Linking environmental factors and the reproductive success of *Acartia tonsa* DANA (Copepoda: Calanoida)

Dissertation

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SUMMARY

The aim of this thesis was to further our understanding of the calanoid copepod *Acartia tonsa*. Manuscript 1 (chapter II) focuses on the trophodynamic interactions between primary producers and *A. tonsa*. It was examined how nutrient availability influenced the growth dynamics and biochemical content of the marine diatom *Thalassiosira weissflogii* and how these biochemical changes impacted the vital rates of *A. tonsa*. Changes in biochemistry (protein, carbohydrate, fatty acids) were compared in diatom cultures that simulated bloom conditions (*B*-algae) and those maintained in near exponential growth (*E*-algae) over the course of a 16d experiment. Egg production rates (*EPR*, eggs female^{-1}d^{-1}) and the developmental success of copepodite stages of *A. tonsa* fed these different diets were quantified. Copepod *EPR* was significantly lower (reduced by half) when *B*-algae entered the senescent phase due to silicate limitation. In a crossover (diet switch) experiment, *EPR* increased when copepods fed *B*-algae were switched to *E*-algae and vice versa. Copepodites of *A. tonsa* developed normally and reached the adult (CVI) stage when fed *E*-algae but ceased development (approximately at stage CII) when reared on senescent phase *B*-algae. Given the importance of copepods as prey for higher trophic levels, our results highlight how nutritional changes that naturally occur during a phytoplankton bloom may influence the productivity of copepods and higher trophic levels.

Chapter III (manuscript 2) centers on its life history strategy and especially on the production of *RE* for overwintering. Potential resting egg (*RE*) production in *A. tonsa* was examined and evidence for phenotypic plasticity among eggs as well as maternal effects on egg hatching characteristics was found. It was tested whether incubation photoperiod (6, 11, 16h) and/or temperature (15, 20, 25°C) affected the proportion of eggs that, between 72 hrs and 3-weeks of age, displayed specific hatching characteristics and whether the 'origin temperature' at which males and females were reared (15, 20, 25°C) also influenced egg development. Exposure to a long photoperiod (*LP*) resulted in a significantly lower proportion of eggs displaying a delayed hatch (*DHE*). A warm origin temperature (25°C) led to significantly less *DHE* whereas a cold incubation temperature (15°C) resulted in significantly lower and higher

proportions of *DHE* and potential resting eggs (*RE*), respectively. The production of potential resting eggs by *A. tonsa* appears to result from photothermal cues experienced by both the parents as well as their eggs and demonstrate, at least for this SW Baltic Sea population, a plasticity in response to local environmental conditions experienced prior to or shortly after reproduction.

Chapter IV (manuscript 3) summarizes the aspects of the life history strategy of *A. tonsa* and compares it with other dominating calanoid copepods in the Baltic Sea. The results of laboratory experiments on reproductive success and field time series data on adult abundance to explore factors controlling the seasonality of *Acartia* spp., *Eurytemora affinis* and *Temora longicornis*, key copepods of brackish, coastal, temperate environments were combined. Over a 5-yr period, changes in adult abundance within two southwest Baltic field sites (Kiel Fjord *Pier*, 54°19´89N, 10°09´06E, 12 to 21 psu, and North / Baltic Sea Canal *NOK*, 54°20´45N, 9°57´02E, 4 to 10 psu) were evaluated with respect to changes in temperature, salinity, day length, chlorophyll *a* concentration (*Chl a*). *Acartia* spp. dominated the copepod assemblage at both sites (up to 16764 and 21771 females m^{-3} at *NOK* and *Pier*) and was 4- to 10-times more abundant than *E. affinis* (to 2939 m^{-3} at *NOK*) and *T. longicornis* (to 1959 m^{-3} at *Pier*), respectively. Species-specific salinity tolerance explains differences in adult abundance between sampling sites whereas phenological differences among species are best explained by the influence of species-specific thermal windows and prey requirements supporting survival and egg production. Multiple intrinsic and extrinsic (environmental) factors influence the production of different egg types (normal and resting), regulate life history strategies, and influence match-mismatch dynamics.

In Chapter V (manuscript 4) daily measurements of the reproductive characteristics (egg production rate (*EPR*) and egg hatching success (*HS*)) of *Acartia tonsa* (Copepoda:Calanoida) were made during 12, relatively long-term (6 to 12-d), laboratory trials. The influence of culture conditions including subtle differences in photoperiod, temperature and food quantity (diatom, *Thalassiosira weissflogii*), and more marked differences in salinity (8, 18, 33 psu) and algal quality (wide ranges of algal growth rates and protein-carbohydrate ratios) as well as female age on reproduction was examined. We

report the daily magnitudes and the time course of changes in *EPR* and *HS* and relate these, when possible, to intrinsic and extrinsic factors. Based upon observed trends in *EPR* and *HS*, practical recommendations on the protocols used to measure copepod *EPR* for ecological studies, toxicity bioassays, and aquaculture production are provided. For example, consistent changes in *EPR* with time (9 of 12 trials) suggest that short-term (1- to 4-day) measurements of copepod *EPR* will likely not capture maximum reproduction rates. Depending upon the absolute test temperature, subtle changes in temperature (±2°C) may have little impact on *EPR* but large effects on short-term *HS*. The *HS* was often consistently high (e.g., with no variability among replicates) but, in some trials, became unexpectedly variable or consistently low. These changes were associated with a switch in feeding methods. These trials demonstrate that a combination of interacting factors can be expected to influence both the mean and variance of *EPR* and *HS* in calanoid copepods demanding tight control of rearing and measurement protocols.

ZUSAMMENFASSUNG

Mit dieser Doktorarbeit sollte das Wissen über den calanoiden Copepoden *Acartia tonsa* erweitert werden. *A. tonsa* hat in den Jahren seit seiner ersten Beschreibung in europäischen Gewässern in den 20er Jahren des 20. Jahrhunderts eine wichtige Position im Nahrungsnetz und bei der Artenverteilung eingenommen. Als ursprünglich tropische Art wird diese Art voraussichtlich zukünftig noch weiter an Bedeutung gewinnen und von der prognostizierten Erwärmung profitieren können.

In Manuskript 1 (Kapitel II) dieser Doktorarbeit werden die Verknüpfungen und Veränderungen im Nahrungsnetz thematisiert. Dabei wurde untersucht wie sich die verfügbaren Nährstoffe auf das Wachstum und die biochemische Zusammensetzung der marinen Diatomee *Thalassiosira weissflogii* auswirkt und wie die Veränderungen der biochemischen Zusammensetzung der Alge *A. tonsa* beeinflusst. Die biochemische Zusammensetzung der Alge, bezogen auf Proteine, Kohlenhydrate und Fettsäuren, wurde über einen Zeitraum von 16 Tagen zwischen Algenkulturen, mit denen eine Blüte simuliert wurde (*B*-Algen), und solchen, die in nahezu exponentiellem Wachstum gehalten wurden (*E*-Algen) verglichen. Außerdem wurde untersucht, wie sich die Eiproduktionsrate (*EPR*, Eier Weibchen^{-1} Tag^{-1}) und der Entwicklungserfolg der Copepoditenstadien zwischen Gruppen von *A. tonsa*, die mit den unterschiedlichen Algen gefüttert wurden, unterschied. Dabei hat sich ergeben, dass die Eiproduktion signifikant niedriger war (um die Hälfte reduziert) nachdem die *B*-Algen die seneszente Phase der Blüte erreicht hatten. Limitiert waren die *B*-Algen in diesem Fall durch Silikat. Ein Kreuzexperiment hat gezeigt, dass die Eiproduktion wieder anstieg, wenn ursprünglich mit *B*-Algen gefütterte Copepoden stattdessen mit *E*-Algen gefüttert wurden. Dies galt auch umgekehrt: Die Eiproduktion von mit *E*-Algen gefütterten Copepoden ist drastisch gesunken, wenn diese statt mit *E*-Algen mit *B*-Algen gefüttert wurden. Die Copepodite von *A. tonsa* haben sich normal zum Adulten entwickelt, wenn sie mit *E*-Algen gefüttert wurden, verstarben jedoch ungefähr im CII-Stadium, wenn sie mit den seneszenten *B*-Algen gefüttert wurden. Da Copepoden das wichtigste Futter für höhere trophische Ebenen, wie z.B. Fischlarven, darstellen, zeigen diese Ergebnisse, wie

natürliche Veränderungen an der Basis der Nahrungskette, bei den Primärproduzenten, höhere trophische Ebenen in ihrer Produktivität beeinflussen können.

Kapitel III (Manuskript 2) dieser Doktorarbeit behandelt die “life history strategy” und insbesondere die Produktion von Dauereiern („resting eggs“, *RE*) durch *A. tonsa*. Potenzielle Dauereier von *A. tonsa* wurden gefunden sowie Hinweise für die phänotypische Plastizität der Eier selber. Auch gab es einen Effekt von mütterlicher Erfahrung bestimmter Umweltbedingungen auf die Schlüpfeigenschaften der Eier. Getestet wurde, ob die Inkubations-Photoperiode (6, 11, 16h) und/oder Temperatur (15, 20, 25°C) einen Einfluss darauf hatte, wie viel Prozent der Eier, zwischen 72h und drei Wochen nachdem sie gelegt wurden, bestimmte Schlüpfeigenschaften aufwiesen. Außerdem wurde untersucht, ob auch die Ursprungstemperatur, in welcher die Männchen und Weibchen gehältert wurden (15, 20, 25°C) einen Einfluss auf die Eientwicklung hatte. Dabei hat sich ergeben, dass unter einer langen Photoperiode (*LP*) signifikant weniger Eier verspätet schlüpfen („delayed hatch eggs“, *DHE*). Eine warme Ursprungstemperatur (25°C) hat ebenso zu signifikant weniger *DHE* geführt, während die Inkubation bei nur 15°C zu signifikant weniger *DHE*, aber zu mehr potenziellen Dauereiern (*RE*) geführt hat. Daher kann man schlussfolgern, dass die Produktion von Dauereiern durch photothermale Auslöser hin erfolgt, wobei diese sowohl von den Eltern als auch von den Eiern selbst erfahren sein können. Dies zeigt zumindest für diese Population aus der südwestlichen Ostsee eine Plastizität als Antwort auf die lokalen Umweltbedingungen, die vor oder kurz nach der Reproduktion vorherrschen.

In Kapitel IV (Manuskript 3) werden die Aspekte der “life history strategy” von *A. tonsa* zusammengefasst und mit anderen dominierenden Arten der Ostsee verglichen. Dafür wurden Labordaten von Experimenten zum reproduktiven Erfolg und Felddaten einer Zeitserie zur Abundanz der adulten Copepoden untersucht. Es sollten Faktoren herausgefunden werden, welche die Saisonalität der Schlüssel-Copepodenarten der küstennahen Brackwassergebiete der gemäßigten Zonen (*Acartia* spp., *Eurytemora affinis* und *Temora longicornis*) erklären. Die Abundanzen der adulten Copepoden wurden über den Zeitraum von fünf Jahren an zwei Stellen der südwestlichen

Ostsee (Kiel Fjord *Pier*, 54°19´89N, 10°09´06E, 12 bis 21 psu, und Nord-Ostsee-Kanal *NOK*, 54°20´45N, 9°57´02E, 4 bis 10 psu) zusammen mit Daten über die Temperatur, Salinität, Photoperiode und Chlorophyll *a* Konzentration erfasst. Es hat sich gezeigt, dass *Acartia* spp. an beiden Probennahmestellen dominierend war (bis zu 16764 Weibchen m^{-3} am *NOK* und bis zu 21771 Weibchen m^{-3} am *Pier*). Damit war *Acartia* spp. in 4- bis 10-mal höheren Abundanzen vorhanden als *E. affinis* (bis zu 2939 Weibchen m^{-3} am *NOK*) und *T. longicornis* (bis zu 1959 Weibchen m^{-3} am *Pier*). Die artenspezifischen Toleranzen gegenüber der Salinität erklären die Unterschiede der Abundanzen der verschiedenen Arten an den beiden unterschiedlichen Probenahmestellen. Phenologische Unterschiede zwischen den Arten aber werden am besten durch artenspezifische Temperaturfenster und Futteransprüche erklärt. Es wirken also multiple intrinsische und extrinsische Faktoren auf die Produktion verschiedener Eitypen (subitan und Dauereier), regulieren die "life-history-strategy" und beeinflussen "match-mismatch dynamics".

In Kapitel V (Manuskript 4) wurden tägliche Messungen der Reproduktion (Eiproduktionsrate und Schlüpferfolg) von *Acartia tonsa* in zwölf relativ langen (6 bis 12 Tage) Laborversuchen durchgeführt. Es wurde untersucht wie sich geringe Unterschiede Photoperioden, Temperaturen, Futtermengen der Diatomee Thalassiosira weissflogii und deutlichere Unterschiede in Salinität (8, 18 und 33 psu) und Futterqualität sowie Alter der Weibchen auf die Reproduktion von *A. tonsa* auswirken. Anhand der von uns untersuchten Trends der Eiproduktionsrate und des Schlüpferfolges geben wir praktische Anweisungen für Eiproduktionsmessungen von Copepoden für ökologische und ökotoxikologische Studien sowie für die Aquakultur. Beispielsweise zeigt sich in 9 der 12 Versuche, dass in Kurzzeitmessungen (1 bis 4 Tage) der Eiproduktionsrate vermutlich nicht die maximal möglichen Reproduktionsraten erreicht werden können. Abhängig von der absoluten Temperatur im Versuch können geringe Veränderungen in der Temperatur wenig Einfluss auf die Eiproduktionsrate haben, jedoch großen Einfluss auf den Schlüpferfolg. Dieser war meistens stabil und hoch (ohne viel Varianz), zeigte aber in manchen Versuchen eine hohe Varianz oder sehr niedrige Werte. Die hohe Varianz und die niedrigen Schlupfraten waren verknüpft mit einer Veränderung im

Futtermodus. Insgesamt hat sich ergeben, dass eine Kombination verschiedener interagierender Faktoren bei calanoiden Copepoden sowohl die mittlere Eiproduktion und den mittleren Schlüpferfolg beeinflussen als auch deren Variabilität.

OUTLINE OF PUBLICATIONS

In the following an outline of the manuscripts included in this thesis and the contribution of the authors to the manuscripts is given.

CHAPTER II

Manuscript 1) Variation in diatom biochemical composition during a simulated bloom and its effect on copepod production

A. Berenike S. Diekmann, Myron A. Peck , Linda Holste, Michael A. St. John and Robert W. Campbell

Berenike Tietjen (née Diekmann) designed the experiments with the help of Prof. Myron Peck, Prof. Michael St. John and Robert Campbell. Berenike Tietjen conducted the experiments, analysed the data and wrote the manuscript. A specific sub-set of the experiment (the copepodite growth experiment) was designed, conducted and analysed by Linda Holste. This work was funded by a grant from the Germany Science Foundation (DFG) in the AQUASHIFT priority program 1162 (“RECONN” grant) awarded to Prof. Myron Peck and Prof. Michael St. John. Robert Campbell helped by an introduction to the laboratory and some of the methods. All co-authors provided editorial comments to earlier versions of this manuscript. This manuscript was published in the *Journal of Plankton Research* (2009), a peer-reviewed journal.

CHAPTER III:

Manuscript 2) Photothermal conditions and plasticity in resting egg dynamics of *Acartia tonsa* (Copepoda: Calanoida)

A. Berenike S. Tietjen, Silke Laakmann, Klaus Huebert and Myron A. Peck

Berenike Tietjen and Prof. Myron Peck designed the experiments. Berenike Tietjen conducted the experiment together with Silke Laakmann. Berenike Tietjen analysed the data and wrote the manuscript. Klaus Huebert helped with some data analysing and produced the figure used in this manuscript. This work was funded by a grant from the Germany Science Foundation (DFG) in the AQUASHIFT priority program 1162 (“RECONN” grant) awarded to Prof. Myron Peck and by a fellowship from the project Pro Exzellenzia funded by the European Social Fund and the agency for science and research Hamburg awarded to Berenike Tietjen. All co- authors provided editorial comments to earlier versions of this manuscript. This manuscript is submitted to *Aquatic Biology*, a peer-reviewed journal.

CHAPTER IV:

Manuscript 3) Environmental cues and constraints affecting the seasonality of dominant calanoid copepods in brackish, coastal waters: A case study of *Acartia*, *Temora* and *Eurytemora* species in the Southwest Baltic

A. Berenike S. Diekmann, Catriona Clemmesen, Michael A. St. John, Matthias Paulsen and Myron A. Peck

Berenike Tietjen analysed the data and wrote the manuscript. Catriona Clemmesen and Matthias Paulsen provided the field data. This work was funded by a grant from the Germany Science Foundation (DFG) in the AQUASHIFT priority program 1162 (“RECONN” grant) awarded to Prof. Myron Peck, Dr. Catriona Clemmesen and Prof. Michael St. John. All co-authors provided editorial comments to earlier versions of this manuscript. This manuscript was published (2012) in *Marine Biology*, a peer-reviewed journal.

CHAPTER V:

Manuscript 4) Changes in the Reproductive Potential of Calanoid Copepods during Laboratory Trials

A. Berenike S. Tietjen, Klaus Huebert and Myron A. Peck

Berenike Tietjen and Prof. Myron Peck designed the experiments. Berenike Tietjen conducted the experiments and analysed the biochemical data. Klaus Huebert helped with additional data analysing and produced the figures used in this manuscript. This work was funded by a grant from the Germany Science Foundation (DFG) in the AQUASHIFT priority program 1162 (“RECONN” grant) awarded to Prof. Myron Peck and by a fellowship from the project Pro Exzellenzia funded by the European Social Fund and the agency for science and research Hamburg awarded to Berenike Tietjen. All co- authors provided text passages and editorial comments to earlier versions of this manuscript. This manuscript is for submission to PLoS ONE, a peer-reviewed journal.

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Certification of Written English Quality

I certify the the thesis by A. Berenike S. Tietjen titled „Linking environmental factors and the reproductive success of *Acartia tonsa* DANA (Copepoda: Calanoida)„ has been prepared according to excellent written English language standards.

Sincerely,

Prof. PhD. Myron A. Peck

Hamburg 15.12.12

CHAPTER I

GENERAL INTRODUCTION

The calanoid copepod *Acartia tonsa* Dana

Origin and range

Reproduction

Salinity and Temperature ranges

Food requirements

Predation effects

Match – mismatch scenarios in temperate waters

Aims of the study

GENERAL INTRODUCTION

THE CALANOID COPEPOD *ACARTIA TONSA* DANA

Origin and range

Acartia tonsa is originally a tropical species and has been reported in European waters since the early 20th century (Brylinski 1981 and references therein). Genetic analyses performed by Drillet et al. (2008) suggested that the Baltic population of *A. tonsa* probably originated from a population in Narragansett Bay, Rhode Island (USA) or adjacent waters. Originating from tropic waters, *A. tonsa* is generally adapted to warm temperatures and it occurs throughout the whole year e.g. in Chesapeake Bay where it dominates the crustacean zooplankton most of the year (Heinle 1966). Fig. I–1 summarises findings on the seasonality of *Acartia tonsa* adults within different estuaries and bays along the east coast of the USA. In some of these locations, *A. tonsa* is found year-round but it is most abundant during warmer months (May to October).

In contrast to warm water environments, in temperate/sub-Arctic waters such as the Baltic Sea, *A. tonsa* occurs at high abundance only during short periods of the year, e.g. starting in late spring, (Fig. I–2), when the first generation arises from the hatching of resting eggs from the sediments in response to favourable conditions (Christiansen 1988). In this and other temperate/sub-Arctic marine and estuarine systems, *A. tonsa* populations persist only as resting eggs (*RE*) during the winter, when virtually no eggs, nauplii, copepodites or adults are found in the water column (e.g. Sullivan and McManus 1986, Gubanova 2000, Katajisto 2006). Unfortunately it is difficult to distinguish *A. tonsa* from other *Acartia* species residing in the North and Baltic Seas making it difficult to find field data that separate *A. tonsa* from the other *Acartia* congeners. Moreover, the various *Acartia* species are adapted to very different environmental conditions. For example, *Acartia longiremis* females undergo dormancy during winter (Davis 1976, Norrbin 1996) while *A. bifilosa* and *A. clausi* are adapted to colder temperatures (Schnack 1975, Uye and Fleminger 1976) and oversummer as resting eggs in the North Sea (Chinnery and Williams 2003).

Although estuarine habitats often contain a large number of copepod species, *A. tonsa* is one of the most numerically dominant members of the copepod community (Mauchline 1998). For Apalachicola Estuary (Florida, USA), Marcus (1991) reported *A. tonsa* to be the dominating planktonic copepod species throughout the year, always exceeding 50% of the total numerical abundance of copepod (adults and copepodites). Heinle (1966) reported *A. tonsa* to comprise more than 50% of the crustacean zooplankton during 7 months of the year within the Patuxent River a tributary of Chesapeake Bay, USA. In nearshore coastal waters off of La Jolla, California, USA, *A. tonsa* was found to be one of the dominant copepods throughout the year (Esterly 1928, Fleminger 1967). During summer months, Jeffries (1962) reported that adult *A. tonsa* formed only a small percentage (10%) of the total planktonic copepods but this percentage increased to 90% when nauplii and copepodites were also included.

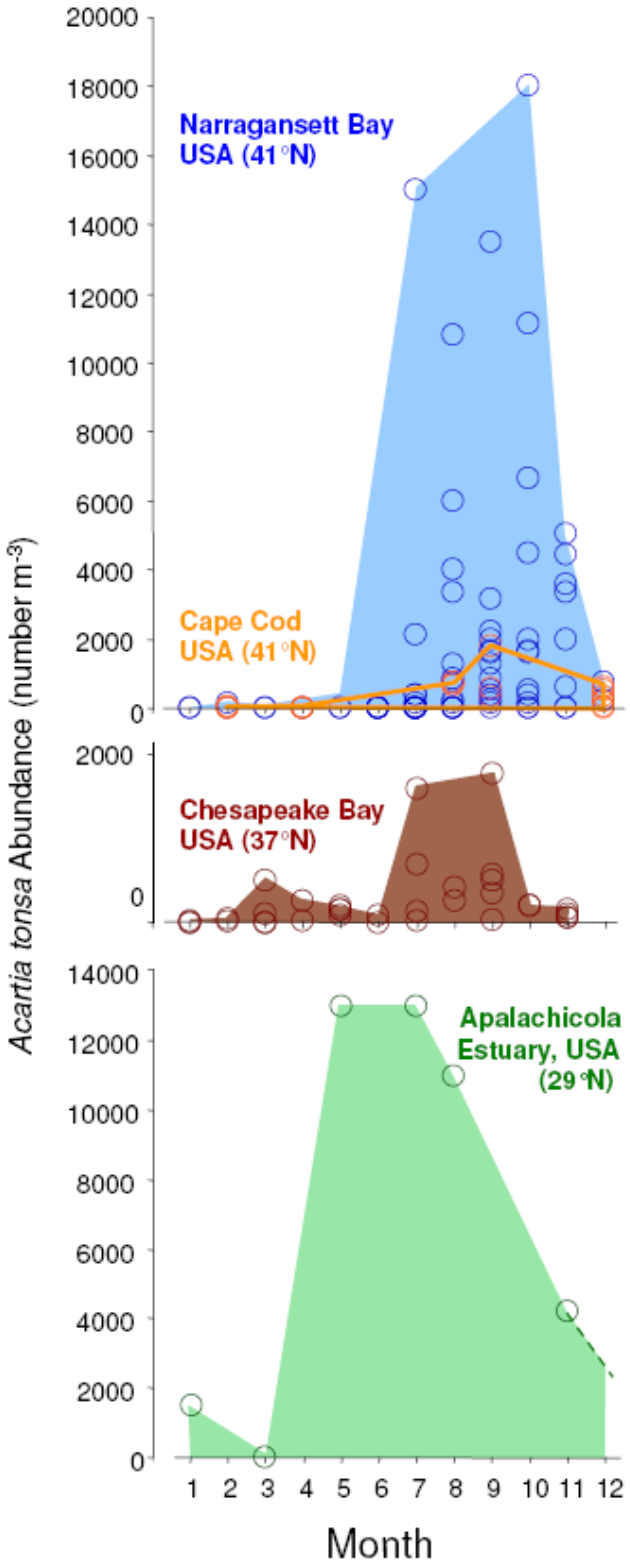

Fig. I–1 Seasonal trend of adult *Acartia tonsa* concentrations in field measurements at different locations along the east coast of the USA: A Narragansett Bay and Cape Cod (41°N), B Chesapeake Bay (37°N) and C Apalachicola Estuary (29°N). The data for Apalachicola Estuary and parts of the data for Narragansett Bay include adults and copepodites. Data from Narragansett Bay come from Sullivan and McManus (1986) and Sullivan et al. (2007), data from Cape Cod Bay come from Anraku (1964), data from Chesapeake Bay come from Elliott and Tang (2011b) and data from Apalachicola estuary come from Marcus (1991)

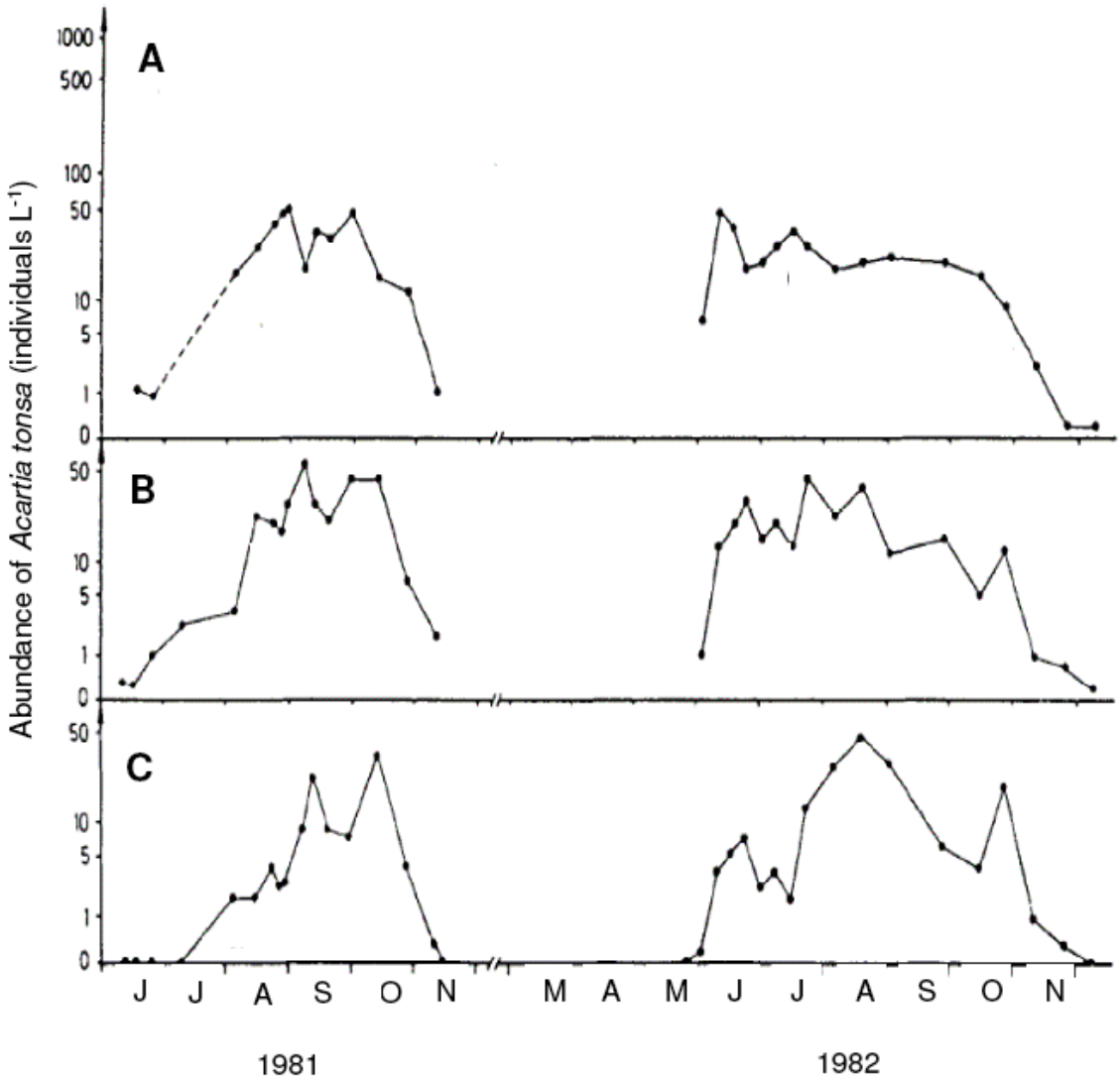

Fig. I–2 Seasonal abundances of *Acartia tonsa* (A) nauplii, (B) copepodites and (C) adults of the years 1981 and 1982 at the Schlei Estuary, Baltic Sea, Germany plotted on log scale. After Christiansen 1988

Reproduction

Acartia tonsa as is an opportunistic feeder and adults do not build up large energy reserves but, rather, invest most assimilated energy into egg production (Dagg 1977; Durbin et al. 1983; Kiørboe et al. 1985a). Moreover, the energetic response of *A. tonsa* (such as changes in egg production rate) to a new food source appears to be rapid (<24h: Dagg 1977; Kiørboe et al. 1985a; Kiørboe et al. 1985b; Stearns et al. 1989; Tester and Turner 1990).

In the laboratory *A. tonsa* was found to be reproductively active and to produce eggs at high rates for ≥ 31 days at 18°C (Parrish and Wilson 1978). Stearns and Forward (1984) reported that females migrated into surface waters at night and Stearns et al. (1989) reported that eggs were released at night. Broadcast spawning eggs at the surface may maximize the probability that eggs will hatch before being deposited onto bottom sediments. The egg production rate (eggs female^{-1} day^{-1}; *EPR*) of *A. tonsa* can be used as a proxy for the condition of the female and is influenced by a number of environmental factors including salinity (Peck and Holste 2006), temperature (Holste and Peck 2006, Hansen et al. 2010) and the quantity and quality of food (Durbin et al. 1983, Drillet et al. 2011). Furthermore, copepod *EPR* may initially increase and then subsequently decrease with increasing age of females (Durbin et al. 1992, Jónasdóttir 1994, Jónasdóttir and Kiørboe 1996). Maximum *EPR* of *A. tonsa* are reported to be about 50 eggs female^{-1} day^{-1} (Dagg 1977).

The *EPR* is not the only factor to be considered when assessing population-level consequences of copepod reproduction. Viability of offspring is important to assess and, due to natural mortality, not all eggs hatch and not all nauplii are viable after hatching. For *A. tonsa* in the field, the hatching success (*HS*) was reported to be linked to *EPR* such that when *EPR* was high (in the earlier season), *HS* was also high and when *EPR* was low (later in the season), *HS* was found to be quite variable (0-90%, Tang and Dam 2001). Bottom-up factors have been assumed to operate when mortality has been observed during naupliar stages in other species.

For example, although a high percentage of the eggs of *Pseudocalanus newmani* hatched (up to 80%), a relatively large percentage of the nauplii (20 to 40%) were deformed (Ban et al. 2000). In this and other studies, the occurrence of deformed copepod nauplii has been discussed in light of potential toxic effects of diatoms as prey (Laabir et al. 1995, Poulet et al. 1995, Ceballos and Ianora 2003, Ianora et al. 2004). Non-viable eggs (eggs that die and disintegrate) have also been reported. The underlying cause(s) for non-viable eggs is(are) thought to be either 1) nutritional deficiency of the diets which induces the female to produce low-quality eggs, 2) the transfer of harmful substances from adult females to their eggs that suppress normal development, and/or 3) the release of extracellular substances by phytoplankton which inhibit normal egg hatching (Tang and Dam 2001 and references therein). Another potential reason for non-viable eggs is 4) lack of egg fertilization, or 5) fertilization but mortality due to polyspermy (due to multiple attachments of spermatophores to the females, Parrish and Wilson 1978).

Clearly, a distinction needs to be made between eggs that do not hatch and eggs that are non-viable. Four different egg types have been reported for calanoid copepods:

1) Subitaneous eggs, which hatch directly within the first days after being laid;
2) Quiescence eggs, which pause facultatively, when conditions are unfavourable, but hatch as soon as conditions get well again;
3) Diapause eggs, which do not hatch within an obligate refractory phase;
3) Delayed hatching eggs, which do not directly hatch despite favourable conditions, but which also do not show a longer cessation of development displayed by diapause eggs

The triggers of the production of the different egg types are still not completely investigated. As in most studies eggs are only incubated for a few days it is not known if at least some proportions of the unhatched eggs found in these studies might have been resting, i.e. quiescence, diapause or delayed hatching eggs. Table I–1 gives an overview over triggers for resting egg production reported in the literature.

Table I–1 Summary of triggers found to induce resting eggs (as dormant, quiescence or diapausing)/delayed hatching eggs (*DHE*) and to terminate the resting period

Species	Type of egg	Period of resting	Effect on egg type	By an	Parameter	Location	Reference
A. tonsa	quiescent	laboratory	induced	increase	salinities below 5 psu	Øresund, Denmark	Højgaard et al. 2008
A. tonsa	dormant		decreased	decrease	photoperiod	Narragansett Bay, USA	McManus 1986
A. tonsa	*DHE*	laboratory	increase	low	food availability	Baltic Sea	Drillet et al. 2011
A. hudsonica	dormant	summer	increased	increase	*T*	Rhode Island, USA	Avery 2005b
A. hudsonica	dormant	summer	unaffected	increase	*T* and photoperiod	Maine, USA	Avery 2005b
A. hudsonica	dormant	summer	increased	increase	*T*	Narragansett Bay, USA	Avery 2005a
A. hudsonica	resting/*DHE*	summer	induced		*T* >16°C	Narragansett Bay, USA	Sullivan and McManus 1986
A. hudsonica	*DHE*	summer	termination of rest	decrease	*T*	Narragansett Bay, USA	Sullivan and McManus 1986
A. bifilosa	diapause	summer	increase	increase	*T*	Southampton Waters, UK	Castro-Longoria + Williams 1999
A. clausi			termination of rest	induction of light after being held in darkness	light	San Juan Island, USA	Landry 1975
A. clausi	diapause	summer	induced	increase	*T* and photoperiod	Inland Sea of Japan	Uye 1985
Centropages hamatus	diapause	summer	termination of rest	decrease	of *T* after a warm period	Alligator Harbor region, Florida, USA	Marcus 1989
Eurytemora affinis	diapause	winter	induced	decrease	*T* and photoperiod	Lake Ohnuma, Japan	Ban 1992
E. affinis	diapause	winter	induced	increase	population density	Lake Ohnuma, Japan	Ban 1992
Labidocera aestiva	diapause	winter	termination of rest	increase	of *T* to 25°C after 1-2 months of chilling at 5°C		Marcus 1980
Labidocera aestiva	diapause	winter	induced	decrease	light and *T*	Virginia, USA	Marcus 1984

Optimal and suboptimal ranges in abiotic conditions

Similar to many invertebrates, *Acartia tonsa* is an osmoconformer, but it is an extremely euryhaline species. Cervetto et al. (1999) reported that *A. tonsa* from a Mediterranean estuary tolerated a wide range of salinities (1 to 72 psu) for short time periods (<10h) but that, longer-term (72h) optimal salinities were between 15 and 22 psu. Within tolerable (non-lethal) limits of salinity, sub-lethal effects of the increased metabolic load associated with osmotic stress at sub-optimal salinities results in lower *EPR*. For example, when acclimated and measured at 6, 10, 14, 20, and 30 psu, the *EPR* by *A. tonsa* was highest at 14 psu (Peck and Holste 2006). Furthermore, the salinity changes experienced by adult copepods could be passed on to the eggs, resulting in a difference in the specific gravity of eggs spawned at different salinities (Lance 1963). At extreme levels, hyper- or hyposmostic conditions cause death, but the ability to cope with osmotic stress can be influenced by other factors. Lance (1963) was the first to demonstrate that temperature affected low salinity tolerance of *A. tonsa*. In that study, using a UK coastal population, this species could tolerate lower salinities (to 3.6 psu) at 16 to 20°C but adults could only tolerate higher salinities outside this temperature optimum (e.g., 5.4 psu at 10 and 24°C or 9 psu at 4.5°C). The ability of *A. tonsa* to utilize free amino acids in hyposmoregulation (Farmer and Reeve 1978, copepods off Florida) appears to be limited at cold (winter) temperatures. Without sufficient time to acclimate to low temperatures (e.g., during periods of rapid cooling), mass mortality could occur in areas of low ambient salinity (0 to 6 psu).

Optimum temperature (T_{opt}) for *EPR* of *A. tonsa* collected from the southwest Baltic Sea was found to be 23°C and 100% mortality was reported at 34°C for *A. tonsa* (Holste and Peck 2006). Working with a population collected in the Gulf of Mexico (off the coast of Texas), Ambler (1985) found the prosome length decreased from 0.79 to 0.61 mm at 21 and 29°C, respectively. Hansen et al. (2010) reported that *A. tonsa* eggs also decrease in size with increasing temperature.

Food requirements

While various studies have focused on temperature as the main factor controlling copepod production, match-mismatch dynamics with phytoplankton often control the productivity of species such as *A. tonsa* in the field (Durbin et al. 1983). In Narragansett Bay, Durbin et al. (1983) reported that *EPR* by *A. tonsa* decreased to negligible levels when chlorophyll *a* (*Chl a*) was 1 to 2µg L^{-1}. The biochemical composition of phytoplankton partially depends upon nutrient status and may play an important role in its quality as a food to grazers. The biochemical composition of marine algae varies with changes in growth rate due to variation in temperature, light and nutrient availability (Redalje and Laws 1983, Palmisano et al. 1988, Mortensen et al. 1988, Hayakawa et al. 1996). Under nutrient-rich conditions, diatoms usually dominate phytoplankton communities and can account for 40% of the total primary production in the oceans (Sarthou et al. 2005). The biochemical composition of diatoms is characterized by high concentrations of fatty acids (*FA*) of 16 carbon chain length (C16), especially C16:1(n-7), but low amounts of C18 *FA* and polyunsaturated fatty acids (*PUFA*) (Arendt et al. 2005 and references therein). The 16:1(n-7) to 16:0 ratio is used as a biomarker for a diatom diet in trophodynamic studies (St. John and Lund 1996, Arendt et al. 2005, Silina and Zhukova 2007). During high growth rates, such as during the onset of a bloom, diatoms tend to have low quantities of *PUFA* and it is assumed that this is due to the lack of time to synthesize complex long chain *PUFA* while growing fast (Morris et al. 1985). As a consequence of the high growth rates during the first phase of a bloom (exponential growth), nutrients are depleted and growth rates decreases. During this phase (stationary phase) the amount of *PUFA* increases in diatoms. *PUFA* are particularly prone to oxidation and oxidation products have toxic effects (Hardwick et al. 1997) maybe also on copepods. It was reported, that within 12h of silicate depletion the mass of lipid/cell doubled (Shifrin and Chisholm 1981). During post-bloom decay (third phase) saturated fatty acids (*SAFA*) and monounsaturated fatty acids (*MUFA*) characterize the lipid composition of diatoms (Mayzaud et al. 1989). With lower growth rates protein contents decrease, while during exponential growth phases protein levels are high as

DNA is synthesized continuously (Vaulot et al. 1987). In contrast, carbohydrate levels increase with decreasing growth rates (Harrison et al. 1977, Morris et al. 1985, Lynn et al. 2000).

Herbivorous zooplankton transfer energy and organic materials up the foodweb. As differences in quality and quantity of the phytoplankton influences the copepods vital rates (Scott 1980, Jónasdóttir 1994), organisms feeding on the copepods, like fish larvae, will indirectly also be affected by the interactions between phytoplankton and copepods (St. John et al. 2001). *A. tonsa* (Støttrup and Jensen 1990) and *A. omorii* (Shin 2003), require (n-3) polyunsaturated fatty acids (*PUFA*) for egg production. Broglio et al. (2003) assumed a minimum amount of essential fatty acids, which the copepods cannot synthesise themselves, to reach >60% *HS*. Furthermore, the amount of protein in the algal cell was positively correlated to copepod *EPR* in a study by Jónasdóttir (1994).

Match – mismatch scenarios in temperate waters

All autotrophs depend on the availability of nutrients and enough light to enable photosynthesis and positive population growth rates. Within temperate areas, waters are normally well-mixed during fall and winter due to storms and the water column is rich in nutrients. Algal growth is inhibited by a lack of light (Sverdrup 1953, Atkins 1930, Colebrook 1982) until the onset of stratification and, in spring, increasing temperatures and irradiance together with decreasing wind intensities establish a thermocline, stratifying the water column (Sommer 1998). As the thermocline usually occurs within the euphotic zone, above the critical depth (the depth where positive net primary production is possible), the conditions for the onset of a phytoplankton bloom are given. A large standing stock of phytoplankton represents a large stock of food for herbivorous zooplankton. Therefore a peak in phytoplankton standing stock is followed by an increase in the zooplankton abundance. Heavy predation of the micro- and macro-zooplankton can lead to top-down control of the phytoplankton stock. Besides this, large losses in phytoplankton during bloom conditions may also occur through viruses (e.g., Furman and Noble 1995, Steward et al. 1996).

Since *A. tonsa* overwinters as resting eggs in many systems, it is crucial that the eggs begin hatching from the sediments at a time when nauplii can take advantage of the high abundance of phytoplankton (Fig. I–3). Specifically, resting eggs of *A. tonsa* would need to hatch early enough in spring to match the timing of high phytoplankton stock, despite temperatures being colder than the optimal for this warm-water species. If eggs hatch too late, a mismatch might occur between nauplii and sufficient phytoplankton availability. Furthermore, *A. tonsa* will compete with a variety of other copepods and zooplankton for phytoplankton so, a spatiotemporal match with phytoplankton resources is important. Naturally, match-mismatch dynamics extend to higher (e.g., tertiary) trophic levels such as the need for young fish larvae to find their key prey items (copepod nauplii and other organisms, Peck et al. 2012).

