely limit offspring survival. In contrast to *A. tonsa*, *T. longicornis*

does not produce resting eggs within the Baltic Sea (Mathupratap *et al.* 1996). The reason for this could be due to the fact that *T. longicornis* originates from higher latitudes than *A. tonsa* and is better adapted to the environmental conditions (*e.g.,* low winter temperatures) in the Baltic Sea and a diapausing life stage (resting egg) is not necessary for overwinter survival. However, within the North Sea, *T. longicornis* has been reported to produce resting eggs (Lindley 1986, Engel and Hirche 2004) and that resting egg production takes mainly place in late summer/early fall during the second phytoplankton bloom (Wesche *et al*. 2007), when food conditions are good and temperatures decline. In general *EP* of *T. longicornis* is higher in full strength seawater (North Sea) than in brackish waters such as the Baltic Sea. The limited reproductive success in habitats with low salinities may not allow the production of resting eggs but forces this species to overwinter at a relatively high standing stock consisting mainly of copepodites and adults, thus enabling females to match their reproduction with the timing of the spring diatom bloom. The other explanation would be that due to the eutrophic character of the Baltic Sea (especially in near shore habitats due to river runoff), the standing stock of food items (ciliates) is allowing an active late copepodite/adult overwintering stage and therefore resting egg production is unnecessary.

Broader Comparison and Habitat Partitioning:

Within the central Baltic Sea, several key species of copepods are found and habitat partitioning appears evident due to differences in preferred/optimal (or tolerable) temperatures and salinities. For example, while *Pseudocalanus acuspes*, inhabits the deeper basins (such as Bornholm Basin and Godland Deep), several *Acartia* congeners are distributed throughout the Baltic Sea and exhibit species-specific salinity preference such as *A. bifilosa* and *A. longiremis* in the northern (low salinity) part of the Baltic Sea or *A. clausii* in deeper, more saline basin waters. *Eurytemora affinis* is found in very low abundance within the basins but is the dominant copepod within the northern parts of the Baltic Sea.
When *EP* by *T. longicornis* and some of the other (aforementioned) Baltic copepod species are displayed within plots of temperature versus and salinity (Fig. 1) two things become apparent. First, reproductive effort of these different species are often segregated either horizontal (within different temperatures) and/or vertically (within different salinities). Second, large differences exist in egg production rates by these different species. For instance *E. affinis* is dominating the northeastern, shallow part of the Baltic Sea with salinities around 4 psu being very productive also at temperatures up to 20°C. The same is true for *A. bifilosa*. Both species are also found in the more central basins, but in lower abundances. *T. longicornis* dominates upper water masses of the central basins that have a slightly higher salinity (ca. 8 psu) and relatively high summer temperatures (from 15 to 20°C in the surface waters), whereas *P. acuspes* spends the entire year dwelling within the deeper water masses of the basins with higher salinity (up to 15 psu) and lower temperatures. *P. acuspes* is thought to be stenohaline and stenothermic which restricts this species to the deeper water masses. The rate of egg production of the different species differs immensely. *E. affinis* is known to be an estuarine species with a preference of low salinities, even invading into freshwater (*e.g.,* Lee and Petersen 2002), and therefore its high productivity in the northeastern Baltic Sea with 4 psu is not surprising. In comparison to *T. longicornis,* the optimal salinity seems to match the need of this species. The latter one is known to be even more productive in terms of total *EP* (egg female^{-1} d^{-1}) in other regions

with higher salinity, but probably because of its reduced size due to salinity (see comment above) it is limited in the production of eggs.

Table III: Summary of maximal egg production of Baltic key copepod species and rearing/field conditions.

Species	Study	Acclimation			Temperature	References
Name	Lab/Field	T (°C)	S (psu)	Food	at EP_{MAX} (eggs female^{-1} d^{-1})	
T. longicornis	Field	1.8 to 12.6	7.5 to 7.9	natural food	3.81	Peters 2006
P.acuspes	Field	2.9 to 6.5	7.6 to 14.7	natural food	4.0	Renz et al. 2007
E. affinis	Field	2.1 to 20.2	3.7 to 4.6	natural food	6.73	Ask et al. 2006
A. bifilosa	Lab	4.0 to 24.0	4.0	natural food	18.0	Koski and Kuosa 1999

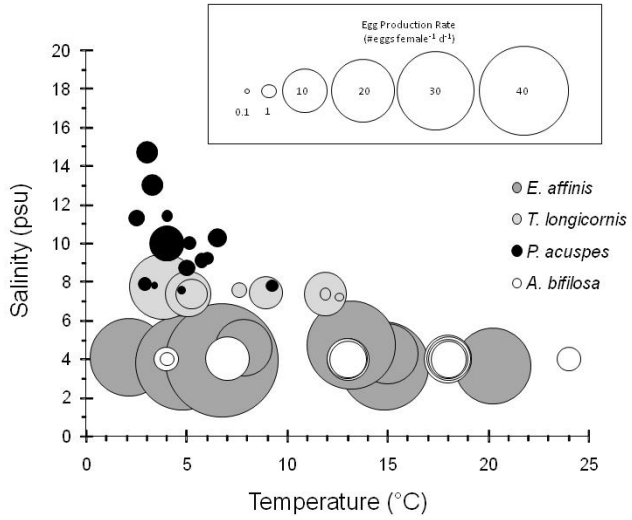

Fig.1: Egg production (# eggs female^{-1} d^{-1}) of four different key copepod species in the Baltic Sea affected by temperature and salinity.

