Zooplankton growth and trophic linkages: Implications for fish feeding conditions in the Baltic Sea

Towe Holmborn
To my precious
ABSTRACT

The aim of this Thesis was to improve our understanding and assessment of feeding conditions for zooplanktivorous fish in the Baltic Sea.

We investigated (papers I, II) the usefulness of biochemical proxies for assessments of growth and metabolic rates in the dominant Baltic copepod *Acartia bifilosa*. A predictive model (paper I) for egg production rate (EPR), based on body size, RNA content, and water temperature, was established using females of different geographical origin. This model demonstrates the usefulness of RNA content as a proxy for growth in zooplankton and, together with abundance data, it could be used to evaluate fish feeding conditions. Further (paper II), using *A. bifilosa* exposed to a food gradient, we evaluated responses of physiological rates and other biochemical proxies for growth and established correlations between physiological and biochemical variables. EPR and ingestion rate were most significantly correlated with RNA content. As assayed variables saturated at different food concentrations, food availability may affect assessments of physiological rates using proxies. In paper III, we explored the effect of high EPR and ingestion rate on astaxanthin content in *A. bifilosa*. We found that the astaxanthin content decreased at high feeding rates, most likely due to decreased assimilation efficiency. This may impact the quality of zooplankton as prey.

The invasion of *Cercopagis pengoi*, a zooplanktivorous cladoceran, has altered the trophic linkages in the Baltic Sea food web. In paper IV, we evaluated the feeding of zooplanktivorous fish on *C. pengoi* and found that irrespective of size both herring and sprat feed on it, with large herring being more selective. In turn, *C. pengoi* feeds mainly on older copepods (paper V), which are acknowledged important in fish nutrition. These results indicate that *C. pengoi* may compete with fish due to the diet overlap.

Keywords: AARS activity, biochemical markers, *Clupea harengus*, copepod physiology, food web interactions, non-indigenous species, RNA-based indices, *Sprattus sprattus*, stable isotopes.
SAMMANFATTNING

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<table>
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<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>AARS</td>
<td>Aminoacyl-tRNA synthetases</td>
</tr>
<tr>
<td>EPR</td>
<td>Egg production rate (eggs ind$^{-1}$ day$^{-1}$)</td>
</tr>
<tr>
<td>NIS</td>
<td>Non-indigenous species</td>
</tr>
<tr>
<td>PL</td>
<td>Prosome length (mm)</td>
</tr>
<tr>
<td>RNA content</td>
<td>RNA content in females (µg ind$^{-1}$)</td>
</tr>
<tr>
<td>RNA:DNA</td>
<td>Ratio of RNA and DNA content</td>
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<tr>
<td>RNA:protein</td>
<td>Ratio of RNA and protein content</td>
</tr>
<tr>
<td>spAARS</td>
<td>Protein specific AARS activity (nmol PPI mg protein$^{-1}$ h$^{-1}$)</td>
</tr>
<tr>
<td>T</td>
<td>Temperature (°C)</td>
</tr>
<tr>
<td>YOY</td>
<td>Young-of-the-year</td>
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PROSPECT OF THESIS

The overall aim of my Thesis was to improve assessments of fish feeding conditions in the Baltic Sea and to increase our understanding of the link between zooplankton and fish. To do this, I conducted studies on (1) physiological and biochemical determinants of quantity and quality of copepod production, and (2) effects of a non-indigenous zooplankton species on the pelagic food web and fish feeding conditions in the Baltic Sea. This was, more specifically, done by:

- Exploring the possibilities of using biochemical proxies to assess growth and metabolic status in copepods (papers I, II). This approach could, for example, provide us with useful analytical tools for assessments of in situ zooplankton growth and nutrition, which are necessary for evaluating fish feeding conditions and to refine food web models.

- Investigating the consequence of high feeding and production rates on the quality of zooplankton as prey. The quality aspect in this study (paper III) concerned the important antioxidant astaxanthin. Studies of this kind will help us understand connections between quantity of zooplankton and its quality as a prey for higher trophic levels, including fish. In the Baltic Sea, this is relevant with respect to the eutrophication that has altered the general productivity status but also to the natural seasonal variations in productivity.