The ultimate trigger(s) for resting egg production of copepods is(are) not well understood. For overwintering, resting eggs would need to be produced early enough in one year to ensure that the obligatory refractory period of resting eggs is completed before conditions are optimal for population increase in the following year. Some copepods might have a strategy of continuously producing a certain proportion of resting eggs or at least during periods of high *EPR* (e.g. *Temora longicornis*, Castellani and Lucas 2003) while others might react to some environmental trigger(s) and switch to producing resting eggs (e.g. *Eurytemora affinis*, Ban 1992). There are also some species that overwinter in the adult stage (e.g. *Acartia longiremis*, Norrbin 1994). Interestingly, some copepod species can synchronize the hatching of *RE* as eggs produced later in the season have a shorter refractory phase (*Labidocera aestiva*, Marcus 1987 and *Eurytemora affinis* Ban and Minoda 1991).

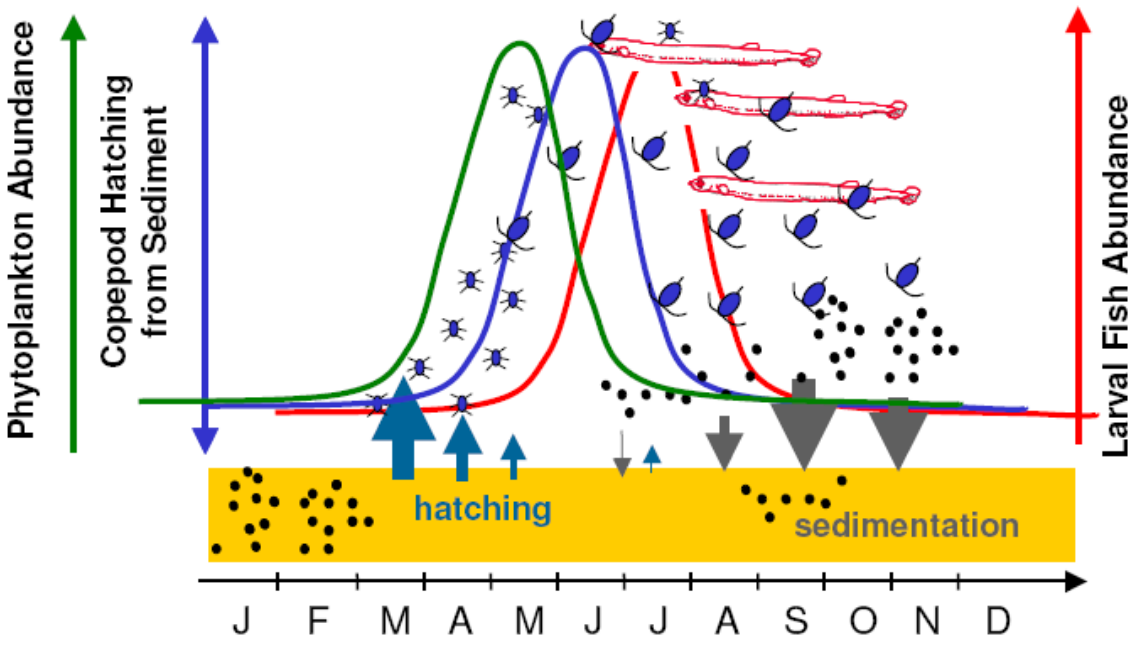

Fig. I–3 Scenario of a match of copepods (blue) in hatching from resting eggs (•, *RE*) to take advantage of a high phytoplankton stock (green) and a match of fish larvae (red) hatching in time to feed on a high copepod standing stock. After Cushing (1990)

The role of Predation in controlling *Acartia tonsa* population dynamics

Coastal areas represent important spawning sites for forage fish that are zooplanktivorous throughout all life stages such as clupeid fishes and it can be assumed that fish larvae and juveniles likely have a severe predation effect on the copepod populations in these areas (Aro 1989). Sprat (*Sprattus sprattus*) is the dominant clupeid in Baltic waters where evidence suggest that this species can exert top-down control of its copepod prey (Rudstam 1988, Möllmann et al. 2008). Gut content analyses indicated that *Acartia* spp. and *T. longicornis* formed the highest proportions of prey within the guts of larval sprat in the Baltic Sea (Dickmann et al. 2007). Trophic cascades documented in offshore areas of the Baltic Sea highlight the tight inter-dependence of copepods and clupeid fishes (e.g., Möllmann et al. 2008, Casini et al. 2011). Furthermore, ctenophores and other gelatinous zooplankton can be important predators of copepods populations during summer months (Javidpour et al. 2009).

Copepods form the most important prey for fish larvae in temperate waters and, because of this, copepods have also been grown for use in marine finfish aquaculture. Several fish species such as Atlantic cod (*Gadus morhua*), sea bream (family Sparidae) and European seabass (Dicentrarchus labrax) cannot be grown effectively using only artificial food (Drillet 2010). *Artemia* and rotifers are commonly used as live feed in aquaculture despite the need to use lipid enrichment to enhance their biochemical quality to sufficient levels for larval fish survival, development and growth (Gracia et al. 2008). Copepod nauplii could serve as a natural food source of high biochemical quality. Resting stages of copepods also be sold to aquaculture companies (similar to *Artemia* resting cysts) eliminating the need to have extensive cultures at companies specializing in early larval rearing of marine fish.

Aims of the study

The research within this thesis was conducted to provide a more comprehensive picture of the biotic and abiotic factors influencing the reproduction and life history strategy of the calanoid copepod *Acartia tonsa*. As *A. tonsa* origins from tropical waters, it can be assumed that the populations of this species that have become established within temperate and sub-Arctic systems will benefit climate-driven warming projected. For example, the population examined in this thesis stems from the southwest Baltic Sea, a brackish area that is projected to freshen and warm by 2 to 3 psu and 3.0 to 3.5°C, respectively in the next 80 years (Meier 2006).

Although *A. tonsa* is one of the best-studied, marine calanoid copepods (Mauchline 1998), large gaps in knowledge still exist regarding its trophodynamic role and life history strategy within temperate and sub-Arctic waters. Manuscript 1 (chapter II) helps to elucidate the trophodynamic interactions between primary producers and *A. tonsa*. Chapter III (manuscript 2) centres on this species' life history strategy and especially on the production of *RE* for overwintering in the southwest Baltic Sea. Chapter IV (manuscript 3) summarises aspects of the life history strategy of *A. tonsa* and compares the seasonal dynamics of *Acartia* to other key calanoid copepods in Baltic Sea coastal areas. Furthermore, in this chapter field and laboratory findings are linked. Finally, the results of 12 laboratory trials *EPR* and *HS* are presented in Chapter V (manuscript 4). In the following, a summary of the aims of each manuscript is given.

Manuscript 1)

Changes in the biochemical composition of the diatom *Thalassiosira weissflogii* Grunow (Phycophyta, Bacillariophyceae, Centrales) are tracked during a simulated bloom (*B*-algae). These changes are compared to those occurring in cultures maintained in semi continuous (exponential) growth phase (*E*-algae) in the laboratory. Furthermore, it is assessed how biochemical changes in this diatom influence its nutritional quality as a prey for *A. tonsa* by quantifying daily egg production rates (*EPR*, eggs female^{-1}d^{-1}) and copepodite development rate (*DR*, stages d^{-}1). In this manner 1) measurements of the biochemical changes occurring each day during the different phases (exponential, stationary and senescent) of diatom growth are made as well as 2) the relevance of these biochemical changes to secondary production of copepods are identified.

Manuscript 2)

To elucidate potential trigger(s) for the *RE* production the egg production, hatching and development of eggs of *A. tonsa* at three different photoperiods (short, medium and long) and three different temperatures (15, 20 and 25°C) of either parental origin or eggs incubation are analysed. The development of unhatched eggs into delayed hatched eggs (*DHE*), dead/disintegrating eggs (*DE*) and potential resting eggs (*RE*) is compared at these different light and temperature regimes.

Manuscript 3)

This manuscript summarizes the present knowledge on how temperature, salinity, day length, phytoplankton production, and the interactions among those factors, affect reproductive success and population productivity of dominant calanoid copepods inhabiting coastal waters of the southwest Baltic Sea. Information from controlled laboratory studies is compared to site-specific changes in the abundance of adult *Acartia* spp., *T. longicornis* and *E. affinis* at two field locations in the southwest Baltic Sea. Based upon the classification of Fry (1971), it is distinguished among “controlling” factors that govern metabolic rate by affecting molecular kinetics (e.g., temperature), “masking” factors that modify the effect of other environmental

factors by influencing energy allocation (e.g., salinity affecting osmoregulation), and “directive” factors that cue some response affecting energy partitioning typically via hormone induction (e.g., day length on reproduction). Differences in life cycle strategies are highlighted and it is speculated on how potential climate-driven changes in key factors would influence the copepod community and lower trophic level match-mismatch dynamics in the southwest Baltic region.

Manuscript 4)

The results of 12 laboratory trials examining *EPR* and *HS* are presented in this manuscript. These relatively long-term (8 to 15-d), well-replicated (n = 6 to 15) measurements allow practical information on variability in the reproductive success of this species. This descriptive study recommends culturing techniques to be used when *A. tonsa* is used as a test species in ecological, toxicological or aquaculture investigations.

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CHAPTER II

MANUSCRIPT 1)

Variation in diatom biochemical composition during a simulated bloom and its effect on copepod production

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MANUSCRIPT 1)

Variation in diatom biochemical composition during a simulated bloom and its effect on copepod production

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ABSTRACT

The biochemical quality of phytoplankton depends in part upon nutrient availability, which has implications for the population dynamics of grazers. Here, we examined how nutrient availability influenced the growth dynamics and biochemical content of the marine diatom *Thalassiosira weissflogii* and how these biochemical changes impacted the vital rates of a calanoid copepod (*Acartia tonsa*). Changes in biochemistry (protein, carbohydrate, fatty acids) were compared in diatom cultures that simulated bloom conditions (*B*-algae) and those maintained in near exponential growth (*E*-algae) over the course of a 16d experiment. Egg production rates (*EPR*, eggs female^{-1}d^{-1}) and the developmental success of copepodite stages of *A. tonsa* fed these different diets were quantified. Copepod *EPR* was significantly lower (reduced by half) when *B*-algae entered the senescent phase due to silicate limitation. In a crossover (diet switch) experiment, *EPR* increased when copepods fed *B*-algae were switched to *E*-algae and vice versa. Copepodites of *A. tonsa* developed normally and reached the adult (CVI) stage when fed *E*-algae but ceased development (approximately at stage CII) when reared on senescent phase *B*-algae. Given the importance of copepods as prey for higher trophic levels, our results highlight how nutritional changes that naturally occur during a phytoplankton bloom may influence the productivity of copepods and higher trophic levels.

INTRODUCTION

Potentially one of the most important issues for furthering our mechanistic understanding of the functioning of marine ecosystems is resolving and parameterizing the processes regulating the transfer of autotrophic production to higher trophic levels. Changes in transfer efficiency due to food chain length and biochemical constraints on growth and reproductive potential have the potential to regulate the dynamics of higher trophic levels as well as the flow of materials within marine food webs. This represents a critical gap for the evolution of coupled biogeochemical models developed to understand production at higher trophic levels and to further the development of end to end food web understanding presently being fostered by international initiatives such as IMBER and GLOBEC.

One of the key biological features in many temperate oceans is the annual spring bloom. This bloom typically commences at the onset of stratification, leading to large standing stocks and high rates of primary production by diatoms in the euphotic zone. These blooms subsequently deplete the major nutrients limiting phytoplankton production thereby influencing not only phytoplankton growth rates but also the biochemical composition (Palmisano et al., 1988; Mortensen et al., 1988; Hayakawa et al., 1996). During these blooms, protein synthesis appears to dominate during the initial exponential growth phase while amounts of cellular protein decrease with increasing nutrient reduction (Mayzaud et al., 1990). Arts et al. (1997) indicated that periods of nutrient deficiency typically concurrent with the senescent phase may intensify lipid synthesis in some phytoplankton species and thereby enhance the rate of lipid biomass that is transferred from phytoplankton to zooplankton. However, changes in lipid content due to nitrogen depletion in diatoms appears to be species-specific (Shifrin and Chisholm, 1981). Working with the diatom *Thalassiosira pseudonana*, Tonon et al. (2002) reported increases in the total fatty acid content, and in particular triacylglycerols, after the onset of a stationary growth phase. Finally, Mague et al. (1980) and Barlow (1982) suggest that carbohydrates function as energy reserves for diatoms and will increase if carbon compounds fixed during photosynthesis are not used in protein synthesis and growth.

These biochemical changes influence both the quantity and quality of unicellular marine algae which subsequently impact upon the reproductive success of herbivorous copepods via changes in both the rate of egg production and the success of egg hatching (Jónasdóttir, 1994; Tang and Dam, 2001; Broglio et al., 2003; Koski et al., 2006). The timing of the bloom and overlap with the early life stages of fish stocks has been proposed to be critical for the recruitment of key fish species (Cushing, 1974). As changes in the nutritional quality of microalgae influence copepod populations (i.e. secondary production: e.g. Kleppel and Hazzard, 2000; Badylak and Philips, 2008) and given the importance of copepods as prey items for early larval stages of many marine fish species (e.g. MacKenzie et al., 1990; Pepin and Penney, 2000; Morote et al., 2008), biochemical changes in marine unicellular algae can cascade up a foodweb, eventually influencing top predators including humans (Claustre and Gostan, 1987). For these reasons, it is important to understand the factors causing variations in chemical composition of phytoplankton over the growth cycle.

In nature, a phytoplankton “bloom” is a period of rapid growth, followed by nutrient depletion and reduced growth rates (Gran, 1931). When culturing phytoplankton *in vitro*, a batch culture is generally considered to simulate a bloom (with initial exponential growth, a cessation of growth upon nutrient depletion, and a final senescent phase: Fogg and Thake, 1987). Beyond the biochemical changes reviewed above, investigating changes in phytoplankton lipid production throughout a bloom is of particular interest as lipids provide a primary source of energy and essential fatty acids to higher trophic levels (Brett and Müller-Navarra, 1997; Broglio et al., 2003; Kainz et al,. 2004). In particular, polyunsaturated fatty acids (*PUFA*) have important physiological functions (Brett and Müller-Navarra, 1997; Spector, 1999; Jump, 2002) and levels of specific dietary fatty acids have been well correlated with changes in calanoid copepod egg production (Jónasdóttir, 1994).

In order to further our understanding of the influence of bloom phase we tracked changes in biochemical composition of the diatom *Thalassiosira weissflogii* Grunow (Phycophyta, Bacillariophyceae, Centrales) during a

simulated bloom (*B*-algae) and compared these changes to those occurring in cultures maintained in semi continuous (exponential) growth phase (*E*-algae) in the laboratory. We also assessed how biochemical changes in this diatom influenced its nutritional quality as a prey for copepods by quantifying daily egg production rates (*EPR*, eggs female^{-1}d^{-1}) and copepodite development rate (*DR*, stages d^{-1}) of the calanoid copepod *Acartia tonsa* Dana fed these different source algae. We chose *A. tonsa* as our model copepod because it is an opportunistic feeder and adults do not build up large energy reserves but, rather, invest most assimilated energy into egg production (Dagg, 1977; Durbin et al., 1983; Kiørboe et al., 1985a). Moreover, the response of *A. tonsa* to a new food source appears to be rapid (<24h: Dagg, 1977; Kiørboe et al., 1985a; Kiørboe et al., 1985b; Stearns et al., 1989; Tester and Turner, 1990). In this manner we could 1) measure biochemical changes occurring each day during the different phases (exponential, stationary and senescent) of diatom growth as well as 2) identify the relevance of these biochemical changes to secondary production of copepods.

METHODS

Phytoplankton cultures

Thalassiosira weissflogii (strain CCMP 1336), were obtained from the Provasoli-Guillard National Centre for the Culture of Marine Phytoplankton. Cultures were maintained in sterilized North Sea seawater (~ 33 psu) in 10L culture bags at 19°C (± 1.5°C) under a 11:13 light:dark cycle, and received about 100µEm^{-2}s^{-1} during the light phase. Cultures were continuously mixed using aeration to minimize light limitation through self shading. The seawater was enriched with 17mL of Walne's medium (McVej, 1993), with 50mg L^{-1} biotin added (Støttrup and Jensen, 1990). Two days prior the start of the copepod feeding experiment six cultures of *T. weissflogii* were established: four of these cultures simulated a bloom (*B*-algae) while two cultures were continuously maintained in a continuous (i.e. exponential) growth phase (*E*-algae). To accomplish this, *B*-algae were initially enriched with nutrients, vitamins and silicate, while half of each *E*-algae was removed every day and replaced with seawater enriched with the nutrient mixture described above. Water was removed in all cultures via the aeration tubes and new water was added to *E*-algae using sterilized tubes, in order to avoid contamination.

Each day, equal volumes of algae were collected from each of the four *B*-algae and each of the two *E*-algae. To exclude any bag effect and to ensure that enough algae were available for daily feedings and samples required for measurements, treatment- specific samples from different bags were combined. Cell concentrations were determined using a Beckman Multisizer 3 Coulter Counter and sub samples taken and stored for measurements of biochemical composition and nutrient concentrations in the culture. The size range of *T. weissflogii* used for calculations of food concentration and sample volume was 8-20µm ESD, representing alive and intact cells.

Copepod cultures

Acartia tonsa (isolated from the Danish Sound) eggs were hatched at 20°C and 25 psu and the developing cohort of nauplii was acclimated to 33 ±1 psu and 18.5±1.5°C (mean ±range) within one week. This copepod species is euryhaline, inhabits marine areas (e.g. Martens, 1980) and the specific strain

used has relatively high *EPR* at full strength seawater at temperatures above 17°C and photoperiods longer than 8h (Holste and Peck, 2006; Peck and Holste, 2006). The culture was maintained under a 11:13 L:D light regime (2µEm^{-2}s^{-1} water surface light intensity during daytime) and fed *ad libitum* portions of *Rhodomonas baltica*. The temperature, salinity and light regime of the copepod cultures matched those used to grow the diatom cultures. Once the culture developed to late copepodite stages (C4-C5) the cohort was acclimated to *T. weissflogii* for four days prior to the start of experiments (earlier stages are not able to feed efficiently on *T. weissflogii* by virtue of its size). During this acclimation period (and during the experiment), copepods were fed *T. weissflogii* at 10000 cells mL^{-1} which is equivalent to ~1830µg C mL^{-1} (Støttrup and Jensen, 1990). This copepod attains maximum ingestion rates and *EPR* at ~1500µg C mL^{-1} and thus copepod ingestion and *EPR* were not considered to be food limited. Oxygen saturation was ensured by daily water changes.

Egg Production Experiment

Four adult females and one male were placed into one of 30 holding chambers (8.4cm in height, 4.5cm diameter and with 130µm nitex mesh bottom). Each holding chamber was placed within a 250mL beaker filled with filtered 33 psu seawater. Daytime surface light intensities were about 3µEm^{-2}s^{-1} and the same 11:13 L:D light regime as the phytoplankton. Eggs produced by the copepods passed through the sieve and settled onto the bottom of the glass beaker, thus eliminating the possibility of egg cannibalism. Each day of the experiment, each holding chamber was transferred to a new beaker and the eggs from the previous 24h were collected onto a 35µm sieve, transferred to a Bogorov tray, and counted. Of these 30 replicates, 15 were fed *B*-algae (*B*-treatment) and 15 were fed *E*-algae (*E*-treatment). The copepods within each holding chamber were counted each day and any dead copepods were removed and the sex determined. The experiment lasted for a total of 16 days (the last four of which contained a diet switch – described below) and was started when both the *B*- and *E*-algae entered the exponential phase and the *A. tonsa* cohort had just reached the adult (C6) stage. At the completion of the experiment, all

copepods within the beakers were sexed again, to ensure correct enumeration of females.

Crossover egg production experiment

On day 13 of the adult egg production experiment, when the bloom was in a senescent phase, crossover treatments were conducted. The algae fed to copepods in 18 beakers were switched: nine of them previously provided *B*-algae were switched to *E*-algae (*BE*) and vice versa (*EB*). The new food was supplied for three days. As a control, the diet of copepods in the remaining 12 beakers (six *EE* and six *BB* beakers) remained unchanged.

Copepodite growth experiment

Offspring produced during the experiment were used to measure the impact of diet treatment (and parental feeding history) on copepodite development. Nauplii hatched from eggs produced on day 5 of the experiment were distributed on well plates (Sarstedt, 24 chambers per plate, each was 1.5cm in diameter and 2cm in height). A total of 10 to 15 nauplii were placed within each well and 12 replicates were used for each of the four treatment groups. Every 24h, half of the water within the chambers was carefully removed with a pipette and new 33 psu seawater with suspended algae (*Rhodomonas baltica*) was added (50 000 cells mL^{-1}). New diet treatments using *T. weissflogii* (10,000 cells mL^{-1}) were established as soon as 50% of the individuals within each replicate reached the C1 stage (day 14 of the simulated diatom bloom). A 2x2 experimental design was used with progeny of adults fed either *E*- or *B*-algae reared using *E*- and *B*-algae. The different treatments were identified by the parental food-offspring food (i.e. *E* - *E*, *E* - *B*, *B* - *B*, *B* - *E*). Copepodites were transferred into new wells with 33 psu seawater and suspended *T. weissflogii* (10 000 cells mL^{-1}). Each day, half of the water was replaced as described above, adding *B*- (now in senescent phase) or *E*-algae. Daily routine checks were performed during which every copepod was staged (and dead animals were removed). Total experimental time of the copepodite growth experiment was the time required to develop from C1 to the adult stage (9 days, from day 14 until day 22). Mean stage was calculated for every replicate

by enumerating the copepodite stages (1 to 6) and multiplying the stage with the number of those appearing in each replicate (Koski et al., 1998).

Biochemical Analyses

Fatty acids

Duplicate samples for fatty acid analysis were obtained daily from *E*- and *B*-cultures. Samples were filtered onto precombusted filters (Whatman GF/C) and immediately frozen at –80°C. Sample aliquots contained at least 2.3 x 10^6 cells, which was a sufficient number of cells for analysis based on pilot experiments. Fatty acids were determined using the methods described by Folch et al. (1957) and Kattner and Fricke (1986) and measured using a GC (Agilent Technologies 6890N Network System) with an MPS2 autosampler and a DB-WAX column (30m long, 0.32mm inner diameter). Helium was the carrier gas and fatty acid methyl esters were detected by flame ionisation detection at 250°C.

Protein

For the protein analyses, algae samples were collected onto a precombusted filter (Whatman GF/C) and immediately frozen at -80°C. A minimum of 500,000 cells were sampled to ensure sufficient biomass for analyses. A Sigma-Aldrich protein assay kit (#BCA-1 and B 9643) was used to measure protein content. In this method, proteins reduce alkaline Cu(II) to Cu(I) in a concentration dependent manner (Lowry, 1951). Bicinchoninic acid is a highly specific chromogenic reagent for Cu(I), forming a purple complex with an absorbance maximum at 562nm (Smith et al., 1985). Bovine Serum Albumin (BSA) was used as the protein standard. Absorbance of the solution was measured at 562nm made using a SAFAS flx-xenius spectrofluorometer.

Carbohydrate

Samples for carbohydrate analyses were taken as described under “protein analysis”. The determinations were made based on the method of Herbert et al. (1971) and Dubois et al. (1956). Following these techniques, furan derivates are formed by adding 96% sulphuric acid to the sample and pentoses are converted to α-furfurylaldehyde while hexoses are transformed

to 5-(hydroxymethyl)-furfurol. These aldehydes react with phenol to produce characteristically coloured products. Measurements of carbohydrates were expressed as glucose equivalents. A 10mg L^{-1} solution of D (+) glucosemonohydrate was used as a primary standard and samples were measured photometrically at 490 nm.

Dissolved nutrient analyses

Concentrations of silicate, phosphate and nitrate were measured spectrophotometrically on both *E*- and *B*-cultures. Each day water samples (50mL) were collected after filtering through a Whatman glass fibre filter (GF/F) and stored at –18°C within acid stripped LDPE bottles. Silicate was measured following the Hansen and Koroleff (1999) method where silica and molybdic ions form a blue silica molybdic complex in the presence of ascorbic acid that was quantified via absorbance at 810nm. Dilutions of a Si stock solution MERCK Titrisol (1g Si in 14% NaOH) were used as standards. For phosphate analysis, methods described by Sternik (1978; 1983) were employed. An acid solution of orthophosphate ions and molybdic ions complex forms in the presence of antimony ions. This is reduced by ascorbic acid to phosphormolybdenum blue and then measured at 880nm. Standards in various dilutions were made of a phosphate stock solution containing KH_2PO_4 and sulphuric acid. Finally, nitrate was measured using a MERCK Spectroquant nitrate cell test kit (# 1.14556.0001). Nitrate ions in a strong sulphuric acid solution and in the presence of chloride react with resorcinol to form a red violet indophenol dye that was quantified via absorbance at 505nm.

Statistical analyses

All statistical analyses were performed using SPSS (windows version 13.0.1) and included linear regression analyses (carbohydrate per cell versus time) as well as the use of multivariate General Linear Model (GLM) with a Bonferroni correction to account for the effects of multiple comparisons (different days) to test for significant differences 1) in levels of individual fatty acids between *B*- and *E*-cultures on each day, and 2) copepod *EPR* between *B*- and *E*-treatments on each day, 3) treatment impacts in *EPR* during the crossover experiment, and 5) development stage versus treatment on each day.

Correlations among fatty acids and copepod *EPR* were analysed using a bivariate Pearsons test with a Bonferroni correction to account for the effects of multiple comparisons.

RESULTS

Water chemistry

Based upon changes in daily cell concentrations, the *B*-algae exhibited the three characteristic growth phases of a bloom: an exponential phase (days 1-3), a stationary phase (days 4-8) and a senescent (declining) phase (from day 9 onward) (Fig. II–1A). High concentrations of phosphate and nitrate were noted throughout the experiment with the former and latter were never < 359µM and 0.93µM, respectively (Fig. II–1B, Fig. II–1C). Silica concentrations decreased with time and were reduced t o 2-3µM after day 2 (Fig. II–1B, Fig. II–1C).

Phytoplankton biochemistry

Changes in protein content were observed in both cultures (Fig. II–2). In the *B*-algae, protein content rapidly increased during the exponential growth phase but decreased on day 10 (one day after cell concentrations started to decline). The decrease in protein content was rapid until day 14 and changed little on the following two days. The *E*-algae exhibited a slight increase in protein content but no significant change in carbohydrate concentration during the experiment. In contrast, a clear increase in carbohydrate content in the *B*-algae was observed (Fig. II–2, slopes are nonzero: R^2= 0.44; p< 0.05). Carbohydrate concentration was always higher in the *B*-algae compared to the *E*-algae after the initial exponential growth phase (after day 3).

Mean amounts of saturated fatty acids (*SAFA*) and monounsaturated fatty acids (*MUFA*) increased in the *B*-algae on days 4/5 and 10/11 (Fig. II–3A, Fig. II–3B) time periods representing the transition in growth phases. Amounts of *B*-algae *SAFA*s were higher than those measured in *E*-algae on these days while amounts of *B*-algae *MUFA*s were consistently higher than those in the *E*-algae after day 3. Amounts of polyunsaturated fatty acids (*PUFA*, Fig. II–3C) remained quite stable during the experiment compared to those of *SAFA* and *MUFA*. In total, 18 of the 33 measured fatty acids were significantly different between *B*- and *E*-algae on at least one day. 12 of these fatty acids were significantly different (p≤0.05) between treatments on day 4 and/or 5 (Table II–1). Significant differences in fatty acids also occurred during the senescent

phase (Table II–1, days 10-16), but never during the exponential growth phase (Table II–1, days 1-3).

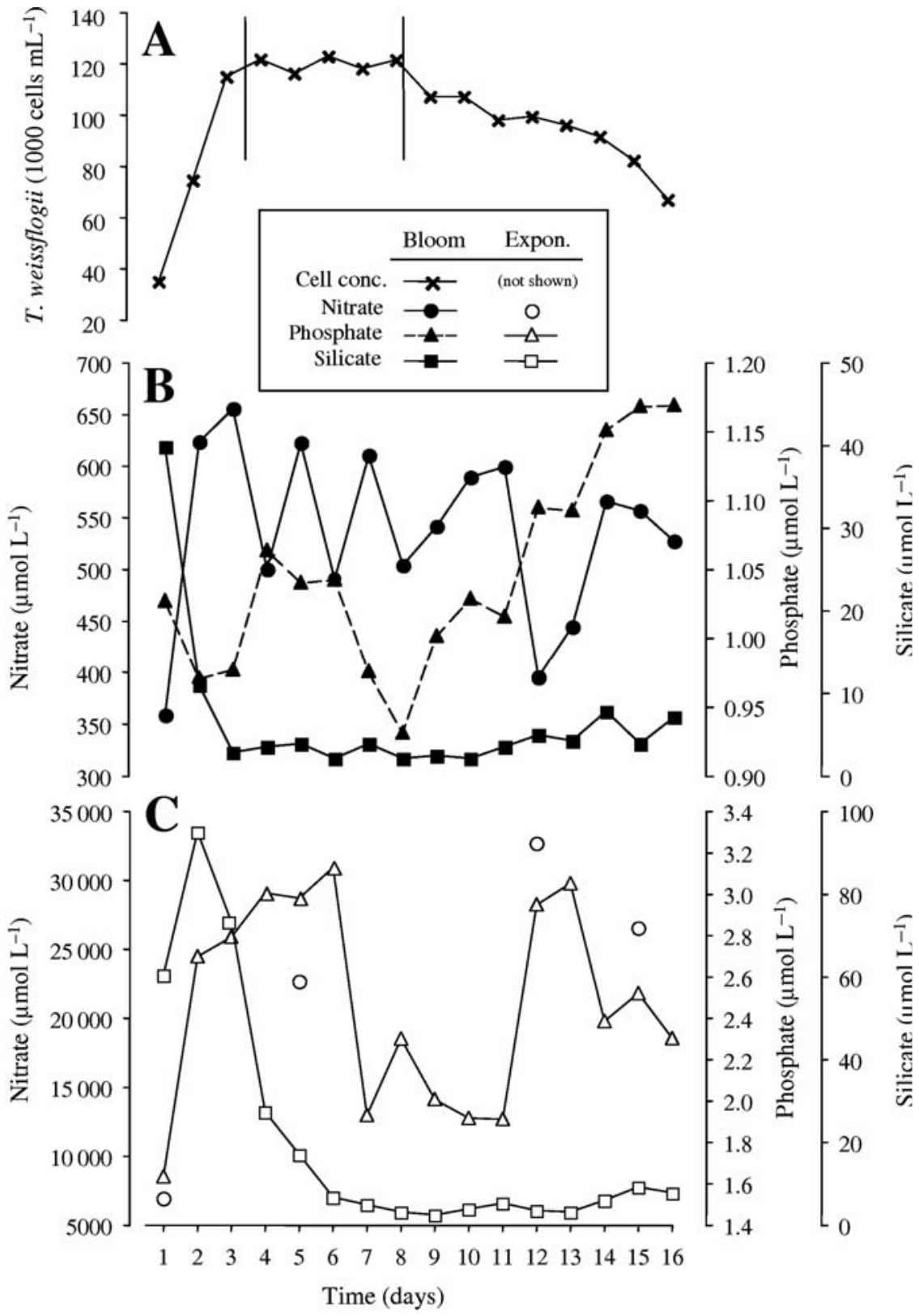

Fig. II–1 Time course of changes in *T. weissflogii* cultures during the experiments. (A) Cell concentration of the *B*-algae. Vertical lines denote the different growth phases of the *B*-algae: days 1–3 exponential phase, days 4–8 stationary phase and days 9–16 senescent phase. (B) Concentrations of nitrate (circles), phosphate (triangles) and silicate (squares) for the *B*-algae. (C) Concentrations of nitrate, phosphate and silicate for the *E*-algae, symbols as in (B).

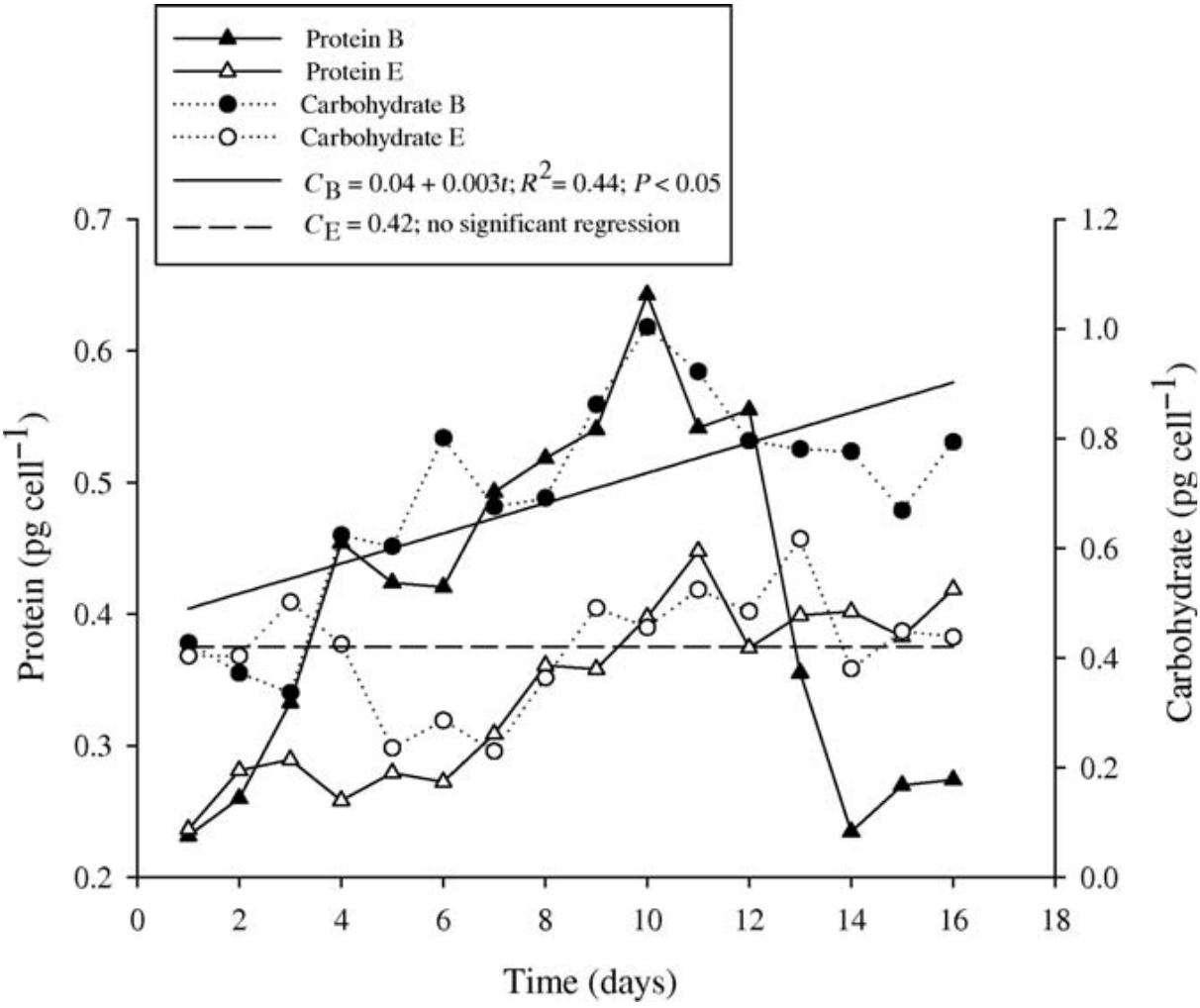

Fig. II–2 Cellular concentration of protein and carbohydrate in *T. weissflogii* in the *B*- and *E*-algae versus time. Linear regression lines are shown for carbohydrate values in the *B*-algae. A line representing the mean value is shown for carbohydrate values for the *E*-algae as it did not show a significant regression.

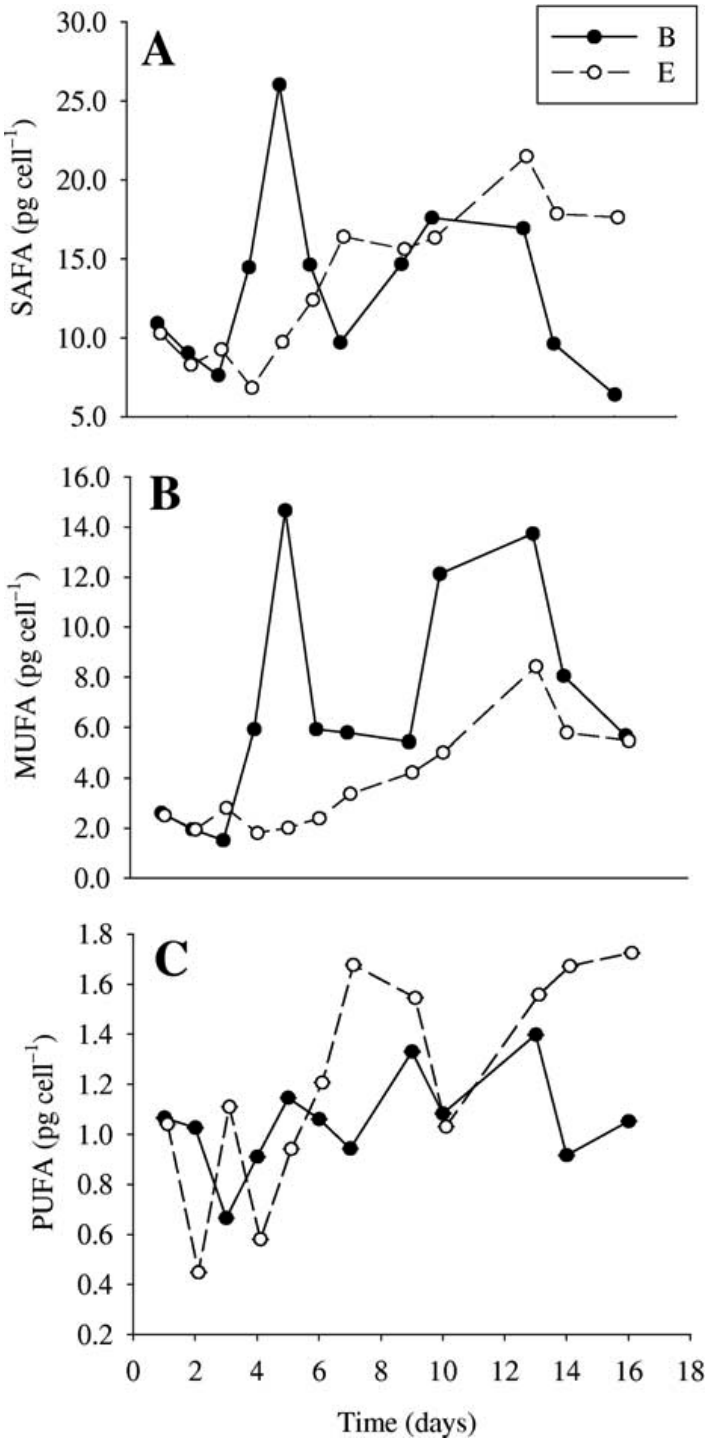

Fig. II–3 Mean concentrations of different fatty acid types versus time in the *B*- and *E*-algae of *T. weissflogii*. Note that mean values are provided but the range in SE is smaller than the width of the symbols. (A) Saturated fatty acids (*SAFA*s); (B) monounsaturated fatty acids (*MUFA*s); (C) polyunsaturated fatty acids (*PUFA*s). No data were available for days 8, 11 and 15. Note different ordinate scales are used in each panel.

Table II–1 Summary list of specific fatty acids that had significantly different ($P \leq 0.003$) concentrations between diatom (*T. weissflogii*) cultures maintained in exponential phase (*E*) and those allowed to proceed through exponential, stationary, and senescent bloom (*B*) phases

day	fatty acids
1	
2	
3	
4	C13:0 C15:0 C15:1 C16:0 C16:1 C20:3n6c
5	C13:0 C18:2n6t C18:3n6c C18:3n3 C20:3n6c C21:0 C20:5n3 C22:6n3
6	C18:2n6t
7	
9	
10	C18:0 C18:2n6c C20:4n6c C20:5n3
12	C18:0
13	C20:4n6c
14	C18:0 C18:1n9t C18:3n3 C20:3n3
16	C18:1n9t C20:1 C20:3n3

Copepod egg production

Mean egg production rate (*EPR*) increased with time in the *E*-treatment group but decreased with time in the *B*-treatment (Fig. II–4B). *EPR* was significantly different between these two treatments (Table II–2) with significant differences occurring on days 10, 11, 12 and 13 (Bonferroni-corrected multivariate GLM, $p \leq 0.01$). Mean(±SE) *EPR* in the *E*-treatment was 37.0(±1.7) eggs female^{-1} d^{-1} with the highest mean rate (49.0 eggs female^{-1} d^{-1}) observed on day nine. Mean(±SE) *EPR* in the *B*-treatment was 30.0(±2.3) eggs female^{-1} d^{-1} with the highest mean rate (42.0 eggs female^{-1} d^{-1}) observed on day three and the lowest mean rate (17.0 eggs female^{-1} d^{-1}) on day 11. Changes in phytoplankton fatty acids including C20:5n3 (eicosapentaenoic acid, *EPA*), C16:0, C16:1 and C22:0 were not significantly correlated with *EPR* (Table II–3). Furthermore, several fatty acid ratios (16:1/16:0; 20:5n-3/22:6n3; 18:2n6/18:3n3; 22:6n3/20:5n3) were tested to correlate with *EPR*, but did not show any correlation. Female mortality during the 13-d experiment was low (5.8%).