In contrast to *A. tonsa*, its congeners found in the Baltic Sea are present throughout the year. The standing stocks of *A. bifilosa* and *A. longiremis* consists mainly of nauplii with a peak in April/May. Highest abundances of copepodites and adults are found in June/July (GLOBAN Data base, GLOBEC Germany). Recent studies (Dutz 2007) have found that prevailing temperatures are the driving mechanism for *A. longiremis* abundance. High naupliar abundance is due to the hatching of resting eggs from the sediments when temperatures increase in late spring (Dutz *et al.* 2004). The match of optimal conditions (including besides temperature also food availability) leads to high recruitment. Egg production rates are highest in summer, consisting partly of resting eggs. For *A. bifilosa*, temperature did not appear to directly influence recruitment potential. Egg production rates are highest in early spring. Resting eggs seem to hatch earlier at the beginning of the year, when temperatures are relatively low. A mismatch situation would cause severe losses of recruits due to unfavourable conditions. The highest adult abundance is found in fall. Both species are not restricted to a specific salinity and dwell in the upper water column down to the phytoplankton maximum (GLOBAN data base, GLOBEC Germany). In contrast, *A. clausii* is mostly restricted to the deeper waters in the halocline, not being able to withstand low

salinities in the upper water column. Reproduction and abundances are low throughout the year.

Aquaculture: Advancements and Outlook for viable Species

The heavy reliance on e newly hatched brine shrimp (*Artemia* sp.) nauplii for live food in aquaculture led to shortages of the availability of cysts (the "Artemia crisis") and, at the present time, the demand greatly exceeds the availability of *Artemia* cysts). Since the late 1970s, a research began focusing on finding an alternative, convenient, economical live food source. Additionally, aquaculture species have been added (*e.g.,* subtropical species and marine ornamental fish, see Payne and Rippingale 2000) that have relatively small mouth sizes (*e.g.,* grouper, Toledo *et al*. 1999). *Artemia* and many strains of rotifers are too large for the first-feeding larvae of those species.

Specific, attractive qualities to any potential species to be used as live food in aquaculture include: 1) ease of laboratory culture, 2) short generation times, 3) high fecundity, 4) consistency of nutritional quality, 5) small early life stages (for feeding fish larvae having small mouth gapes) and, finally, 6) the possibility to store harvested eggs/cysts and the high hatching of those eggs/cysts after storage. *A. tonsa* is a good alternative to other live feeds used in marine fish aquaculture because it fulfils all of these requirements. The information gained in this thesis on the responses of *A. tonsa* to various environmental factors and to rearing/harvesting procedures (MANUSCRIPT 1, 3, 4 and 5), advances the ability to effectively culture this species on scales relevant to the aquaculture industry cultivation. Some environmental factors appear particularly relevant to intensive culture protocols including photoperiod, light intensity and stocking density due to their impacts on egg harvest and egg hatching. Nonetheless, the triggers for resting egg production of this species were not explicitly examined (a logical next step). Results of the present studies suggest that low photoperiod and low temperature may cue the production of resting eggs, but this has to be tested in future investigations employing longer incubation times required to study resting egg hatching. Moreover, studies that include the potential interactive effects of both photoperiod and temperature will hopefully help disentangle the mechanisms responsible for resting egg production. The ultimate goal is the production of either high quality subitaneous eggs that hatch rapidly (for feeding during production periods within intensive or extensive culture facilities) or resting eggs for storage (between production cycles).

Although much experience has been gained in culturing different calanoid species, there is still a need for more progress. This includes the optimal feeding regime for cultures, the optimal quality (size and nutrition), and how to ensure the food is available for the copepods to maximise production. Finally tank hygiene needs to be maintained and eggs or nauplii harvested. A method that includes all these features in the most efficient manner, requiring a minimum of labour and ensuring stability would be a great step forward towards the introduction of calanoid cultures in mariculture.

Quiecent eggs of *A. tonsa* can be collected and stored for later use. Although the storage time is limited, these stored eggs are useful as a supplement during low periods of eggs production or during rearing when larger quantities of nauplii are required than afforded by

the production system on a daily basis. Work towards improving the quality of the cold-stored non-diapause eggs may help to increase their benefits.

Table 2: Summary of optimal environmental conditions for rearing *A. tonsa* and *T. longicornis* in intensive cultures as life feed for fish.

Factor	*Acartia tonsa*			*Temora longicornis*		
	EP	*HS*	*SaS*	*EP*	*HS*	*NS*
T_{opt} (°C)	24.7	≥ 23.0	na	16.6	na	12.0
S_{opt} (psu)	14.0	≥ 13.0	na	20.0	≥ 20.0	20.0
F_{opt} (cells mL^{-1})	na	na	≥ 12500	na	na	na
Ph_{opt} (h)	12:12	24.0	na	na	na	na
StD_{opt} (ind L^{-1})	65.0	no effect	na	na	na	na

EP =Egg Production
HS =Hatching Success
NS =Naupliar Survival
SaS =SizeatStage
T_{opt}=Optimal Temperature
S_{opt}=Optimal Salinity
F_{opt}=Optimal Foodquantity
Ph_{opt}=optimal Photoperiod
LI_{opt}=optimal Light Intensity
StD_{opt}=optimal Stocking Density

Modelling: Improvements for Parameterizations of abiotic Factors

One of the keys to success in building trophodynamic models representing complex marine ecosystem will be to formulate models to include 1) reasonable aggregations of species into plankton functional groups (PFTs) of similar life history traits and with similar (inter-) relationships with abiotic factors and other PFTs (*e.g.*, trophic interactions), and 2) complex parameterizations required to simulate key species/genera (individual based models – IBMs, structured population models – SPMs). These two criteria should allow modellers to adequately (yet parsimoniously) represent trophic links and both approaches require knowledge of key components, processes and vital rates.