- Investigating top-down and bottom-up effects of the non-indigenous predatory cladoceran *Cercopagis pengoi* in the Baltic Sea pelagic food web (papers IV, V). Studying trophic interactions of this recently introduced species with native prey and predators is crucial for understanding and predicting the impacts of this non-indigenous species on the Baltic Sea pelagic food web and fish feeding conditions.
LIST OF PAPERS

The Thesis is based on the following papers, referred to in the text by their Roman numerals (published papers are reprinted with kind permissions of the publishers):


My contributions to the papers were:

I  Participation in planning of the study, all fieldwork, all analysis and data processing, main part of the writing.

II  Participation in planning and execution of the experiment (responsible for the egg production and egg viability analyses), most data processing, main part of writing.

III Participation in planning and execution of the experiment (responsible for the egg production and egg viability analyses), commenting on the manuscript.

IV Participation in planning of the study, all analysis, except the one of the zooplankton community, most data processing, writing of the first draft.

V Participation in planning of the study, microscopic analysis of the zooplankton community, commenting on the manuscript.
AN INTRODUCTION TO THE STUDY SYSTEM

The study area – The Baltic Sea

The Baltic Sea is one of the largest brackish water seas in the world. It has a natural salinity gradient with higher salinities in the south (Jansson and Velner 1995), and therefore, the organisms living in the Baltic Sea is a mixture of freshwater and marine species. Compared to fully saline or fresh water areas, the species diversity is very low in areas with a salinity of 6–7 (PSU, A. Remane, according to Ackefors 1965), a salinity that corresponds to the conditions of my study areas; coastal regions of the northern Baltic proper (Fig. 1).

![Map of the Baltic Sea](image)

Figure 1. Schematic map of the Baltic Sea. The black dots indicate areas in the northern Baltic proper used for field sampling that provided data for this Thesis. The Roman numerals denote the paper(s) in question for the sampling site. For a more detailed description of the sampling sites see the respective papers.

Crustacean mesozooplankton in the Baltic proper

Zooplankton is a diverse group with respect to e.g., taxonomy, life history traits, behavior, and size. The size group of zooplankton called mesozooplankton (0.2–2 mm) consists mainly of small crustaceans, such as copepods and cladocerans. They constitute a major link between primary producers (e.g., planktonic algae) and higher trophic levels (e.g., mysids and fish).
The calanoid copepods are among the most abundant and productive zooplankton groups in the Baltic proper (Ackefors 1971, Ackefors and Hernroth 1972). They are generally present in the water column throughout the year being more numerous during summer (Ackefors and Hernroth 1972). There are several species of calanoid copepods abundant in the northern Baltic proper; however, in coastal areas, only a few species are found in great numbers, e.g. *Acartia bifilosa* and *Eurytemora affinis* (Hessle and Vallin 1934, Ackefors 1981). The copepods of genus *Acartia* are broadly distributed around the world (Bradford 1976) and are numerically dominant or co-dominant copepods in most estuaries (Turner 1981, and references therein). *Acartia* are free-spawning, opportunistic omnivores (Castel 1981) that feed on phytoplankton, detritus and small heterotrophs depending on availability (Turner 1984, and references therein).

*A. bifilosa* (paper I, II, III; Fig. 2a) is a dominant species in many European estuaries (e.g., Irigoien and Castel 1995, Burdlof et al. 2002) and can also be found elsewhere in the northern hemisphere (Razouls et al. 2005–2009). In the Baltic Sea, it occurs in salinities between 4 and 8 (Hessle and Vallin 1934, Viitasalo et al. 1994) and tolerates a wide range of temperatures. However, it is rarely found in cold waters or below 50 m depth (Hessle and Vallin 1934). Therefore, in the Baltic Sea, *A. bifilosa* is abundant in offshore areas but is far more numerous and important in coastal areas (Hessle and Vallin 1934, Hernroth and Ackefors 1979, Ackefors 1981). In fact, it has been suggested that *A. bifilosa* is the most important copepod in coastal areas of the Baltic proper (Ackefors 1981) and it is also recognized as an important prey for fish (Turner 1984, and references therein, Arrhenius 1996, Voss et al. 2003).