Table II–2 Results of a two-factor, repeated-measures ANOVA of *A. tonsa* egg production rate between the diet treatments of bloom (*B*)- and exponential growth (*E*)-diatom (*T. weissflogii*) cultures versus time (13 days)

Source	df	Mean Square (MS)	F (MS regression)	p
Treatment	1	5103.3	4.2	0.05
Error	28	1217.8		
Time	7.4	1064.3	11.4	<0.001
Time x Treatment	7.4	1504.5	16.2	<0.001
Error	208.1	93.1		

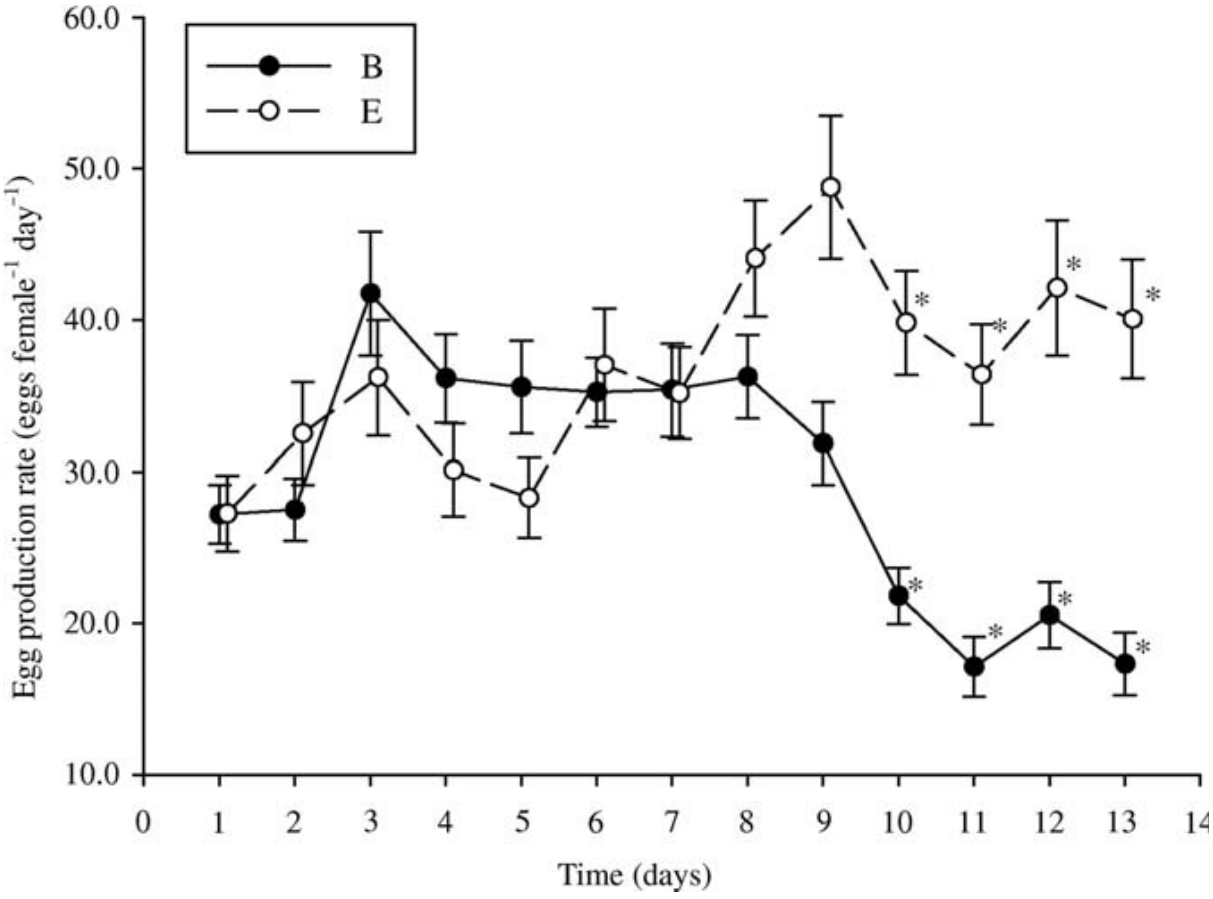

Fig. II–4 Mean (±SE, N=15) egg production rate (no. of eggs female^{-1} d^{-1}) of *A. tonsa* versus time. Diet treatments of copepods were either *B*- or *E*-algae of *T. weissflogii*. Asterisks indicate a difference significant (GLM, Bonferroni corrected; $P \leq 0.003$). Note that symbols were shifted slightly along the abscissa for visual clarity.

Table II–3 Summary of Pearson correlation coefficient (r) values between rates of egg production (*EPR*) with levels of various fatty acids in diatoms (*Thalassiosira weissflogii*) cultured to simulate a bloom (*B*) and maintained in an exponential growth phase (*E*). After a Bonferroni correction (P ≤ 0.001) no significant correlations were found

Food signal	***B***		***E***	
	EPR		*EPR*	
	r	p	r	p
C12:0	-0.615	0.044	0.005	0.988
C14:0	-0.319	0.34	0.547	0.082
C15:0	-0.235	0.486	0.64	0.034
C15:1	0.443	0.173	0.722	0.012
C16:0	-0.148	0.664	0.593	0.055
C16:1	-0.352	0.288	0.518	0.103
C16:1/C16:0	-0.426	0.191	0.594	0.054
C17:0	0.227	0.502	0.719	0.013
C18:1n9t	-0.661	0.027	-0.049	0.885
C18:2n6t	-0.461	0.153	0.514	0.106
C18:3n3	-0.588	0.057	0.512	0.107
C18:3n6c	-0.588	0.057	0.573	0.065
C20:0	0.094	0.783	0.206	0.544
C20:1	-0.615	0.044	-0.202	0.552
C20:2	-0.206	0.544	-0.439	0.176
C20:3n3	-0.631	0.037	-0.281	0.402
C20:3n6c	0.143	0.674	0.449	0.166
C21:0	-0.288	0.391	0.607	0.047
C22:0	0.058	0.865	0.68	0.021
C22:2	0.110	0.748	0.309	0.355
C23:0	0.225	0.505	0.25	0.459
C24:1	-0.56	0.073	0.571	0.66
SAFA	-0.03	0.929	0.687	0.02
MUFA	-0.356	0.283	0.519	0.102
PUFA	-0.373	0.258	0.633	0.037
Sum amount of *FA*	-0.192	0.571	0.641	0.034

During the crossover experiment, mean *EPR* in most treatments decreased with time, however, switching food sources from *E*- to *B*-algae (*EB*) and vice versa (*BE*) clearly impacted *EPR* (Fig. II–5). Mean relative *EPR* (*EPR* normalized to mean value of *EPR* obtained for the groups of copepods that were continuously fed *E*-algae, *EE*) markedly decreased in the *E*- to *B*-treatment (*EB*, Fig II–5 insert). In contrast, mean relative *EPR* increased in the *B*- to *E*-fed group (*BE*, Fig. II–5 insert). At the beginning of the crossover experiment (prior to diet shift), mean *EPR* in beakers chosen for *BB* (no change, control group) and *BE* (switched) were not significantly different (Bonferroni corrected; $p \leq 0.01$) and the *EPR* measured in the *EE* (no change, control) and *EB* (switched) were also not significantly different (Bonferroni corrected; $p \leq 0.01$). On the final day of the experiment (day 16) mean values of *EPR* in the groups fed *B*-algae (the *BB* control and *EB* group) were significantly different (Bonferroni corrected; $p \leq 0.01$) from those measured in both groups fed *E*-algae (both the *EE* control and *BE* group).

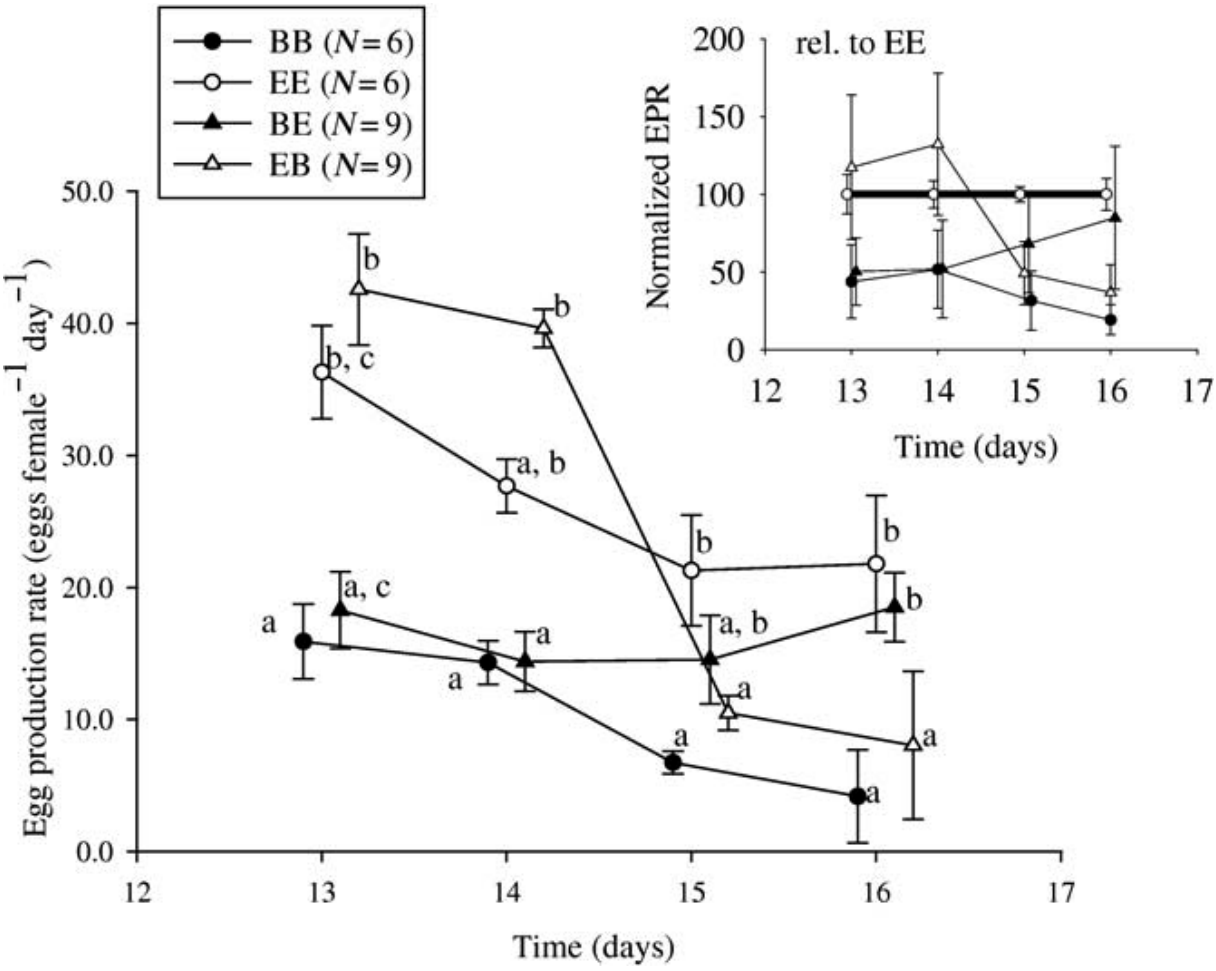

Fig. II–5 Mean(+SE) egg production rate (no. of eggs female^{-1} d^{-1}) versus time during the crossover experiment. *EE*: *E*-control; *EB*: originally *E*-fed, changed to *B*-fed; *BE*: originally B-fed, changed to *E*-fed; *BB*: B-control. N, number of replicates. Insert: Relative (+SE) egg production rate (%, normalized to daily value for control *EE*) versus time. Note that the symbols were moved slightly along the abscissa for visual clarity. Symbols with the same letters were not significantly different on the given day (GLM, Bonferroni corrected; P ≤ 0.01).

Copepod growth

No significant differences in development stage were noted among the four treatment groups during the first five days of the copepod growth experiment (Fig. II–6). However, statistically significant differences occurred from the sixth day onward. After the fifth day, treatment groups fed *E*-algae were significantly more advanced in mean developmental stage than treatments fed *B*-algae. After 17 days, copepodites in both groups fed *E*-algae reached the adult (C6) stage, while the development of both groups fed *B*-algae were significantly ($p<0.001$) retarded by a mean of 3.3 (*B*-*B* group) and 4.0 (*E*-*B* group) stages, respectively.

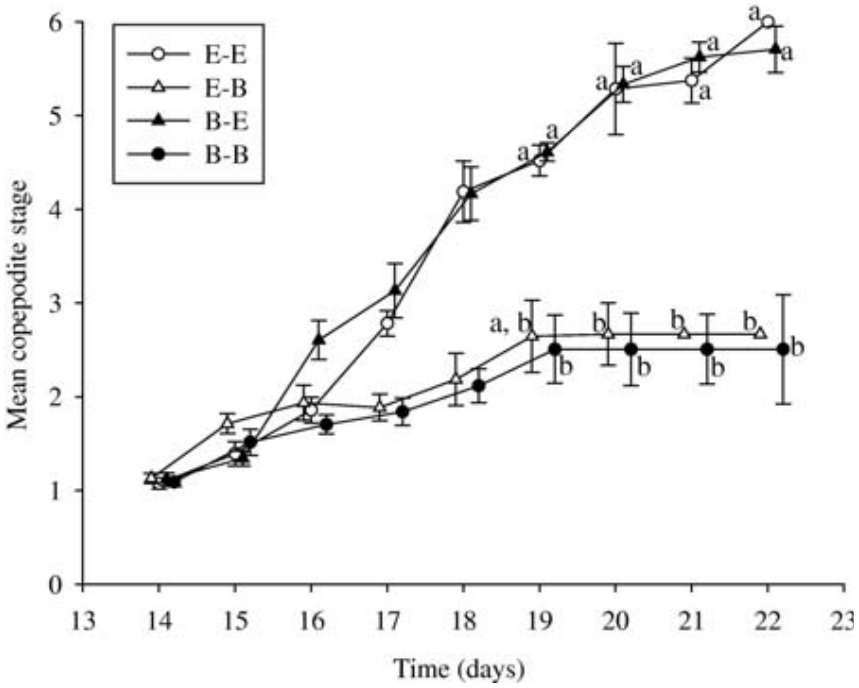

Fig. II–6 Mean(+SE) copepodite stages versus time for *A. tonsa*. Nauplii that were hatched from both *E*- and *B*-treatments were reared on either *E*-algae (*E*-*E*, or *B*-*E*) or late *B*-algae (*E*-*B*, or *B*-*B*). On days when significant differences were observed, symbols labelled with the same letter were not significantly different (GLM, Bonferroni corrected; $P \leq 0.005$). Note that symbols were shifted slightly along the abscissa for visual clarity.

DISCUSSION

Nutrients and phytoplankton growth rate

In general, silicate and phosphate concentrations of 3.9µM and 0.24µM, respectively, are adequate for phytoplankton (Sarthou, 2005). Nitrate concentrations were more than 300 times higher than required, concentrations of 1.6µM are adequate (Sarthou, 2005). Based upon this, phosphate and nitrate were present in surplus amounts during the entire experiment while silicate concentrations were reduced to growth limiting concentrations for the *B*-algae from day 3 onward. Since diatoms require silicate to form their cell walls (Coombs, 1967) it is reasonable that the silicate limitation in this study resulted in a change from an exponential growth phase to a stationary phase in the *B*-algae. This is consistent with findings of Shifrin and Chisholm (1981) who studied various phytoplankton species and Parslow et al. (1984) who studied *T. pseudonana* and found an immediate decrease in cell division following the depletion of external silicate. Lund (1950) reported that the end of a spring bloom of *Asterionella formosa* frequently corresponded to the silica depletion. Working with *Chaetoceros gracilis*, Lombardi and Wangersky (1991) reported a threshold silicon concentration below which uptake was minimal. This might explain why there was no silicate uptake from day 3 onwards in the present study. As the *B*-cultures entered into senescent phase, we observed a large secondary peak that was considerably smaller than what we would expect for our *T. weissflogii* (<6 µm ESD). The Multisizer III instrument used in this study also allows us to examine the pulse width and pulse height for each observation. That smaller peak was easily removed from cell counts when looking at both pulse height and pulse width, and we interpreted that as fragments of dead cells. Therefore, measurements indicating higher silicate concentrations on days 14 and 16, (7.83µM, nearly twice of those measured after day 3) may be explained by the degrading dead cells inside the bags of the *B*-cultures during the senescence phase which might have lead to a small silicate enrichment from partly dissolved valves. On day 15 the amount of silicate was half that measured the previous day, suggesting a rapid uptake of silicate.

E-algae were enriched with nutrients each day so that amounts of phosphate and nitrate in the water were consistently very high. Although silicate

concentrations were also low in the *E*-algae (see Fig. II–1C), water samples were taken directly prior to the daily silicate enrichment so measured values represent daily minima. Our results indicate that silicate was rapidly taken up by *E*-algae and agrees with observations from starvation experiments conducted by Parslow et al. (1984) in which uptake rates of silicate by *T. pseudonana* increased rapidly and within two hours of silicate addition. Adding silicate in higher quantities and more frequent water sampling (at least after enrichment) would have improved the design of the present experiment. Nevertheless, given the increase in cell concentrations measured in *E*-algae, it appears doubtful that any short phases of silicate limitation markedly impacted the growth biochemistry of algae in this treatment group.

Phytoplankton biochemistry in relation to nutrient availability and bloom phase

In the present study, *B*-algae protein content increased during the exponential phase, rapidly decreased during the stationary phase and finally slowly decreased during the senescent phase. These results are similar to those reported by Morris et al. (1985) who simulated a spring phytoplankton bloom in an enclosed experimental ecosystem. In that study, protein levels increased during the pre bloom and bloom period but then markedly decreased at the end of the bloom. Mayzaud et al. (1990) characterized the exponential growth phase by an initial dominance of protein synthesis, with protein decreases following nutrient depletion. Similarly, Scott (1980) and Cowles et al. (1988) indicated that faster growing cells contained more total protein which concurs with the increase in protein content observed in the *E*-algae in the present study.

Vaulot et al. (1987) demonstrated that silicon was required for the initiation of DNA synthesis in *T. weissflogii*. Therefore it is plausible that with slower cell-division rates due to silicon limitation, protein contents will be low because they regulate translation while, during the exponential growth phase (when cells are dividing rapidly), protein contents will be high because protein for DNA production is continuously being synthesised. The relatively stable (and high) concentrations of phosphate and nitrate in the bloom cultures of this study suggest that no luxury consumption of these nutrients occurred.

Therefore, it can be assumed nitrogen limitation did not limit the production of protein, a nitrogenous compound (e.g. Conover, 1975).

As expected, the carbohydrate content of the *B*-algae clearly increased while only modest increases in cellular carbohydrate concentration were observed in the *E*-algae. After the initial 3 day exponential growth phase, carbohydrate content was always higher in the *B*- compared to the *E*-algae which agrees with the inverse relationship observed between carbohydrate concentration and diatom growth rate reported by Scott (1980). The sharp increase in carbohydrates in the *B*-algae on days 3 to 4 could be connected to silicate depletion as previously reported by e.g. Morris et al. (1985), Harrison et al. (1977) or Lynn et al. (2000) where carbohydrate levels were observed to increase under silicate limitation.

Increases in carbohydrate content of *T. weissflogii* observed in the present study (upon silica limitation) have been shown to increase intracellular density and sinking rates of cells (Richardson and Cullen, 1995). Although recent pilot studies indicated that treatment-specific differences in *EPR* were not explained by direct food limitation via sinking (unpublished data), future studies are needed to quantify sinking rates and examine whether copepods experience both direct and indirect (quality) food-limitation when utilizing algae in different stages of a bloom. This would have important consequences for laboratory measurements (e.g. within mesh-bottom containers in this study) and for the *in situ* productivity of copepods feeding in specific depth layers.

Temporal changes in levels of fatty acids were also observed in this study in relation to algae growth phase. Lipid content (the sum of all fatty acids measured), increased rapidly in the *B*-algae on days 4 and 5 corresponding to a shift from exponential to stationary phase also evidenced on days 9-11 corresponding to a change from stationary to senescent phase. Significant differences in individual fatty acids between the two treatments occurred at day 4 and/or 5 and during senescent phase indicating the importance of growth rate to biochemical composition. The increase in the lipid content from day 3, when exponential growth stopped, to day 5 agrees with the findings of

Shifrin and Chisholm (1981), who observed that the mass of lipid per cell doubled within 12 h of silicate depletion. As was observed here, Hayakawa et al. (1996) reported that cellular concentrations of *PUFA* increased when diatoms were in active growth under nutrient rich conditions, and declined when nutrients were exhausted. This is also consistent with Mayzaud et al. (1989) in which saturated fatty acids (*SAFA*) and monounsaturated fatty acids (*MUFA*) were characteristic of post bloom decay.

Bloom dynamics and copepod vital rates

In the present study, copepod *EPR* in the *E*-treatment increased during the first 13 days of the experiment and mean values (37.0±1.7) are within the confidence limits of mean values obtained in other investigations (e.g., Ederington et al., 1995; 48.0±11.9 eggs female^{-1} d^{-1} for *A. tonsa* fed *T. weissflogii* at 19°C). A rapid decline in *EPR* of the *B*-treatment was noted during the senescent growth phase (lowest *EPR* on day 11, 17.0 eggs female^{-1}d^{-1}) suggesting reduced nutritional quality of senescence phase *B*-algae in comparison to *E*-algae. Similar to the decline of *EPR* in the *B*-treatment at approximately the same time as the algae moved into stationary/senescence phase, Kiørboe (1989) reported *A. tonsa EPR* to respond to variations in phytoplankton growth rate by increasing ingestion rates. But as Durbin et al. (1983) reported, mean *EPR* of *A. tonsa* in Narragansett Bay was 25.3±4.13 while mean *EPR* in this study was slightly higher in the *B*- and clearly higher in the and *E*-treatment groups. Therefore, we assume that food conditions in both treatments were still superior to those in a field situation. Nevertheless, it is possible that changes in biochemical composition of algae that occurred during the various bloom phases changed the palatability of the algae to *A. tonsa* and that food consumption rates by copepods were impacted. Therefore, changes in egg production rates observed in this study may have resulted from the interplay between food quality and quantity. This remains to be tested by adding measures of grazing rates in future experiments.

Despite measured differences in phytoplankton protein during the senescent growth phase, no relationship between *A. tonsa EPR* and protein concentration of the food were observed in the present study. This was an unexpected result since eggs are primarily formed of protein and a previous

study found significant correlations among *EPR* and phytoplankton protein (Jónasdóttir, 1994). Similarly, Kleppel and Hazzard (2000) found *A. tonsa EPR* to depend upon nutrient and particularly on protein availability in the seston while the correlation between *EPR* and seston lipid concentration was weak. High *EPR* in the *E*-treatment agree with earlier studies (e.g. Ederington et al., 1995), so we can assume that the *E*-algae were a good quality food source and an appropriate control compared to the *B*-algae.

During our crossover experiment, *EPR* by copepods previously fed *E*-algae sharply decreased when fed *B*-algae in the senescent growth phase. In contrast, *EPR* increased (albeit more slowly than the decrease in the former) when copepods were switched from *B*- to *E*-algae. Given the general decline in *EPR* with time in all treatments, which is a general feature associated with ageing in *A. tonsa* adults (Durbin et al., 1992; Carlotti et al., 1997), the small increase in *EPR* likely indicated a strong improvement in food quality. Therefore, *EPR* were unrelated to the previous 13-d feeding, an effect that was more easily observed when *EPR* was normalized to values obtained in the control (*EE*) treatment (Fig. II–5, insert). This short crossover experiment underscores the findings of Dagg (1977), Durbin et al. (1983) and Kiørboe et al. (1985a) indicating that *A. tonsa* is an opportunistic feeder that does not build up energy reserves but invests all excess energy into egg production. Our results and those of others (Dagg, 1977; Kiørboe et al., 1985a&b; Stearns et al., 1989; Tester and Turner, 1990) clearly supported our assumption that *A. tonsa* would need < 24h to respond to changes in their food source.

Surprisingly, although differences in the amounts and composition of fatty acids were apparent during the senescent phase, no significant correlations were found between *EPR* and fatty acids during this period. In fact, none of the fatty acids previously correlated to *A. tonsa EPR* in various studies (e.g. Jónasdóttir, 1994 and Kleppel et al., 1998) were significantly correlated with *EPR* in the present study. For example, Støttrup and Jensen (1990) and Shin (2003) reported that copepods require (n-3) *PUFA*s for egg production. The lack of correlation between n-3 fatty acid levels and *EPR* in the present study might indicate the ability of *A. tonsa* to synthesize (n-3) *PUFA*s from precursors when their diet lacks (n-3) *PUFA*. A similar finding was reported by

Shin et al. (2003) where *A. omorii* seemed to synthesize C20:5n3 or C22:6n3 from C16 and C18 *PUFA*s. In the present study only small amounts of *EPA* (C20:5n3) were found in both *B*- and *E*-algae, despite *EPA* being considered a major compound of diatom fatty acids in general (e.g. Opute, 1974; Sargent et al., 1987). Based on our results, we recommend that the potential conversion of precursors to *PUFA* be examined more closely. Given the strategy of adult *A. tonsa* to allocate ingested energy to egg production, this might be best accomplished by measuring the biochemical composition of eggs compared to those of the adult food source.

The role of diatoms as an adequate food source has also been specifically questioned. Ban et al. (1997) examined several diatom species including *T. weissflogii* and concluded that some species had negative impacts on copepod reproduction by reducing fecundity (*EPR*) and/or egg hatching success. In their study, they hypothesized that either a lack of essential nutrients modified copepod reproduction or that some unidentified inhibitory compounds blocked copepod embryogenesis (representing a defence mechanism by diatoms against grazing by copepod offspring). Furthermore, Miralto et al. (1999b) reported that extracts from *T. weissflogii* above a threshold concentration of 10^4 cells mL^{-1} blocked embryonic development in copepod, sea urchin and ascidian eggs. However, that threshold concentration was ten times higher than concentrations employed in the present study. Ban et al. (1997), Ban et al. (2000) and Miralto et al. (1999b) did not analyse diatom biochemistry and their ideas concerning nutritional deficiencies or toxic compounds leading to the reduced grazer fecundity were not tested. More recently, polyunsaturated aldehydes (*PUA*) appeared to induce apoptosis in copepods (e.g. Miralto et al., 1999a; Romano et al., 2003) but *T. weissflogii* does not produce *PUA* (Wichard et al., 2005). We therefore conclude that nutritional deficiencies in the *B*-algae existed during the senescence growth phase and this deficiency resulted in the marked decline in *EPR* compared to copepods fed *E*-algae. Our suite of biochemical measurements could not detect the limiting constituent(s) for *EPR*.

One key finding of this study was the cessation of copepod development when fed senescent phase *T. weissflogii*. This finding establishes that nutritional deficiency is not only copepod *EPR* but also copepodite development which was drastically impacted when senescent phase *T. weissflogii* was used as food. Here, when fed senescent phase *B*-algae, copepodite development ceased at stage C2 while development proceeded normally when copepodites were fed the same algae maintained in exponential growth phase. Developmental cessation at the same stage (C2) has been observed for the calanoid copepod (*Pseudocalanus elongatus*) when it was provided an alga (*Dunaliella* spp.) known to be of poor nutritional quality (Koski et al., 1998). Klein Breteler et al. (2005) found that P- and N-limited *T. weissflogii* were also inadequate for optimal development in *Temora longicornis* and *Pseudocalanus elongatus* and explained their findings based upon an inferior lipid composition of the algae during nutrient limitation.

Given the measurements and results of the present study, we recommend that future analyses include a battery of measurements that encompasses not only those measured here (protein, carbohydrate and fatty acids) but other compounds including amino acids and various elemental ratios (e.g., C:N and C:P) all having the potential to impact on copepod development. We also suggest that further effort be put into the examination of bloom dynamics and copepod *EPR* and development during field programs. Typically field programs examine egg production rate as a measure of population growth rate, a metric which maybe questionable given our findings here. It should be noted however that *in situ* copepods are typically able to exploit a more diverse diet which has the potential to depending upon the limiting agent to mask the effects found in this study.

CONCLUSIONS

Considerable changes in the biochemical composition of the diatom *T. weissflogii* occurred upon nutrient (silicate) limitation and entry of diatom cultures into different (exponential, stationary and senescent) bloom stages. Nutrient limited, senescent stage algae reduced secondary production in terms of both egg production rate and copepodite development rate compared to when copepods were fed diatoms maintained in nutrient replete conditions. Our results suggest that reductions in copepod production and standing stocks often observed at the end of diatom blooms may not only be due to reductions in food quantity (and losses due to predators) but also that remaining diatoms may be an exceptionally poor quality food source for grazers. Given the vast importance of marine copepods as prey items, changes in food quality of diatoms during the latter portions of a spring bloom will undoubtedly impact productivity at higher trophic levels (e.g. St. John et al., 2001; Malzahn et al., 2007) and overall system productivity.

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CHAPTER III

MANUSCRIPT 2)

Photothermal conditions and plasticity in resting egg dynamics of *Acartia tonsa* (Copepoda: Calanoida)

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MANUSCRIPT 2)

Photothermal conditions and plasticity in resting egg dynamics of *Acartia tonsa* (Copepoda: Calanoida)

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ABSTRACT

Many marine invertebrates have developed a resting (diapause) stage as a life history strategy and understanding the intrinsic and extrinsic (environmental) triggers controlling the dynamics of these life stages is critical to understanding the factors influencing population seasonality and persistence. Here, we examined potential resting egg (*RE*) production in *Acartia tonsa* (Copepoda: Calanoida) and present evidence for phenotypic plasticity among eggs as well as maternal effects on egg hatching characteristics. We tested whether incubation photoperiod (6, 11, 16h) and/or temperature (15, 20, 25°C) affected the proportion of eggs that, between 72 hrs and 3-weeks of age, displayed specific hatching characteristics and whether the 'origin temperature' at which males and females were reared (15, 20, 25°C) also influenced egg development. Exposure to a long photoperiod (*LP*) resulted in a significantly lower proportion of eggs displaying a delayed hatch (*DHE*). A warm origin temperature (25°C) led to significantly less *DHE* whereas a cold incubation temperature (15°C) resulted in significantly lower and higher proportions of *DHE* and potential resting eggs (*RE*), respectively. The production of potential resting eggs by *A. tonsa* appears to result from photothermal cues experienced by both the parents as well as their eggs and demonstrate, at least for this SW Baltic Sea population, a plasticity in response to local environmental conditions experienced prior to or shortly after reproduction.

INTRODUCTION

Diapause is a common strategy employed by invertebrates for population persistence during unfavorable periods, especially in temperate zones. In calanoid copepods, although diapause can also occur in other life stages, around 40 species have been reported to produce diapausing, or resting eggs (*RE*) (Mauchline 1998). Zillioux and Gonzalez (1972) first documented resting eggs in *Acartia tonsa*, a species that originated from tropical waters where it occurs throughout the year (e.g. Chen and Marcus 1997). This species now has a cosmopolitan distribution and occurs only during warmer months at higher latitudes (e.g., Katajisto 1998, Diekmann et al. 2012). In temperate waters such as the Baltic Sea, *A. tonsa* occurs at high abundance for only a short period of time, with the first generation arising from the hatching of resting eggs from the sediments during periods which are favorable for hatching (e.g. from spring on) (Christiansen 1988). In this and other temperate marine and estuarine systems, *A. tonsa* is virtually absent from the water column during the winter (e.g., Sullivan and McManus 1986, Gubanova 2000, Katajisto 2006), a period of time when *A. tonsa* populations persist as benthic *RE* in these regions. Since *RE* of marine and limnic copepods can be viable for tens to hundreds of years within sediments (Hairstone et al. 1995, Katajisto 1996, Chan et al. 2008), *RE* production also contributes to genetic diversity.

Previous studies on calanoid copepods have reported up to four different egg types:

Subitaneous eggs, which hatch directly (within a few days) independent of environmental conditions;

Quiescence eggs, which facultatively pause development under unfavorable conditions, but hatch when conditions improve;

Diapause eggs, which will not hatch until after an obligate refractory phase;

Delayed hatching eggs, which neither rapidly hatch under favorable conditions, nor display the obligate refractory phase of diapause eggs

Within calanoid copepods, the production of *RE* may be triggered by a number of (potentially interacting) intrinsic and extrinsic factors including temperature, photoperiod, prey availability, population density, predation pressure, salinity and/or female age (Zillioux and Gonzalez 1972, Marcus 1980 & 1982, Ban and

Minoda 1994, Walton 1985, Acheampong et al. 2011, Drillet et al. 2011, Ji 2011, Diekmann et al. 2012). Avery (2005a) pointed out that these factors can have direct physiological effects or function indirectly as cues for behavior. For example, absolute temperature can directly influence egg production rate (*EPR*), while seasonal changes in temperature can indirectly trigger the synchronization of hatching. Furthermore, Avery (2005a) argued that changes in temperature or photoperiod or accumulations of these cues might be more important than simple absolute values of either factor.

To elucidate potential triggers for *RE* production, we analyzed the development of *A. tonsa* eggs from three different photoperiods, parental origin temperatures, and incubation temperatures. Different proportions of eggs that did not rapidly hatch (within 72 hrs) either developed into delayed hatched eggs (*DHE*) or potential resting eggs (*RE*), or disintegrated (*DE*) during incubation at these different light and temperature regimes.

METHODS

Three experiments were successively conducted using three photoperiods: short (*SP*), 6h; medium (*MP*), 11h; and long (*LP*), 16h light. Within each of the experiments, copepod cohorts and eggs produced were maintained at three different temperatures (15, 20 and 25°C) while the same salinity (18 psu), light source, and photoperiod were used. Adult *Acartia tonsa* from Baltic Sea cultures were incubated (Thermo Scientific Heraeus BK800) at 25°C in an 8-L Plexiglas beaker and fed *ad libitum* using *Rhodomonas* spp.. After 24h the adults were removed and the eggs were allowed to hatch. After two more days, nauplii were distributed to nominal incubation temperatures of 15, 20 and 25°C. These temperatures were defined as 'origin' temperatures for experiments on egg developmental characteristics. The actual mean (± standard deviation, *SD*) temperatures were 14.7±0.3°C, 20.3±0.6°C and 24.9±0.4°C (Hygrosens temperature logging system). Every day, one third to one half of the water was replaced and the cultures were fed a cryptophyte (*Rhodomonas* spp.) at *ad libitum* concentrations. When copepods of one origin temperature treatment developed to the adult stage, 12 females and 4 males were incubated in each of three replicate 880-ml holding chambers with 100 µm mesh bottom sieves that were suspended within 1-L glass beakers. Eggs produced by the copepods passed through the sieve and settled onto the bottom of the glass beaker, thus eliminating egg cannibalism. After three days of acclimation, 24-h egg production (*EP*) was measured on each of three subsequent days by collecting the eggs on a 35 µm sieve, and counting them in a Bogoroff tray at 16x magnification (Wild Herrbrugg dissecting scope).

Eggs were allowed to hatch for 72h in 20-ml vials (32 mm diameter and 56 mm height) maintained at their respective origin temperatures. Additionally, nauplii, from eggs that hatched within the preceding 24h, were counted in the 20 and 25°C *LP* experiments, allowing us to quantify total *EPR* and egg hatching success (*HS*) for those treatments.

After the counting procedure, individual unhatched eggs were isolated in 24-well plates (Sarstedt, wells were 1.5-cm diameter and 2.0-cm high). Three well plates were loaded with eggs from each replicate and each plate was incubated at a different temperature (see Table III–4 for actual numbers of eggs incubated per treatment). Well plates were checked for potential resting

eggs (*RE)*, delayed hatched eggs (*DHE*) and disintegrated eggs (*DE*) every week for a period of three weeks. *RE* were defined according to Augustin and Boersma (2006) who described *RE* as those, which did not disintegrate within three weeks.

Since the data did not meet the assumptions required for parametric statistical analysis, two non-parametric approaches were taken to test for significant effects on the distribution of egg types. Rank-based statistical analyses were performed to test for significant differences in the arcsine transformed proportion *DHE*, *RE* or *DE* types observed at the three photoperiods (*LP*, *MP*, *SP*) and origin and incubation temperatures (15, 20 and 25°C). This included a Kruskal-Wallis test as well as a Mann-Whitney test with a Bonferroni correction ($p < 0.0167$) (SPSS, version 15.0). Additionally, a custom permutation test was performed using the statistical software *R* (version 2.14.2). For the permutation test, the mean proportions of egg types (e.g., 20% *DE*, 30% *DHE*, 50% *RE*) among repeated measures (3 days in 68 cases, 2 days in 13 cases) were calculated for the n=81 different experiments (3 photoperiods x 3 origin temperatures x 3 replicates x 3 incubation temperatures). Then, the observed mean proportions for each photoperiod, origin, and incubation treatment among the respective group of n=27 experiments was determined. Finally, mean proportions were calculated for 100,000 random samples of n=27 drawn (without replacement) from the n=81 experiments. The influence of individual treatment factors was considered statistically significant if the observed group mean lay outside 99% of randomized means ($p < 0.01$), in terms of Euclidian distance in *DE*, *DHE*, and *RE* ternary coordinates.

RESULTS

At long photoperiod conditions (*LP*), mean (± standard error, *SE*) egg production rate (*EPR*) of *A. tonsa* was higher at 25°C than at 20°C (49±3.3 and 20.5±3.3 eggs female^{-1} day^{-1} respectively), while mean±*SE* hatching success (*HS*) was rather similar at both temperatures (80.7±2.4 and 84.4±3.56%, at 25 and 20°C, respectively).

In comparison to randomly permuted data, *LP* and 25°C origin treatments resulted in significantly less delayed hatched eggs (*DHE*) and more disintegrated eggs (*DE*) (Fig. III–1). Furthermore, egg incubation at 15°C led to the production of significantly more *RE* and less *DHE* while significantly less *DE* and more *DHE* were produced in the *SP* treatment (Fig. III–1). Eggs laid at origin temperatures of 15 and 20°C, incubated at 20 and 25°C, and/or produced under medium photoperiod (*MP*) conditions, displayed no significant differences from the randomly permuted data (i.e. data points are distributed within the blue area in Fig. III–1).

Significant differences among different photoperiods, incubation temperatures and origin temperatures were found for *DHE*, *RE* and *DE* respectively (Kruskal-Wallis test, $p<0.05$). Eggs from the *LP* treatment did not show any significant differences in their further development into *DHE*, *RE*, or *DE* respectively, independent of incubation temperature (Bonferroni corrected Mann-Whitney test, $p<0.0167$, Table III–1). In contrast, eggs in the *LP* treatment originating from 15°C showed significantly smaller proportions of *RE* compared to those originating from 20°C for all incubation temperatures (Fig. III–2, Table III–2 and Table III–5).

Eggs from the *SP* and *MP* treatment incubated at 15 and 20°C showed significant differences in their proportions of *DHE* and *RE*, but not *DE* (Fig. III–2, Table III–1). Significantly more eggs incubated at 15°C developed to *DHE* at *SP* and *MP* from an origin temperature of 20°C compared to 15°C, while more eggs developed to *RE* that were produced at the coldest (15°C) origin temperature (Fig. III–2, Table III–1 and Table III–5).