Estimates of physiological traits in models are often based on single reports or observations. On one hand, biological rates obtained from laboratory studies are usually species-specific and not necessarily representative for a functional type. On the other hand, rates derived from field observations strongly depend upon sampling time and location and the prevailing environmental conditions and species assemblages. Hence, rates from field studies can just be considered as a rough approximation of the relevant value. Generally, there is still a need to improve our knowledge about underlying processes connecting environmental factors with species or functional groups. All attempts to understand and simulate the dynamics of ecosystems will remain inconclusive in case of imprecise parameterisations and inadequate spatial and temporal scales for the target organism (De Young *et al.* 2004).
Most extensive data collections were made for marine copepods in terms of global rates for growth, production and mortality (Huntley and Lopez 1992, Hirst and Kiørboe 2002, Hirst and Bunker 2005). As an example, relatively little is known about the functional response (reaction norms) of copepod reproductive success (*i.e.* egg production and hatching success) to temperature, light intensity, photoperiod and salinity based on a wide enough range in (and a sufficiently large number of different levels of) those abiotic factors (MANUSCRIPT 1, 2, 3, and 4) -the latter factor being especially important in brackish

and estuarine systems like the Baltic Sea. Furthermore, for the parameterisation of regional models, the local or seasonal (mass) variability of certain zooplankton taxa needs to be considered as well as the possibility of intra-specific adaptation processes. Here, parameter estimates from the same geographical areas rather than global rates are more appropriate. Hence, when needed, models should be parameterised so that they are not only species- but also population- specific. Specific populations may have adapted to local and/or regional conditions, thus shifting (changing) responses to external factors. The impacts of a certain environmental factor on a specific vital rate may be so different to warrant completely new functions and parameter estimates (see MANUSCRIPT 2). Issues related to acclimation and phenotypic plasticity are at the present time, not implemented within models targeting copepods.

Authentic and reliable predictions with models used nowadays (no matter what type) are not possible yet. But with the help of models valuable hindcasts can be done to help to understand processes. Therefore models serve as useful tools when trying to find explanation for *e.g.,* regime shifts and the impact of climate change on ecosystem dynamics.

Perspectives: Gabs in Knowledge and next Steps

A variety of gaps in knowledge remain concerning all three of the topics addressed within this thesis. For being able to model copepods as well as for optimizing mass cultures in aquaculture, the need of understanding the ecology of the target species is essential. This and other studies are mainly focused on one or in exception two factors. To really understand target species, the interactions of factors (abiotic as well as biotic) have to be investigated in the future. The Baltic Sea is characterized by strong vertical gradients of temperature, salinity and oxygen as well as a great horizontal salinity gradient. Therefore this system serves as a very good study side since inhabitants of this system might show a different recruitment behavior or life cycle strategy than in other system where conditions are more homogeneous. For realistic modeling the local challenge of environmental factors (horizontally and vertically) of species and/or population has to be taken into account. Data collected in the Bornholm Basin give evidence for vertical migration of *Temora longicornis* (Schmidt 2006)-a behavior from which copepods are forced to undergo sudden changes in environmental conditions (*i.e.* temperature, salinity, oxygen- and food concentration). Understanding the potential trait-off of benefitting from higher temperatures in the upper water column and taking into account a limitation due to salinity versus vice versa (Fig.2) would be disentangle able by long and short term acclimation experiments.

Therefore the understanding of interaction of temperature, salinity and oxygen may play a very important role on this` species vital rates. As seen in MANUSCRIPT 2in this thesis, *Temora longicornis* does not profit from exposure to higher salinity when it has been reared at low salinities before. So to really understand the life strategy, longer term measurements on cohorts reared for more than one generation have to be done. Short term versus long term shifts in gradients would then give a proxy for adaptation or acclimation potential.

A recent study examined how temperature and salinity gradients (*e.g.,* due to movement of copepods through thermo- or haloclines) impacted the swimming activity of *A. tonsa* (Peck and Holste, unpubl. Data, for methods see Appendix). The percentage of time moving (%)

was found to be strongly linked to temperature copepods only experienced a change in temperature (Fig.3A).

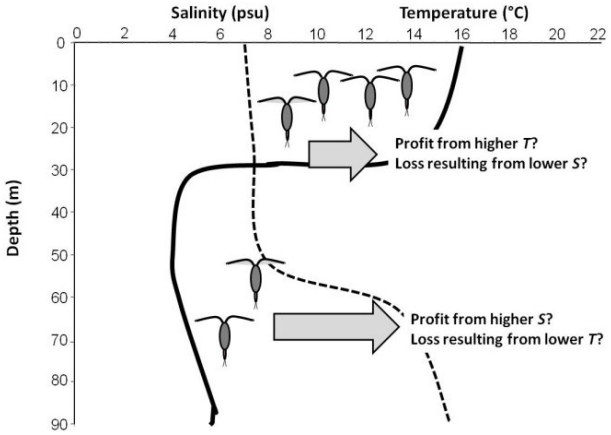

Fig.2: Schematic depiction of trade offs in the Baltic Sea due to strong halo- and thermoclines.

Simulations of a Baltic Sea situation, where copepods were faced with changes from low temperature and high salinity to high temperature and low salinity (simulating movements from below to above halo- and thermoclines) exhibited a less pronounced increase in activity. This leads to the suggestion that the reduced salinity is negatively masking the positive impact of temperature on activity (Fig.3B). This underscores the need to examine the interaction of key factors to reveal parameterizations most relevant for depicting field situations encountered by copepods (*e.g.,* temperature x salinity x O_2 concentration in the Baltic Sea).

Besides the special hydrography, biotic factors such as food availability and -quantity and mortality due to predation must also be investigated. Mortality (natural mortality including mortality due to predation) estimates are made by using the vertical or horizontal life table approach (*e.g.,* Ohman *et al.* 2002). These methods require a precise determination of species-specific development times and field collections that take into account advection of water masses (and copepods within them). Mortality functions estimated in the laboratory would help field and modeling approaches.

As indicated in the discussion, food availability causes variability in slow and fast growers that are characterized by larger or smaller body size. The effect of body size caused by temperature on egg production is well demonstrated (*e.g.,* Durbin *et al*. 1983). To investigate the effect of fast or slow growth on egg production would disentangle the question on what life strategy a species chooses in poor/good feeding conditions.