The cladocerans found in the northern Baltic proper are usually only present in the water column during the warm season and overwinter as diapause eggs (Ackefors 1971). However, during the warm season they can be found in large numbers (Hessle and Vallin 1934, Ackefors 1981). In archipelago areas, *Pleopsis polyphemoides*, *Bosmina maritima*, and *Cercopagis pengoi* are the most abundant species (Hessle and Vallin 1934, Litvinchuk and Telesh 2006).

*C. pengoi* (paper IV, V; Fig. 2b) is a predatory cladoceran native to the Ponto-Caspian region (Rivier 1998). It was probably introduced form the Black Sea (Cristescu et al. 2001) via the Volga-Baltic waterway (Leppäkoski and Olenin 2000). In 1992, the first specimens were recorded in the Gulfs of Finland and Riga (Ojaveer and Lumberg 1995, Krylov et al. 1999), and in 1997, the first specimen on the Swedish coast was found (Stockholm archipelago and Gotland basin; Gorokhova et al. 2000). It has since spread to the southern regions of the Baltic proper (Bielecka et al. 2000, Litvinchuk and Telesh 2006, Olszewska 2006), and from the Baltic Sea
to the North American Great Lakes (MacIsaac et al. 1999, Cristescu et al. 2001). In the Baltic Sea, *C. pengoi* is especially abundant in coastal areas (Litvinchuk and Telesh 2006) and it is generally more numerous in the upper part of the water column (down to 10 m depth) than in deeper layers (Krylov et al. 1999), which may indicate its preference for warmer temperatures.

Figure 2. Species in focus in this Thesis: a) *Acartia bifilosa*, adult (CVI) female, the model organism in paper I, II, and III; b) *Cercopagis pengoi*, gamogenetic female (barb stage III) carrying two diapause eggs, the species in focus in paper IV and V.

**Zooplankton in the Baltic Sea pelagic food web**

Generally, the population dynamics of fish stocks depends on their reproductive success, i.e. successful spawning and survival of embryos and larvae (Fogarty and O’Brien 2009). The reproductive success, in turn, depends on a variety of abiotic variables, but also on feeding conditions for larvae (Kalejs and Ojaveer 1989, Ojaveer and Lehtonen 2001). Nearly all fish species, including piscivorous fish, feed and depend on zooplankton in their early life, as larvae and/or juveniles. The major zooplanktivorous fish in the Baltic Sea are sprat (*Sprattus sprattus*) which is a holozooplanktivore, and herring (*Clupea harengus*), whose diet also includes mysids, amphipods, and small fish (Rudstam et al. 1992, Thurow 1997).

Due to the central position that zooplankton have in the Baltic Sea pelagic food web, studies on zooplankton production, in terms of both quality and quantity, would facilitate assessments of energy flow through the pelagic ecosystem, increase our understanding of pelagic interactions and enhance predictive capacity of fish stock modeling. However, during 1990–2006, the importance of zooplankton dynamics and production was somewhat ignored and zooplankton monitoring was excluded from the national monitoring programs. Recently, however, as a result of drastic changes in the reproductive success, population dynamics, and physical condition of many commercially important fish species, such as herring, sprat, cod, pike and perch, there has been a growing interest in the dynamics and production of zooplankton.
ASSESSING ZOOPLANKTON GROWTH AND METABOLIC ACTIVITY

Production assessments in continuously reproducing wild populations (such as most zooplankton; Winberg et al. 1971) includes sampling, identification and counting of different life stages (Winberg et al. 1971, Runge and Roff 2000), and calculations based on an estimated or measured growth rate. Consequently, the production estimate is a function of the growth rate estimate. Although assessment of in situ growth may help us understand food web structure and dynamics, few such studies on zooplankton exist. The reason for this is partly the lack of reliable and convenient methods.