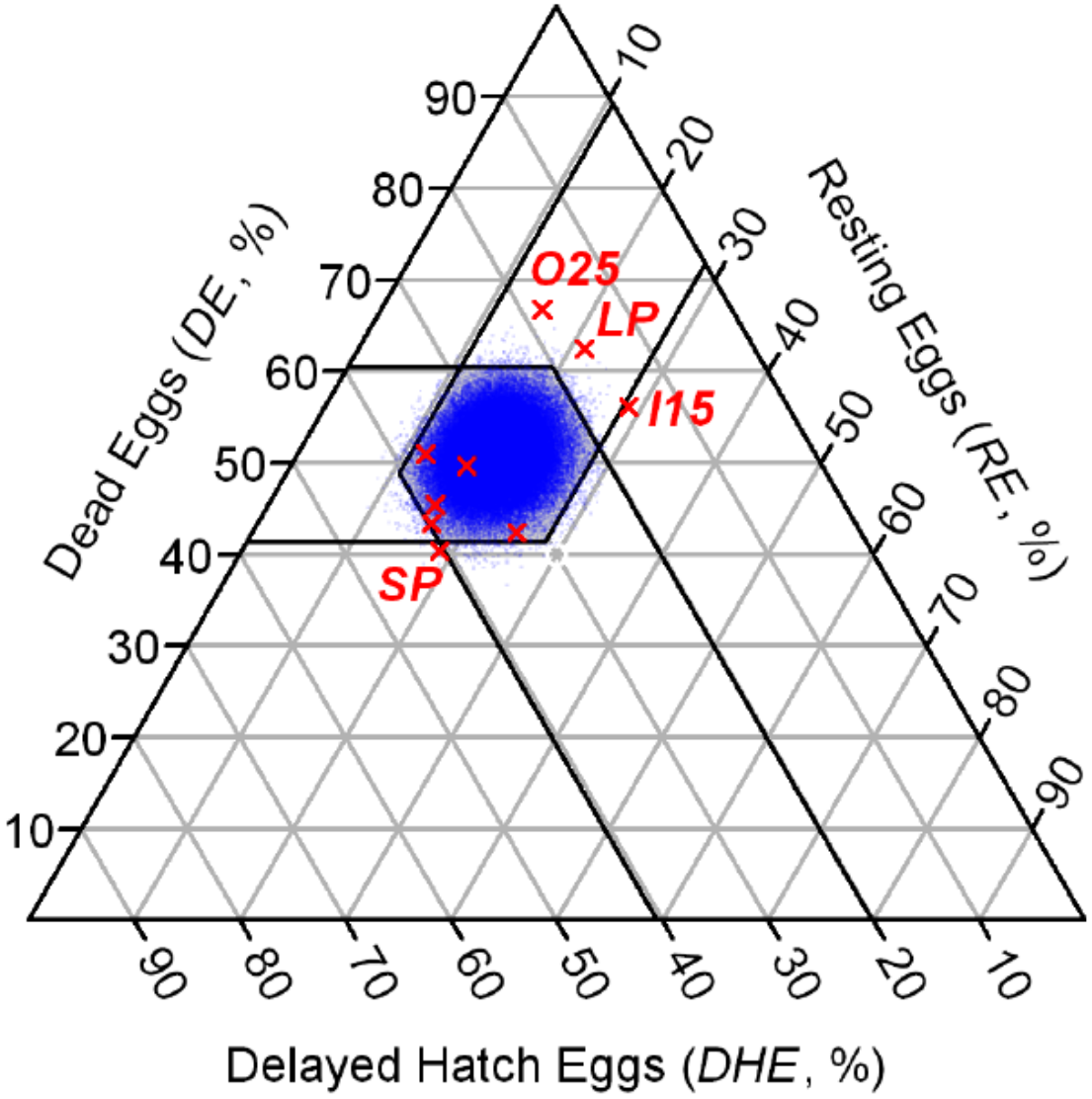

Fig. III–1 Mean (n = 27) percentages (%) of delayed hatched eggs (*DHE*), potential resting eggs (*RE)* and disintegrated eggs (*DE*). Red crosses represent experiments grouped by nine different treatment factors (origin temperatures of 15, 20 and 25°C, incubation temperatures of 15, 20 and 25°C and long, medium and short photoperiod). Blue points represent 100,000 randomly permuted subsets of experiments. Over 99% of random samples fall inside the outlined confidence polygon. Lines extending from the 99% confidence polygon indicate confidence intervals for each axis. Labels identify statistically significant treatment effects ($p<0.01$), i.e. groups falling outside the confidence polygon. Note that the three axes are labeled from low to high in a clockwise manner. *LP*: long photoperiod, *SP*: short photoperiod, *O*: origin temperature, *I*: incubation temperature

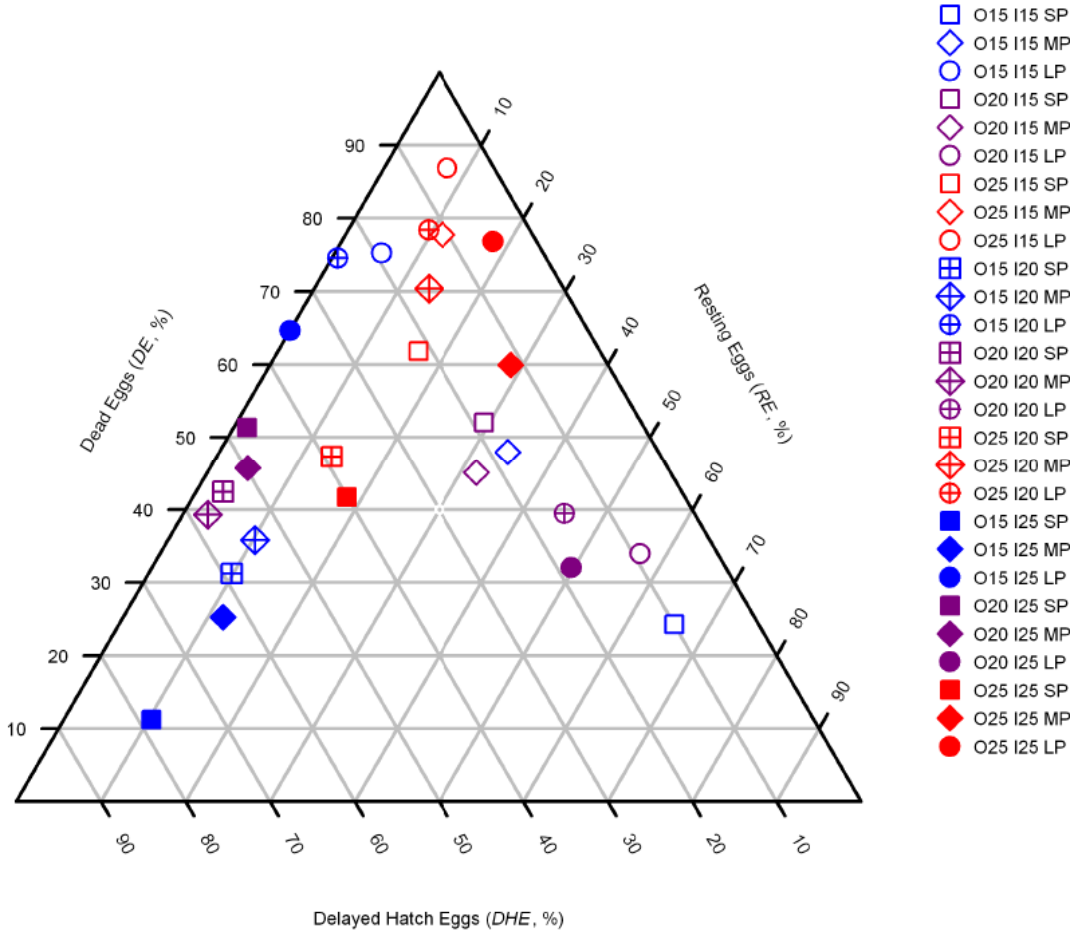

Fig. III–2 Mean (n = 9) percentages (%) of delayed hatched eggs (*DHE*), potential resting eggs (*RE*) and disintegrated eggs (*DE*). Each of the 27 points represents a different combination of experimental treatment categories (photoperiod, origin temperature, incubation temperature). Note that the three axes are labeled from low to high in a clockwise manner. □: short photoperiod, ◊: medium photoperiod, ○: long photoperiod, blue: 15°C origin, violet: 20°C origin, red: 25°C origin, empty: 15°C incubation, cross: 20°C incubation, full: 25°C incubation

No significant differences occurred in the development of eggs with an origin temperature of 20 and 25°C at the *SP* treatment independent of incubation temperature (Table III–2). In the *MP* treatment, an origin temperature of 20°C led to significantly more *DHE* compared to an origin temperature of 25°C at all incubation temperatures (Fig. III–2, Table III–2 and Table III–5).

In the *LP* treatment <1% of the eggs originating from 15°C were *RE* at incubation temperatures of 20 and 25°C and <10% were *RE* at an incubation temperature of 15°C (Fig. III–2 and Table III–5). Therefore, the statistical analysis also showed significantly higher proportions of *RE* in the eggs originating from 15°C at the *MP* treatment compared to the *LP* treatment independent of incubation temperature (Fig. III–2, Table III–3 and Table III–5). This was also true for eggs originating from 15°C at the *SP* treatment compared to the *LP* treatment, but only for incubation temperatures of 15 and 25°C (Fig. III–2, Table III–3 and Table III–5). In contrast, eggs originating from 20°C showed significantly higher proportions of *RE* at the *LP* treatment compared to the *SP* and *MP* treatments when incubated at 20 and 25°C (Fig. III–2, Table III–3 and Table III–5). No differences between the three light regimes occurred for proportions of *DE* at an origin temperature of 20°C independent of incubation temperature (Table III–3).

Most of the delayed hatched eggs (*DHE*) hatched within the first week (Table III–4), except for those in the *LP* 25°C treatment, where at all incubation temperatures most eggs hatched within the second week. In the third week comparatively many eggs hatched still, except for the *LP* treatment. Further, more than four times as many eggs hatched delayed under *MP* and *SP* compared to the *LP* treatment (mean proportions of 12.8, 14.6 and 3.2% of the incubated eggs respectively). With respect to incubation temperatures, the least eggs hatched delayed at 15°C (mean proportions of 4.9% of the incubated eggs), while more than twice as many hatched delayed at 20°C and 25°C (mean proportions of 13.4% and 13.2% respectively). An origin temperature of 15°C led to a mean hatching of 14.4% of the incubated eggs, compared to 11 and 7.6% at 20 and 25°C respectively, regardless of actual incubation temperature.

Table III–1 Summary of significant differences among the different incubation temperatures based on a Bonferroni corrected Mann-Whitney test ($p < 0.0167$). Abbreviations: *LP*: long photoperiod, *MP*: medium photoperiod, *SP*: short photoperiod, *DHE*: delayed hatched eggs, *RE*: potential resting eggs, *DE*: disintegrated eggs

		Photoperiod, Origin T, Egg type
Incubation T (°C)	15°C compared to 20°C	*SP*15*DHE*
		*SP*20*DHE*
		*SP*15*RE*
		*MP*15*DHE*
		*MP*20*DHE*
		*MP*15*RE*
		*MP*20*RE*
	15°C compared to 25°C	*SP*15*DHE*
		*SP*20*DHE*
		*SP*15*RE*
		*SP*20*RE*
		*MP*15*DHE*
		*MP*20*DHE*
		*MP*15*RE*
		*MP*20*RE*
		*MP*15*DE*
	20°C compared to 25°C	*SP*15*DE*

Table III–2 Summary of significant differences among the different origin temperatures based on a Bonferroni corrected Mann-Whitney test ($p < 0.0167$). Abbreviations: *LP*: long photoperiod, *MP*: medium photoperiod, *SP*: short photoperiod, *DHE*: delayed hatched eggs, *RE*: potential resting eggs, *DE*: disintegrated eggs

		Photoperiod, Incubation T, Egg type
Origin temperature (°C)	15°C compared to 20°C	*SP*25*DHE*
		*SP*15*RE*
		*SP*25*RE*
		*SP*15*DE*
		*SP*25*DE*
		*MP*20*RE*
		*MP*25*RE*
		*MP*25*DE*
		*LP*15*RE*
		*LP*20*RE*
		*LP*25*RE*
		*LP*15*DE*
	15°C compared to 25°C	*SP*25*DHE*
		*SP*15*RE*
		*SP*15*DE*
		*SP*25*DE*
		*MP*15*DHE*
		*MP*20*DHE*
		*MP*25*DHE*
		*MP*15*RE*
		*MP*15*DE*
		*MP*20*DE*
		*MP*25*DE*
		*LP*15*DHE*
		*LP*25*RE*
	20°C compared to 25°C	*MP*15*DHE*
		*MP*20*DHE*
		*MP*25*DHE*
		MP15RE
		MP15DE
		MP20DE
		LP15RE
		LP15DE
		LP20DE
		LP25DE

Table III–3 Summary of significant differences among the different photoperiods based on a Bonferroni corrected Mann-Whitney test (p< 0.0167). Abbreviations: *LP*: long photoperiod, *MP*: medium photoperiod, *SP*: short photoperiod, *DHE*: delayed hatched eggs, *RE*: potential resting eggs, *DE*: disintegrated eggs

		Incubation T, origin T, egg type
Photoperiod	*SP* compared to *MP*	1525*DHE*
		2025*DHE*
		1515*RE*
		1515*DE*
		1525*DE*
	SP compared to *LP*	1525*DHE*
		2020*DHE*
		2025*DHE*
		1515*RE*
		1525*RE*
		2020*RE*
		2025*RE*
		1515*DE*
		1520*DE*
		1525*DE*
		2525*DE*
		2525 *DE*
	MP compared to *LP*	2020*DHE*
		2025*DHE*
		1515*RE*
		1520*RE*
		1525*RE*
		2020*RE*
		2025*RE*
		1515*DE*
		1520*DE*
		1525*DE*

DISCUSSION

Calanoid copepods form the bulk of secondary production in marine systems and understanding the cues for the production of resting life stages is a fundamental step towards understanding and predicting what regulates plankton seasonality and food web dynamics. Among calanoid copepod species, obvious differences exist in resting stage dynamics and various cues have been postulated for the entry into diapause including temperature, photoperiod, prey availability, population density, predation pressure, salinity and/or female age (Zillioux and Gonzalez 1972, Marcus 1980 & 1982, Ban and Minoda 1994, Walton 1985, Acheampong et al. 2011, Drillet et al. 2011, Ji 2011, Diekmann et al. 2012). Intra-specific differences also exist as exemplified by the geographic differences in the initiation of diapause in *Calanus finmarchicus* (Ji 2011). Species occurring within semi-enclosed systems also display population differences in resting life stage dynamics such as the production of resting eggs by *Acartia hudsonica* (Avery 2005b). Clearly, whether or not a species and/or population utilizes resting (diapause) stages and the specific level of a (the) cue(s) initiating diapause are traits potentially modified by local environmental conditions. The present study documented phenotypic plasticity in the production of different egg types by *Acartia tonsa* and also demonstrated the eggs have some degree of plasticity in hatching characteristics that depends upon environmental conditions experienced by both females and the eggs that they have produced.

The finding of phenotypic plasticity, the change in the expressed phenotype of a genotype as a function of the environment (Bradshaw 1965), in resting egg production is not unexpected since calanoid copepods can have high dispersal rates and species such as *A. tonsa* inhabit estuarine systems that can undergo rapid changes in key environmental factors (e.g., temperature, salinity and food concentration/quality, Diekmann et al. (2009, 2012)). Thus, although a high degree of genetic (e.g., mitochondrial 16S rRNA and cytochrome C oxidase subunit I (COI)) differences have been reported among *Acartia tonsa* populations (Caudill and Bucklin 2004, Chen and Hare 2008 & 2011, Drillet et al. 2008), no differences on COI level were found between *A. tonsa* inhabiting the North (marine) and Baltic (brackish) Seas (Laakmann unpublished data). This suggests (at least at the population level) that a variety of environments

may be experienced by the population examined in this study and that a flexible life history strategy is likely to be favoured (Scheiner et al. 2012, Scheiner and Holt 2012). In the following, we compare the results of this and other studies examining *Acartia tonsa* and discuss the proximate and potentially ultimate causes for changes in the proportion of the three different egg types including photothermal cues.

Comparison of Reproductive Rate and Egg Hatching Success

We obtained egg production rate (*EPR*) and hatching success (*HS*) data at both 20 and 25°C for adults at *LP*. The warmer temperature supported higher *EPR* (mean (±*SE*) than the colder one (49 (±3.3) versus 20.5 (±3.3) eggs female^{-1} day^{-1}, respectively). These results correspond well to the findings of Holste and Peck (2006) where Baltic Sea *A. tonsa* fed the same cryptophyte (*Rhodomonas baltica*) exhibited a mean *EPR* of 29.7 (±7.7) eggs female^{-1} day^{-1} at 21°C, 18 psu and 13:11 L:D. Holste and Peck (2006) calculated 24.7°C as the optimum temperature that at which mean *EPR* was maximal (40 eggs female^{-1} day^{-1}). According to their calculations, *EPR* at 15°C was expected to be ~ 10 eggs female^{-1} day^{-1} which is a bit lower than the *EPR* we calculated at that temperature (25.4±2.0). Furthermore, the latter is an underestimate since nauplii hatched within our 72-h incubation at 15°C were not included. Our hatching success of eggs at 20 and 25°C was 84.4(±3.7) and 80.7(±2.4)%, respectively which agrees well with the findings of Chinnery and Williams (2004) who reported a *HS* of 85.4% at 20°C for *A. tonsa* eggs from Southampton waters. The agreement of *EPR* and *HS* between the studies suggests that our copepods were in good condition in all treatments during our experiments.

Relatively few systematic studies have been performed to examine how environmental factors might interact to affect resting stage dynamics in calanoid copepods. Marcus (1982) performed a series of experiments on potential interactions between temperature and photoperiod on the hatching characteristics of another calanoid copepod species (*Labidocera aestiva).* In that study, the greatest percentages of subitaneous eggs (89.3% ±1.4) were produced at the longest photoperiod tested (18h) regardless of temperature

(15 to 25°C). Furthermore, the percentage of subitaneous eggs decreased and the percentage of diapause eggs increased with decreasing photoperiod but that this trend was influenced by temperature; short photoperiods and warm temperatures lead to proportionally more subitaneous eggs (Marcus 1982). That study utilized *L. aestiva* females collected in the field, where such short photoperiods occur from mid-September until the vernal equinox (Marcus 1982). Therefore, exceptionally warm water temperatures in fall would postpone the decline in subitaneous egg production. Furthermore, Marcus (1982) speculated that exceptionally cold temperatures would lead to an earlier production of diapause eggs. As subitaneous egg production is unaffected by temperature at long photoperiods (Marcus 1982), even cold temperatures during spring and early summer would not be expected to delay the increase in population size. Tang and Dam (2001) reported for *A. tonsa* from Long Island Sound that when *EPR* was high (early in the production season), *HS* was also high, while at low *EPR* (later in the season), *HS* was extremely variable (0 to 90%). Based on the aforementioned studies, it seems likely that the *EPR* and *HS* measurements from the *LP* treatment at warm temperatures corresponded to the highest rates in our study. Working at 20.5°C, Peck et al. (2008) reported that the photoperiod used to rear adults as well as the photoperiod used during eggs incubation significantly influenced the egg *HS* of *A. tonsa* from a SW Baltic population.

Table III–4 Total amounts and proportions (% of incubated eggs) of delayed hatched eggs (*DHE*) at the different light (*LP*: long photoperiod; *MP*: medium photoperiod, *SP*: short photoperiod) and temperature treatments given for each of the three weeks of incubation. (X: no data)

Photoperiod	Origin T (°C)	Incubation T (°C)	No. Incubated Eggs	1. week		2. week		3. week	
				No. Eggs (total)	% of Incubated Eggs	No. Eggs (total)	% of Incubated Eggs	No. Eggs (total)	% of Incubated Eggs
LP	15	15	217	8	03.7	X	X	1	00.5
		20	216	28	13.0	X	X	0	00.0
		25	216	35	16.2	X	X	0	00.0
	20	15	216	6	02.8	0	00.0	2	00.9
		20	216	9	04.2	1	00.5	0	00.0
		25	216	21	09.7	0	00.0	0	00.0
	25	15	209	0	00.0	11	05.3	0	00.0
		20	208	0	00.0	20	09.6	2	01.0
		25	198	0	00.0	10	05.1	1	00.5
MP	15	15	224	20	08.9	9	04.0	11	04.9
		20	224	116	51.8	16	07.1	11	04.9
		25	221	127	57.5	7	03.2	5	02.3
	20	15	207	13	06.3	16	07.7	16	07.7
		20	205	93	45.4	23	11.2	1	00.5
		25	200	93	46.5	10	05.0	1	00.5
	25	15	120	17	14.2	5	04.2	0	00.0
		20	122	26	21.3	4	03.3	0	00.0
		25	124	16	12.9	3	02.4	0	00.0
SP	15	15	234	3	01.3	8	03.4	12	05.1
		20	233	56	24.0	64	27.5	28	12.0
		25	228	165	72.4	13	05.7	1	00.4
	20	15	177	21	11.9	8	04.5	4	02.3
		20	177	60	33.9	42	23.7	2	01.1
		25	178	84	47.2	5	02.8	1	00.6
	25	15	57	5	08.8	4	07.0	4	07.0
		20	57	10	17.5	6	10.5	6	10.5
		25	63	15	23.8	5	07.9	4	06.3

Table III–5 Proportions (% of incubated eggs) of delayed hatched eggs (*DHE*), resting eggs (*RE*) and disintegrated eggs (*DE*) at the different light (*LP*: long photoperiod; *MP*: medium photoperiod, *SP*: short photoperiod) and temperature treatments after three weeks of incubation

Photoperiod	Origin T (°C)	Incubation T (°C)	Experiment			
			Start	End (after 3 weeks incubation)		
			Incubated eggs (total no.)	*DHE* (%)	*RE* (%)	*DE* (%)
LP	15	15	217	20.59	5.57	73.84
		20	216	27.06	0.60	72.35
		25	216	35.76	0.00	64.24
	20	15	216	9.43	56.15	34.42
		20	216	16.41	42.31	41.28
		25	216	19.53	46.49	33.99
	25	15	209	5.63	7.52	86.85
		20	208	12.00	9.18	78.82
		25	198	5.14	18.00	76.86
MP	15	15	224	17.82	34.20	47.98
		20	224	53.89	10.29	35.82
		25	221	62.95	11.80	25.25
	20	15	207	23.00	31.91	45.09
		20	205	57.69	3.00	39.30
		25	200	49.76	4.48	45.76
	25	15	120	10.75	11.50	77.75
		20	122	16.03	13.58	70.39
		25	124	11.48	28.58	59.94
SP	15	15	234	9.97	65.75	24.27
		20	233	58.92	9.86	31.23
		25	228	78.41	10.37	11.22
	20	15	177	18.61	29.35	52.04
		20	177	54.32	3.22	42.47
		25	178	47.04	1.62	51.34
	25	15	57	21.14	15.54	63.32
		20	57	39.90	13.42	46.68
		25	63	40.05	18.80	41.16

Egg Types I: Delayed hatched eggs (*DHE*)

Our approach to describe all eggs hatching later than 72h after being produced as delayed hatched eggs (*DHE*) is supported by the temperature-dependent embryonic durations reported by McLaren (1969). At our coldest temperature (15°C), subitaneous eggs would have been expected to hatch within three days. Katajisto (2006) reported for Baltic Sea *A. tonsa* an egg type which appeared to be an intermediate between subitaneous and diapause, and defined it as a 'delayed-hatching' egg type. These delayed-hatching eggs lacked the characteristic, fixed refractory phase of diapause eggs and were found in autumn but were rare in summer (Katajisto 2006). In our study, significantly lower proportions of *DHE* were observed in the *LP* treatment, 25°C origin, and 15°C incubation treatments in comparison to randomly permuted data, while in the *SP* treatment significantly more eggs developed to *DHE* (Fig. III–1). Eggs in the *SP* and *MP* treatments originating from 15 and 20°C showed significantly more *DHE* when incubated at 20 or 25°C compared to 15°C (Fig. III–2, Table III–1 and Table III–5). These results suggest that an increase in ambient temperature might be a trigger for eggs to hatch despite the fact that it might take much longer than usual depending on the previous environment (e.g., temperatures) experienced during egg production.

An origin temperature of 25°C (compared to an origin of 15°C and 20°C) led to significantly lower proportions of *DHE* in the *MP* treatment at all incubation temperatures (Fig. III–2, Table III–2 and Table III–5). The fact that eggs stemming from *SP* and 15°C origin (as potential *RE*) successfully hatched when the incubation temperature was increased (Table III–5), suggests that eggs also have an inherent flexibility or physiological plasticity. As the photoperiod combined with the temperature gives information about the season it is ecologically reasonable to hatch under *SP* and *MP* when temperatures are increasing (indicating spring conditions, so light will also increase in the near future) but not to hatch when temperatures are decreasing (indicating the approach of winter). As environmental temperature is subject to random fluctuations, while light levels change gradually and predictably with the seasons, long photoperiods might be a general indicator of favorable conditions for *A. tonsa*. Nevertheless, maternal effects also appear to be important, as eggs from females at either 25°C and/or *LP* did not show

significant differences in *DHE* regardless of incubation temperatures (Table III–1).

Chen and Marcus (1997) defined *DHE* as eggs that need a longer time to hatch compared to subitaneous eggs, even under good environmental conditions, but lack a clear refractory phase. In our study, neither incubation temperature nor temperature at egg production (origin) influenced delayed hatching within the *LP* treatment, with one exception (15°C compared to 25°C origin at an incubation temperature of 15°C, Table III–2). In the *MP* treatment, no differences occurred in proportions of *DHE* between incubation in 20 or 25°C. One might therefore hypothesize that the effect of increasing temperature depends upon the photoperiod: At short (long) photoperiods a strong (subtle) effect of temperature.

Egg Types II: Potential resting eggs (*RE*)

Since we could not determine that proportion of *RE* that were true “diapause” eggs (requiring a refractory period) and the proportion that were merely quiescent for our 3 week observations, our usage of *RE* should be more formally defined as ‘potential resting eggs’.

In terms of the overall effects of single treatment factors, only incubation at 15°C led to significantly more potential *RE*, compared to 100,000 random subsets of the data (Fig. III–1). The other photoperiod, origin and incubation treatments fell within the expected range of randomly chosen subsets. Specifically, within the *SP* and *MP* treatments, significantly more eggs originating from 15 and 20°C developed to *RE* when incubated in 15°C, as opposed to when incubated at 20 and 25°C (Fig. III–2, Table III–1 and Table III–5). For eggs originating from 15°C and incubated at 15°C, significant differences occurred between all three photoperiod treatments, with a maximum of 66% *RE* in the *SP* treatment (Fig. III–2, Table III–3 and Table III–5). Among those eggs originating from 20°C the proportions of *RE* were significantly higher for eggs in the *LP* treatment when incubated in 20 and 25°C compared to eggs from the *MP* and *SP* treatments (Fig. III–2, Table III–3 and Table III–5).

As was already discussed for *DHE*, eggs laid by the same females appear to have the potential to change their hatching characteristics depending on incubation temperature and photoperiod. This is consistent with the findings of Uye and Fleminger (1976) working with *A. tonsa* collected from Southern California waters, where eggs laid at 6.5°C hatched at temperatures below 10.3°C somewhat more rapidly than eggs laid at 17.5°C. Plasticity among nauplii and females of *A. tonsa* and other calanoids related to temperature and photoperiod appears to be common. For example, the females raised from *Eurytemora affinis* nauplii collected in late autumn (November) and held under spring conditions (15°C, 12h photoperiod) in the laboratory, produced exclusively subitaneous eggs, whereas those raised from nauplii collected in spring (May) and incubated under late autumn conditions (10°C, 10h) produced exclusively diapause eggs (Ban 1992). When *A. tonsa* eggs were collected from the Baltic Sea a smaller proportion of them entered quiescence when the adults were acclimatized to colder temperatures for one or more generations (Hansen et al. 2010). In *L. aestiva*, no diapause eggs were produced under long photoperiods (18:6 L:D), indicating that photoperiod affects the induction of egg diapause (Marcus 1982). For the colder-water congener *Acartia hudsonica* Avery (2005a) found a sudden increase in the proportion of dormant eggs in late June at temperatures around 18°C and a maximal photoperiod. For adults collected in Maine waters, resting egg production was unaffected by temperature or photoperiod while temperature was a trigger to produce dormant eggs in females collected from warmer, temperate waters (Rhode Island) (Avery 2005b). Beyond this, Diekmann et al. (2012) reported salinity to effect *RE* production. In their study significantly more *RE* were produced at 8 psu compared to 18 psu and the proportion of *RE* increased with decreasing *EPR*. Ji (2011) suggested that phenotypic plasticity explained most of the differences in the diapause response to local environments of *Calanus finmarchicus* populations from the Northwest Atlantic as it can be assumed that the different populations are genetically connected.

Egg Types III: Disintegrating Eggs (*DE*)

Unfertilized eggs produced by unmated *A. tonsa* females can remain intact for ≤ two weeks before disintegrating (Parrish and Wilson 1978). Given this time

course, Augustin and Boersma (2006) described *RE* as those which did hatch nor disintegrate after three weeks of incubation. Drillet et al. (2011) found that *A. tonsa* eggs appeared viable after 14 months of cold and dark storage, but, did not hatch when incubated at 17°C and disintegrated within one month at that temperature. In contrast to the definitions provided by Parrish and Wilson (1978) and Augustin and Boersma (2006), Drillet et al. (2011) assumed that eggs disintegrated due to the lack of a relevant environmental cue for hatching. Drillet et al. (2011) argued that nonviable eggs would have degraded much more rapidly and speculated that those eggs lacked ecodysteroids, molting hormones which seem to be important for regulating diapause processes (Johnson 2003). In our study, eggs were not stored before the experiments and high proportions of subitaneous eggs hatched before the experiments started. We therefore assume that the eggs disintegrating in our study were not viable, potentially because they were not fertilized. It still remains unclear why a long photoperiod and a high origin temperature (25°C) led to significantly more *DE,* or eggs that were probably not fertilized. As a warm incubation temperature (25°C) of the eggs did not show this trend we assume that the high proportions of *DE* in the *LP* treatments or at least those at an origin temperature of 25°C might occur due to a reduced male fertilization capacity. Another reason could be polyspermy due to multiple attachments of spermatophores to the females as discussed by Parrish and Wilson (1978). This might have occurred significantly more often at an origin temperature of 25°C and a long photoperiod as these might be the treatments optimal for males. Nevertheless, as we cannot say if the eggs were dead already before we incubated them, it is still possible that they died during their development, i.e. during incubation. This lack of knowledge is unfortunate as otherwise we could have excluded *DE* from our statistical analysis, as eggs, which were already dead when we incubated them, never had the chance to develop into any of the other categories (*DHE* and *RE*).

CONCLUSIONS

Our study is among the first to report that the environmental conditions experienced by both the adults as well as their eggs influence egg hatching characteristics in calanoid copepods. The two main results of our study were that a relatively low (high) proportion of eggs developed within the *DHE* (*RE*) category when females experienced long photoperiods and a cold temperature (15°C). These results suggest a flexible hatching character of *A. tonsa* eggs not only due to plasticity among individual females but also among the eggs produced on any one day. Our findings also suggest that the importance of temperature as a cue and directive factor for the developmental trajectory of eggs of this species may change depending upon the photoperiod (season).

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CHAPTER IV

MANUSCRIPT 3)

Environmental cues and constraints affecting the seasonality of dominant calanoid copepods in brackish, coastal waters: A case study of *Acartia*, *Temora* and *Eurytemora* species in the Southwest Baltic

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Environmental cues and constraints affecting the seasonality of dominant calanoid copepods in brackish, coastal waters: A case study of *Acartia*, *Temora* and *Eurytemora* species in the Southwest Baltic

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ABSTRACT

Information on physiological rates and tolerances helps one gain a cause-and-effect understanding of the role that some environmental (bottom-up) factors play in regulating the seasonality and productivity of key species. We combined the results of laboratory experiments on reproductive success and field time series data on adult abundance to explore factors controlling the seasonality of *Acartia* spp., *Eurytemora affinis* and *Temora longicornis*, key copepods of brackish, coastal, temperate environments. Patterns in laboratory and field data were discussed using a metabolic framework that included the effects of "controlling", "masking" and "directive" environmental factors. Over a 5-yr period, changes in adult abundance within two southwest Baltic field sites (Kiel Fjord *Pier*, 54°19´89N, 10°09´06E, 12 to 21 psu, and North / Baltic Sea Canal *NOK*, 54°20´45N, 9°57´02E, 4 to 10 psu) were evaluated with respect to changes in temperature, salinity, day length, chlorophyll *a* concentration (*Chl a*). *Acartia* spp. dominated the copepod assemblage at both sites (up to 16764 and 21771 females m^{-3} at *NOK* and *Pier*) and was 4- to 10-times more abundant than *E. affinis* (to 2939 m^{-3} at *NOK*) and *T. longicornis* (to 1959 m^{-3} at *Pier*), respectively. Species-specific salinity tolerance explains differences in adult abundance between sampling sites whereas phenological differences among species are best explained by the influence of species-specific thermal windows and prey requirements supporting survival and egg production. Multiple intrinsic and extrinsic (environmental) factors influence the production of different egg types (normal and resting), regulate life history strategies, and influence match-mismatch dynamics.

INTRODUCTION

Understanding the physiological-basis behind the distribution and productivity of marine organisms is important if we hope to increase our ability to predict how anthropogenic changes to environments will affect key species and the functioning of ecosystems (Pörtner and Farrell 2008; Pörtner and Peck 2010; Somero 2010). At the most basic level, suitable environments are those where organisms have excess energy available to fuel growth and other essential activities (e.g., Huey and Kingsolver 1993; Pörtner and Knust 2007). Changes in key environmental factors can affect the amounts of available energy and influence the seasonal timing and spatial extent of environments that are optimal for the productivity of predators and their prey, influencing match-mismatch dynamics and the functioning of marine systems (Rijnsdorp et al. 2009; Pörtner and Farrell 2008). Compared to deeper, marine habitats, estuaries and other shallow coastal zones can display relatively rapid changes in key abiotic factors (e.g. temperature, salinity, nutrient concentration). These rapid changes place added physiological demands and constraints on local organisms.

Small-bodied copepods form the bulk of secondary production in coastal, estuarine environments and, in northern temperate latitudes, species are characterized by short generation times and overlapping broods (Conover 1988; Mauchline 1998). Calanoid species within the genera *Acartia*, *Temora*, and *Eurytemora* dominate the copepod biomass of many estuaries (Mauchline 1998) and key species within the Baltic Sea include *Eurytemora affinis, Acartia tonsa*, *A. bifilosa, A. longiremis,* and *Temora longicornis*. These species have local populations within shallow waters of Kiel fjord and the Kiel Canal within the southwest Baltic Sea that display different phenologies due to species-specific differences in temperature optima as well as feeding strategies. For example, *E. affinis* dominates in the spring (to 15°C) but is succeeded by *A. tonsa* in warmer periods (Hirche 1992 and references therein). *Acartia* spp. utilize raptorial and suspension feeding and ingest a wider size spectrum of food than *E. affinis* which only utilizes suspension feeding (Gyllenberg 1980; Adrian et al. 1999 and references therein). In late summer, large decreases in the copepod assemblage result from the intensive predation by fish and

mysids (Adrian et al. 1999). Adrian et al. (1999) also reported a larger decline during winter in the biomass of *E. affinis* compared to *Acartia* spp. (here: *A. bifilosa* and *A. longiremis*) which was explained by the differences in feeding efficiency. In the winter, *A. tonsa* is reported to be absent (Christiansen 1988) but the other congeners (*A. bifilosa* and *A. longiremis*) can be found all year round with maximal abundances in nauplii in February/March and November (Hernroth and Ackefors, 1979). Working in offshore areas of the Baltic Sea, Schulz et al. (2007) also demonstrated that populations of *A. longiremis* as well as *Temora longicornis* persisted throughout most of the year but that these species did not utilise the warmer, surface waters. In that study, *A. bifilosa* inhabited warm surface waters where its egg production rate was more controlled by food availability than temperature (Schulz et al. 2007).

In the following, we summarize present knowledge on how temperature, salinity, day length, phytoplankton production, and the interactions among those factors, affect reproductive success and population productivity of dominant calanoid copepods inhabiting coastal waters of the southwest Baltic Sea. Information from controlled laboratory studies was compared to site-specific changes in the abundance of adult *Acartia* spp., *T. longicornis* and *E. affinis* at the two field locations in the southwest Baltic Sea. Based upon the classification of Fry (1971), we distinguished among “controlling” factors that govern metabolic rate by affecting molecular kinetics (e.g., temperature), “masking” factors that modify the effect of other environmental factors by influencing energy allocation (e.g., salinity affecting osmoregulation), and “directive” factors that cue some response affecting energy partitioning typically via hormone induction (e.g., day length on reproduction). Based upon previous research on these copepods, we hypothesize that 1) *Acartia* spp. should be found throughout the year (albeit as varying proportions of the three congeners), 2) temperatures at should be suitable for *T. longicornis* and *E. affinis* throughout the year but that salinity will affect abundance since *T. longicornis* (*E. affinis*) is not adapted to low (high) salinities (Holste et al. 2009, Viitasalo et al. 1994), and 3) *E. affinis* will occur at highest abundance during spring and at lower abundance during the rest of the year. Unless otherwise stated, literature data summarized in subsequent sections were collected on

Southwest Baltic Sea populations of these copepods. We highlight differences in life cycle strategies and speculate on how potential climate-driven changes in key factors would influence the copepod community and lower trophic level match-mismatch dynamics in the southwest Baltic region .

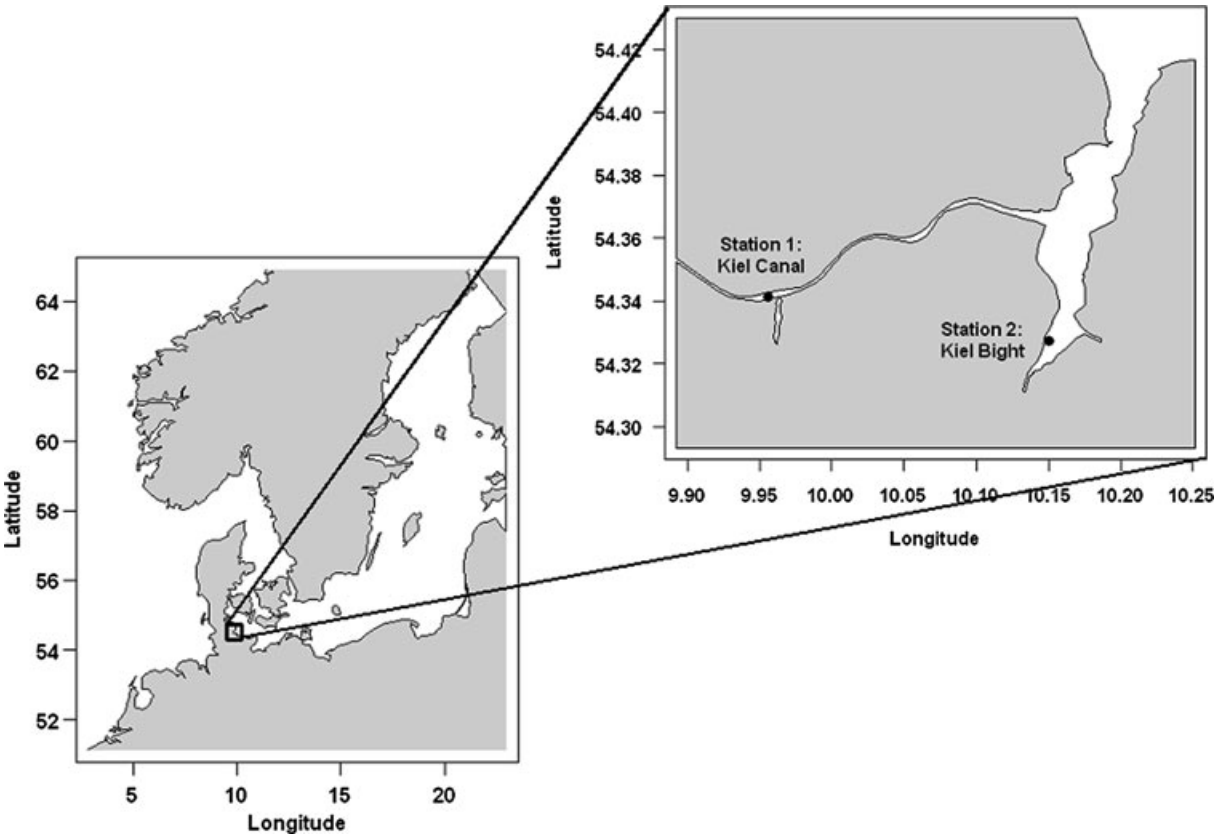

Fig. IV–1 Location of the two field sampling sites utilized in this study: North Sea and Baltic Canal (*NOK*, 54°20'45N, 9°57'02E) and Kiel Fjord (*Pier*, 54°19'89N, 10°09'06E), Baltic Sea, Germany. Note that the coordinates are given in decimals: *NOK* (latitude: 54.34; longitude: 9.96) and *Pier* (latitude: 54.33; longitude: 10.15).

METHODS

Laboratory data

Egg production rate (*EPR;* eggs female^{-1} day^{-1}) and hatching success (*HS; %*) were measured in a 16-d trial conducted with *A. tonsa*. The methods employed were the same as those discussed by Diekmann et al. (2009) examining different algal treatments on *EPR* except for the following changes. First, *EPR* and *HS* are presented from control groups of copepods - those fed with algae maintained in exponential growth-phase. Second, trials were conducted at each of two Baltic Sea salinities (8 and 18 psu), using a day length of 13h and 16°C water. Third, three different egg types were distinguished: subitaneous eggs (*SBE*) which rapidly hatched, resting eggs (*RE*) which neither hatched nor disintegrated within a 3-week incubation, and dead eggs (*DE*) which did not hatch but disintegrated within this 3-wk period. In terms of *RE*, we did not distinguish between diapause eggs that require an obligatory refractory phase before hatching and quiescence eggs which have a facultative pause in development under unfavourable conditions, but hatch as soon as conditions ameliorate.

Field data

From March 2005 until September 2010, a regular survey aimed at determining the abundance and species composition of zooplankton in relation to environmental data has been performed at two sampling sites (Kiel Fjord (*Pier,* 54°19`89N, 10°09`06E) and the North /Baltic Sea Canal (*NOK*, 54°20`45N, 09°57`02E), Fig. IV–1). For exact sampling periods see Table IV–1. All samples were collected aboard the RV “Polarfuchs”. For this study, temperature (*T*), salinity (*S*), and chlorophyll a (*Chl a*), and adult female copepod abundance data were utilized. Measurements of *T* and *S* were made at 7m (WTW senor through 2006 then Sea and Sun Technology, CTD48M). and concentrations of *Chl a* were measured in water collected at 7m using a, a 5L Nanson water sampler deployed at both sites. A 250-ml water sample was filtered through a GF/F glassfiber filter (25 mm diameter, Whatman) and *Chl a* was determined using the method of Jeffrey and Humphrey (1975). Copepods were sampled with a WP2 net (200-µm mesh size) hauled vertically from 3m above the bottom to the surface. Contents were preserved with a 4% formalin

solution. In the laboratory, a plankton splitter was used to obtain a representative sub-sample that contained at least 100 individuals of the most abundant copepod species. The sub-sample counts were converted to total abundance (individuals m^{-3}). *Eurytemora* and *Temora* were identified to the species level (*E. affinis*; *T. longicornis*), *Acartia* to the genus level. In a few instances (specific dates and species), we back-calculated the hatch date of adult females based upon previously published, species-specific, temperature-dependent development times (e.g., Ban 1994; Klein Breteler and Gonzalez 1982, Mauchline 1998). For these rough estimates, we assumed that females were one week old when captured and that temperature changed in a linear fashion between (weekly) measurements.

Table IV–1 Data set sampling periods per station and year in calendar week (CW) and month

	Dates of *Pier* sampling		Dates of *NOK* sampling	
	First (CW/month)	Last (CW/month)	First (CW/month)	Last (CW/month)
2005	13/March	51/December	–	–
2006	09/March	42/October	–	–
2007	01/January	51/December	17/April	39/September
2008	02/January	43/October	–	–
2009	06/February	51/December	14/April	51/December
2010	05/February	32/August	11/March	37/September

RESULTS

Literature and laboratory based findings

Thermal windows

A summary of *EPR* measurements reported in various studies and at various study sites in the Baltic and North Sea suggest an optimum temperature (T_{opt}) for *A. tonsa* and *T. longicornis* at 23 and 17°C, respectively (Fig. IV–2). Upper and lower "pejus" temperatures were calculated to be equal to the points were *EPR* was 50% that at T_{opt}. Temperatures between lower and upper pejus thresholds (T_P) can be considered to represent the window of optimal thermal conditions. When thermal windows were calculated in this manner, both *A. tonsa* and *T. longicornis* could be considered thermal generalists with the former slightly more stenothermal (12.5°C range between upper and lower T_P) compared to the latter (15°C range). For *A. tonsa* a lethal temperature of 34°C has been reported.