Overall these challenges of understanding the ecology of copepods the question remains of how adaptive single species are and where the tolerances of external forcing end. In times of global climate change with a relatively rapid change of abiotic and biotic environments only species with a high potential for acclimation and adaptation will be able to remain within their original system and/or spread to new habitats. Therefore the investigation of phenotypic

plasticity and plasticity in life cycle strategy, including resting egg dynamics and other overwintering strategies will be essential for the understanding of the changing systems all over the world. Common garden experiments on species inhabiting different systems (*e.g., A. longiremis* or *T. longicornis*) with different life cycle strategies will give valuable hints on 1) triggers causing certain behavior and 2) the plasticity of species to adapt to changing environmental conditions.

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ACKNOWLEDGEMENTS:

Finally I would like to thank all the people that supported me during my studies in many different ways.

First of all I would like to express my deep gratitude to my supervisor and mentor Prof. Ph.D. Myron Peck. He taught me everything! Most of all, he taught me to stay curious, interested and open-minded and to never stop asking questions. He fed me with inexhaustible ideas and never hesitated to help me realizing - at least the makeable amount of - them. With his trust in me and my capabilities it was a lot easier to also cross one or the other "dark valley" of science. Myron, thank you for your friendship and patience!

I would like to thank Prof. Ph.D. Michael St John for always reminding me of the "big picture", valuable comments on a variety of scientific topics and for philosophical comments on marine science. Thank you for being there when needed (not only scientifically), for your patience and trust!

Prof. Dr. Axel Temming is to thank for many constructive scientific comments, for enduring encouragement and for his (almost) always sunny mind that always exhilarates my mood.

I also would like to thank Prof. Dr. Christian Möllmann for valuable comments on the *Temora* research, for guiding my focus into the right direction and for teaching me that one should not take oneself (and sometimes others) too seriously.

I am beholden to Dr. Janna Peters for scientific as well as personal help, encouragement, good words, strength and many other things in all circumstances! You are an all-round talent - a rock star- you make my day!

I want to thank Dr. Ute Daewel for being for over three years the best office mate in the world, for sharing science and family issues - for becoming a really good friend!

Also I have to thank Dr. Rabea Diekmann - not only for helpful comments on research but also for fruitful and encouraging "daily routine" discussions.

Patricia Gorre is to be gratefully thanked for never refusing help to get through the administrative jungle! Without your patience and friendliness many days would have been wasted.

Sven Stäcker, Jochen Lütke and Michael Grossmann are to thank for all the effort they put into providing equipment, to help realizing experiments, maintaining cultures, soccer debates and many more things.

Klas Möller, Philipp Kanstinger and Stefan Meyer I have to thank for sharing office (Klas, I promise I will cheer you up the same way you did with me) and many hours in the laboratory (Philipp and Stefan: Thank you for taking over my aquarium duties in the "hot phase" of this thesis) rearing critters and helping out when experiments were designed slightly too big (Philipp) for one person.

Dr. Hannes Baumann I want to thank for endless discussions about graphs that will make us rich, about fish and copepods, about programs and many many more topics. Most of all for a deep friendship!

I am deeply grateful to my parents, Inge and Ewald Fiedler who always supported me and my wish to become a scientist. They never questioned my way and always backed me unconditionally in difficult situations. I have to thank my brother Till, his girlfriend Swantje and especially my sister Laura for helping out with encouraging phone calls or babysitting. My parents in law, Christa und Giselher Holste, are to be thanked for supporting me with babysitting and a lot of faith in me and my work.

Last but not least, I am in greatest gratitude to my husband Erdwig and my children Leonard and Hannah. They always grounded me, strengthen me and teach me day by day that science is not everything worth living for. Without you all this would be senseless!

CHAPTER V:

APPENDIX:

a) Swimming activity of *Acartia tonsa* in thermo- and haloclines:

INTRODUCTION

Spatiotemporal changes in species composition can be driven by processes occurring over short time intervals and at small spatial scales such as accumulations and interspecific interactions (*e.g.,* differences in escape responses to predators) at "hot spots" (Kimoto 1988, Kils 1992, Viitasalo et al. 1998). Such small-scale topics were among a short list of recommended avenues for future research on marine zooplankton (see review by MZC2 2001). As part of proposed research within the "RECONN" AQUASHIFT (DFG) project, changes in predator-prey dynamics were to be explored. This was an especially germane topic due to the partitioning of Baltic Sea habitats not only due to narrow ranges in physiological tolerances of animals that encounter sharp thermo- and haloclines, but also in terms of foraging strategies and risks of predation. Costs and tradeoffs of Baltic habitat partitioning might be revealed by examining changes in swimming activity rates of copepods and the foraging efficiency of their larval fish predators at different levels (or gradients) in temperature and salinity.

In order to develop methods to track the individual activity of copepods to assess how patterns of swimming activity changed in response to changes in abiotic conditions, two pilot studies were conducted using *A. tonsa*. Patterns of swimming activity have previously been examined in *A. tonsa* to assess, among other things, hydrodynamic signalling (Kiørboe *et al.* 1999), mate encounter rates (Kiørboe and Bagøien 2005) and escape responses from predators (Buskey *et al.* 2002). The following is a brief description of two pilot studies conducted to assess the impacts of thermo- and haloclines on *A. tonsa* swimming activity.

METHODS:

Experimental set up:

Measurements of the swimming activity of *A. tonsa* were made by capturing digital movies of the movements of individuals within a group of copepods that had been acclimated to experimental glass aquaria (25 x 25 x 30 cm). The glass aquarium (30 x 30 cm) was held within a temperature-controlled water bath (40 x 50 cm). Then having two underwater cameras mounted at 90° angles to one another on a 30x 30 cm rack. The cameras were the same height and captured images of copepods swimming within a 2.5 cm^3 water volume (Fig.1). The camera system was designed for 3-D video observations.