Today, common methods to assess growth include temperature-dependent models, weight-dependent models, physiological or laboratory-derived budget models, cohort or artificial cohort weight increase or moult rate, and (for e.g. copepods and rotifers) egg production assessment (reviewed by Winberg et al. 1971, and Runge and Roff 2000). All these methods have shortcomings and their accuracy and reliability for in situ growth assessment are often questioned. The temperature and weight dependent models are simplified empirical growth models based on temperature and/or body weight. The major problems with these models therefore, is that the independent variables are limited to temperature and/or body weight only, while other variables, such as food quantity and quality (Kleppel 1993), salinity (Calliari et al. 2006), and physiological condition (health; e.g., Fahmi and Hussain 2003), age/life stage; Calbet et al. 2000) also may affect zooplankton growth. The physiological method, on the other hand, which relies on previously determined relationships between growth and e.g. weight, temperature, food availability, respiration, and assimilation for in situ extrapolation have other problems. The major problem, as suggested by Huntley and Lopez (1992) and Huntley (1996) is difficulty in parameterizing the impact of different variables in the combined physiological equation. The cohort or artificial cohort weight increase and moult rate methods use the dynamics of a single stage, cohort or a whole population to establish weight increase or moult rate over time in field sampled specimens incubated in the laboratory (Runge and Roff 2000). The same rationale is employed in the egg production method, where egg production in adult females represents the growth (Runge and Roff 2000). In juvenile copepods, growth consists of somatic growth (i.e., increase in body-mass and building exoskeleton), while in adults the growth is generative (i.e., formation of sexual products; Carlotti and Nival 1992). The egg production method can therefore be used only for assessing production in adult female copepods, while the cohort or artificial cohort weight increase or moult rate methods should be used to assess growth in juvenile life stages. However,
assuming a constant specific growth over different life stages during non limiting food conditions, as suggested by Sekiguchi et al. (1980) and tested by e.g. Berggren et al. (1988), the egg production method can also be used to approximate juvenile growth. This assumption and therefore applicability of the egg production method for the copepod production assessment has been challenged by many physiologists (e.g., Richardson and Verhey 1999, Calbet et al. 2000). The major drawbacks with the egg production and molting methods are related to the risk of introducing artifacts due to handling of the animals and to the enormous amount of labor inherent to the methods. Nevertheless, the egg production method is generally the least laborious of these methods, and is probably the most employed method for direct measurement of copepod growth in field and laboratory studies (Runge and Roff 2000).

Although direct growth measurements are crucial for production assessments, other physiological variables are also useful for interpreting the condition and production status of zooplankton. For example, a number of variables of metabolic activity such as the rate of which an individual ingest food, egests or excrete non-assimilative food products, and respire, all give us clues about what might inhibit or enhance growth (Blažka 1971, Fig. 3).

![Diagram of energy flow through a copepod.][1]

**Figure 3.** Schematic figure of energy flow through a copepod. The shaded boxes represent variables assayed in paper I and II of this Thesis.
Of particular interest is the ingestion rate as it sets the upper limit for all physiological processes including growth (Fig. 3). Although these metabolic indicators are valuable for understanding key mechanisms of growth-related responses, the assessment of these variables are by nature as laborious as direct growth measurements and often difficult to employ in field studies. Therefore, alternative, easy to employ, yet reliable, methods for direct assessment of in situ growth and metabolic activity in zooplankton could facilitate the accuracy of zooplankton production estimates. This, in turn, would improve our ability to produce more data, which would allow for a better understanding of food web interactions; a prerequisite for reliable predictions of ecological models including those evaluating fish feeding conditions.