Egg development/hatching

Hatching and/or development of the three different egg types was found to be influenced by day length and salinity (Fig. IV–3). The proportion of *SBE* increased with increasing day length but remained relatively unchanged at $\geq$16h of light (Fig. IV–3A). A key difference between 8 and 18 psu was that the proportion of RE produced was significantly higher at the former versus the latter salinity (Kruskal-Wallis and Mann-Whitney; arcsine transformed; $p \leq 0.001$) (Fig. IV–3B). At 8 psu, the proportion of *SBE* (*RE*) decreased (increased) with decreasing *EPR* (Fig. IV–3B).

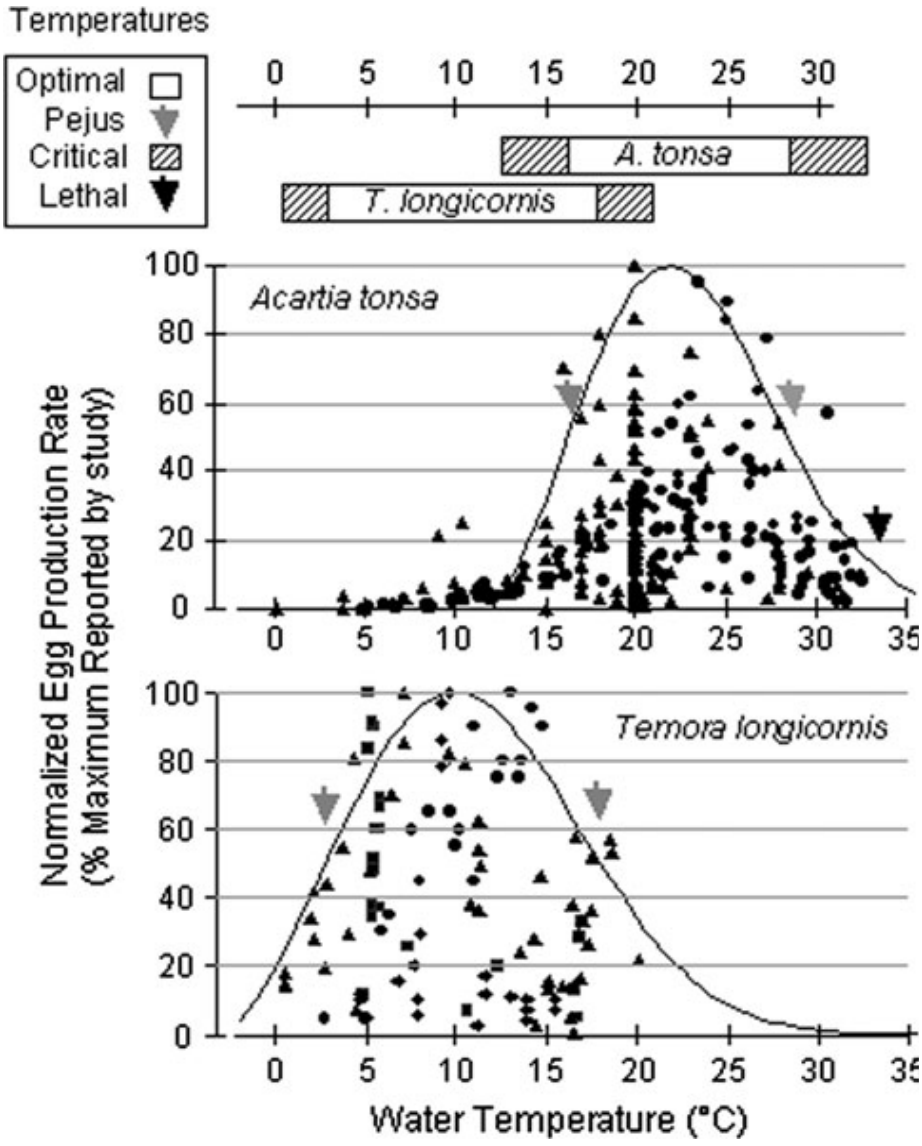

Fig. IV–2 Normalized egg production rate (% maximum rate (eggs female^{-1} d^{-1}) reported by study) of *Acartia tonsa* and *Temora longicornis* versus water temperature (°C) showing optimal (T_{OPT}), pejus (T_P) and critical (T_{CRIT}) temperatures. Lower and upper T_P were set equal to 50% maximum egg production rate For *A. tonsa*, data were pooled and normalized from > 10 laboratory and field studies (see Holste 2010). For *T. longicornis*, the data from 3 studies were utilized (Fransz and Gonzalez 1990; Halsband and Hirche 2001; Holste et al. 2009).

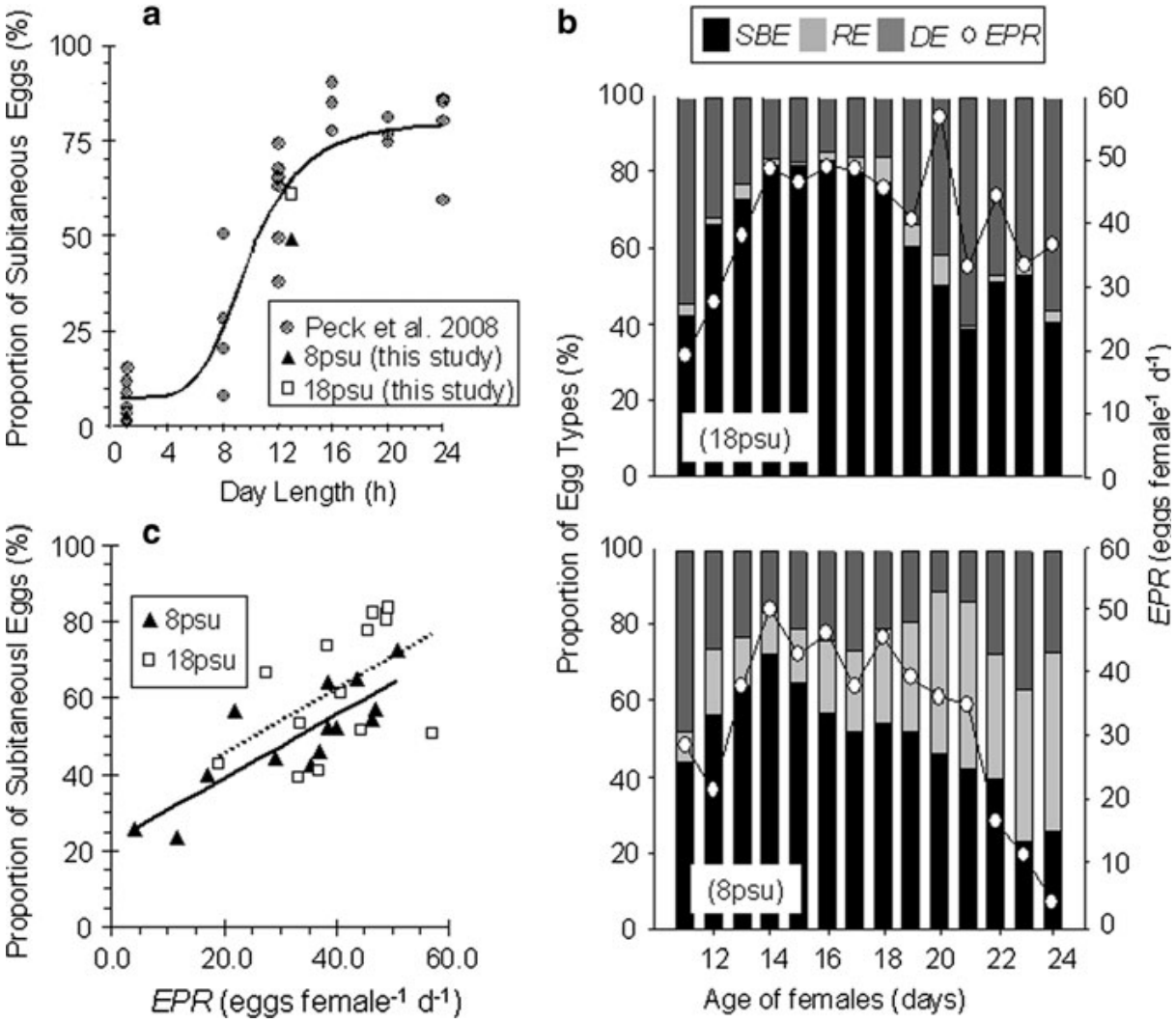

Fig. IV–3 The relationships between the types of eggs produced by *Acartia tonsa* when females were reared at different day lengths (h) (Panel A), versus time (female age) at 8 psu and 18 psu (Panel B), and in relation to egg production rate (eggs female^{-1} d^{-1}) (Panel C). In panels A and C, the proportion of subitaneous eggs (*SBE*, those hatching within 48 to 72h) are shown (modified after Peck et al. 2008). In panel B, three egg types are shown: 1) *SBE*, 2) potential resting eggs (*RE*) that did not hatch or disintegrate after 3 weeks), and 3) "*Other*", eggs which disintegrated within 3 weeks. In panel C: Regression equations are: SBE_{18psu}=0.854*EPR*+28.4, r^2=0.25, p=0.055; SBE_{8psu}=0.837*EPR*+22.1, r^2=0.7, p<0.0001.

Field data based findings

Among adult females of the studied copepods, *Acartia* spp. was most abundant at both sites and obtained higher peak abundance at *Pier* compared to *NOK* (Fig. IV–5A, Table IV–2). At *NOK E. affinis* was often abundant but was rare or absent at *Pier* (Fig. IV–5B, Table IV–2). At *Pier*, peak abundances of *E. affinis* were between 4 to 435m^{-3}, and their temporal occurrence was more variable (end of April until August) compared to *NOK*. Finally, *T. longicornis* was never found at *NOK* but was regularly encountered at *Pier* (Fig. IV–5C, Table IV–2). Irrespective of the sampling site, peak abundances of *E. affinis* and *T. longicornis* were similar and were 10 to 25% those of *Acartia* spp. (Fig. IV–5A, B and C).

Although *Acartia* spp. were abundant at both sites (Fig. IV–5D), the different salinity conditions between *NOK* (4.9 to 12.4 psu) and *Pier* (11.9 to 21.4 psu) coincided with differences between presence/absence and/or abundance of *T. longicornis* (Fig. IV–5E) and *E. affinis*, (Fig. IV–5F). Despite warmer temperatures and higher *Chl a* concentrations at *NOK*, A*cartia* spp. were most abundant at *Pier* (Figs. IV–4, IV–5) where *Acartia* spp. females were found at 0°C at *Chl a* concentrations generally <5µg L^{-1} (Fig. IV–6A). In contrast, at the *NOK* site, *Acartia* spp. was absent when samples were collected at <10°C and occurred infrequently at <15°C (Fig. IV–6A). Peak abundances of *E. affinis* occurred at ~15°C at *NOK* (Fig. IV–6B). Given the low occurrence of *E. affinis* at the *Pier* site, no trends were described. At *Pier*, the peak abundance of *T. longicornis* occurred in waters colder than 15°C when *Chl a* concentrations were < 5µg L^{-1} (Fig. IV–6C).

The mean abundance of *Acartia* spp. females increased with increasing day length (7 to 17h) and water temperature at *Pier* (Fig. IV–7A) but, despite equal if not warmer temperatures later in the summer, mean abundance sharply declined as soon as day lengths started to decrease. At the same temperature, the abundance of *Acartia* spp. females at *Pier* was always higher at increasing as opposed to decreasing day lengths (with the exception of one sampling point equally high at ~16°C) (Fig. IV–7A). At *NOK*, the increase in mean *Acartia* spp. abundance was delayed until much longer day lengths

compared to the *Pier* site and the decrease in abundance with decreasing day length was much slower. Consequently, peak abundance occurred when day lengths were decreasing at *NOK*. At this site, *E. affinis* abundance increased with increasing day length and when waters were colder than ~15°C (Fig. IV–7B). Longer day lengths and warmer temperatures were associated with dramatic decreases in the abundance of *E. affinis*. Interestingly, the abundance of *E. affinis* did not increase in the autumn when temperatures declined below 15°C. Lastly, the mean abundance of *T. longicornis* females was highest at day lengths between 10 and 17h and when both day length and water temperature were increasing (Fig. IV–7C).

Back-calculations of the time of hatch could be made for *T. longicornis* and *Acartia* spp. at *Pier* in 2007. In that year, peak abundances occurred in calendar weeks 22 (May 29th) and 25 (June 18th). Based upon *in situ* temperatures, development times needed for *T. longicornis* and *Acartia* spp. to reach the adult stage were estimated to be 25 and 53 days, respectively. Hatch dates would be approximately May 5th and April 27th, respectively. For *E. affinis and Acartia* spp. at *NOK* in 2009, peak abundances occurred in calendar weeks 15 (May 25th) and 23 (June 2nd) and calculations suggested that development times of 20 and 52 days would have been required for eggs to each the adult stage. At *NOK*, therefore, hatching of *E. affinis* and *Acartia* spp. was predicted to have occurred on May 5th and April 11th, respectively. These dates at the two sites suggested that eggs hatched at temperatures of approximately 9.2°C, 9.9°C, and 12.7°C for *Acartia* spp., *T. longicornis*, and *E. affinis*, respectively.

Table IV–2 Summary of maximal abundances of *Acartia* spp., *T. longicornis*, *E. affinis* and environmental conditions

		Maximal abundances					
		Calender week	Day length (h)	Temperature (°C)	Salinity (psu)	*Chl a* (μg L^{-1})	Abundance (No. m^{-3})
Acartia spp.	2007 *Pier*	25	17.25	16.8	14.8	9.3	1,524
	2007 *NOK*	34	14.5	19.1	8	15.9	16,764
	2008 *Pier*	35	14.25	16.6	16.7	6.7	1,023
	2009 *Pier*	24	17	15.4	14.6	9.4	7,620
	2009 *NOK*	23	17	16.7	8.8	10.5	3,576
	2010 *Pier*	26	17.25	17.5	13.4	19.9	21,771
	2010 *NOK*	20	16.25	10.3	9.3	3.5	980
T. longicornis (only *Pier*)	2007	23	17	14.3	14.8	10.6	871
	2009	16	14	6	16	2.4	435
	2010	12	12.5	1.5	16.3	4	1,959
E. affinis (only *NOK*)	2007	20	16	13.9	6.2	10.1	2,047
	2009	20	16	13.3	8.2	7.4	2,939
	2010	21	16.5	12.6	8.9	4.5	686

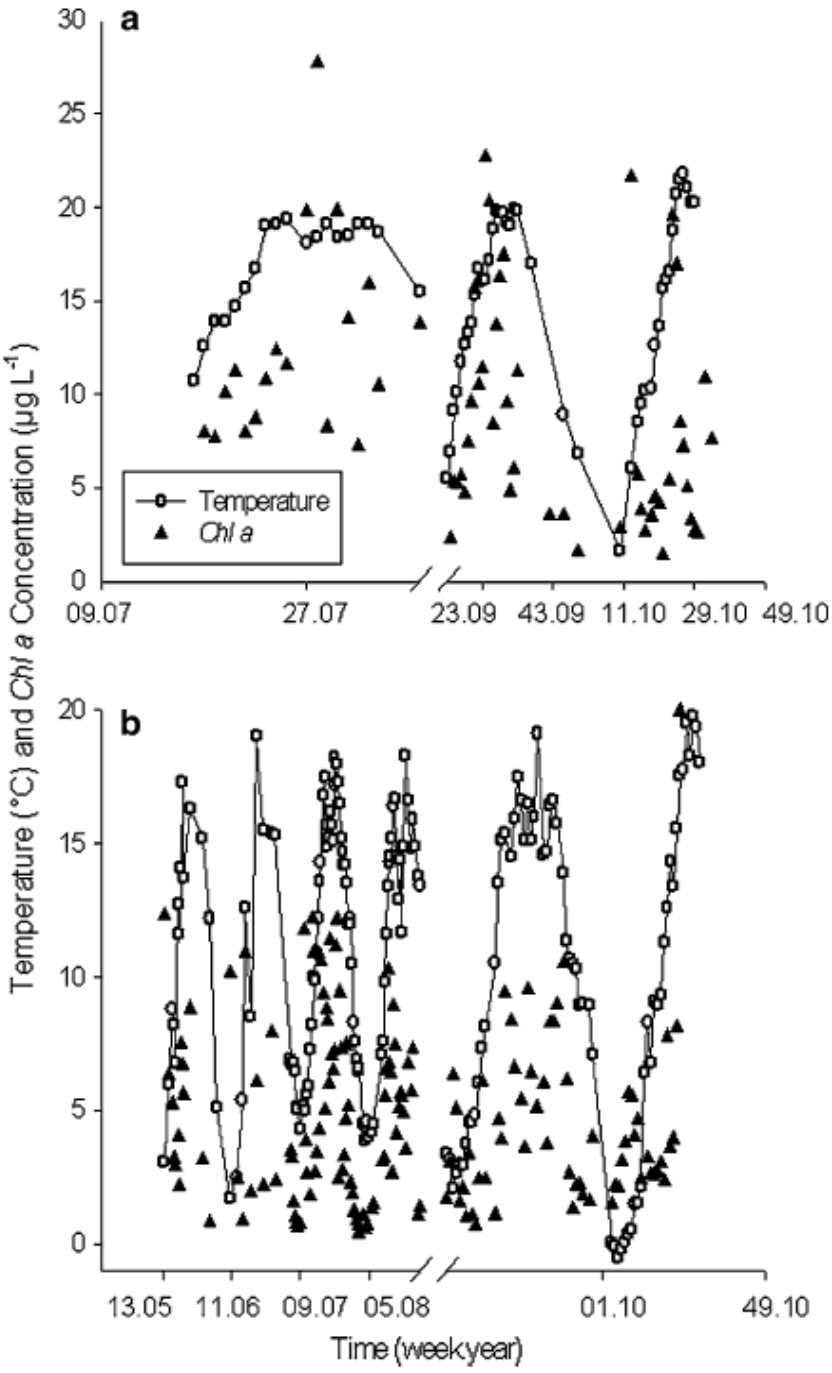

Fig. IV–4 *Chl a* concentration (µg L^{-1}) and water temperature (°C) measured at 7 m depth at *NOK* (Panel A) and *Pier* (Panel B) sampling sites versus time (shown as year and week, yyww) from March 2005 until September 2010. Note, sampling was not continuously performed at either location.

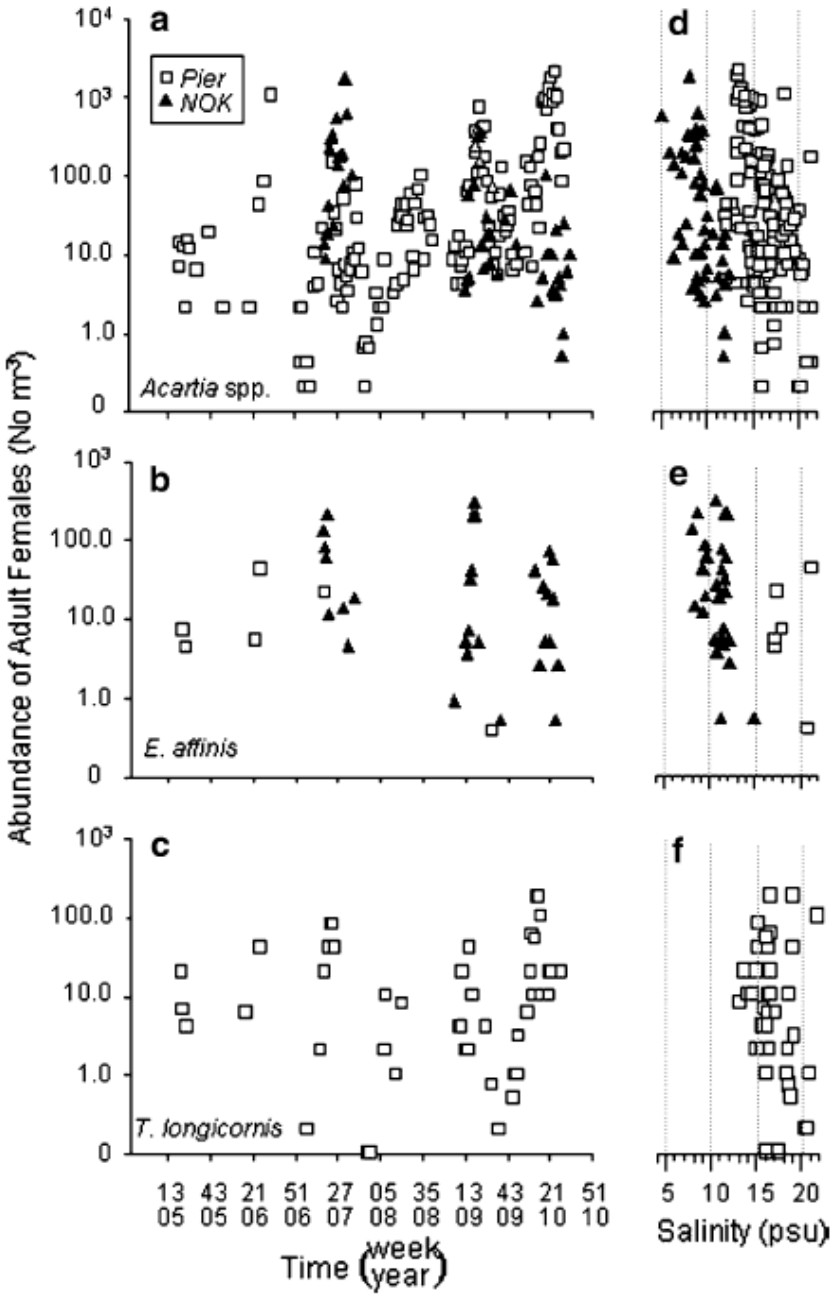

Fig. IV–5 The abundance (individuals m^{-3}) of female copepods of *Acartia* spp. (panels A and D), *Eurytemora affinis* (panels B and D) and *Temora longicornis* (panels C and E) at the two sampling sites (*NOK* and *Pier*) versus time (shown as week and year) as well as water salinity at capture. Note, sampling was not continuously performed at either location.

DISCUSSION

Understanding species-specific differences in physiological optima and tolerances can provide insight regarding how climate-driven changes in bottom-up factors can lead to changes in species abundance, distribution and composition (Pörtner and Peck 2010; Somero 2010). Once robust estimates of these physiological limits have obtained, changes in populations can then be assessed with respect to other (trophodynamic) processes such as the role of key predators. The present study reviews how important environmental factors affect the reproductive success and abundance of calanoid copepods inhabiting shallow, brackish waters of the southwest Baltic Sea. After a brief overview of the *Acartia* species complex in the Baltic Sea, we review how various controlling, masking and directive factors affect copepod reproductive rate and survival and reproductive strategy (production of different egg types). In each case, a brief summary of previous field and laboratory findings is provided as a backdrop for patterns observed in our field data. Moreover, within each sub-section, we first discuss *Acartia* spp., then *T. longicornis*, and finally *E. affinis*. If not otherwise stated, all literature data discussed were collected on populations inhabiting the Baltic Sea.

Acartia spp. in the Baltic Sea

In the present study, copepods in the *Acartia* genus were not identified to species and some general remarks are needed with respect to distribution and physiology of different species. Three congeners (*Acartia bifilosa*, *A. longiremis* and *A. tonsa*) have been reported by Christiansen (1988) and Postel (2005) from seasonal collections made in our study region (the Schlei estuary (northwest of Kiel Bight) and northeast of Kiel Bight up to the Gotland Sea, respectively). Thus, we assume the *Acartia* spp. category in our study consists of these three species. Previous field collections reported that *A. bifilosa* and *A. longiremis* occur throughout the year while *A. tonsa* was absent in the cooler months (e.g., December through May, (Christiansen 1988)). *Acartia longiremis* females undergo dormancy during winter (Davis 1976; Norrbin 1996) while *A. bifilosa* is adapted to colder temperatures (Schnack 1975) and over-summers as resting eggs (*RE*) in the North Sea (Chinnery and Williams 2003). *Acartia tonsa* is originally a tropical species and has been

reported in the Baltic since the 1930's (Brylinski 1981 and references therein). Genetic analyses by Drillet et al. (2008) suggested that the Baltic population of *A. tonsa* probably originated from a population in Narragansett Bay, Rhode Island (USA) or adjacent waters. This species is adapted to warmer temperatures and produces *RE* as an overwintering strategy (Zillioux and Gonzalez 1972; Sullivan and McManus 1986; Drillet et al. 2011). The previous work by Postel (2005) suggested that the three species tend to vary in dominance depending upon the year. However, we assume that *A. tonsa* represented the largest part of the *Acartia* spp. stock at least during some periods of each year because its *EPR* (20-41 eggs female^{-1} d^{-1}) can be 2- to >5-times higher than that of *A. bifilosa* (up to 22 eggs female^{-1} d^{-1}) and *A. longiremis* (5 eggs female^{-1} d^{-1}) in the Baltic Sea (Dutz et al. 2004a; Peck and Holste 2006).

Reproductive Rate and Survival

Controlling and directive Factors: Thermal Windows, Prey Concentrations and Day Length

The majority of net energy available for growth of adult copepods, after allocating energy for metabolic costs is allocated to the production of eggs (reproductive tissue). Therefore, thermal windows supporting growth can be gauged by examining *EPR* at different temperatures. There are only a few studies that have measured *EPR* over a wide enough range of temperatures to properly estimate thermal windows. Optimum temperature (T_{opt}) for *A. tonsa* in the Baltic and North Sea was 23 °C (Fig. IV–2). Studies examining wider ranges in temperatures and working with southwest Baltic populations reported 100% mortality at 34 °C for *A. tonsa* (Holste and Peck 2006). In contrast, *A. bifilosa* is adapted to colder temperatures with Koski and Kuosa (1999) reporting highest *EPR* between 13 to 18°C and high mortality at 24°C. *Temora longicornis* has a similar thermal window as *A. bifilosa* with highest *EPR* occurring at 17°C and 100% mortality at 24°C (Holste et al. 2009). Although using *EPR* is a good approximation of the energy available for growth and the shape of thermal windows, adult body size decreases at warmer temperatures which reduces absolute *EPR* (eggs per female) (see Landry 1978). The relationship can be complex since the size of eggs also

decreases at warmer temperatures (Hansen et al. 2010). Future studies exploring thermal windows should employ mass- and/or carbon-specific *EPR* for a the most robust estimate of the relative physiological benefit accrued at optimal compared to sub-optimal temperatures.

As opposed to *Acartia* spp. and *T. longicornis* that are broadcast spawners, *E. affinis* produces fewer eggs that are brooded in separate clutches in an egg pouch. It is more difficult to estimate the thermal window supporting *EPR* in *E. affinis* compared to *A. tonsa* and *T. longicornis*. Ban (1994, study site: Lake Ohnuma, Japan) and Hirche (1992) estimated *E. affinis EPR* from measurements of clutch size (number of eggs) and inter-clutch duration. Laboratory-derived estimates of *EPR* were 19.0, 34.2, and 34.1 eggs female^{-1} d^{-1}, at 10, 15, and 20°C, respectively (Ban 1994). Field-derived estimates of *EPR* reported by Hirche (1992) were lower (5.5 to 7.8 eggs female^{-1} d^{-1} from 5 to 20°C) Hirche (1992). After combining temperature-dependent functions of clutch size, egg development rate, and female size, those field data (collected in the same system investigated in this study) suggested that *E. affinis* had its peak *EPR* at ~12°C. Controlled laboratory experiments conducted on *E. affinis* suggest a broader thermal window. For example, successful egg hatching in *E. affinis* is quite broad (0 to 25°C) (see Andersen and Nielsen (1997, study site: North Sea) and references therein). This agrees with findings by Devreker et al. (2009) who found relatively high hatching success of *E. affinis* eggs in the Seine estuary at 10 and 15°C, but embryonic development time, inter clutch time, and cumulative clutch size were markedly decreased at 10°C. They showed that *EPR* was significantly higher at 15°C with 13 eggs female^{-1} d^{-1} compared to 10°C (4 eggs female^{-1} d^{-1}). An individual-based model parameterised for *E. affinis* in the Seine estimated maximal *EPR* between 17 and 19°C (Dur et al. 2009).

Temperatures optimal for *EPR* in *T. longicornis* and *E. affinis* in the laboratory occurred at our field sites but these species were nearly absent at temperatures warmer than 15°C (Fig. IV–6). At those warmer temperatures, *Acartia* spp. dominated samples. In general *Acartia* spp. were clearly (4- to 10-times) more abundant than *E. affinis* and *T. longicornis* at both field sites

throughout the year which was contrary to our expectations that *T. longicornis* and especially *E. affinis* would dominate the copepod assemblage at least during specific times of the year. The fact that the highest peaks in abundance of *Acartia* spp. females occurred between mid May and mid October were most likely due to increased productivity of *A. tonsa*, but data with increased taxonomic resolution are needed to confirm this.

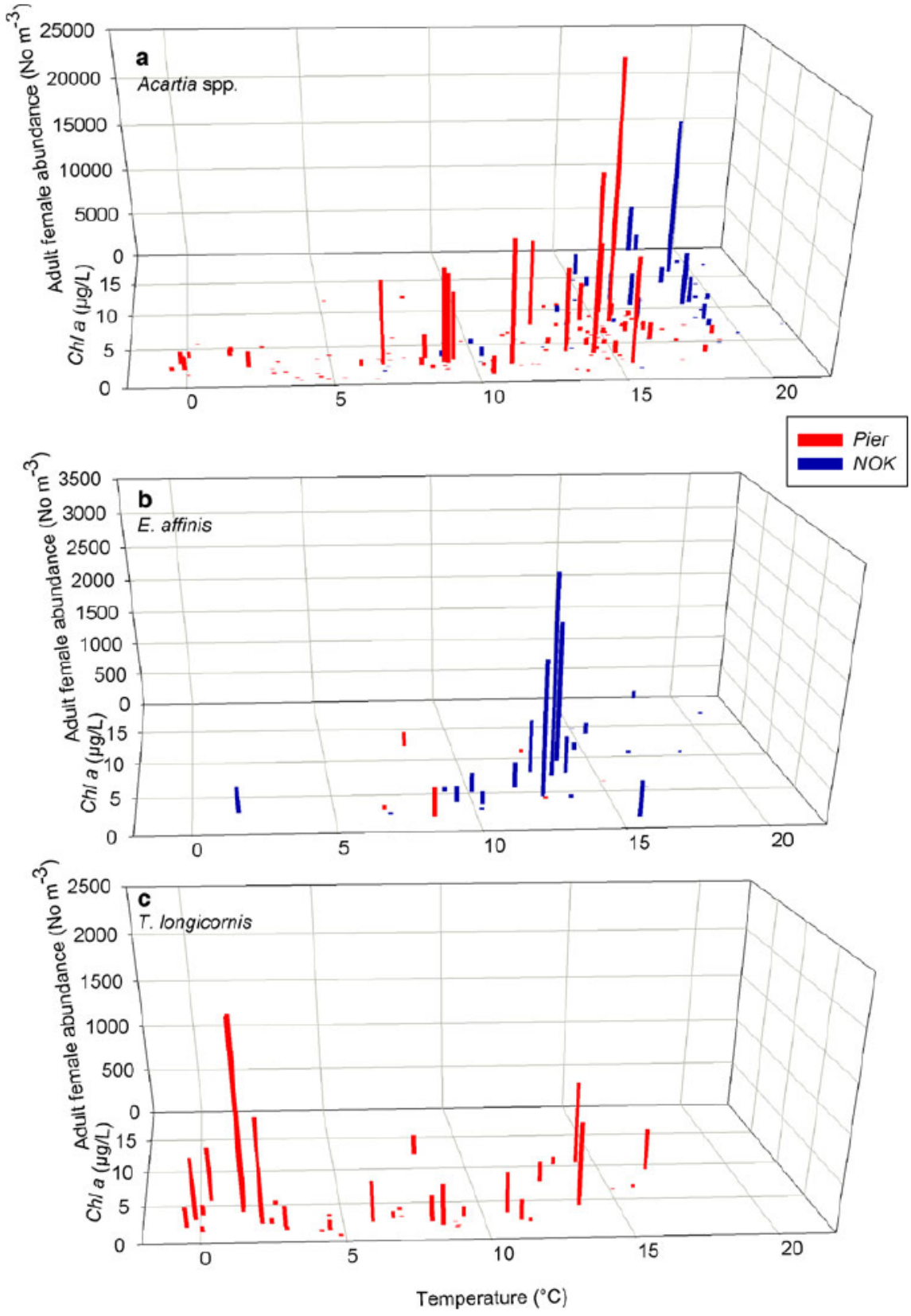

Fig. IV–6 The abundance (individuals m^{-3}) of female copepods of *Acartia* spp. (panel A), *Eurytemora affinis* (Panel B), and *Temora longicornis* (Panel C) versus water temperature (°C) and *Chl a* concentration (µg L^{-1}) at the two sampling sites. Note, the data from five years of sampling are pooled.

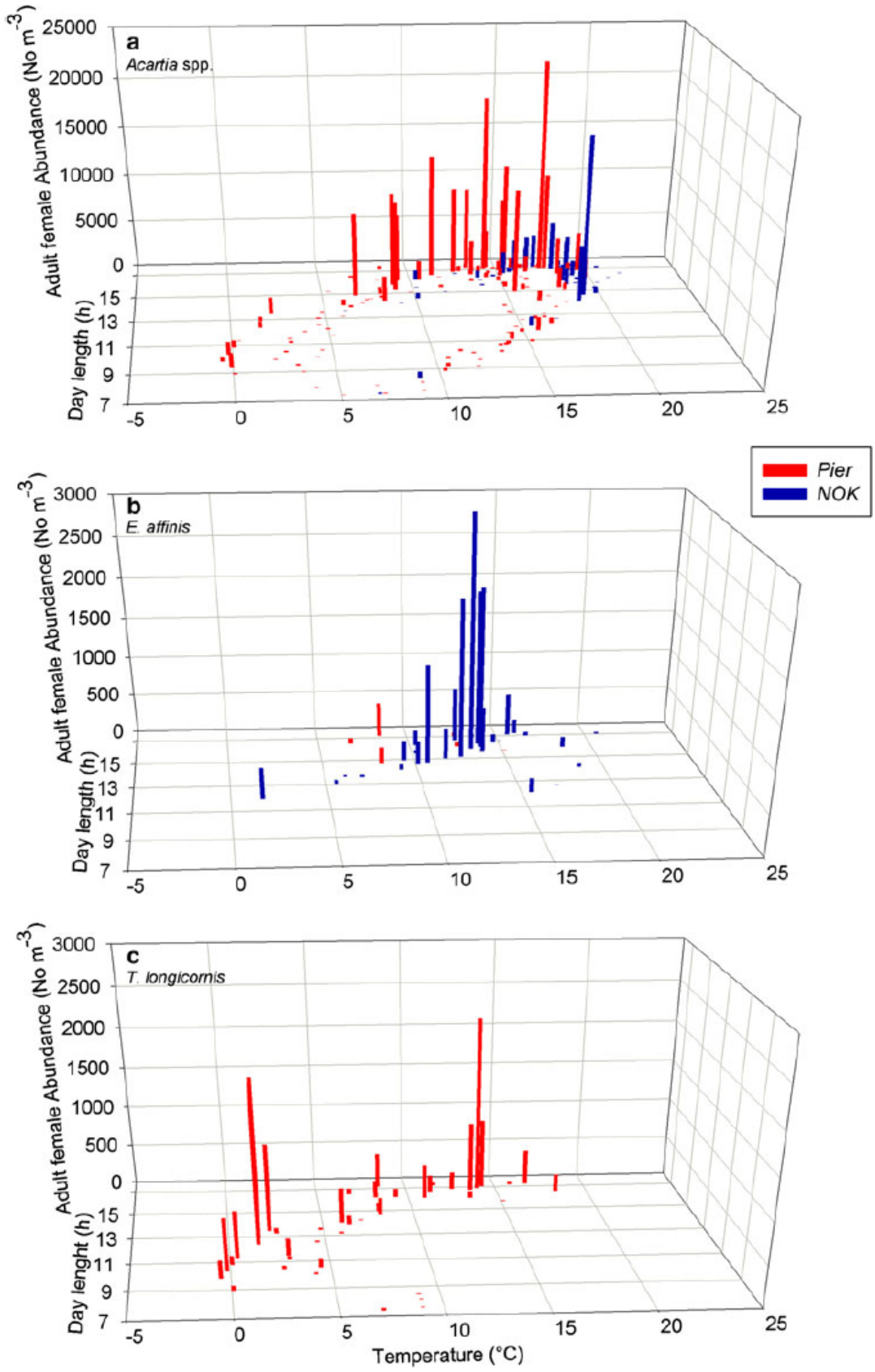

Fig. IV–7 The abundance (individuals m^{-3}) of female copepods of *Acartia* spp. (panel A), *Eurytemora affinis* (Panel B), and *Temora longicornis* (Panel C) versus water temperature (°C) and day length (h) at the two sampling sites. Note, the data from five years of sampling are pooled.

As previously mentioned, seasonality in copepod productivity (within temperate systems) will also be affected by changes in the size of females within cohorts produced during relatively warm and cold periods of the year, an aspect that was not measured in our time series samples. Ambler (1985) found the prosome length of *A. tonsa* off the coast of Texas to decrease from 0.79 to 0.61mm between 21 and 29°C, respectively. Hansen et al. (2010) reported also *A. tonsa* eggs decreased in size with increasing temperatures. Nevertheless, eggs were 6% bigger at 6°C compared to 24°C while the size of females in 9°C was 18% bigger compared to females in 24°C. Hirche (1992) reported nearly 2-fold differences in *E. affinis* adult prosome length between the winter (0.93mm, 5°C) and summer (0.55 mm, 20°C) for *E. affinis*. Ban (1994) reported that adult body size of *E. affinis* was more influenced by food shortage than by temperature; female prosome length decreased by 10% between 10 and 20°C, but decreased by 25% between *ad libitum* and low phytoplankton concentrations. Prey concentrations < 9.5µg *Chl a* L^{-1} caused more dramatic decreases in adult body size in that field study. That finding highlights the importance of feeding conditions as a controlling factor of copepod productivity.

While various studies have focused on temperature as the main factor controlling copepod production, match-mismatch dynamics with phytoplankton often control the productivity of species such as *A. tonsa* in the field (Durbin et al. 1983, study site: Narragansett Bay). Koski and Kuosa (1999) reported that *A. bifilosa* had maximum *EPR* at food concentrations > 14 to 20µg *Chl a* L^{-1} . However, *A. bifilosa* seemed well adapted to changes in *Chl a* concentrations and displayed relatively high *EPR* even at 5µg *Chl a* L^{-1}, a critically low threshold food concentration for *EPR* in *A. tonsa* (Ambler 1986). In Narragansett Bay, Durbin et al. (1983) reported that *EPR* by *A. tonsa* decreased to negligible levels when *Chl a* was 1 to 2µg L^{-1}. Peterson and Bellantoni (1987) reported that *T. longicornis* off central Chile required higher concentrations of *Chl a* than *A. tonsa* to obtain maximal *EPR*. Castellani and Altunbaş (2006) measured highest *EPR* of *T. longicornis* in the Irish Sea when *Chl a* increased above 1 up to 3.5µg *Chl a* L^{-1}.

Our field measurements indicated that *Chl a* concentrations were rarely <2µg L^{-1} at *NOK* (only on two sampling dates) but were often lower than this at the *Pier* site (22% of all sampled dates). *Acartia* spp. was abundant at *Pier* even when *Chl a* concentrations were relatively low. Similarly, the majority of *T. longicornis* was found at *Chl a* concentrations <5µg L^{-1}. Finally, despite reports that *E. affinis* is able to effectively select food in turbid conditions (Gasparini and Castel 1997, study site: Gironde estuary; Tackx et al. 2003, study site: North Sea) most samples containing *E. affinis* in our study were collected when *Chl a* was >5µg L^{-1}. Although such threshold concentrations of *Chl a* are interesting to compare among species and years, it is important to note that *Chl a* is only a proxy of the available prey resources available to copepods. Not only food quantity but also quality (size, biochemical composition) are critical features affecting growth and reproductive success of calanoid copepods (e.g. Houde and Roman 1987, study site: Chesapeake Bay; Dutz et al. 2008, copepods from the North Sea; Diekmann et al. 2009). To better understand the role of phytoplankton as a controlling factor of copepod production, studies of food quality at our study site are needed.

Many environmental factors co-vary and, in this respect it is difficult (and often impossible) to disentangle the direct influence of day length from effects of temperature and prey in field data. Patterns in our field data on adult females abundance suggested that *Acartia* spp. abundance was more linked to increasing day length despite experiencing warmer water temperatures when day lengths started to decline after the summer solstice. Note, predation likely plays an important role in controlling zooplankton populations at this time of the year and is discussed in a later section. For *T. longicornis*, other authors have reported maximum abundances from autumn until spring (Rae and Fraser 1941; Fransz 1975, study site: North Sea) which contrasts with patterns in our field data. At the *Pier* site, *T. longicornis* only occurred under medium and high day lengths and when day lengths were increasing (despite very few exceptions with low abundances). Furthermore, the abundance of adult *E. affinis* did not increase again with decreasing day lengths in the autumn at similar temperatures as in spring. This agrees with previous field studies reporting dominance of *E. affinis* during March – June (Devreker et al. 2007;

Mouny and Dauvin 2002, study sites: Seine estuary). The beginning of decreasing day length might therefore be a cue in *E. affinis'* life history.