Procedure during filming:

Adult *A. tonsa* were reared using methods described within MANUSCRIPTS 1, 3 and 4 and removed from tanks by sieving through a 280 µm sieve. Approximately 20 individuals L^{-1} were carefully transferred using a pipette in order to keep the water as clean as possible. Individuals were allowed to acclimate to the test conditions for 24 h. Afterwards, a total of 300 to 420 min of activity were recorded. Two preliminary trials were conducted, a thermocline trial (Trial 1) and a thermo-halocline trial (Trial 2). During the former, the activity was recorded at 20°C for 30 min and then the temperature was decreased to 10°C

over the course of 3.5 hrs. The temperature was then increased at the same rate until approximately 20°C. In Trial 2, activity during a change in both temperature and salinity was recorded. In that trial, copepods were acclimated for 24 hrs to the test chamber at 9°C and activity was recorded at that temperature for 30 minutes and then the temperature was increased to 19°C over the course of 2.25 hrs. At the same time that the temperature was increasing, the salinity was reduced from 18 to 8 psu. The latter was accomplished by carefully reducing the water with a reverse filter and adding freshwater to the tank.

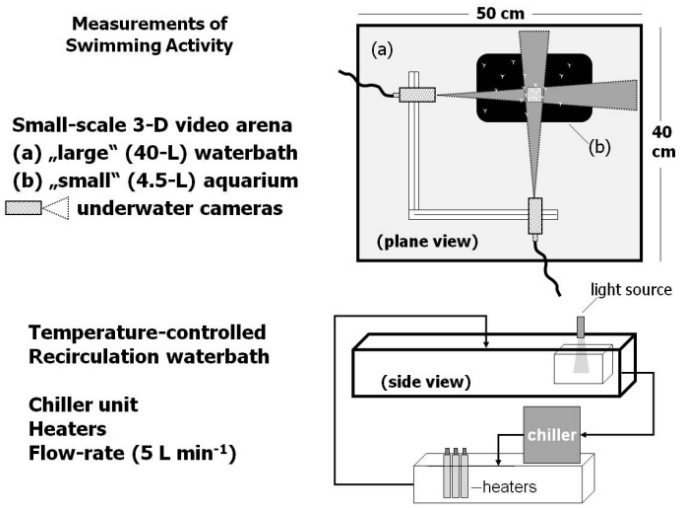

Fig. 1: Schematic drawing of experimental set up for testing the effect of temperature and temperature/salinity interaction on *A. tonsa* swimming activity.

Procedure with collected film material:

The digital film was converted from .mov into .avi format and then individual copepods were tracked using approximately every 10 min a sequence of 20 sec that was cut out to be analyzed with the help of Lab Track. With the help of this program particles can be tracked with time and the covered distance and speed of a copepod within a certain number of frames can be calculated.

RESULTS: see conclusive discussion

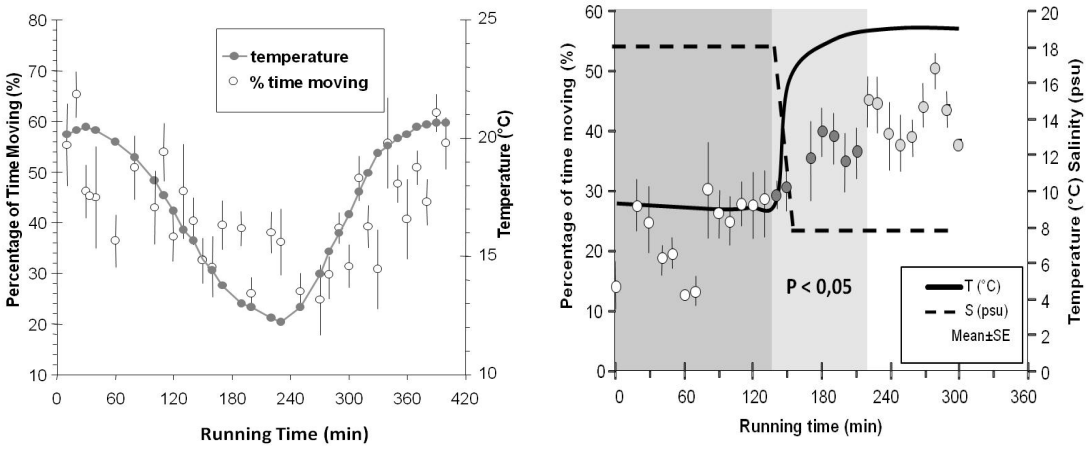

Fig.3: Activity (percentage time moving) of *A. tonsa* affected by A) temperature and B) by temperature and salinity interactions.

b) "Faster smaller" and "slower larger": Intra-cohort growth variability in a calanoid copepod (*Acartia tonsa*)

INTRODUCTION

A general feature the becomes apparent during the intensive rearing of *Acartia tonsa* for aquaculture (200 L tanks) is the large, intra-cohort variability that exists in the development rate (stage versus age) of copepods grown within the same tank. Large mesocosm studies (*e.g.,* Klein-Breteler 1994, this study) capture the variability in development better than laboratory experiments conducted using low numbers (10's) of individuals within relatively small volumes (a few liters). Unfortunately, the vast majority of studies has been made under the latter conditions where the dynamics of cohort development, including the variability in stages and lengths of individuals of the same age, are likely not captured.

The effects of feeding level on growth rate and bioenergetics of *A. tonsa* have been previous studied (*e.g.,* Kiørboe et al. 1985). However, with respect to intra-cohort growth variability, intriguing questions remain unanswered including: 1) What physiological mechanisms create such vast differences in growth rate among individuals? And 2) why is marked growth variability maintained within certain populations/strains and is this an heritable trait? The following study was conducted to examine whether intra-cohort variability in size- (and stage-) at-age (growth and development rate) was influenced by feeding level. If cohorts of copepods maintained at low feeding levels exhibited the same degree of variability in growth as cohorts of copepods maintained at *ad libitum* feeding levels, then differences in growth among individuals may not necessarily be due to merely differences in feeding rate among individuals.