Masking Factors: Salinity

Copepods are osmoconformers and display species-specific differences in their abilities to cope with high and low salinity, and fluctuations in salinity can affect different copepods, even members of the same genus, in different ways (Calliari et al. 2008). Hence, changes in salinity may also be more important than changes in temperature in structuring the copepod community composition and productivity of estuarine regions (Attrill 2002).

At extreme levels, hyper- or hyposmostic conditions cause death, but the ability to cope with osmotic stress can be influenced by other factors. Lance (1963) was the first to demonstrate that temperature affected low salinity tolerance of *A. tonsa*. The species (UK coastal population) was most tolerant to low salinities at 16 to 20°C (3.6 psu) compared to 10 or 24°C (5.4 psu) or 4.5°C (9 psu). The ability of *A. tonsa* to utilize free amino acids in hyposmoregulation (Farmer and Reeve 1978, copepods off Florida) appears to be limited at cold (winter) temperatures. Without sufficient time to acclimate to low temperatures (e.g., during periods of rapid cooling), mass mortality could occur in areas of low ambient salinity (0 to 6 psu). In *E. affinis*, Devreker et al. (2009) reported a very high post-embryonic mortality (85%) at 25 psu when combined with a low temperature (10°C).

Within tolerable limits of salinity, sub-lethal effects of the increased metabolic load associated with osmotic regulation at sub-optimal salinities result in lower *EPR.* For example, when measured at 6, 10, 14, 20, and 30 psu, the *EPR* by *A. tonsa* was highest at 14 psu (Peck and Holste 2006). This agrees with the findings of Cervetto et al. (1999) who reported that *A. tonsa* from a Mediterranean estuary can tolerate a wide range of salinities (1 to 72 psu) for short time periods (<10h) but that, longer-term (72h) optimal salinities were between 15 and 22 psu. Castro-Longoria and Williams (1999, study site: Southampton Water, UK) found *A. bifilosa* hatching success (%) to be highest at 25 psu with lower values occurring at both higher and lower salinities. For *T.*

longicornis, Holste et al. (2009) reported much reduced *EPR* at 8 psu compared to higher salinities. In the Baltic Sea, *T. longicornis* has been collected at 7 psu (Peters 2006) but the species is known to occur at higher (marine) salinities around 33 psu (e.g. in the North Sea: Krause et al. 1995). In a study by Viitasalo et al. (1994), *E. affinis* avoided salinities above 6.5 psu and respiration rates of adult *E. hirundoides* (likely *E. affinis*) suggested metabolic stress at 9 psu, particularly at the warmest of three temperatures tested (6, 11 and 16°C) (Gyllenberg and Lundqvist 1979). Cailleaud et al. (2007) found salinities between 5 and 15 psu to be optimal for maximum expression in enzymatic activities of *E. affinis* from the Seine estuary and Devreker et al. (2009) reported a high salinity of 25 psu to have a negative effect on embryonic development time, inter clutch time, and cumulative clutch size.

The results of laboratory studies suggest that important, stage-specific differences in salinity tolerance can exist within copepod species. For example, the salinity experienced by nauplii of *T. longicornis* may be particularly relevant for survival and population persistence in the Baltic Sea (Holste et al. 2009). Again, an interaction with temperature was apparent with naupliar mortality at lower salinities increasing at warmer temperatures (Holste et al. 2009). A similar temperature x salinity interaction was reported in a laboratory study by Devreker et al. (2004) working on *E. affinis* naupliar survival at 0 to 35 psu. In that study, survival was highest at 15 psu but was only reduced at the extreme values of salinity (0 and 35 psu).

Our field data thus agree with reported tolerances of these six species to salinity. *Acartia* spp. are known to be euryhaline and were abundant across the entire range of salinities at both sites (4.9 to 12.4 psu at *NOK* and 11.9 to 21.4 psu at *Pier*). Of the six species examined here, *T. longicornis* is the least tolerant of low salinity and was absent from the *NOK* site. At the *Pier* site, *T. lonigcornis* was only found at salinities >13 psu, which appears to be the threshold value for population persistence. Maximal abundances of *T. longicornis* occurred at higher salinities (~16 and 19 psu). The abundance of *E. affinis* was highest between 5 and ~10 psu, rapidly declined at higher

salinities, and was absent above 19 psu. At the *Pier* site (11.9 – 21.4 psu), the extremely low and highly variable abundance of *E. affinis* likely indicates that adults were merely, occasionally transported to the site via advection.

Reproductive Strategy

Production of different Egg Types

Most of the species examined here produce different egg types including resting eggs as a strategy to survive unfavourable conditions. Understanding the intrinsic and extrinsic (environmental) triggers for the production of different egg types is critical for understanding patterns in phenology and factors influencing population persistence. In the copepod literature, there is a potentially confusing array of terms given to different egg types but most authors have classified eggs based upon the time required for hatching. “Subitaneous eggs” (*SBE*) hatch rapidly within hours to days (Grice and Marcus 1981), while “delayed hatching eggs” (*DHE*) require days to weeks to hatch (Chen and Marcus 1997). Finally, in our definition resting eggs (*RE*) include quiescent and diapausing eggs which are dormant over a longer period of time. As diapausing eggs require an obligatory refractory phase before hatch, while quiescent eggs hatch when conditions become favourable, Grice and Marcus (1981) define the latter as quiescent subitaneous. A myriad of factors (intrinsic and extrinsic) have been related to the production of different eggs types and, in the following, we discuss the main, directive factors.

Key Directive factors

Most of the controlled laboratory studies examining the triggers for the production of different egg types (*SBE*, *DHE* and *RE*) has been conducted on *A. tonsa* (Holste and Peck 2006; Hansen et al. 2010; Drillet et al. 2011). In terms of eggs that rapidly hatch (*SBE*), Peck et al. (2008) reported a non-linear decrease in rapid (48-h) hatching success of *A. tonsa* eggs with decreasing day length (photoperiod) experienced by adults (see also Fig. IV–3A). In a different study (Diekmann et al., unpublished data) eggs produced at relatively long day lengths (16h), temperature of incubation (and acclimation of adults) had no influence on the proportions of *DHE*, *RE* or *DE* categories.

Second, egg types (categories) produced at the warmest temperature (25°C) did not differ among short (6h), medium (11h), or long (16h) day lengths. When females were acclimated to colder temperatures (9 to 12°C), Holste and Peck (2006) and Hansen et al. (2010) suggested that the production of *RE* was markedly higher. Finally, Drillet et al. (2011) reported that poor feeding conditions induced the production of *DHE* which synchronously hatched after incubation for several months at conditions that mimicked winter (dark, 1.5°C). Taken together, these results suggest that *A. tonsa* is expected to produce resting eggs during unfavourable feeding conditions during short day lengths and at relatively cold (<12°C) temperatures. Furthermore, Holmstrup et al. (2006) reported subitaneous eggs from *A. tonsa* at the Limfjord to enter quiescence when incubated in anoxia and cold temperatures.

Given the brackish environment inhabited by some *Acartia* populations (such as the southwest Baltic), it is important to consider the potential masking effects of salinity on the life history strategy of producing different egg types. In our experiment concerning egg hatching and development, significantly more *RE* were produced at 8 psu compared to 18 psu. Furthermore, at 8 psu the proportions of *SBE* (*RE*) decreased (increased) with decreasing *EPR* (Fig. IV–7C). As previously discussed, other studies have reported increased *RE* production when *EPR* decreased due unfavourable feeding conditions, when copepods experienced unsuitably low temperatures, or when adults experienced short day lengths (Holste and Peck 2006; Peck et al. 2008; Hansen et al. 2010; Drillet et al. 2011). Since temperature and day length were both constant in our experiment, salinity is the most likely factor causing *A. tonsa* to increase the proportion of resting eggs (*RE*) produced.

Female *A. bifilosa* can be found in samples collected during winter in the Baltic Sea and continue to produce eggs at that time, albeit at relatively low rates (Schmidt et al. 1998). *A. bifilosa* do not appear to produce *RE*, at least not as an overwintering strategy. The third congener, *A. longiremis*, is an Arctic relict species that exhibits adult diapause in Norwegian fjords (Norrbin 1994). That strategy may be an adaptation to the deep water habitats that are unsuitable for *RE* (Norrbin 1994). Winter temperatures in the shallow waters of the Baltic

Sea are likely warm enough to support active reproduction and Peters et al. (2007) found high abundances of *A. longiremis* nauplii during winter in the southwest Baltic (Bornholm Basin). The life history strategy of *A. longiremis* in the Baltic Sea is not well studied and it is unknown, for example, how this species survives the summer period.

In contrast, *T. longicornis* appears to utilize a completely different strategy with regard to the production of resting eggs. In the Irish Sea, *T. longicornis* produced mostly *RE* when *EPR* was highest during the spring and early summer shortly after periods of peak *Chl a* concentrations (5 to >20µg L^{-1}) (Castellani and Lucas 2003). Those authors suggested that the timing of *RE* production during peak *Chl a* would be advantageous to the population in terms of avoiding predation pressure. In the northwest Atlantic, Maps et al. (2005) documented marked declines in hatching success of eggs produced and incubated at temperatures warmer than 14°C. In those experiments, *EPR* increased with increases in temperature from 2 to 18°C indicating that, at least for *T. longicornis*, the production of potential resting eggs occurs when *EPR* is relatively high.

Work on *E. affinis* at different sites suggests the production of eggs that did not rapidly hatch were related to short day lengths, cold temperatures, and high copepod population densities (Ban 1992; Flinkman et al. 1994; Katajisto 1996; Andersen and Nielsen 1997; Katajisto et al. 1998; Roman et al. 2001). For example, Ban (1992) collected nauplii of *E. affinis* in lake Ohnuma, Japan, during late autumn (November) and reared them in the laboratory under spring conditions (15°C, 12h). Females raised from these nauplii produced exclusively subitaneous eggs, whereas those raised from nauplii collected in spring (May) and incubated under late autumn conditions (10°C, 10h) produced exclusively diapause eggs (here eggs that did not rapidly hatch). Hence, *E. affinis* appears to have a strategy similar to *A. tonsa* with regard to cues for the production of potential resting eggs.

Although we only examined *in situ* female abundance in our field study, the literature (previously reviewed) suggests that populations of some of these

species should persist during portions of the year as resting eggs at our field sites (Madhupratap et al. 1996). *DHE* would be a good life history strategy to allow populations to persist through seasons of unfavourable conditions as well as to spread to different areas (Drillet et al. 2011). Drillet et al. 2011 reported that 50% of *A. tonsa* females produced subitaneous and *DHE* eggs and that the latter displayed a synchronous, 80% hatch after a 5-month cold storage period but lower *HS* after longer-term storage. In an earlier study Drillet et al. (2006) found no change in the viability and biochemical composition of eggs stored for ≤1 year at 2 to 3°C. Concerning salinity, at least the eggs produced by *A. tonsa* appear unaffected by changes in salinity during their resting period as reported by Hansen et al. (2012). Using short-term (24h) incubations at different salinities they reported that changes in salinity affected egg volume but that the embryo was able to maintain its volume and osmolality as it is protected by an impermeable plasma membrane. Utilizing longer-term incubations, Holmstrup et al. (2006) observed, that *A. tonsa* eggs were viable for up to 20 weeks at salinities between 10 and 50 psu but very low salinities were only tolerated for a short time. Katajisto (2006, study site: Northern Baltic Sea) found that the development of *A. bifilosa* eggs was not affected by darkness and low temperature but that eggs became quiescent under anoxic conditions. The eggs remained viable for one year in anoxic water and for two years in the sediment (Katajisto 2006). After a sufficient amount of time (refractory phase), the triggers for hatching of resting eggs of calanoid copepods are similar to those for cladocerans and rotifers (May 1987; Vandekerkhove et al. 2005) and include specific temperatures and day lengths (Katajisto et al. 1998; Dutz et al. 2004b). Our back-calculated hatching dates of adults at peak abundance suggested that eggs (*RE* or *DHE*) hatched at ~9°C, 10°C, and 13°C for *Acartia* spp., *T. longicornis*, and *E. affinis*, respectively. Thermal thresholds required for egg hatching in these copepods are not known but temperatures between 9 and 12°C agree with those reported in the literature for *Acartia* spp. (10°C by Christiansen (1988)) and *T. longicornis* (~5 to 10°C by Castellani and Lucas (2003)). However, thermal triggers for *RE* hatching in *E. affinis* (if they exist) are expected to be colder than 13°C. Hirche (1992) reported minimum fecundity values at 12°C and decreases in field abundance of adults at temperatures warmer than 12°C. It is

likely that the peak in *E. affinis* occurred earlier in the year when sampling was not conducted Nevertheless, Katajisto (2006) reported, that *E. affinis* produced two types of eggs, subitaneous and diapause eggs, with the latter to hatch after a period of chilling at a warm temperature (13°). Obviously, our back-calculations represent a test of how likely it is that resting eggs occur at our sampling sites. We cannot say if the adults we sampled were hatched from eggs at the same sites or from other locations. A complex hydrography exists in the Baltic Sea and plankton can be advected to coastal areas (Hinrichsen et al. 2001).

The role of predators

Although the present study has focused on the role of abiotic and biotic (bottom-up) factors affecting copepod productivity in shallow coastal water, copepods are the primary diet item of most secondary consumers in marine and estuarine environments and top-down control of their populations is possible, particularly in the Baltic Sea (Möllmann et al. 2008). The present field locations and other coastal areas represent important sites for spring and autumn spawning herring (*Clupea harengus*) and it can be assumed that herring larvae and juveniles likely have a severe predation effect on the copepod populations in these areas (Aro 1989). Sprat (*Sprattus sprattus*) is the dominant clupeid in Baltic waters that can exert top down control of its copepod prey (Rudstam 1988; Möllmann et al. 2008). Trophic cascades documented in offshore areas of the Baltic Sea highlight the tight inter-dependence of copepods and clupeid fishes (e.g., Möllmann et al. 2008; Casini et al. 2011). In offshore areas such as the Gotland Basin, *T. longicornis* was more important than *Acartia* spp. in the diets of sprat (Möllmann and Köster 2002). In the Bornholm Basin and Central Baltic Sea, *Acartia* spp. and *T. longicornis* formed the highest proportions of gut contents in larval sprat (Dickmann et al. 2007). Large schools of sprat have been found in starving condition during late summer (August) at locations close to those to our field sampling sites (Baumann et al. 2007) highlighting the importance of fishes as regulators of the copepod community at that time of year. Furthermore, ctenophores and other gelatinous zooplankton can be important predators of copepods populations during summer months (Javidpour et al. 2009). Thus,

changes in adult copepods documented in our time series, particularly during late spring and summer are attributable to a combination of both bottom-up and top-down processes.

CONCLUSIONS

The present study described how various environmental factors affect key calanoid copepods in shallow, brackish, temperate waters using a metabolic framework originally proposed by Fry (1971). Environmental factors such as temperature, salinity, day length, and prey availability act synergistically (with predation) to influence vital rates. We provide examples of key interactions by reviewing how temperature, salinity, day length and/or prey abundance affect survival, reproductive success (*EPR*), and reproductive strategy (types of eggs produced) in the dominant calanoid copepods inhabiting the southwest Baltic Sea. This framework distinguishing controlling, masking, and directive factors was discussed in light of laboratory research on reproductive strategy and success, and field time series data on seasonal abundance of adult females of *Acartia* spp., *T. longicornis* and *E. affinis*. Our literature review highlights thermal windows that control productivity, the potential affect of day length as a "directive factor" for reproductive strategy, and the importance of salinity as a key "masking" factor interacting with temperature to alter reproduction in adult calanoid copepods. Many species live at the edge of their salinity tolerance in the Baltic Sea and this is highlighted by differences in the abundance of *T. longicornis* and *E. affinis* between two, relatively nearby field locations. Our review also highlights the need to examine earlier life stages due to potential ontogenetic differences in the sensitivity to environmental factors. Furthermore, more research is needed on the physiology, vital rates, and life history strategy of *A. longiremis*, a copepod highly abundant in the Baltic, but poorly understood.

To understand how interactions among different metabolic stressors (e.g. temperature, salinity, prey level) constrain the life history strategy of copepods, estimates of metabolic scope are needed under different combinations of key factors (*T*x*S*, etc.). Such estimates could be made by combining measurements of respiration (energy loss) with grazing (energy gain) to calculate "scope-for-growth", a common measurement technique utilized for decades in ecotoxicological research on invertebrates (Widdows et al. 1981; Maltby et al. 1990) that is now re-emerging in climate research (e.g., Stumpp et al. 2011). The Baltic Sea is predicted to freshen and warm by 3 to 3.5°C in

the next 80 years (Meier 2006) and continuing to collect basic physiological data on the synergistic effects of different environmental factors (including pollutant loads in near-shore areas) will be needed if we hope to understand how climate-driven changes might impact copepods and subsequent match-mismatch dynamics between copepods, their prey, and their predators.

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CHAPTER V

MANUSCRIPT 4)

Changes in the Reproductive Potential of Calanoid Copepods during Laboratory Trials

A. Berenike S. Tietjen, Klaus B. Huebert and Myron A. Peck

MANUSCRIPT 4)

Changes in the Reproductive Potential of Calanoid Copepods during Laboratory Trials

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ABSTRACT

Here, we describe daily measurements of the reproductive characteristics (egg production rate (*EPR*) and egg hatching success (*HS*)) of *Acartia tonsa* (Copepoda:Calanoida) made during 12, relatively long-term (6 to 12-d), laboratory trials. Trials were routinely conducted at our aquarium facility to examine the influence of culture conditions including subtle differences in photoperiod, temperature and food quantity (diatom, *Thalassiosira weissflogii*), and more marked differences in salinity (8, 18, 33 psu) and algal quality (wide ranges of algal growth rates and protein-carbohydrate ratios) as well as female age on reproduction. In each trial, copepods received the same algal source and were maintained in small groups in test chambers (5 to 16 replicate chambers per trial). We report the daily magnitudes and the time course of changes in *EPR* and *HS* and relate these, when possible, to intrinsic and extrinsic factors. The large number of replicates and days allowed us to more thoroughly explore not only mean rates but variability within and among test chambers. Based upon observed trends in *EPR* and *HS*, practical recommendations on the protocols used to measure copepod *EPR* for ecological studies, toxicity bioassays, and aquaculture production are provided. For example, consistent changes in *EPR* with time (9 of 12 trials) suggest that short-term (1- to 4-day) measurements of copepod *EPR* will likely not capture maximum reproduction rates. Depending upon the absolute test temperature, subtle changes in temperature (±2°C) may have little impact on *EPR* but large effects on short-term *HS*. The *HS* was often consistently high (e.g., with no variability among replicates) but, in some trials, became unexpectedly variable or consistently low. These changes were associated with a switch in feeding methods. These trials demonstrate that a combination of interacting factors can be expected to influence both the mean and variance of *EPR* and *HS* in calanoid copepods demanding tight control of rearing and measurement protocols.

INTRODUCTION

Due to the abundance and trophodynamic importance of copepods in marine systems, “copepod production” is often synonymous with “secondary production” and understanding the dynamics of the latter demands thorough investigation of how various abiotic and biotic factors influence the former. Copepod egg production rate (*EPR*, # eggs female^{-1} d^{-1}) is commonly measured and can provide estimates of the relative condition or growth “status” of individuals and populations (e.g. Omori and Ikeda 1984, Poulet et al. 1995). Furthermore, controlled measurement of the impacts of different environmental factors on copepod *EPR* may lead to process understanding in terms of the factors responsible for changes in the abundance, productivity, and/or phenology.

Measurements of the reproductive success of calanoid copepods (egg production rate and hatching success of eggs) are commonly made using a series of steps including: 1) collection, identification and sorting of animals 2) acclimatization of animals to novel (test) conditions, 3) transfer of animals into measurement chambers, and 4) measuring *EPR* and, often 5) incubating those eggs (either alone or in groups) to quantify the success of hatching. Runge and Roff (2000) provide a thorough review of these methods. The amount of time (days) required between loading and testing (time between steps 3 and 4 considered an acclimation period) varies considerably among studies and species. Similarly, the number of replicates used on any day (number of test chambers) and the number of determinations of *EPR* and *HS* from the same test chamber (replication in time, days) also vary widely among studies but is normally between 3 to 4 chambers, and 1 to 3 days, respectively (Runge and Roff 2000, Holste 2009). The lack of a high number of replicate tanks and relatively long-term measurements of *EPR* is likely due to both practical constraints (i.e. the large amount of time and effort required to make these measurements - particularly onboard research vessels) and an implicit assumption that the mean values of *EPR* and *HS* calculated from only a few chambers measured during short-term trials will be similar to those obtained from many replicates measured over longer time periods.

Acartia tonsa (Dana), 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 has been very well-studied. Previous studies have examined the influence of key environmental factors on *A. tonsa* growth and reproduction including the effect of 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 between temperature and food quantity (e.g., Klein Breteler and Gonzales 1986, White and Roman 1992), as well as the effect of salinity (Cervetto et al. 1999, Peck and Holste 2006). In the last decades, *Acartia tonsa* has not only been grown for marine fish aquaculture (Støttrup et al. 1986, Peck and Holste 2006), it has also become widely used in standard toxicity tests examining acute endpoints such as mortality (Pinho and Bianchi 2010), semi-chronic endpoints such as immobilization (Rose et al. 2006, Gorbi et al. 2012) and chronic endpoints such as osmotic balance and enzyme activity (Na^+, K^+-ATPase) (Pedroso et al. 2007) and reproductive success (Bielmyer et al. 2006).

This paper describes the results of 12, relatively long-term (8- to 15-d) trials conducted to quantify the mean and variability in fecundity and egg hatching success (*EPR* and *HS*) in *Acartia tonsa*. Trials examined the influence of culture conditions including subtle differences in light regime, temperature and quantity of algal food (diatom, *Thalassiosira weissflogii*), and more marked differences in salinity (8, 18, 33 psu) and algal quality (wide ranges of growth rates and protein-carbohydrate ratios) as well as female age on *EPR* and *HS*. A relatively large number of replicate chambers were included (5 to 15 chambers with small groups of copepods) and the relatively long duration of trials allowing us to discuss sources of variability in reproductive success, a topic rarely explored in research on calanoid copepods (Mauchline 1998). We provide recommendations for measurements of reproductive success in this, and potentially other small neritic copepod species.

METHODS

This study includes the data of twelve trials of feeding experiments using calanoid copepod *Acartia tonsa* feeding on the marine diatom *Thalassiosira weissflogii* (Table V–1). Some of the data reported within two of these trials (3 and 7) were previously published by Diekmann et al. (2009).

The culturing of the phytoplankton and copepods, quantification of egg production rates and egg hatching success, and the biochemical analyses of algal carbohydrate and protein were made using the methods described in Diekmann et al. (2009). However, differences existed in the number of replicates, water salinities, temperatures, light regimes, feeding regimes, and ages of the females among the trials. All copepods were fed one time each day in each trial except after day 7 in trials 2, 4, 10 and 11, when copepods were fed three times a day. Furthermore, in trials 6 and 12, gentle aeration was used in each copepod chamber to keep the algae well mixed within the chamber. The sinking of algae was sometimes observed depending upon the culture salinity, and those trials helped identify whether *EPR* and *HS* might be affected by the presence or absence of mixing (e.g., an increase in *EPR* would suggest that, all other culture aspects being equal, copepods might have been food limited due to the sinking of algae to the bottom of chambers where it was unavailable to copepods).

Phytoplankton cultures

Cultures of the diatom *Thalassiosira weissflogii* (strain CCMP 1336) were obtained from the Provasoli-Guillard National Centre for the Culture of Marine Phytoplankton. Cultures were maintained in sterilized North Sea seawater diluted with freshwater to one of three salinities (8, 18 or 33 psu), one of two light regimes (11:13 or 13:11 L:D), and one of three temperatures (15, 16 or 19 °C). (Table V–1). The cultures received about 100 $\mu Em^{-2}s^{-1}$ during the light phase. Cultures were continuously mixed using aeration to minimize light limitation through self shading. The seawater was enriched with 17mL of Walne's medium (McVej 1993), with 50mg L^{-1} biotin added (Støttrup and Jensen 1990).

Each day, equal volumes of algae were collected from each culture. To exclude any bag effect and to ensure that enough algae were available for daily feedings and samples required for measurements samples were combined. Cell concentrations were determined using a Beckman Multisizer 3 Coulter Counter and sub samples taken and stored for measurements of protein and carbohydrates. The size range of *T. weissflogii* used for calculations of food concentration and sample volume was 8-20µm ESD, representing alive and intact cells.

Copepod cultures used in trials

Acartia tonsa (isolated from the Danish Sound) eggs were hatched in water of the temperature used in the trial and a salinity of 18 psu. The developing cohort of nauplii was acclimated to the salinity used in the trial if that differed from 18 psu within one week. The copepod cultures were maintained under either 11:13 L:D or 13:11 L:D light regimes (depending upon the trial) with a 2µE m^{-2} s^{-1} water surface light intensity during daytime. Copepods were fed *ad libitum* portions of *Rhodomonas baltica*. The temperature, salinity and light regime of the copepod cultures matched those used to grow the diatom cultures in each trial. Once the culture developed to late copepodite stages (C4-C5) the cohort was acclimated to *T. weissflogii* for four days prior to the start of experiments (earlier stages are not able to feed efficiently on *T. weissflogii* by virtue of its size). During this acclimation period (and during the experiment), copepods were fed *T. weissflogii* at 10000 cells mL^{-1} which is equivalent to ~1830µg C mL^{-1} (Støttrup and Jensen 1990). This copepod attains maximum ingestion rates and *EPR* at ~1500 µg C mL^{-1} and, thus, copepod ingestion and *EPR* were not considered to be limited by the quantity of food supplied each day in each trial. Oxygen saturation was ensured by daily water changes in each trial (aeration was also used in trials 6 and 12 to keep the seawater and algae more thoroughly mixed).

Egg Production and Hatching Measurements

Four adult females and one male were placed into each holding chamber (8.4cm in height, 4.5cm diameter and with 130µm nitex mesh bottom). Each holding chamber was placed within a 250-mL beaker filled with seawater.

Daytime surface light intensities were about 3 μE m^{-2} s^{-1}. Eggs produced by the copepods passed through the sieve and settled onto the bottom of the glass beaker, thus minimizing the possibility of egg cannibalism. Each day of the experiment, each holding chamber was transferred to a new beaker and the eggs from the previous 24h were collected onto a 35μm sieve, transferred to a Bogorov tray, and counted. Eggs were then placed within 200 ml of culture water (algae and seawater). The number of unhatched eggs was counted 72-hr later. This quantified the percentage (%) of eggs that rapidly hatched (hatch success, *HS*). The copepods within each holding chamber were counted each day and any dead copepods were removed and the sex determined. At the completion of the experiment, all copepods within the beakers were sexed again, to ensure correct enumeration of females. Copepods were maintained for three days using these conditions prior to the start of data collection – to allow time for the copepods to acclimate to test chambers.

Biochemical Analyses

Protein

For the protein analyses, algae samples were collected onto a pre-combusted filter (Whatman GF/C) and immediately frozen at -80°C. A minimum of 500,000 cells were sampled to ensure sufficient biomass for analyses. A Sigma-Aldrich protein assay kit (#BCA-1 and B 9643) was used to measure protein content. In this method, proteins reduce alkaline Cu(II) to Cu(I) in a concentration dependent manner (Lowry, 1951). Bicinchoninic acid is a highly specific chromogenic reagent for Cu(I), forming a purple complex with an absorbance maximum at 562nm (Smith et al. 1985). Bovine Serum Albumin (BSA) was used as the protein standard. Absorbance of the solution was measured at 562nm made using a SAFAS flx-xenius spectrofluorometer.

Carbohydrate

Samples for carbohydrate analyses were taken as described under “protein analysis”. The determinations were made based on the method of Herbert et al. (1971) and Dubois et al. (1956). Following these techniques, furan derivates are formed by adding 96% sulphuric acid to the sample and

pentoses are converted to α-furfurylaldehyde while hexoses are transformed to 5-(hydroxymethyl)-furfurol. These aldehydes react with phenol to produce characteristically coloured products. Measurements of carbohydrates were expressed as glucose equivalents. A 10mg L^{-1} solution of D (+) glucosemonohydrate was used as a primary standard and samples were measured photometrically at 490 nm.

Statistical analyses

For each trial, the overall mean and standard deviation of egg production rate and hatch success were determined using equal weighting of all combinations of experimental day and replicate chamber. To show the development of each trial over time, daily means and standard deviations were determined. Further, the standard deviation among daily means around the overall mean was used to quantify the magnitude of day-to-day variability. Equivalently, the standard deviation among different means for each replicate around the overall mean was used to measure among-replicate variability. Finally, the relative contribution of day effects and replicate effects to total variability was quantified by the coefficient of determination (R^2) between individual measurements and daily means or replicate means, respectively.

Table V–1 Summary information for culture conditions, *Thalassiosira weissflogii* growth rates and P-C ratios and *Acartia tonsa* egg production rate (*EPR*, eggs female^{-1} day^{-1}) and hatching success (*HS*) in each of the 12 laboratory trials. The mean and standard error (SE) are provided (replicates averaged on each day). [a] copepods were fed three times instead of once a day from day 8 on; [b] copepod beakers were aerated

Trial	Culture Conditions			*Thalassiosira weissflogii*						*Acartia tonsa*						
ID	Salinity	T	Light Regime	Duration	Replicate	Algal Growth Rate		Algal P-C ratio		Duration	Replicate	Initial female age	EPR		HS	
	(psu)	(°C)	(L:D)	(days)	(no.)	(d^{-1})				(days)	(no.)	(days)	(eggs female^{-1} d^{-1})		(%)	
						Mean	SE	Mean	SE				Mean	SE	Mean	SE
1	18	16	13:11	11	2	0.14	0.05	0.12	0.01	8	6	18	17.52	2.06	90.66	3.89
2[a]	8	16	13:11	18	2	0.06	0.06	0.04	0.02	15	6	28	27.73	2.66	60.33	8.49
3	33	19	11:13	13	4	x	x	0.61	0.04	10	15	15	29.52	2.53	91.99	1.9
4[a]	8	16	13:11	17	1	0.05	0.04	0.02	0.01	14	6	28	32.71	3.35	51.43	7.79
5	8	16	13:11	14	2	0.1	0.03	0.08	0.01	11	6	18	34.11	2.13	68.84	3.57
6[b]	18	15	11:13	11	6	-0.01	0.01	0.13	0.01	8	6	20	36	3.07	70.6	8.55
7	33	19	11:13	16	2	x	x	0.74	0.07	13	15	15	36.75	3.52	94.93	1.47
8	18	16	13:11	14	1	0.21	0.05	0.16	0.34	11	6	18	39.38	3.46	56.01	3.86
9	8	16	13:11	14	1	0.22	0.07	0.09	0.01	11	6	18	40.37	3.35	59.59	4.18
10[a]	18	16	13:11	18	1	0.07	0.03	0.05	0.02	15	6	28	40.44	5.46	61.23	8.01
11[a]	18	16	13:11	18	2	0.06	0.03	0.09	0.01	15	5	28	40.65	6.72	70.43	7.82
12[b]	18	15	11:13	11	2	0	0.05	0.14	0.01	8	6	20	41.11	4.03	69.25	4.88

RESULTS

Summary of trends with time in each trial

The following provides a summary of the time course of changes observed in key algal (growth rate and quality, i.e. P-C ratio) and copepod reproductive (*EPR*, *HS*) characteristics in each of the 12 trials. The trials are organized by increasing mean *EPR* (Table V–1).

Trial 1: Two cultures of *T. weissflogii* were grown and fed to copepods within six replicate chambers. Algae and copepods were maintained in 18 psu water at 16°C and a light regime of 13:11 L:D for 11 days. The algal culture water was initially enriched with nutrients and then cultures were grown in static conditions (no water exchange and no further nutrient addition). The cell concentrations (data not shown) increased from day 1 to 5 and then remained more or less constant until the end of the trial (data not shown). Therefore, algal growth rates were positive until day 5 and negative or very low after day 5. The P-C ratio changed little during this trial and ranged from 0.09 to 0.15. The copepod mean *EPR* was lowest in this trial (17.5 ±11.4 eggs female^{-1} d^{-1}) and displayed temporal variability. *EPR* decreased to < 10 eggs female^{-1} d^{-1} after day 4 but then increased from day 7 onward to ~20 eggs female^{-1} d^{-1}. The mean (±SD) egg hatching success (*HS*) was high and not variable (90.7±11.5%) across all days. Female mortality was 35% in this trial.

Trial 2: Temperature, light and nutrient conditions of the two algal cultures fed to copepods in this trial were as described in trial 1 except that the salinity was 8 psu (Table V–1). This trial was conducted for 18 days. Algal growth rate was positive (the cell numbers increased with some variability) until day 12 and then cell concentrations were stable until day 15. The P-C ratio varied from 0.07 to 0.15 over the experimental period but without any clear trend (note, only five daily measurements were available). The mean (±SD) *EPR* of the six replicates was 27.9(±11.5) eggs female^{-1} d^{-1}. The mean *EPR* increased within the first four days from 20 to 40 eggs female^{-1} d^{-1}, was stable on days 5 to 8, and slightly declined to a value of 20 eggs female^{-1} d^{-1}. The *HS* displayed the same trend, increasing from 40 to 75% until day 4 and decreasing to 49% by the end of the trial on day 18. There was no clear response of mean *EPR* and

mean *HS* due to the additional daily feedings (and mixing) provided from day 8 onward. A total of 17% of the females died during this trial.

Trial 3: The algal culture and copepods were maintained at 19°C under a light regime of 11:13 L:D and at a salinity of 33 psu. The algal culture nutrient conditions were the same as described for trial 1. Algal cell concentrations (data not shown) increased during the first three days, and were more or less constant for the following five days. From day 4 onward, algal growth rate varied little. The P-C ratio varied from 0.45 to a maximum of 0.95 on day 3 but was fairly constant with time. The mean *EPR* of the 15 replicates showed the same trend as the algae growth rate, increasing during the first three days (from 7.2 to 41.8 eggs female^{-1} d^{-1}) and then remaining fairly constant (~36 eggs female^{-1} d^{-1}) from days four to eight and then sharply declined to 17 eggs female^{-1} d^{-1} by the end of the trial (day 13). Female mortality (5%) was the lowest for all trials.

Trial 4: Two cultures of *T. weissflogii* were grown and fed to copepods within six replicate chambers. Algae and copepods were maintained using 8 psu water at 16°C and a light regime of 13:11 L:D (the temperature, light and salinity conditions of this trial were the same as described for trial 2). A notable difference was that, unlike trial 2, half of the water of the algal cultures was replaced every other day and nutrients were added after the addition of new water. Therefore, algal growth was not expected to be nutrient-limited. Algal instantaneous growth rate varied between -0.31 and 0.23 d^{-1} and was generally positive and relatively higher at the beginning of the 15-day experiment. Not all of the P and C samples could be analysed and measurements were made on only 4 different days. Those P-C ratios varied between 0.08 and 0.12. The mean *EPR* of the six replicates increased from 20 to 60 eggs female^{-1} d^{-1} during the first four days then rapidly declined from day 11 to 14 (from ~40 to ~4 eggs female^{-1} d^{-1}). The mean *HS* was maximal on the fourth trial day (88%) and decreased to zero (0%) over the following 11 days. The additional daily feeding made on day 8 onward had no positive effect on mean *EPR* and *HS*, but were associated with increase daily variability in *EPR*

and a sharp reduction mean *HS*. This trial had the highest female mortality of all trials (96%).

Trial 5: The conditions during this trial were the same as those described for trial 1 (16°C, 13:11 L:D) except that a lower salinity (8 psu) was used. The algal cell concentrations increased from day 1 to 4 and were fairly constant from day 10 until the end of the trial (day 14) except for one increase observed on day 11 (data not shown). The P-C ratios varied between 0.04 and 0.15 showing no clear trend with time. The mean *EPR* of the six replicates increased from day 1 to 3 from 10 to 45 eggs female^{-1} d^{-1} and was stable from days 4 to 8 before declining to 30 eggs female^{-1} d^{-1} at the end of the trial (day 11). The mean *HS* was quite stable (~70%) across all days but slightly declined from day eight onward. In this trial, 9% of the females died.

Trial 6: This trial was conducted at 15°C using 18 psu water and a light regime of 11:13 L:D. The nutrient conditions equal to those described for trial 1. During this 8-day trial, the algal growth rates were near zero or negative and P-C ratios ranged from 0.09 to 0.19, displaying no trend with time. The mean *EPR* of the six replicates slightly declined with time from 44 to 27 eggs female^{-1} d^{-1}. Mean *HS* was more stable, varying from 62 to 77%. During this trial 13% of the females died.

Trial 7: The algal culturing for this trial was the same as that described for trial 3 except that water replacement occurred and nutrient conditions were expected to be similar to those described in trial 4. The P-C ratios in this 13-day trial were the highest compared to all other experiments and showed values from 0.4 to 1.34 with no trend in time. The mean *EPR* of the 13 replicates in this trial showed a general increasing trend and were variable. The mean *EPR* was minimal on day 1 (27 eggs female^{-1} d^{-1}) and maximal on day 9 (49 eggs female^{-1} d^{-1}) of the trial. *HS* was generally very high, varying from 89.6 to 98.6%. A total of 16% of the females died during this trial.

Trial 8: The conditions in this trial were the same as those described for trial 1 except that water replacement and nutrient additions occurred (similar to trial

4). The algal growth rates ranged from -0.11 to 0.5 and were mostly positive during the 14 days of the trial. The P-C ratio varied from 0.01 to 0.53 showing no clear trend with time. During the eleven days of measurements, the mean *EPR* of the six replicates increasing from ≤10 to 58 eggs female^{-1} d^{-1} from day 1 to day 8, respectively. The mean *HS* was maximal on day 1 (72.4%) and, afterwards, remained fairly stable between 50 and 60%. A total of 13% of the females died in this trial.

Trial 9: The algal culturing conditions and copepods were maintained in the same manner as trial 2 except that a salinity of 18 psu was used. Here, the algal growth rates varied from -0.06 to 0.35 and the P-C ratio varied from 0 to 0.16 showing no trend with time (18 days). The mean *EPR* of the five replicates increased during the first five days from 20 to 50 eggs female^{-1} d^{-1}, then slightly declined until day 9 (34 eggs female^{-1} d^{-1}). During the final five days of this trial, mean *EPR* displayed marked day-to-day variability and mean *EPR* repeatedly fluctuated on consecutive days between 34 and nearly 60 eggs female^{-1} d^{-1}. The mean *HS* increased from 43 on day 1 to 88% on day 4 then slowly declined until the end of the trial when it was 46%. Similar to trial 10, the decrease in *HS* occurred simultaneously with change in feeding regime (additional feedings) from day 8 onward. Across all replicates and days, female mortality was 29% in this trial.

Trial 10: This trial closely resembled trial 6, it used 18 psu water, an 11:13 L:D and was conducted at 15°C. The algal instantaneous growth rates were considerably variable (from -0.22 to 0.26 d^{-1}) during this 8-day trial while the P-C ratio was relatively constant, ranging from 0.12 to 0.19. Mean *EPR* slightly increased with time. The mean *EPR* was lowest on day 1 (38 eggs female^{-1} d^{-1}) and highest on day 6 (45 eggs female^{-1} d^{-1}). The mean *HS* of the six replicates was more stable and ranged from 58 to 72% across all days. Female mortality was 25% in this trial.

Trial 11: This trial was conducted as described for trial 5 (16°C, 13:11 L:D, 8 psu) except that water replacement and nutrient additions were used for the algal culture (similar to trial 4). Algal growth rate was negative (-0.03 d^{-1}) on

only the last day of the trial (day 14) and was zero or positive (up to 0.95) the other days. The P-C ratios varied between 0.04 and 0.13. Mean *EPR* of the six replicates rapidly increase during the first three days (from <10 to 40 eggs female^{-1} d^{-1}), followed by a slight increase until day eight (up to 55 eggs female^{-1} d^{-1}). Afterwards the mean *EPR* declined again to < 40 eggs female^{-1} d^{-1}. The mean *HS* was highest on day 1 (80%) and stable (50 to 60%) for the rest of the 11-day trial. Female mortality was 15% in this trial.

Trial 12: This trial was conducted in exactly the same manner as trial 4 except that, instead of 8 psu water, 18 psu water was used. The growth rates were quite variable, ranging from -0.17 to 0.28 with a tendency of decreasing growth rates over the 18-day period. The P-C ratio varied from 0.02 to 0.22. Mean *EPR* of the six replicates was minimal on day 1 (20 eggs female^{-1} d^{-1}) and maximal on day 10 (62 eggs female^{-1} d^{-1}). During the 15 days of measurements, mean *EPR* increased from day 1 to 4, remained stable until day 9, increased on day 10 and then declining again to relatively stable values between 30 and 40 eggs female^{-1} for the rest of the trial. The mean *HS* increased during the first four days from 42 to 83% and then was stable until day 8 before it decreased to 35% until the end of the trial. The increase in mean *EPR* on day 10 onward and the decrease in mean *HS* from day 8 onward coincide with the day (day 8) when copepods were switched from being fed algae one- to three-times per day. A total of 35% of the females died in this trial.

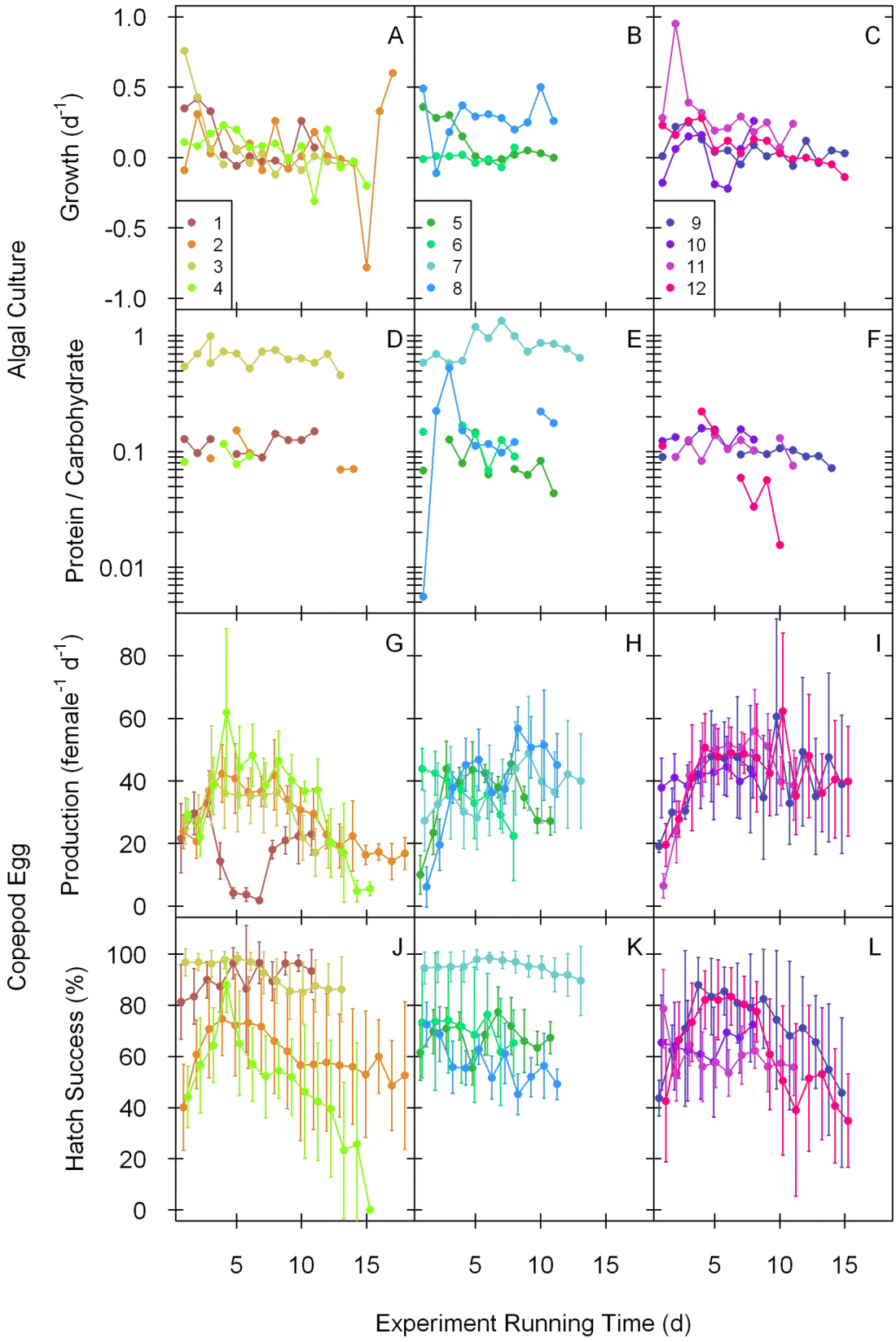

Fig. V–1 Time course of changes in algal instantaneous growth rate (day^{-1}), the algal protein-carbohydrate ratio, mean (±SD) egg production rate (*EPR*, eggs female^{-1} d^{-1}) and mean(±SD) egg hatch success (%). Each of the three columns displays data collected in four trials (indicated by color, see legends). The trials are organized in columns based upon mean *EPR*: leftmost (lowest mean *EPR*), middle (intermediate mean *EPR*), right (highest mean *EPR*)

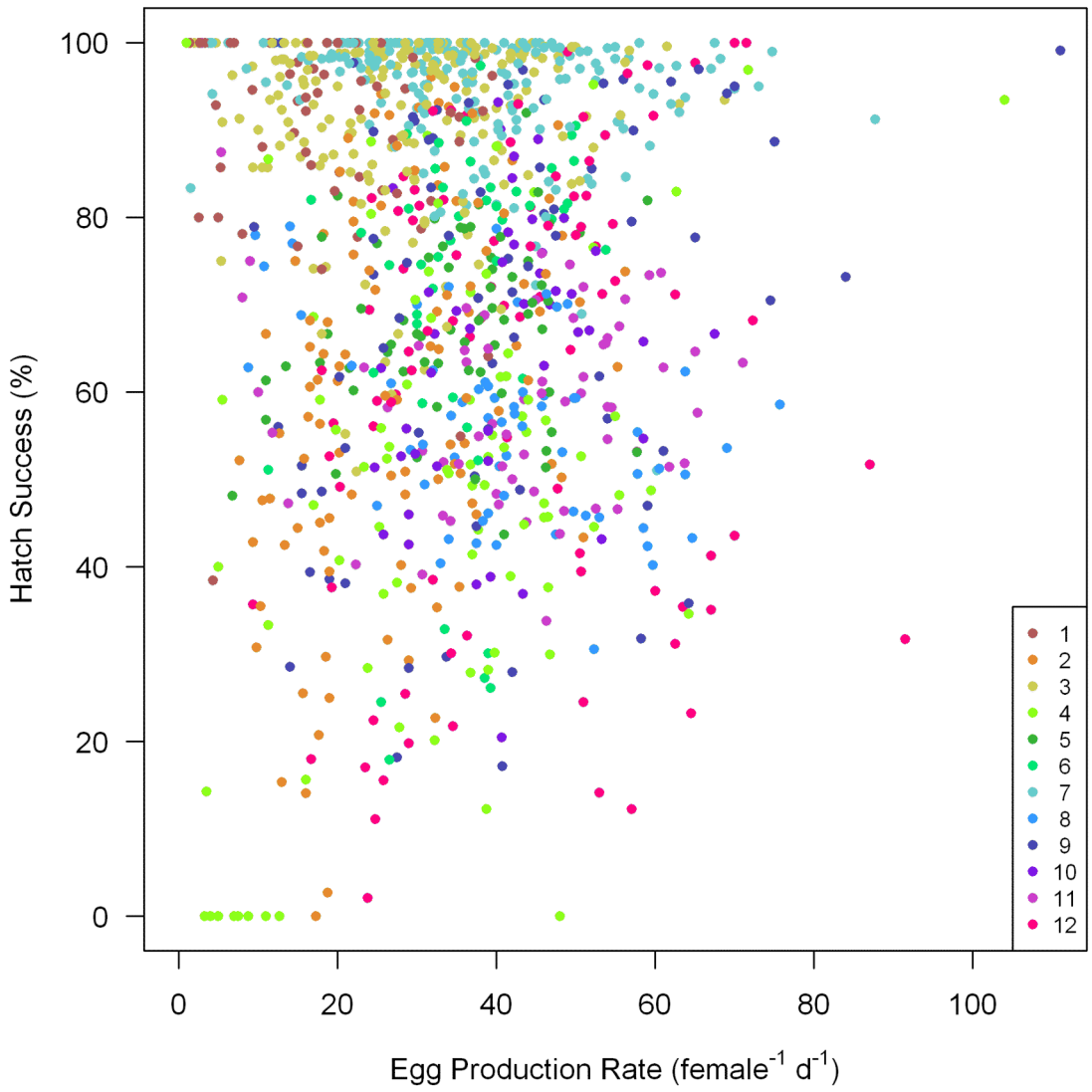

Fig. V–2 Relationship between 72-hr egg hatching success (*HS,* %) and egg production rate (*EPR*, eggs female^{-1} day^{-1}) in *Acartia tonsa* cultured within each of 12 trials (colors, see legend). All combinations of X-Y replicates and X-Y days are shown

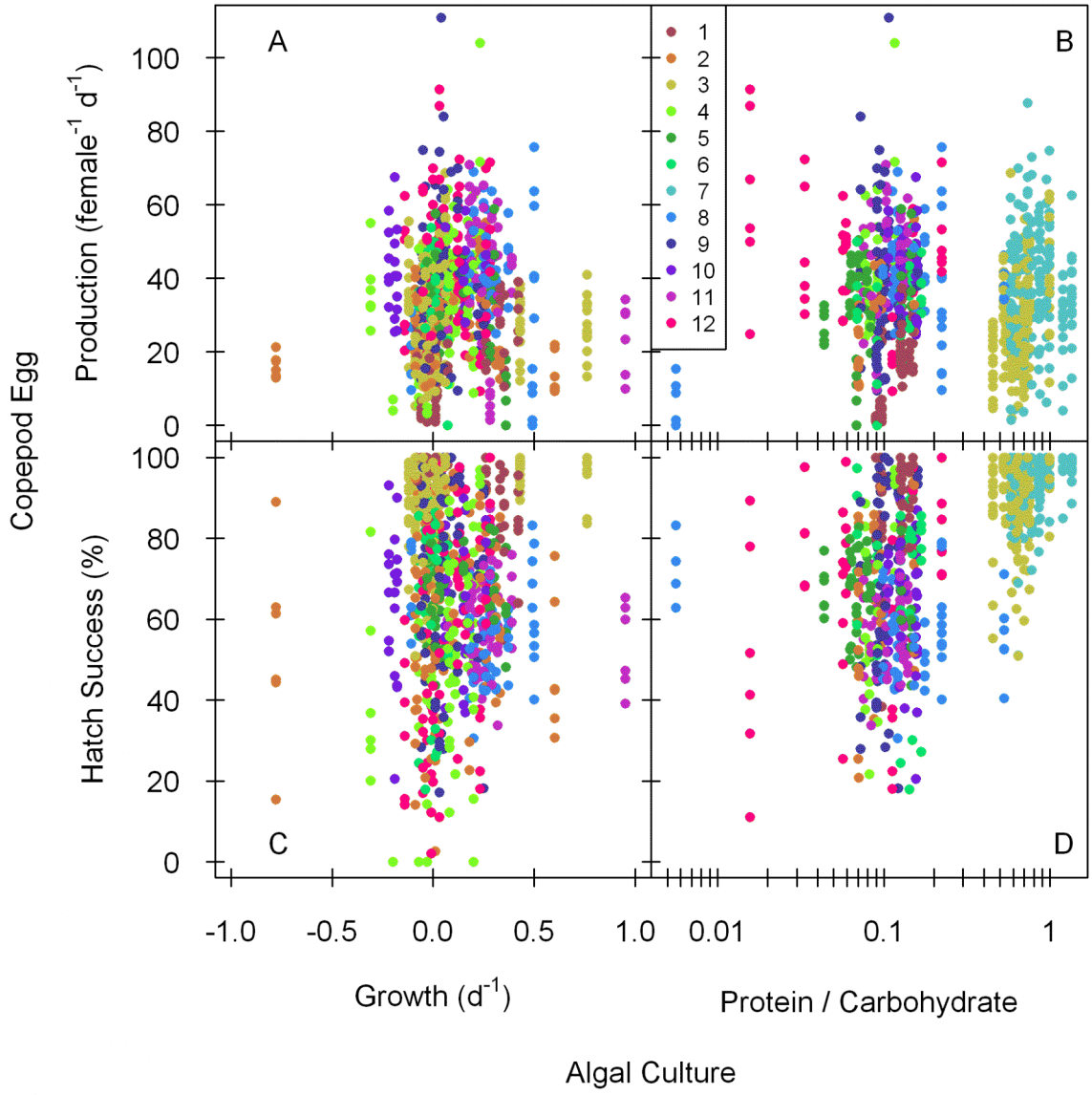

Fig. V–3 Measurements of egg production rate (top panels, A&B) and egg hatching success (bottom panels, C&D) in *Acartia tonsa* versus algal growth rate (left) and algal protein to carbohydrate ratio (right). Each daily measurement is shown for all replicates and all days in each of the 12 trials (colors)

Relationship between *EPR*, *HS* and Culture Conditions

HS vs *EPR*: Although mean values of *EPR* and *HS* tended to be between 30 to 40 eggs female^{-1} d^{-1}, and 50 to 95%, respectively (Table V–1), these values varied more widely (*EPR* from 0 to >80, *HS* from 0 to 100%) when data from individuals days and replicates are viewed (Fig. V–2). When data from all trials are pooled, there was no significantly correlation between *HS* and *EPR*, although several trials had values of *EPR* and *HS* that were consistently low (trial 4) or high (trials 3 and 7) in one or both measures. However, there was no clear clustering by trials suggested large variability in *EPR* and *HS*.

Algal Characteristics: When all measurements of *EPR* and *HS* were plotted versus algal growth rate (Fig. V–3A&C) and algal quality, expressed as the P-C ratio (Fig. V–3B&D), only a few clear patterns were detected. First, there was a tendency for *EPR* to be relatively low when algal growth rates were extremely low (~-0.2 d^{-1}, trial 2) and high (~1.0 d^{-1}, Trials 1 and 3) indicating either a complex or no relationship between *EPR* and algal growth rate (Fig. V–3A). Second, *HS* was relatively high and less variable when copepods were fed algal cultures having a P-C ratio that was > 0.5 (trials 3 and 7) (Fig. V–3D). Third, high *HS* was not only associated with high P-C ratios but was also observed in trial 1 (P-C ratios between 0.1 and 0.5) (Fig. V–3D). That trial also tended to have consistently low *EPR* (Fig. V–3B). Finally, at the lowest P-C ratio, (Trial 8) *EPR* was consistently low (Fig. V–3B) but hatching success was not particularly low (>60%). Unfortunately, no measurements of C-P ratios were available for the trial where hatch success was extremely low (trial 4, final half of the time). Given the high degree of variability in *EPR* and *HS* observed at intermediate levels of algal growth rates and algal P-C ratios, it is likely that *EPR* and *HS* were affected by a combination of factors other than algal growth characteristics.

Temperature and salinity: When *EPR* values are pooled across trials and plotted against the three temperatures (Fig. V–4A) and three salinities (Fig. V–4B), few clear patterns emerge. Intermediate, high and low values of *EPR* were observed at the three levels of each of these abiotic factors. Trials 3 and 7 were conducted at the warmest temperature and highest salinity and had the

highest *HS* (Fig. V–3C&D). However, trial 1 also had consistently high *HS* and was performed at 18 psu and 16°C, suggesting that a mixture of factors combined to influence *HS* and that day-to-day variability was high.

Variability: Trends with time in *EPR* and *HS* (Fig. V–1) display clear differences in the strength of changes with time (temporal effect) and the size of the error bars on any given day (inter-replicate variability). When both of these sources of variability are visualized (as ±1 SD values around means), clear differences in cultures are apparent. In most trials, ±1 SD of total variability in *EPR* ranged between ~20 eggs female^{-1} d^{-1} around mean values (Trials 1-3, 6, 10) and ~45 eggs female^{-1} d^{-1} or two-times more variance (Trials 4, 8, 9, 11, 12) (Fig. V–5A). In the latter trials (displaying the highest total variance), a considerable amount of variance was attributable to temporal (day-to-day) variation but, in some of those cases, only small amounts of variance existed among replicates (e.g., Trials 4 and 8). Trials 6 and 10 displayed the smallest amounts of total variance in *EPR*. Those were the only trials that used aeration.

As opposed to total variance in *EPR*, more marked differences existed in patterns of total variability (and sources of variability) in *HS* among the 12 trials (Fig. V–5B). Values for ±1 SD in *HS* ranged from <15% to >50%. The smallest total variability was observed in trials 7, 3, 1 and 8 and three of those trials (1, 3 and 7) had the highest mean values of *HS* (~90%). The greatest variability was observed in trials 4, 9 and 12 (Fig. V–3B). Those three trials were the ones where the algae were switched from being fed once per day to three-times per day at the mid-point of the measurement period.

There tended to be a clear pattern when the variance associated among replicates (pooled across time for each replicate) and the variance associated with time (pooled across replicates on each day) were plotted against each other (Fig. V–6). In some trials, a large portion of the variance could be explained by differences among replicates and almost no variance could be explained by variability with time, in other words, trials where the right (left) bar was large (small) in Fig. V–5A, and vice versa (Fig. V–6A). The same pattern

was observed in *HS* except that R^2 values tended to be higher for among-replicate variance as opposed to among-day variance Fig. V–6B). Finally, trials with a high day effect R^2 for *EPR* were not necessarily those with a high day effect R^2 for *HS* and vice versa. Trials where variance associated with replicates and time were both relatively high in both *EPR* and *HS* (Trials 4, 9 and 12) were those having the oldest females (28 days) at the start of the experiment and were among the longest trials (15 days of duration).

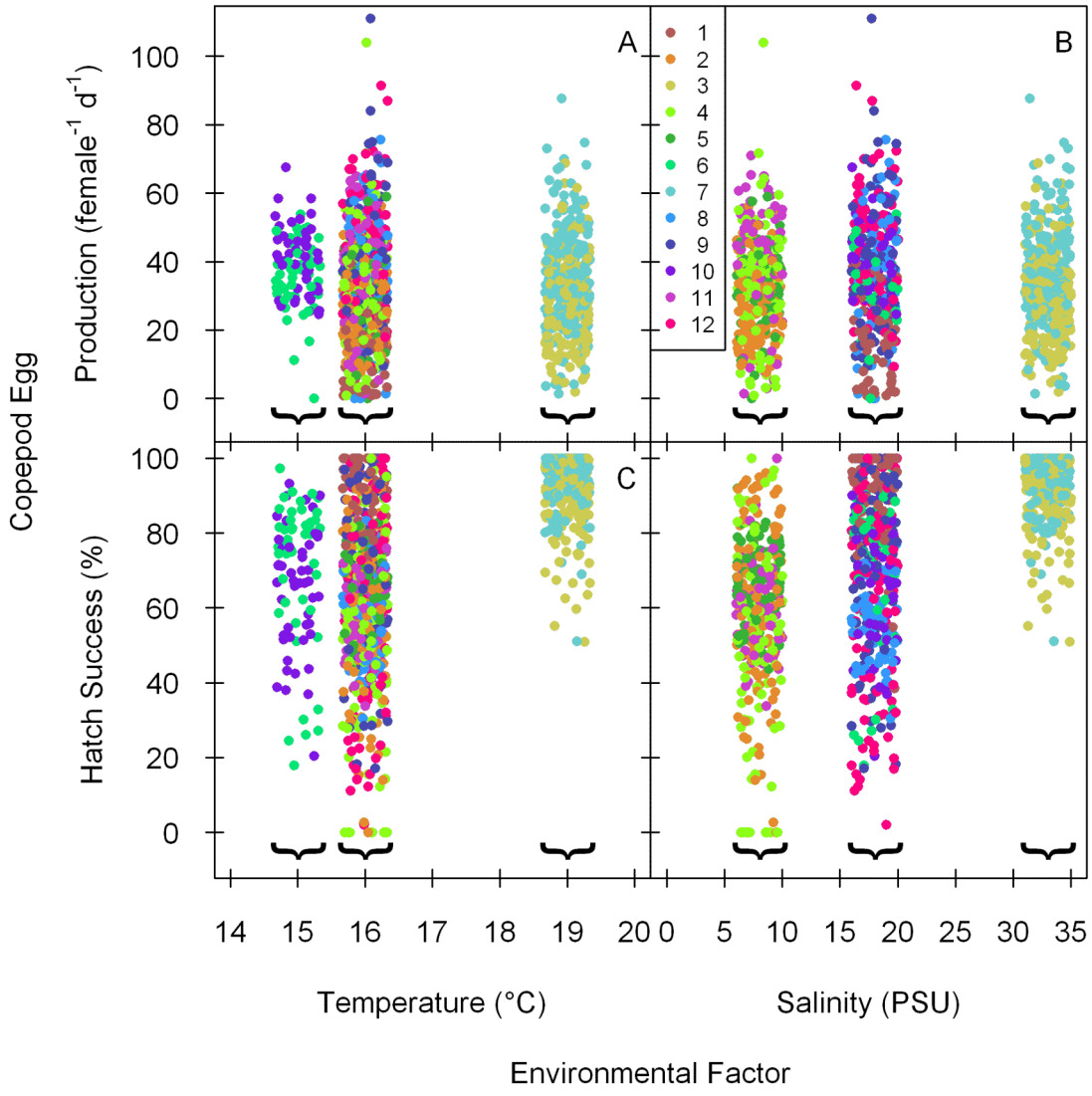

Fig. V–4 Egg production rate (top panels, A&B) and egg hatching success (bottom panels, C&D) data collected for *Acartia tonsa* at different temperatures (left) and water salinities (right). Along the x-axis, data within the three temperatures (15, 16 and 19°C) and three salinities (8, 18, and 33 psu) were jittered (by adding small random numbers) to reveal more data points

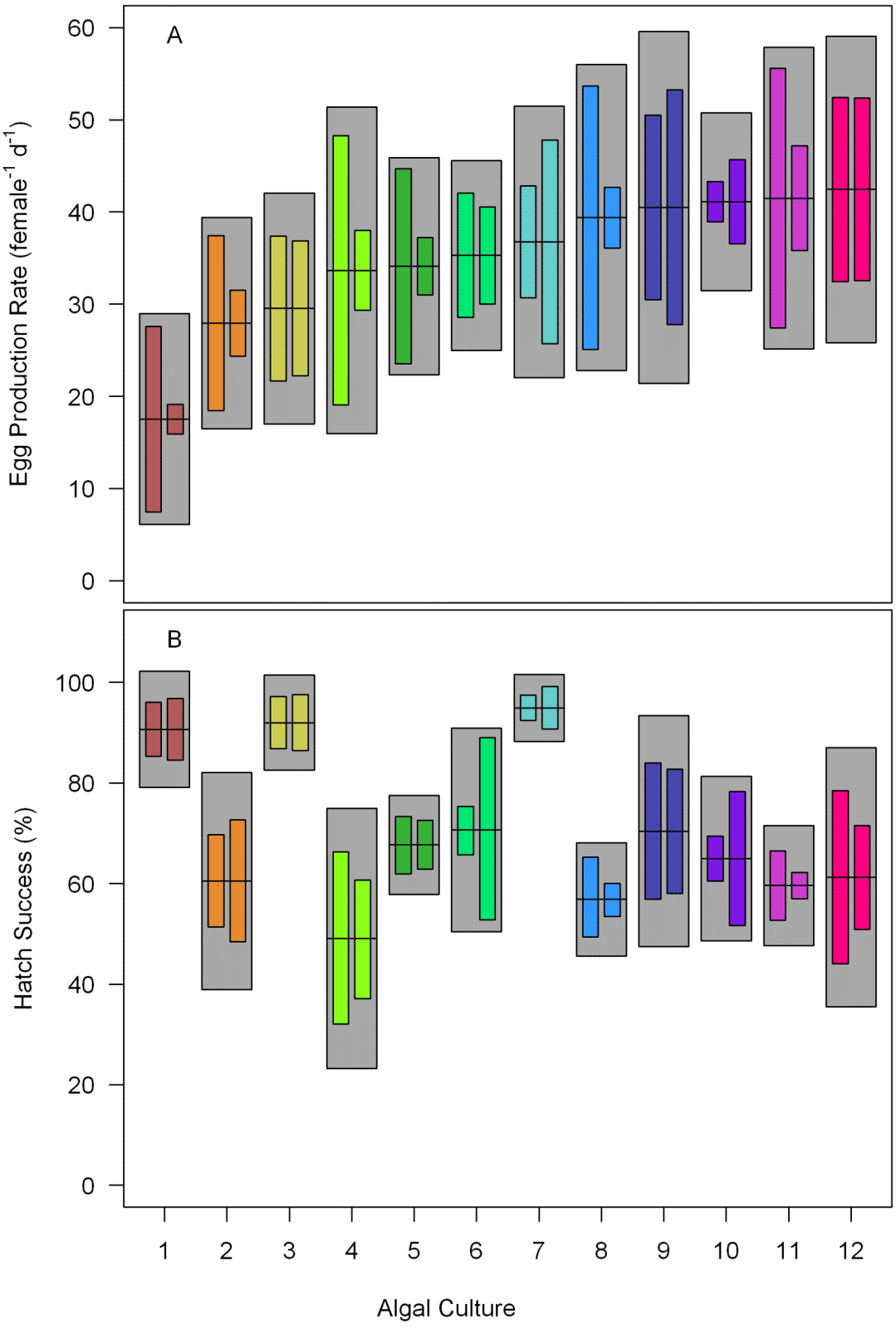

Fig. V–5 Reproductive characteristics observed in 12 trials examining egg production rate (panel A) and egg hatching success (panel B) of *Acartia tonsa*. In each panel, gray boxes display the overall mean ± overall SD in measurements. The colored bars display temporal (day-to-day) variability in terms of the SD of daily means (left bar, replicates pooled on each day) and variability in terms of the SD of replicate means (right bar, days pooled for each replicate). Note, variability displayed in this manner is not additive (the two bars do not sum to the total variance)

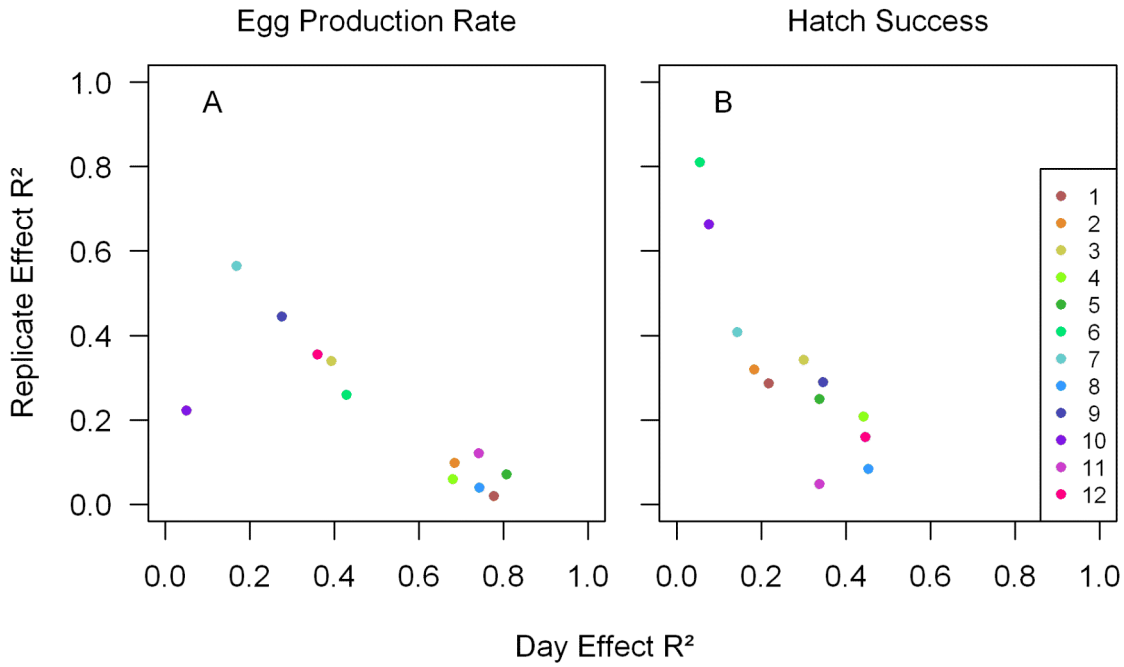

Fig. V–6 Relative contribution of experimental day and replicate chamber towards the total variability in egg production rate (panel A) and hatch success (panel B) of *Acartia tonsa*. Points represent 12 trials, indicated by color (see legend). Axes indicate the coefficient of determination (R^2) between measurements for each day and replicate vs. daily means (x) and replicate means (y), respectively

DISCUSSION

Protocols for culturing *Acartia tonsa* have been published in light of the need of sufficient quantities of healthy adults for use in standard toxicity testing (Medina and Barata 2004) and marine finfish aquaculture (Peck and Holste 2006). In either case, a semi-static culture technique has been recommended whereby either all of the water (in the case of small, 1.2-L cultures) or 20% of the water (in the case of larger, 250-L cultures) is replaced every 24 to 48 hrs. A range of stocking densities from 300 to 2000 copepods L^{-1} appears to be suitable for successful growth and reproduction. Researchers have also posed the question of whether copepods reared in the laboratory for many generations were suitable candidates for toxicity and/or basic ecophysiological and behaviour studies and, in general, very few differences have been noted when various aspects of wild-caught and long-term laboratory cultured copepods were compared (Sosnowski and Gentile 1978, Tiselius et al. 1995). For example, when examining acute (LC_{50}) values to heavy metals, Sosnowski and Gentile (1978) found no differences in wild-caught individuals and those that were cultured and successive tested for 6 generations in the laboratory.

Although considerable literature exists describing the methods to use to grow *Acartia tonsa* in the laboratory (e.g., see Peck and Holste 2006, Marcus and Wilcox 2007) and several thorough synopses have been published on how *EPR* and *HS* measurements have been performed (Runge and Roff 2000), there is no thorough critique and “best practice” guide available. This is surprising given that these reproductive parameters are fundamental characteristics of population status. In *Acartia tonsa*, these reproduction metrics can also provide an ideal metric for short-term energy / growth status (for use in chronic toxicity testing) since *Acartia tonsa* has few lipid / energy stores and its reproductive rate responds rapidly to environmental changes (Kiørboe 1989). In the following, we discuss a number of factors that could potentially influence measurements of *EPR* and *HS* in this (and potentially other) calanoid copepod species. We do this in the hope of providing practical information to researchers using this species.

Environmental Factors Important to Copepod Reproduction

Temperature responses

The results of our trials conducted at 15 to 19°C suggest little effect of temperature on *EPR* but *HS* was highest in two trials conducted at the warmest temperature (Table V–1). This could suggest that a relatively small range in temperatures (4°C) could have a larger effect on mean (and variance) in hatching success compared to egg production rate. Although definitive conclusions on the role of temperature cannot be drawn here (since salinity was also highest in the same two trials), when planning laboratory trials, it is important to consider that conditions supporting the highest *EPR* may not be those supporting the highest *HS*. While *EPR* tends to be highest at 25°C (Holste and Peck 2006), the combined data from a great number of laboratory and field studies suggested that a relatively wide range in temperatures supported high rates of egg production rate; a 50% reduction in *EPR* was not expected until temperatures were <16°C or > 28°C (Diekmann et al. 2012). On the other hand, temperature appears to be one of the cues associated with the production of potential resting eggs (Tietjen et al. submitted) or eggs that do not hatch nor disintegrate within three weeks of being produced. A large proportion of potential resting eggs was produced by *Acartia tonsa* females grown and tested at 15°C compared to either 20 or 25°C. Thus, subtle differences in temperatures (±2°C around 18°C) can lead to marked differences in 72-h *HS* (more properly, the percentage of eggs that do not hatch within 72-h) but only modest changes in *EPR*.

Light Regime

In species such as *Acartia tonsa* that produce “normal”, “delayed hatching” and “resting” eggs (Tietjen et al. submitted), short-term (48- to 72-h) hatch success will depend upon the type of eggs produced. As previously mentioned, photothermal cues are thought to affect the relative proportion of these egg types produced on any given day by various copepod species (Marcus 1982, Mauchline 1998). *Acartia tonsa* from the southwest Baltic Sea (the population studied here) produces a relatively constant and large proportion of eggs that rapidly hatched (<72 hr) at photoperiods >16 hrs and the proportion of eggs that do not rapidly hatch increases with decreasing

photoperiod (used to culture adults and incubate eggs) (Peck et al. 2008). Peck et al. (2008) documented the most variability in 48-h hatching success at an intermediate photoperiod (12 h). Thus, light regimes utilized in laboratory bioassays will affect both the mean *HS* and variance around that mean. In *Acartia tonsa*, photoperiods longer than 13 hrs are recommended to simplify the interpretation of *HS* obtained when the effects of other environmental factors (food quality, toxicants, etc.) are examined. Furthermore, egg incubation conditions need to be the same as those during egg production since eggs display a certain amount of phenotypic plasticity by changing their development in response to changes in temperature and/or photoperiod (Tietjen et al., submitted).

Diets of Microalgae Provided to Copepods

Both food quality and food quantity affect survival, growth and reproductive success of copepods (Kiørboe et al. 1985, Arendt et al. 2005). Although some researchers report that feeding mixtures of algal diets yield higher reproductive success, most studies have utilized single algal species such as the cryptomonad *Rhodomonas* spp. Støttrup et al. (1986) list the microalgae successfully used to culture *Acartia tonsa*. When microalgae are of adequate quality, threshold concentrations of phytoplankton C required to maintain maximum *EPR* are on the order of 1500 µg C L^{-1} (Kiørboe et al. 1985). This is approximately equal to 10 000 cells ml^{-1}, of the diatom *T. weissflogii* based on a carbon content of 183 pg C $cell^{-1}$ (Støttrup and Jensen 1990).

Houde and Roman (1987) characterized the feeding response of *Acartia tonsa* to senescent and actively growing *T. weissflogii* that had 16.3 and 40.2 pg $cell^{-1}$ of carbohydrate and 51.6 and 105.0 pg $cell^{-1}$ of protein, respectively, leading to P-C ratios (2.6 to 3.1) that were higher than mean ratios (0.02 to 0.71) for the same algae in the present study (Table V–1). Houde and Roman (1987) speculated that higher cellular contents of protein may be detected by copepods by an increase in the concentration of amino acids in the zone surrounding medium leading to higher ingestion rates of actively growing cells at relatively lower concentrations. Copepod egg production is proportional to the intake of N suggesting that copepods tend to be N- and not C-limited in

their diets (Kiørboe 1989). Copepod ingestion rates were not examined here but changes in ingestion rate may explain at least some of the potential differences in reproduction characteristics of copepods observed in our 12 trials.

A clear result from several studies examining *EPR* and *HS* in *Acartia tonsa* is the need to feed algae with a high ratio of the polyunsaturated fatty acids (PUFAs) 22:6(n-3) and 20:5(n-3). Thor et al. (2007) reported that 18:3(n-3) (or 16:4[n-3]) and 22:6(n-3) are essential long chain fatty acids for high rates of egg production in this species and that feeding algae devoid of both 20:5(n-3) and 22:6(n-3) (e.g., *Dunaliella tertiolecta*) leads to poor egg hatching success. Thus, once saturating levels of food concentrations are reached, both algal growth rate and fatty acid composition needs to be considered. Although fatty acids were not examined in this study, *HS* clearly responded to changes in algal quality (expressed as P-C ratios).

Practical Recommendations for Reproduction Measurements

Some of the reproduction trials reported in this study are among the longest in duration for *Acartia tonsa*. An acclimation time of several days may be needed to obtain maximum, treatment-specific *EPR*. A review of six different experiments performed on *Acartia tonsa* revealed that *EPR* was consistently lower during the first 1 to 2 days after loading animals (Holste 2009). Dutz et al. (2008) observed an increase in *Temora longicornis EPR* during the first three to four days in four of their six treatment groups. In that study, *EPR* increased ~80% within the first three days for copepods fed high-quality food (*Thalassiosira weissflogii*) but this temporal trend was absent in copepods fed poor-quality food (*Leptocylindricus danicus* and *Skeletonema costatum*) that limited *EPR*. Within both of these studies, not only transfer/handling stress but also a diet shift may have contributed to temporal trends in *EPR*. In both studies, the potential effect of copepod age can be discounted since females were an optimal age for reproduction (~5 days in adult stage at the beginning of the experiment) (Parrish and Wilson 1978). Working with *Calanus finmarchicus*, Poulet et al. (1995, and references therein) found no significant differences in *EPR* 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, suggesting that copepods required time to adjust to experimental conditions.

Observations from this and several of the aforementioned studies suggest that it may be important to allow an acclimation time for copepods to adjust to test conditions prior to measuring *EPR* and *HS*. Copepods may experience a novel environment within the test chambers (compared to pre-test, rearing conditions) and "transfer stress" may result from both handling and adjustments needed within the new environment (Holste 2009). The general point of view is that stress which might affect the measurement is avoided (or minimal) when animals are carefully treated during step 1, a conclusion that stems from discussions started >50 years ago (Marshall and Orr 1955; Hargrave and Geen 1970).

In 9 of the 12 trials conducted here, egg production rate markedly increased during the first five days of the trial. In another trial (trial 1), *EPR* started to increase during the first 3 days but then rapidly declined (in all replicates) for four days for unknown reasons. In all cases, a 3-day acclimation period to test chambers was provided, thus copepods had been within test conditions for at least a week before *EPR* reached peak levels in most of these trials. In general, if researchers wish to capture the highest rates of egg production (which may be important in distinguishing subtle treatment effects), daily measurements should be conducted for at least a week or the potential effect of time (increase or decrease in reproductive measures) should be examined in pilot experiments.

Some researchers have acclimated copepods at concentrations ≥200 individuals L^{-1} (Teixeira et al. 2010). However, high concentrations may cause stress to individuals with unknown consequences on egg production rate and egg hatching success. Støttrup et al. (1986) indicated that *Acartia tonsa* reduced their egg production rate when maintained at >100 adults L^{-1}. Although some researchers have reported an increase in resting egg production when copepods were maintained at high concentrations, likely due

to the build-up of metabolites (Ban 1992), this has not been demonstrated in *Acartia tonsa*. Although optimal concentrations of copepods will vary (and should be determined prior to testing), a standard concentration of 20 *Acartia tonsa* L^{-1} has the potential to yield maximum egg production rates (Holste and Peck 2006).

The long-term measurements made in our trials demonstrated the extent of changes in mean *EPR* (and *HS*) that can occur with time (or as females and males age) for copepods fed *ad libitum* rations of a high quality diet. Both the duration of the trial and the age of adults are important factors to consider when designing and conducting measurements of egg production rate and egg hatching success of *Acartia tonsa* and likely other calanoid copepods. The lifespan of adult copepods depends to some degree on their mating history and senescence may manifest itself differently between male and female copepods (Ceballos and Kiørboe 2011). Based upon previous work on *Acartia tonsa*, egg production rates are expected to peak at an intermediate age and decline towards old age. In the two instances where *EPR* started at relatively high levels (~40 eggs $female^{-1}$ d^{-1}), females were 20 days within stage (an intermediate starting age in these trials). Interestingly, these two trials displayed a unique pattern of variability in *HS* – they were the only ones in which *HS* variance among test chambers (right-hand bars in Fig. V–5B) was much higher than that associated with time (across test chambers, left-hand bars in Fig. V–5B), suggesting close synchrony in *HS* but little temporal variability. This is one of the few potential links between patterns in *EPR* and *HS* found in the present study.

Naturally, the age of field copepods is not known but, when copepods from laboratory cultures are utilized, laboratory bioassay should standardize methods so that copepods within a narrow range of ages are utilized. This greatly increases the effort required to culture animals. Standard methods are semi-static and allow a mixture of cohorts within copepod rearing tanks (Støttrup et al. 1986; Peck and Holste 2006). However, using females that are the same age will be needed if one hopes to distinguish how intrinsic (e.g.,

energy status) and extrinsic (salinity, temperature, toxicants, etc.) interact to affect the reproductive success of calanoid copepods such as *Acartia tonsa*.

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CHAPTER VI

DISCUSSION

Climate impacts on *Acartia tonsa*

The need of physiology-based studies

The adaptive capacity of *Acartia tonsa*

DISCUSSION

Climate impacts on *Acartia tonsa*

The work of this thesis is embedded in the RECONN cluster of the DFG priority program AQUASHIFT. That program focused on the impacts of climate variability on aquatic (marine and freshwater) ecosystems with specific interest in match/mismatch dynamics resulting from shifts in seasonality and distribution of key species. Within marine ecosystems, copepods play a key role linking primary and tertiary levels of production. This means that the copepod standing stock depends upon the amount and type of primary producers (marine algae) as a food source while the copepods themselves function as the main food source for zooplanktivores such as the larvae of marine fishes. The degree of match or mismatch in these trophic interactions in time and space is affected by changes in the seasonality and distribution.

Copepods within the *Acartia* genus are highly abundant in the south–west Baltic Sea and it can be assumed that, at least during warmer periods of the year, *A. tonsa* forms the majority of the biomass and numerical abundance of the different, coexisting *Acartia* congeners (Manuscript 3). Due to the tropical origin of this species, populations of *A. tonsa* that have effectively spread to temperate and sub-Arctic waters are interesting candidates for investigations of phenotypic plasticity and climate change impacts, particularly when considering projections of climate-driven warming. *Acartia tonsa* displays a wide range of *T* and *S* tolerance (see Manuscript 3) and it is capable of producing resting eggs (*RE*) to survive unfavorable conditions in the water column, such as colder temperatures prevalent during winter at higher latitudes. The projected climate-driven warming of these colder, temperate and sub-Arctic regions is expected, therefore, to increase the importance of *A. tonsa* to the food web dynamics of coastal and shallow water areas.

Despite being eurythermal and euryhaline, *A. tonsa*, during the adult stage, has few energy stores and invests the bulk of its surplus energy directly into egg production (Dagg, 1977; Durbin et al. 1983, Kiørboe et al. 1985). This

energy strategy makes *A. tonsa* particularly responsive to mismatch situations regarding food availability (in terms of food of good quality). For Narragansett Bay, Durbin et al. (1983) argued that while various studies have focused on temperature as the main factor controlling copepod production, match-mismatch dynamics with phytoplankton often control the productivity of species such as *A. tonsa* in the field.

With this thesis, I demonstrated that the *EPR* of *A. tonsa* was drastically impacted by a change in the growth rate of its prey (Manuscript 1). A rapid decrease in *EPR* occurred when it fed on a diatom culture that entered into the senescence growth phase. *A. tonsa* is an opportunistic feeder and is capable of food selection (Cowles et al. 1988) and different mixtures of two food items of the same quality resulted in no significant differences in either *EPR* or biochemical (protein and carbohydrate) composition of *A. tonsa* eggs and females (see Appendix AII). During a diatom bloom, food quality can change within two or three weeks (when the senescence phase is reached) depending upon the environmental situation. This could influence the copepods *EPR* drastically, which was found to be maximal about one week after maturity (Manuscript 4). Furthermore, a slight shift in the hatching of *A. tonsa* resting eggs (*RE*) from the sediment would have marked consequences for the population. Projected warming for the Baltic Sea is on the order of 3.0 to 3.5°C in the next 80 years (Meier 2006) which could lead to an earlier hatch of *A. tonsa RE*. The phenology of phytoplankton is less linked to temperature (directly) and more linked to the availability of light and nutrients and the onset of stratification (affected by wind strength, Sommer 1998). If the light and nutrient dynamics do not change, warming could result in an earlier appearance of hatched nauplii and a mismatch with phytoplankton, suggesting that early hatching nauplii could suffer mortality due to food limitation in the future. The results in Manuscript 2) support flexible hatching behavior not only due to plasticity among individual females but also among the eggs produced on any one day. Also, evidence was found that the importance of temperature for directing the developmental trajectory of eggs in *A. tonsa* might change seasonally. More investigations on *RE* dynamics in terms of triggers of *RE*

production and hatching would be needed to give a more precise picture of potential effects by climate warming on population dynamics in the field.