METHODS:

Acartia tonsa used in this study were cultured as described in MANUSCRIPT 1, 3 and 4.

Refrigerated eggs were hatched and *A. tonsa* cohorts reared within six, 350 L "starter culture" tanks. *A. tonsa* was maintained at a density of 30 to 50 ind. L^{-1} in these tanks and fed *Rhodomonas sp.* at ≥ 50 000 cells mL^{-1} each day. The experiment was conducted within a controlled-environment room having a 12L:12D light regime with surface light intensities of 1 to 5 µE (µmol m^{-2} s^{-1}).

In this experiment, the effect of food availability on cohort development and characteristics was studied using eleven *A. tonsa* cohorts. To produce eleven cohorts of the same characteristics (ages, stages, numbers, etc.), a total of 100,000 eggs was loaded into one 300 L tank containing 275 L filtered (cartridge filter, nominal 1 µm) seawater supplied with gentle aeration to produce a homogeneous hatch. The incubation temperature was 20°C and the salinity was 18 psu and light regime was 13L:11D. These conditions were chosen to decrease hatching time and increase hatching percent (Holste & Peck 2006). After 6 h and 12 h, aeration was removed for 45 min and unhatched eggs were carefully removed by siphoning the bottom of the tank. At 48 h post-hatch, the nauplii were transferred into 11 tanks each containing 140 L of filtered seawater at an initial density of 225 nauplii L^{-1}, a naupliar density used in other studies (*e.g.,* Berggreen *et al.* 1988). The experiment was conducted using a 12L:12D light regime.

A high algal concentration (50 000 cells mL^{-1} *Rhodomonas sp.*) was maintained in each container until > 50% of the nauplii had developed to stage C_1. On this day (day 0 of the experiment), nine of the eleven cultures were randomly chosen to be fed less than 50 000

cells ml-1 of *Rhodomonas*. In total six different feeding levels were created by dilution of the cultures: 1500, 3000, 6000, 12 500, 25 000 and 50 000 cell ml^{-1} (two replicates at each feeding level except 1500 cells ml^{-1}). Algal cell concentrations were estimated daily from three counts (Coulter Counter, TA II) made on three ~60 ml subsamples of water from each control and experimental tank. Previous experiences growing *A. tonsa* cultures with *Rhodomonas* sp. at high food concentrations algal concentrations indicated that detritus and faecal pellets accumulated (personal observations). To minimize grazing on detritus and faecal pellets by copepods, each day the aeration was stopped for 30 min and the bottom of each tank was cleaned using a siphon. Within the daily samples, faecal pellets and detritus rarely occurred, demonstrating that the technique worked well and that grazing on detritus and faecal pellets was minimized during the experiment.
During the experiment, the number of copepods L^{-1} was decreased (by removing a known number) as individuals grew and developed: C_1 = 133 ind. L^{-1}, $C_{2\&3}$ = 100 ind. L^{-1}, $C_{4\&5}$ = 50 ind. L^{-1}, adults = 30 ind. L^{-1}. Daily samples of a total of 10,000 to 900 individuals $tank^{-1}$ were collected from the surface, middle, and bottom portions of the tank using a siphon. The number of individuals sampled depended upon the biomass of individuals. Each of the copepodites sampled was anaesthetised with sparkling mineral water containing carbonic acid to facilitate rapid sorting for further analyses. Samples were taken every day until egg production was noticed for seven days to ensure that generations did not mix.

Determination of prosome length
For the determination of the prosome length, 22 to 95 *A. tonsa* individuals from each tank were videotaped using a Panasonic NV-FS 200 HQ video recorder. To accomplish this, small groups of individuals were placed on a slide in a drop of water and put under a Wild ® Heerbrugg M3 dissecting scope with built-in video camera (Theta system CCD Video camera module) connected to the video recorder. For image analysis, the analogue videos were digitised using the VCR Digitising program (Cyber Link 2.55 SE+). Prosome length was determined via computer image analysis using a second software package (Optimas 6.51). Length measurements made each day were pooled by treatment.

Determination of stage
Daily sampling included 11 to 159 individuals $tank^{-1}$ for stage determination. For this purpose the individuals were fixed in formalin (e.g. Durbin and Durbin, 1978) and staged later with aid of a Leica MZ 95 dissecting microscope using a standard method (Klein Breteler 1982).

Statistic:
A one-way ANOVA was used to test for differences in length and stage between treatments. All statistical tests were performed using SPSS (SPSS 1990) and were considered significant

RESULTS:
Adult individuals with a food availability of ≥ 25 000 cells ml^{-1} of *Rhodomonas* were significantly greater in mean length than adult individuals reared at feeding levels from 3 000 to 12 500 cells ml^{-1}. Only very few individuals reached the adult stage in the lowest feeding level (1 500 cells ml^{-1}). They were significantly shorter than all other treatments (Fig. 2). Variability in all treatments was high and not significantly different.

Comparing the lengths of first adults (fast growing copepods) within a treatment with the adults after one week of egg production (mixture between fast growing copepods and slow growing individuals), one find that independently of feeding level, slow growing copepods are larger in length than fast growing individuals. Interestingly the variability in length of fast growing copepods under high feeding conditions (50 000 cells ml^{-1}) is lower than in the other treatments.

DISCUSSION:

An advantage of the design this study was that it allowed calculations of intra-cohort variability in lengths and stages at daily intervals. In the high feeding treatments (ad libitum feeding), one might expect to find reduced intra-cohort variability in lengths compared to low feeding level treatments. However, this was only the case for fast growing adults. The present study clearly indicates that high intra-cohort variability existed in lengths all treatment groups. In all cohorts, there existed "winners" and "losers" and, until the time of first egg production.