The strength of interaction between different calanoid copepods, in particular different *Acartia* congeners, needs to be better clarified in future studies performed in the field and laboratory and within modeling studies. The fact that the different *Acartia* species are difficult to distinguish often causes these species to be lumped together within one "*Acartia* spp." group in field studies. Given the physiological and life history differences among these *Acartia* species, (e.g., in terms of *T* and *S* optima and tolerances - see Manuscript 3), treating these separate species as one *Acartia* spp. group leads to a huge loss of process knowledge and will lead one to spurious conclusions regarding the dynamics of that portion of the copepod community, particularly as influenced by future scenarios of climate change.

The need of physiology-based studies

Future studies should attempt to provide a mechanistic, physiological-based understanding of how multiple factors interact to influence the vital rates of copepods. One example would be to conduct studies examining how different stressors influence the aerobic scope, which represent the energy available (after maintenance metabolism) to pay for the costs of all additional activities (foraging, reproduction, etc.). In this thesis, aspects of copepod reproduction (*EPR* and *HS*) were used as indicators for *A. tonsa* condition and the availability of surplus energy. One can investigate energy partitioning in a more mechanistic fashion. For example, the effect of interactions of different biotic and abiotic factors (e.g., different combinations of temperature, salinity, O_2 concentrations and food quantity ad quality) on respiration (O_2 consumption) of different copepod stages would yield more insight on mechanisms affecting productivity.

Investigating different stages (from eggs to adults) would elucidate which stages might be most sensitive to different environmental factors. As reported in manuscript 3) adult *A. tonsa* females show a wide tolerance for several factors like *T* and *S*, but it is known that e.g. males are more sensitive. Furthermore, in manuscript 1) it was shown that the development of *A. tonsa*

copepodites ceased development around stage CII when fed food of poor quality. Elliott and Tang (2011) also reported higher proportions of dead nauplii (NI-NIII 30%, NIV-NVI up to 15%) and adult males (40%) compared to other stages (CI-CV up to 8%) and females (9%) indicating a greater susceptibility of nauplii and males to death from environmental stressors. Measurements on respiration should be made with populations from different sites to compare possible differences in their plasticity or adaptation resulting in different e.g. *T* and *S* optima. For *RE* dynamics investigating respiration rates is also of great interest as *RE* are reported to have a reduced metabolic activity (Romano et al. 1996b) at least at relatively warm temperatures (Wu et al. 2009). It could be investigated if this reduced metabolic activity can be used to distinguish subitaneous and resting eggs (e.g. soon after being laid), which might help understanding the plasticity in the response of adults to their environment or the possibility of plasticity by the eggs themselves.

Respiration measurements have been previously made on *A. tonsa* eggs by Nielsen et al. (2007) who also reported an exponentially increase in O_2 consumption with increasing temperature. In that study the O_2 consumption rates between batches of eggs produced on two consecutive days exhibited significant variation. Within the present thesis, measurements of the respiration rate of individual *A. tonsa* eggs were made using a Unisense NanoRespiration System (for Methods see Nielsen et al. 2007) and those rates were compared to differences in the surface structure of eggs. Despite the differences in surface structure (smooth, rough and spiny) of the eggs, no clear differences in respiration rate existed (Fig. VI–1). Respiration rates found were much lower than for other copepod species. In *Anomalocera patersoni* oxygen consumption rates were low in newly spawned eggs kept at 13°C, but increased steadily with time, reaching maximum values of about 2.0 nl O_2 embryo^{-1} h^{-1} at 70 d after deposition (Romano et al. 1996a). In that study, respiration rates slowly decreased to a minimum of about 0.3 nl O_2 embryo^{-1} h^{-1} after 150 days, values that are comparable to those measured here on *A. tonsa*. In the Romano et al. (1996a) study, O_2 consumption for eggs maintained at 20°C exhibited a similar trend as eggs at 13°C, but with values that were almost 2.5 times higher. In another species (*Pontella mediterranea*),

newly-spawned subitaneous embryos exhibited low respiration rates at 5-hrs post-spawning but rates rapidly increased and maximum uptake rates of 5.2 nl O_2 embryo^{-1} h^{-1} were measured after 30 h (Romano et al. 1996b). In diapause embryos, O_2 levels rose rapidly after the first day of spawning and reached mean values of 8.0 nl O_2 embryo^{-1} h^{-1} within 5 days (Romano et al. 1996b). Values then gradually decreased during the period of pre-diapause which lasted for about 25 to 30 days. The true diapause stage was characterized by lower O_2 consumption values that persisted for about 4 to 5 months (ca. 1.8 nl O_2 embryo^{-1} h^{-1}, Romano et al. 1996b). This was followed by a rapid rise in O_2 uptake during post-diapause development with rates as high as those recorded during pre-diapause development (Romano et al. 1996b). This temporal trend in O_2 consumption rate by newly produced eggs was not observed by Nielsen et al. (2007) who examined *A. tonsa*. Nielsen et al. (2007) higher and more constant mean oxygen consumption rates for *A. tonsa* compared to the consumption rate by *P. mediterranea* in the beginning reported by Romano et al. (1996b). Mean O_2 consumption rates (±SD) of *A. tonsa* comparable to those found by Nielsen et al. (2007) were 0.273±0.036 nl O_2 h^{-1} at 34 psu and 0.282±0.014 nl O_2 h^{-1} at 2 psu (Hansen et al. 2012). The lack of a significant difference between these treatments (two-tailed t-test; $p=0.63$) indicated egg metabolism was relatively insensitive to changes in salinity, suggesting that changes in salinity were not followed by active pumping of ions nor by changes in the concentration of osmolytes to maintain homeostasis (Hansen et al. 2012). The mean rates reported by Hansen et al. (2012), who examined individual eggs, agree well with those made as part of the present thesis (0.2 to 0.8 nl O_2 h^{-1}). However, it should be stated that in the present study, only about 60% of the measurements made were successful while about 40% of the single egg trials did not yield measurements (due to technical problems associated with the O_2 probe).

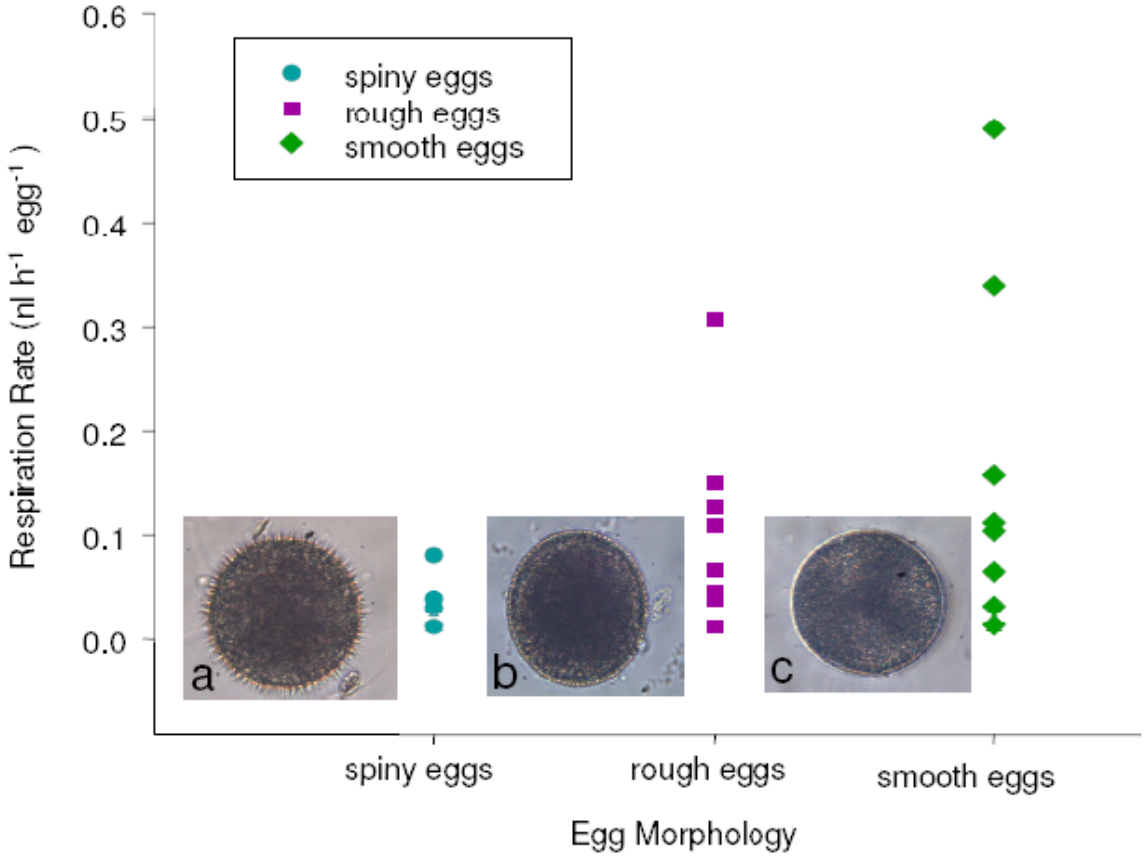

Fig. VI–1 Mean (±*SE*) respiration rates (nl/hr/egg) of spiny, rough and smooth eggs. Note that the *SE* was too small to be distinguished. Pictures of spiny (a), rough (b) and smooth (c) eggs by courtesy of Nadine Peck

Biochemical analyses of different egg types should also be compared as it can be assumed that the different egg types will exhibit differences due to their different requirements / needs to support rapid hatching, delayed hatching or resting. Acheampong et al. (2011, see Appendix AI) found higher protein content in eggs being laid by females in a low food environment. They assumed that food limitation led to the production of resting eggs as protein might be the most appropriate metabolic substrate under low O_2 conditions (as would be experienced after resting eggs sank to and were buried in sediments). Less oxygen is required for the catabolism of protein compared to that of lipids and carbohydrates (Marcus 1996), which represent more a energy reserve for e.g. delayed hatching eggs.

The adaptive capacity of *Acartia tonsa*

Another important aspect for future studies would be a genetic comparison of different *A. tonsa* populations around the world. This could explain how far plasticity is driving the tolerances of this cosmopolitan or if the populations adapt to their specific environments by showing differences in their genetics leading to separation into different species with time. Phenotypic variation within a population is the first step towards speciation and can be detectable at the molecular level prior to changes in morphological characteristics. Consequently, molecular analyses are often the only methods powerful enough to identify/discover sibling and cryptic species (species which are morphologically similar but reproductively isolated) (e.g., de Vargas et al. 1999, Lee 2000, Goetze 2003, Castro-Longoria et al. 2003, Peijnenburg et al. 2004). Recent molecular genetic studies suggest that pelagic species diversity in the open ocean is higher than that inferred from many morphological taxonomies and cryptic species biodiversity is high and well structured in the open ocean (Norris 2000). Thus, genetic diversity (based on any variation in the nucleotides, genes, chromosomes or whole genomes) is not only a fundamental component of biodiversity; it can also serve as an independent indicator of environmental conditions (Bagley et al. 2002).

Furthermore, understanding the adaptive capacity of key marine species will be critical if one hopes to make robust projections of how marine ecosystems are expected to change due to the activity of anthropogenic and/or climate

drivers. Marine holoplankton is generally considered to have a high potential for long-distance, passive dispersal via ocean currents (and hence population mixing). However, the finding of a high degree of cryptic species in that group suggests a high potential for adaptation to surprisingly small-scale oceanographic features, implying either limited dispersal and/or strong selection gradients (Chen and Hare 2008). High genetic differentiation of planktonic species among different sampling locations have been documented e.g. for *Calanus finmarchicus* (Bucklin and Kocher 1996) and *C. pacificus* (Nuwer et al. 2008).

These differences suggest that even copepods which have a potentially high capacity for dispersion (and mixing) can develop genetically-structured populations in the absence of obvious geographic barriers. Extreme variability in the success of reproduction and survivorship of the offspring in marine environments apparently creates the opportunity for rapid changes in the genetic make-up of populations through genetic drift or natural selection. At the population level, genetic diversity varies across spatial and temporal scales (Caudill and Bucklin 2004) and gene flow can provide insights regarding the resilience of a population against sudden or gradual environmental changes. Losses of genetic diversity may compromise the ability of a population to respond to environmental change (O'Brien et al. 1985, Lande 1988, Waples and Teel 1990, Frankham 2005).

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Eidesstattliche Erklärung

(gemäß §7 (5), Promotionsordnung des Fachbereichs Biologie der Universität Hamburg vom 09.02.1999

In Bezug auf meine Dissertationsschrift mit dem Titel: „Linking environmental factors and the reproductive success of *Acartia tonsa* DANA (Copepoda: Calanoida)“ zur Erlangung der Würde des Doktors der Naturwissenschaften des Fachbereichs Biologie, der Fakultät für Mathematik, Informatik und Naturwissenschaften, der Universität Hamburg, bestätige ich hiermit an Eides statt, dass die Arbeit selbständig angefertigt worden ist, ich die wörtlich oder inhaltlich aus anderen Quellen übernommenen Stellen als solche kenntlich gemacht habe und die Inanspruchnahme fremder Hilfen namentlich aufgeführt wurde.

Hamburg, den 18.12.2012

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APPENDIX

AI Co – author manuscript

(Acheampong et al. 2011)

Acheampong E, Campbell RW, Diekmann ABS, St. John MA (2011). Food availability effects on reproductive strategy: the case of *Acartia tonsa* (Copepoda: Calanoida). Mar Ecol Prog Ser 428: 151 – 159

ABSTRACT: Food availability has been linked to changes in the biochemical composition of zooplankton eggs. However, a number of species are capable of resource storage and are thereby able to use accumulated reserves for reproduction during periods of poor food conditions. Conversely, in species such as *Acartia tonsa* with limited storage capacities, there can be a strong dependence of egg composition on ambient food conditions. The aim of this study was to determine the effect of food availability on the carbohydrate, protein and fatty acid composition of *A. tonsa* females and their eggs after being fed with different concentrations of the cryptophyte *Rhodomonas baltica*. During the experiments, no significant differences in the biochemical composition of females were observed, although egg protein composition was higher in food-limited females. We propose that the production of protein-rich eggs by food-limited copepods is a reproductive strategy for ensuring the survival of offspring during poor feeding conditions. In terms of their relative biochemical makeup, there were no significant differences between both adults and eggs of *A. tonsa* and their prey *R. baltica*. However, these biochemical similarities did not influence egg production. Rather, higher biochemical similarities were observed between *R. baltica* and eggs when females were food limited. These findings suggest that food-limited females may moderate the cost of reproduction by producing eggs without much modification to the substrates they ingest.

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Food availability effects on reproductive strategy: the case of *Acartia tonsa* (Copepoda: Calanoida)

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ABSTRACT: Food availability has been linked to changes in the biochemical composition of zooplankton eggs. However, a number of species are capable of resource storage and are thereby able to use accumulated reserves for reproduction during periods of poor food conditions. Conversely, in species such as *Acartia tonsa* with limited storage capacities, there can be a strong dependence of egg composition on ambient food conditions. The aim of this study was to determine the effect of food availability on the carbohydrate, protein and fatty acid composition of *A. tonsa* females and their eggs after being fed with different concentrations of the cryptophyte *Rhodomonas baltica*. During the experiments, no significant differences in the biochemical composition of females were observed, although egg protein composition was higher in food-limited females. We propose that the production of protein-rich eggs by food-limited copepods is a reproductive strategy for ensuring the survival of offspring during poor feeding conditions. In terms of their relative biochemical makeup, there were no significant differences between both adults and eggs of *A. tonsa* and their prey *R. baltica*. However, these biochemical similarities did not influence egg production. Rather, higher biochemical similarities were observed between *R. baltica* and eggs when females were food limited. These findings suggest that food-limited females may moderate the cost of reproduction by producing eggs without much modification to the substrates they ingest.

KEY WORDS: *Acartia tonsa* · Food concentration · Biochemical content · Resting eggs

INTRODUCTION

Copepods occupy a key trophic position in marine food webs and are responsible for the transfer of a large proportion of the energy between primary producers and higher trophic levels (Cushing 1990). Vital for the persistence of copepod populations is the production of viable eggs that ensure the supply of naupliar recruits and hence the survival of individual populations. Among other factors, normal development and subsequent hatching of eggs depends on their chemical constituents. For example, carbohydrates are mainly used as an energy source during hatching (Guisande & Harris 1995), proteins modulate cellular events such as gene expression and growth, and fatty acids (FAs, specifically polyunsaturated FAs) are involved in the metabolism of chemicals responsible for regulating cell differentiation and hatching (Sessler & Ntambi 1998). Often these roles are specific to individual biochemical constituents, and they are typically not interchangeable. Several studies have demonstrated the effect of prey biochemical contents on egg viability (e.g. Jónasdóttir & Kiørboe 1996, Guisande et al. 2000). However, it is not clear whether egg viability is also affected by food availability (e.g. Tang et al. 1998).

Food availability influences egg size, which influences the amount of chemical substances required for embryogenesis and subsequent hatching (Guisande & Harris 1995, Auel 2004). Furthermore, the extent to which ambient food levels influence egg biochemical characteristics may depend on the reproductive strategy of animals. For example, during poor food conditions, species capable of storing lipids rely mostly on

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AII Food composition influencing *Acartia tonsa* (Copepoda: Calanoida) reproduction and egg biochemical composition

Introduction

Different algal components were found to be correlated to high egg production rates (*EPR*, eggs female^{-1} day^{-1}) and hatching success (*HS*) of several copepod species. Therefore, food quality highly influences the reproductive success of copepods. The consequences of food of inferior quality on the copepod's reproduction can be different, e.g. previous studies found *EPR* to decline, *HS* to decline or even both *EPR* and *HS* to decline (Ban et al. 1997). Especially in a situation in which poor food quality leads to a decline in *EPR* but not in *HS*, an examination of the biochemical composition of the copepod's eggs in relation to the composition of the food is of great interest. Might the copepod be able to produce less, but good quality eggs? This might be, at least for opportunistic species like *A. tonsa*, which do not build up energy reserves in the adult stage but instead invest all energy uptake into egg production (Dagg, 1977; Durbin et al. 1983, Kiørboe et al. 1985a).

With three short term experiments the influence of the composition of the food source on the composition of *Acartia tonsa* eggs should be investigated. The experiments were done using different ratios of the two algae in each experiment (75*Tw*/25*Rh*; 50*Tw*/50*Rh*; 25*Tw*/25*Rh*). As well, in each experiment different algae concentrations (high and low food) were used. Samples were taken to determine the Protein and Carbohydrate content of the food and the eggs of the copepod. Also samples of the females were taken in the beginning and at the end of each sub-experiment. With this it should be investigated if the different food combinations led to different eggs in terms of their biochemistry.

Methods

Copepod and Algal cultures

The copepod and two *T. weissflogii* cultures were grown as described in Diekmann et al. (2009). Two 10 L *Rhodomonas* spp. cultures were established accordingly to the *T. weissflogii* cultures. The four algal cultures were maintained in a continuous growth phase by removing half of the water every

day and replace it with nutrient enriched seawater. All cultures were held at 33 ±1 psu and 18.5±1.5°C (mean ±range). Algae and copepods were held in a 13:11 L:D light regime.

Egg production and hatching success experiment

In each experiment six 8 L beakers were filled with filtered 33-psu seawater and set up with 150 females and 40 males of *A. tonsa* sitting a holding chamber. Daytime surface light intensities were 2 µE m^{-2} s^{-1}. Eggs produced by the copepods passed through the sieve and settled onto the bottom of the beaker, thus eliminating the possibility of egg cannibalism. Every 24hrs, each holding chamber was transferred to a new beaker and the eggs from the previous 24h were collected onto a 35µm sieve, transferred to a Bogoroff tray, and counted to determine the egg production rates (*EPR*, eggs $female^{-1}$ day^{-1}). Eggs were incubated for 72h after which hatching success (*HS*, %) was calculated. Each experiment was conducted for three days. Three experiments were conducted each with a different food treatment and two food concentrations:

Experiment 1: The copepods were fed an algae mixture containing 50% *T. weissflogii* and 50% *Rhodomonas* spp. (50*Tw*/50*Rh*)

Experiment 2: The algae mixture consisted of 25% *T. weissflogii* and 75% *Rhodomonas* spp. (25*Tw*/75*Rh*)

Experiment 3: The algae mixture consisted of 75% *T. weissflogii* and 25% *Rhodomonas* spp. (75*Tw*/25*Rh*)

Based on the differences of carbon content in the two algae copepods were fed five times more *Rhodomonas* spp. cells ml^{-1} (50000 cells ml^{-1}, based on 36.9 pg C $cell^{-1}$ (Støttrup and Jensen 1990)) compared to *T. weissflogii* (10 000 cells ml^{-1}, based on a carbon content of 183 pg C $cell^{-1}$ (Støttrup and Jensen 1990)). The two food concentrations were high (H) and low (L) using concentrations of 1500µg C L^{-1} and 250µg C L^{-1}. Cell concentrations were determined using a Beckman Multisizer 3 Couter Counter (range of *Rhodomonas* spp. 4-9µm, range of *T. weissflogii* 9 -17µm).

Biochemical Analyses

The methods employed for the analyses of Protein and Carbohydrate were the same as those discussed by Diekmann et al. (2009). For the analyses of females 20 females were used per sub sample. Each egg sample consisted of 100 eggs.

Results

Comparing all experiments the mean *EPR* (Fig. AII–9) was low and varied from 9.84 (±1.21) in the low food treatment (L) to 21.23 (±1.09) eggs female^{-1} day^{-1}in the high food treatment (H). A Kruskal–Wallis test ($p<0.05$) showed significant differences in *EPR* but not in *HS* (Table AII–4). Nevertheless, using a Mann-Whitney Test with a Bonferroni correction to test for the actual differences in *EPR* neither the comparison between nor within each group met the confidence intervals ($p<0.003$, Table AII–5). *HS* varied between 71.94% (±2.79) in the L treatment and 77.22% (±2.65) in the H treatment (Fig. AII–9).

Significant differences in carbohydrate and protein contents neither occurred between or within the three experiments in the algae (Table AII–4, Fig. AII–10A), nor including the different food treatments (H and L) in the eggs (Table AII–4, Fig. AII–10B) and females (Table AII–4, Fig. AII–10C). The mean carbohydrate content of *T. weissflogii* and *Rhodomonas* spp. was highest in the 75*Tw*/25*Rh* experiment (0.00039±8.11x10^{-5}µg cell^{-1} and 8.73x10^{-5}±1.27x10^{-5}µg cell^{-1}).

In the eggs coming from the low food treatment the mean carbohydrate content was always slightly but not significantly higher than in the eggs from the high food treatments showing a maximal value of 2.23(±0.56)µg egg^{-1} in the 75*Tw*/25*Rh* experiment. Eggs from the high food treatment showed a minimal value at the 75*Tw*/25*Rh* experiment (1.29±0.24µg egg^{-1}) and a maximal mean carbohydrate value at the 50*Tw*/50*Rh* (1.84±0.67µg egg^{-1}).

The females' carbohydrate contents varied between 6.47µg female^{-1} (±0.2, after the 25*Tw*/75*Rh* experiment and the L food treatment) and 13.6µg female^{-1} (±5.5, before the 50*Tw*/50*Rh* experiment).

The mean protein content of *T. weissflogii* and *Rhodomonas* spp. was highest in the 25*Tw*/75*Rh* experiment (6.38x10^{-5}±1.2x10^{-5}µg cell^{-1}) and the 50*Tw*/50*Rh* experiment (3.15x10^{-5}±7.5x10^{-6}µg cell^{-1}), respectively (Fig. AII–11A).

Some of the data on protein content of the eggs (Fig. AII–11B) was lost, but values of the analyzed eggs did not show any significant differences within or between the three experiments including H and L food concentrations. Mean maximal values of eggs coming from the low and high food treatments occurred in the 75*Tw*/25*Rh* experiment and were 0.23(±0.03) and 0.22(±0.06)µg egg^{-1} respectively. Mean initial protein contents as well as protein contents at the end of the low and the high food treatment were highest in the 25*Tw*/75*Rh* experiment with 3.13 (±0.73)µg female^{-1}, 3.8(±1.47)µg female^{-1} and 3.65(±0.76)µg female^{-1}, respectively.

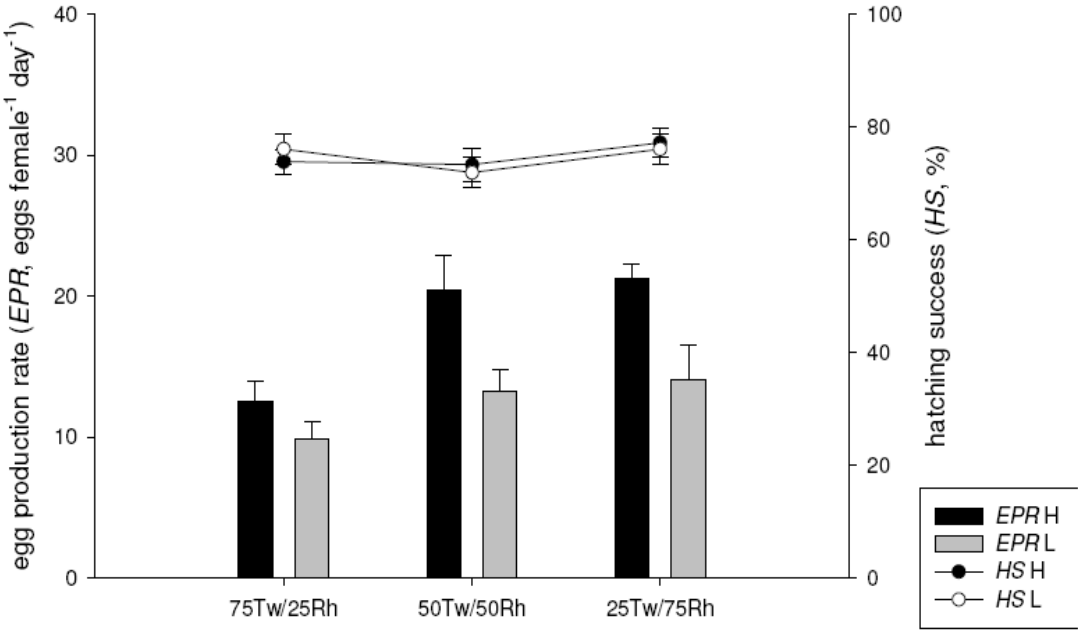

Fig. AII–1 Mean (±SE) egg production rate (*EPR*, no. of eggs female^{-1} d^{-1}) and hatching success (*HS*, %) of *A. tonsa* at the three diet treatments: 75% *T. weissflogii* and 25% *Rhodomonas* spp. (75*Tw*/25*Rh*); 50% *T. weissflogii* and 50% *Rhodomonas* spp. (50*Tw*/50*Rh*); 25% *T. weissflogii* and 75% *Rhodomonas* spp. (25*Tw*/75*Rh*). Food concentrations were either high (H) or low (L)

Table AII–1 Results of the Kruskal-Wallis tests testing for significant differences in egg production rate (*EPR*), hatching success (*HS*), protein and carbohydrate content of *T. weissflogii* (*Tw*), *Rhodomonas* spp. (*Rh*), *A. tonsa* eggs and females. Asteriks indicate significant differences

	chi-square	df	p-value
EPR *	11.058	5	0.05
HS	4.476	5	0.483
Protein *Tw*	0.036	2	0.982
Carbohydrate *Tw*	0.94	2	0.625
Protein *Rh*	4.321	2	0.115
Carbohydrate *Rh*	0.982	2	0.612
Protein eggs	2.509	4	0.643
Carbohydrate eggs	3.053	5	0.692
Protein females	13.116	8	0.108
Carbohydrate females	9.91	8	0.271

Table AII–2 Results of the Mann–Whitney test made to detect the actual differences within egg production rates (*EPR*). Diet treatments: 75% *T. weissflogii* and 25% *Rhodomonas* spp. (75*Tw*/25*Rh*); 50% *T. weissflogii* and 50% *Rhodomonas* spp. (50*Tw*/50*Rh*); 25% *T. weissflogii* and 75% *Rhodomonas* spp. (25*Tw*/75*Rh*). Food concentrations were either high (H) or low (L). Note that differences are only significant with a $p<0.003$ due to a Bonferroni correction

	Mann–Whitney–U	p
H 75*Tw*/25*Rh* compared to L 75*Tw*/25*Rh*	2	0.275
H 75*Tw*/25*Rh* compared to H 50*Tw*/50*Rh*	0	0.05
H 75*Tw*/25*Rh* compared to L 50*Tw*/50*Rh*	4	0.827
H 75*Tw*/25*Rh* compared to H 25*Tw*/75*Rh*	0	0.05
H 75*Tw*/25*Rh* compared to L 25*Tw*/75*Rh*	4	0.827
L 75*Tw*/25*Rh* compared to H 50*Tw*/50*Rh*	0	0.05
L 75*Tw*/25*Rh* compared to L 50*Tw*/50*Rh*	1	0.127
L 75*Tw*/25*Rh* compared to H 25*Tw*/75*Rh*	0	0.05
L 75*Tw*/25*Rh* compared to L 25*Tw*/75*Rh*	2	0.275
H 50*Tw*/50*Rh* compared to L 50*Tw*/50*Rh*	0	0.05
H 50*Tw*/50*Rh* compared to H 25*Tw*/75*Rh*	4	0.827
H 50*Tw*/50*Rh* compared to L 25*Tw*/75*Rh*	1	0.127
L 50*Tw*/50*Rh* compared to H 25*Tw*/75*Rh*	0	0.05
L 50*Tw*/50*Rh* compared to L 25*Tw*/75*Rh*	4	0.827
H 25*Tw*/75*Rh* compared to L 25*Tw*/75*Rh*	2	0.275

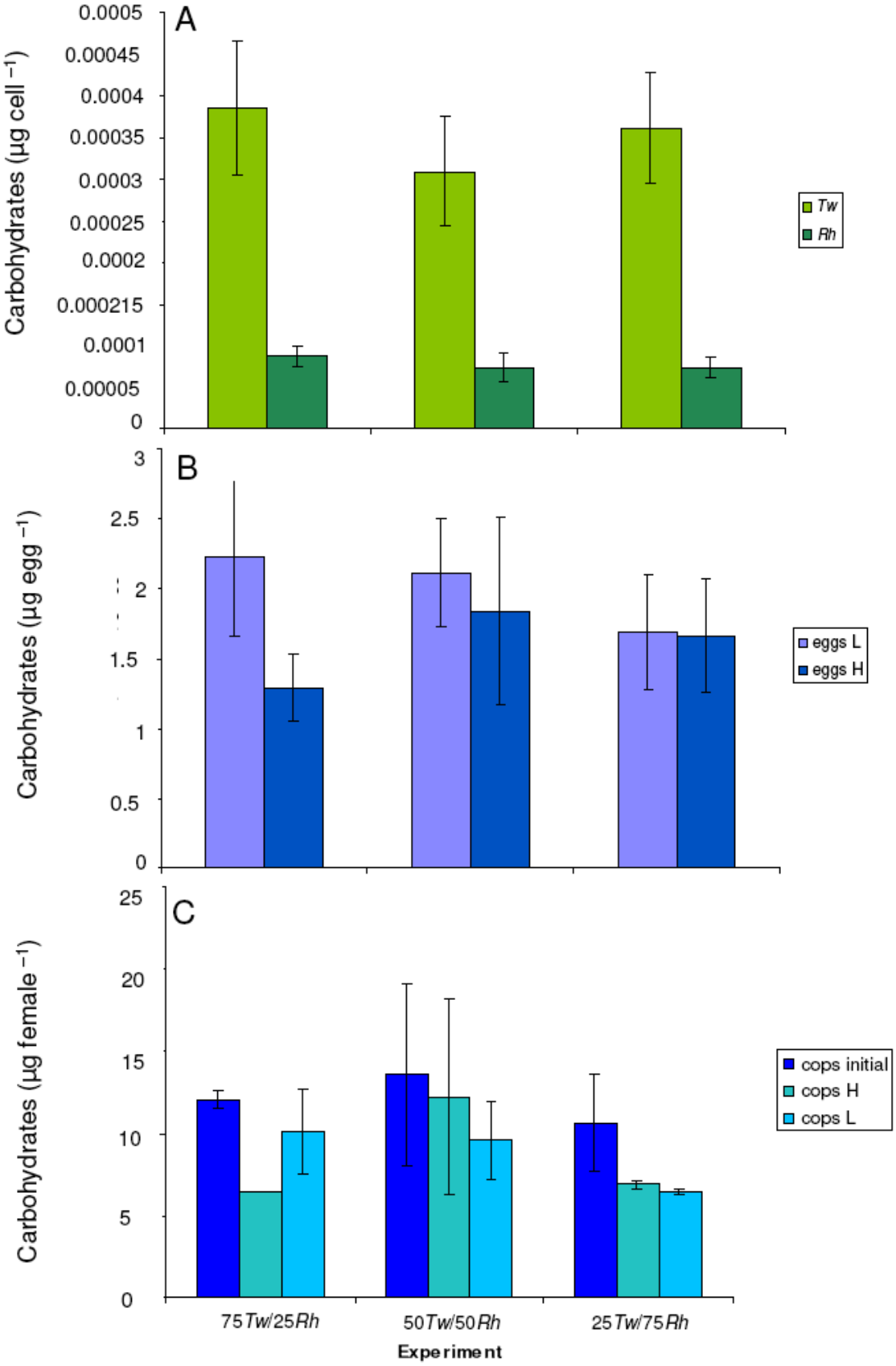

Fig. AII–2 Mean (±SE) carbohydrate content (*µg*) in (**A**) the algae used as food, *Rhodomonas* spp. (*Rh*) and *T. weissflogii* (*Tw*), in each experiment (**B**) in the eggs of *A. tonsa* in each experiment and food treatment (H= high, L= low) (**C**) in the females before the experiments (initial) and after the experiments at each food treatment

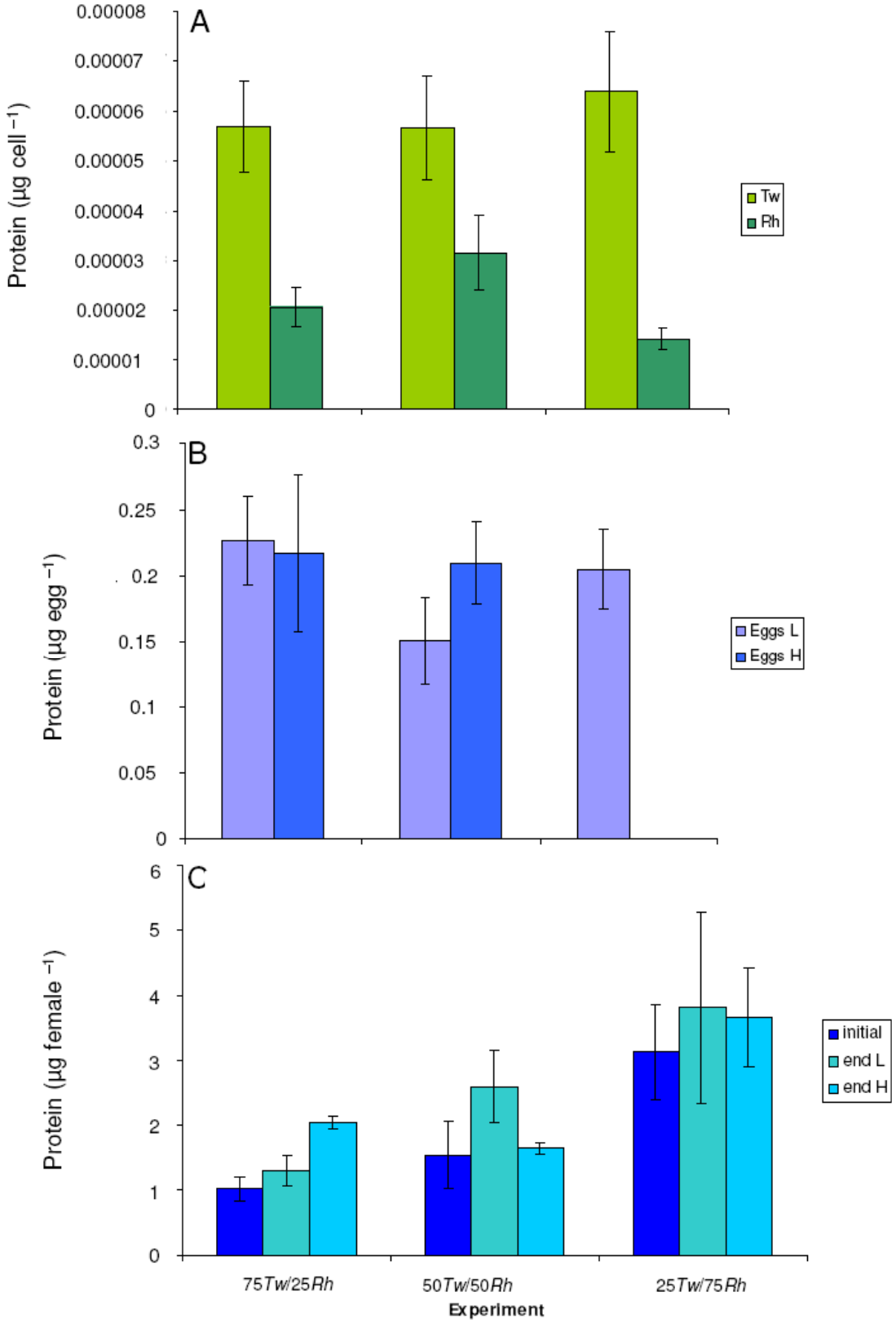

Fig. AII–3 Mean (±SE) protein content (*pg*) in (A) the algae used as food, *Rhodomonas* spp. (*Rh*) and *T. weissflogii* (*Tw*), in each experiment (B) in the eggs of *A. tonsa* in each experiment and food treatment (H= high, L= low) (C) in the females before the experiments (initial) and after the experiments at each food treatment

Discussion

Maximum *EPR* of *A. tonsa* were 21.23 eggs female^{-1} day^{-1} and therefore not as high as assumed under optimal conditions. Holste and Peck (2006) reported *A. tonsa EPR* to be around 40 eggs female^{-1} day^{-1} at optimal conditions and Diekmann et al. (2009, 2012) even reported *EPR* of up to 49 and 57 eggs female^{-1} day^{-1}. *HS* was quite stable in all three experiments (Fig. AII–9), varying from 71.94 to 77.22%, representing a medium *HS* as *A. tonsa* can show a *HS* of >90% (Holste and Peck 2006, Hansen et al 2010).

The obvious differences between carbohydrate and protein content in *T. weissflogii* and *Rhodomonas* spp. cells are due to the differences in cell size. The carbon content of *T. weissflogii* is 183pg C cell^{-1} (Støttrup and Jensen 1990) while that of *Rhodomonas* spp. is 36.7pg C cell^{-1} (Kiørboe et al. 1985a). On the basis of these measurements food concentrations used in these experiments were calculated to compensate for the differences due to the size of the algae. The copepods were fed to achieve an environment with 10,000 *T. weissflogii* cells ml^{-1} (which equals 1830µg C L^{-1}) and 50,000 cells ml^{-1} *Rhodomonas* spp. (which equals 1840µg C L^{-1}). A study by Kiørboe et al. (1985a) showed maximal ingestion rates and *EPR* of *A. tonsa* when it was fed around 1500µg C L^{-1}. In our measurements the differences in carbohydrates and protein between *T. weissflogii* and *Rhodomonas* spp. cell^{-1} were more 3 to 4-fold than 5-fold (as we used for our calculations) therefore we might have overcompensated the lesser content of *Rhodomonas* spp. .

A. tonsa is not capable of building up large energy reserves and rather invests the assimilated energy into egg production (Dagg 1977, Durbin et al. 1983, Kiørboe et al. 1985a,b, Stearns et al. 1989, Tester and Turner 1990). In the high food treatment (H) we expected high *EPR* rates as we ensured a food concentration around 1500µg C L^{-1}. In the L food treatment the food concentration was only 250µg C L^{-1}. Therefore it is unexpected that *EPR* was low in both treatments and did not show significantly differences between food treatments. We assume that either the food quality or some unknown factor inhibited the copepod's egg production in both treatments. Nevertheless, no significant differences in the biochemical composition of the eggs and females occurred within the two food quantities (H and L) at the different food compositions. It might be that our overcompensation of the lesser carbon

content of *Rhodomonas* spp. neglected the differences in food concentration by providing an adequate amount of food. The finding, that no significantly differences in the biochemical composition of the females occurred at the different food compositions agrees well with the findings of Acheampong et al. (2011 and references therein) who reported *A. tonsa* females to maintain homeostasis at the biochemical level possibly by regulating their post-ingestion processes to meet their specific requirement for chemical substances. In contrast to our findings Acheampong et al. (2011) found significantly differences in the biochemical composition, more precisely in the protein content, of *A. tonsa* eggs at different food concentrations. They argue that consumers feed first to meet their maintenance requirements and egg production is only possible when there are surplus resources leading to a decrease in *EPR* in favor of an production of eggs which meet at least the minimum requirements for successful egg development (Anderson and Pond 2000; Acheampong et al. 2011). As we found generally low *EPR* and no differences in the eggs biochemical composition it might be that all eggs produced in our experiments suffered from the same lack of resources independent of food composition.

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