Clearly it was shown, that fast growing individuals are smaller than the slow growers. Since large copepods are able to produce more eggs than smaller individuals (ref), the benefit of growing slow would be a higher egg production $female^{-1}$. On the other hand, fast growing copepods would not produce as many eggs, but the population would be able to undergo more generations per year. The latter strategy would be applicable in warm waters with a low to intermediate food availability, while the first strategy is probably more applied in boreal waters, where phytoplankton blooms occur and seasonal mass abundances have to be produced by high egg production. Within this production, overwintering eggs would have to be formed as well.

Unfortunately, few studies have collected the data required to calculate intra-cohort variability in stage and length, so comparisons of these findings and others are difficult. However, these unexpected results in the present study agree well with those of another mesocosm study (Klein-Breteler 1994) where high intra-cohort variability existed in stage distribution of *Acartia clausi* at the time of occurrence of adults.

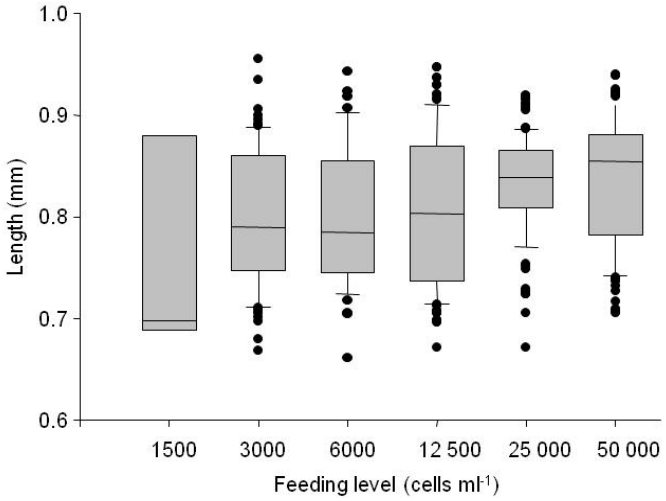

Fig. 2: *A. tonsa* adult size of six different feeding levels (cells ml^{-1}). Data represent groups of individuals that are, depending on developmental rate between, 1 and 7 days (data collected on day after which egg production was observed for one week) old.

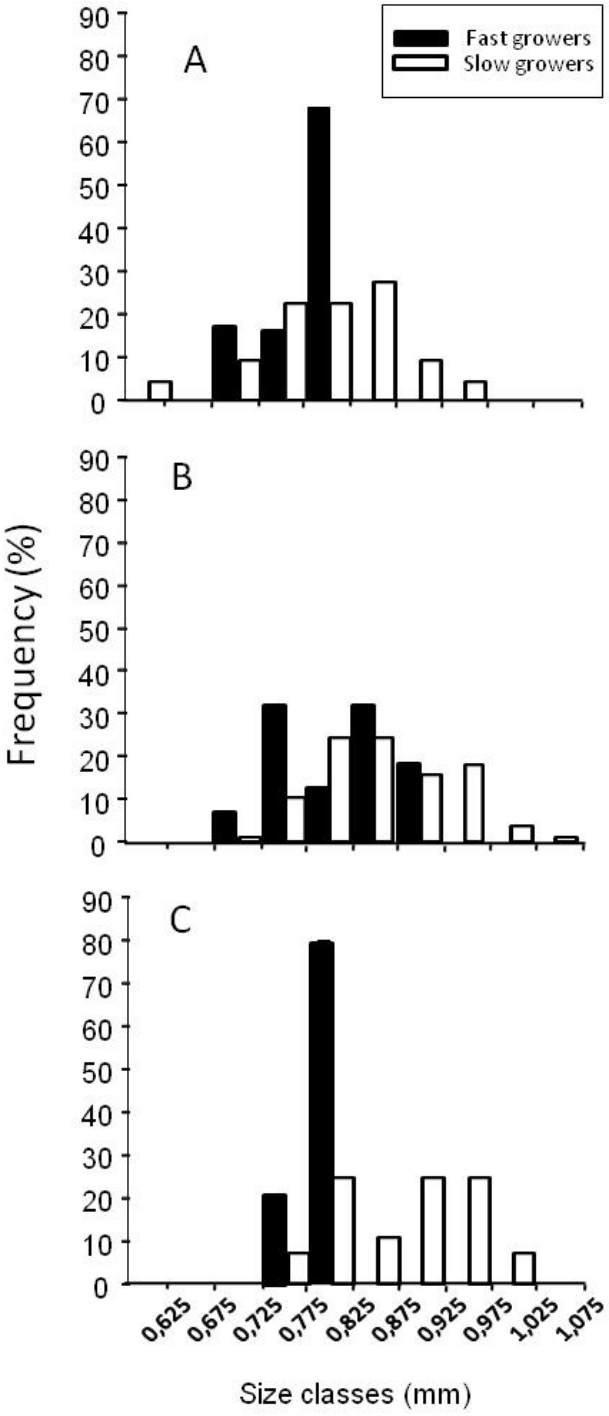

Fig. 3: *A. tonsa* adult size frequency of fast and slow growing individuals. Panel A) 3000 cells ml^{-1}, B) 12 500 cells ml^{-1} and C) 50 000 cells ml^{-1})

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Eidesstattliche Erklärung
(Gem. § 7(d) PromO des Fachbereichs Biologie Universität Hamburg)

Hiermit versichere ich, Linda Holste, an Eides statt, dass ich die vorliegende Arbeit
1. ohne unerlaubte, fremde Hilfe angefertigt habe,
2. keine anderen, als die von mir im Text angegebenen Quellen und Hilfsmittel benutzt habe und
3. die den benutzen Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Desweiteren erkläre ich, dass ich Zuhörer bei der Disputation zulasse.

Hamburg, 9. März 2009

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ZUSAMMENFASSUNG:

In dieser Arbeit wird der Einfluss verschiedener Umweltfaktoren auf die Vitalraten zweier Copepoden-Schlüsselarten (*Acartia tonsa* und *Temora longicornis*) der Ostsee untersucht. Hierbei stand vor allem der Reproduktionserfolg dieser Arten im Vordergrund. Copepoden sind die Hauptnahrungsquelle von allen marine Fischlarven und planktivoren Fischen. Daher ist es von großer Wichtigkeit Wissen über den Einfluss auf Populationen dieser Arten und deren mögliche Reaktion auf veränderte Umweltbedingungen (z.B., klimatische und/oder ökosystematische Veränderungen) zu gewinnen. Diese Arbeit setzt sich aus vier Kapiteln zusammen, von denen zwei aus fünf wissenschaftlichen Artikeln bestehen. Diese beiden Kapitel sind von einer allgemeinen Einleitung und einer abschließenden Diskussion eingerahmt.

In dem ersten MANUSKRIPT "The effects of temperature and salinity on egg production and hatching success of Baltic *Acartia tonsa* (Copepoda: Calanoida): A laboratory investigation" wird der funktionale Zusammenhang zwischen Reproduktionserfolg (Eiproduktion und Eischlupf) und Temperatur sowie Salinität quantifiziert. Um eine Modellierung des Zusammenhangs zu ermöglichen, wurde ein großer Bereich an Temperaturen und Salinitäten abgedeckt. Der optimale Temperaturbereich für die Eiproduktion sowie den Schlupferfolg von *Acartia tonsa* liegt zwischen 22 und 23°C. Bei Temperaturen über 20°C schlüpften 90% der Eier innerhalb der ersten 24 Stunden. Eier, die bei 10°C oder bei geringen Temperaturen produziert wurden, zeigten keinen Schlupferfolg, was vermutlich auf eine Temperatur induzierte Produktion von Dauereiern zurückzuführen ist. Salinitäten höher als 17 psu führten zu einem stark erhöhten Schlupferfolg von *A. tonsa* Eiern. Der hohe Reproduktionserfolg von *Acartia tonsa* bei unterschiedlichsten Umweltbedingungen erklärt möglicherweise die weltweite Verbreitung dieser Art innerhalb verschiedenster produktiver, mariner sowie estuarer Habitate.

In MANUSKRIPT 2: "The effects of temperature and salinity on reproductive success of *Temora longicornis* in the Baltic Sea: a copepod coping with a tough situation" werden die Einflüsse von Temperatur und Salinität auf den Reproduktionserfolg und die Nauplliensterblichkeit von *Temora longicornis* charakterisiert. Die optimale Reproduktionstemperatur für diese Ostseepopulation liegt bei 17°C. Sowohl Eiproduktion als auch –schlupferfolg waren stark von der Salinität (Hälterung- sowie Inkubationssalinität) beeinflußt. Bei Salinitäten über 14 psu fanden sich höchste Eiproduktionen, wenn Hälterungs- und Inkubationssalinität übereinstimmten. Unterhalb von 14 psu konnte keine maximale Eiproduktion erreicht werden und die Weibchen profitierten auch nicht von einer höheren Inkubationssalinität. Maximaler Eischlupf konnte bei Salinitäten höher als 24 psu gemessen werden, jedoch variierte der Schlupferfolg stark mit der Hälterungsalinität der Weibchen. Die Nauplliensterblichkeit bei sechs verschiedenen Temperaturen und zwei Salinitäten (7 und 20 psu) zeigte deutlich, dass hohe Temperature und geringe Salinitäten zu einer erhöhten Mortalität führen. Diese Ergebnisse, im Zusammenhang mit einem Populationsvergleich aus der Literatur, geben Hinweise auf die Reaktion von *T. longicornis* auf unterschiedliche Temperatur- und Salinitätsbedingungen.

MANUSKRIPT 3 und 4 "Effects of salinity, photoperiod and adult stocking density on egg production and egg hatching success in *Acartia tonsa* (Calanoida: Copepoda): Optimizing intensive cultures" und "Impacts of light regime on egg harvests and 48-h egg hatching success of *Acartia tonsa* (Copepoda: Calanoida) within intensive culture" behandeln die

praktische Anwedung von Copepodenkuturen in der Aquakultur als Lebendfutter für Fischlarven. Zur Optimierung der Kulturbedingungen, wurden verschiedene Umweltfaktoren wie Lichtintensität, Photoperiode, Salinität und Individuendichte innerhalb der Kultur und deren Auswirkung auf Eiproduktion bzw Eiernte und Schlupferfolg getestet. Ebenfalls wurde der Einfluß der Langzeit-Eilagerung (unter anoxischen, kühlen und dunklen Bedingungen) quantifiziert. Diese beiden Artikel geben wertvolle Ratschläge, wie man Massenkulturen von *A. tonsa* optimieren und damit kosten- und zeitsparend als Lebendfutter hältern kann.

Im fünften MANUSKRIPT: "Handling Copepods and Egg Production Rates: A Note of Caution" wird auf die Empfindlichkeit der Copepoden im Bezug auf deren Akklimatisierung und auf das Behandeln vor Experimenten eingegangen. Hierzu wurde eine Metaanalyse zur Eiproduktion von *A. tonsa* durchgeführt. Die Eiproduktion steigt aufgrund des nachlassenden Stresses, verursacht durch Umsetzen/handling und der Anpassung an die experimentellen Bedingungen konstant innerhalb der ersten Tage signifikant an. Es sollte unter anderem getestet werden, ab welchem Zeitpunkt signifikante Unterschiede zwischen Temperaturunterschieden nicht mehr durch Akklimatisierungseffekte überlagert werden. Dabei stieg die Anzahl signifikant unterschiedlicher Temperaturen zwischen Tag 3 und 4 von 30 auf 70% an. Es wird deutlich, dass ohne eine Phase der Akklimatisierung und der Erholung nach der Einsetzung der Copepoden in die experimentellen Container viele getestete Faktoren unterschätzt werden können.