Using biochemical macromolecules to assess growth and metabolic activity in zooplankton

There is an increasing recognition that biochemical indices can provide a suitable alternative and/or complement to existing in situ methods for assessment of growth and physiological condition in zooplankton (Runge and Roff 2000). In general, use of these indices to assess physiological performance, such as egg production, somatic growth, ingestion, respiration, and stress, are based on key components of synthetic and/or metabolic pathways. These pathways are usually either directly or indirectly linked to cell growth and synthesis of important biological macromolecules. During recent decades, as a result of significant progress in analytical methods (Caldarone et al. 2006), analysis of bulk ribonucleic acid (RNA) has been increasingly used to estimate growth in a variety of small animals, including cladocerans (Gorokhova and Kyle 2002, Vrede et al. 2002) and copepods (Nakata et al. 1994, Wagner et al. 1998). In copepods, the RNA concentration has proven to be a sensitive indicator of both reproductive (Nakata et al. 1994, Saiz et al. 1998, Gorokhova 2003, papers I and II) and somatic growth (Wagner et al. 2001), as well as a measure of metabolic activity in response to nutritional conditions (Wagner et al. 1998, Gorokhova et al. 2007, paper II), salinity stress (Calliari et al. 2006) and toxic substances (Gardeström et al. 2006). Studies employing RNA-based indices usually standardize the RNA content of an individual to either its DNA or protein contents. The underlying rationale for using RNA-based indices is that RNA concentration, primarily, is a function of ribosome number correlating with protein synthesis and hence with growth rate (Buckley et al. 1999). The amount of DNA, on the other hand, is considered semi-constant and may therefore be an indicator of the cell number, which is a rough estimate of the body size. The RNA:DNA ratio therefore indicates the amount of RNA per cell. The RNA:protein ratio reflects the number of ribosomes per amount of protein and hence the cytoplasmic ribosome concentration (Bremer and Dennis 1996). Moreover, a
wide range of enzymes, such as the metabolic enzymes trypsin, citrate synthase, lactate dehydrogenase, phosphofructokinase, and a moulting enzyme, chitobiase, have been suggested as proxies for somatic growth, and physiological condition in crustaceans (Mayrand et al. 2000, Sastri and Roff 2000, Lemos et al. 2002, Cullen et al. 2003). The rationale of using metabolic enzymes is that the activities of these enzymes are linked to adenosine triphosphate (ATP) production, which is required for elevated growth. The rationale for using the moulting enzyme activity, on the other hand, is related to the moulting cycle as a part of somatic growth process in arthropods. Furthermore, in recent years, another enzymatic indicator of zooplankton growth based on the activity of the aminoacyl-tRNA synthetases (AARS) has been introduced and applied for copepod growth analysis (Yebra et al. 2005, paper II). The AARS are a group of enzymes important in catalyzing a critical step of protein synthesis; i.e. the activation and attachment of amino acids to the tRNA (Ibba and Söll 2000).

Creating a model to assess in situ egg production rate (paper I)
To produce robust predictive models, relationships between growth/physiological condition and biochemical indices must be tested and validated before they can be used for growth assessments of wild populations under varying environmental conditions. In paper I, we evaluated factors regulating the earlier proposed RNA–egg production rate (EPR) relationship in Acartia bifilosa (Gorokhova 2003) and developed a model for in situ EPR assessment. This was done by measuring EPR and RNA content in individual adult females sampled at geographically distant areas (station effect) during spring and summer (season effect). We also measured surface water temperature (T) and prosome length (PL). Multiple linear regressions were generated to create a model that predicts EPR from RNA content, PL, station, season, and T. We found that PL, RNA content, and season explained 53% of the variation in EPR, nearly half of which was explained by RNA content alone. The significance of season indicates the importance of factors varying with season. If RNA content and EPR saturate at different food concentrations (as later found in paper II), it is reasonable to believe that the RNA–EPR relationship is also affected by food availability and possibly also food quality. Therefore, effect of the season variable found in paper I could be, at least partially, related to the differences in food between the seasons. Another factor that varies with season is temperature. Since surface water temperature, which is a continuous and more precise variable than season, was measured in this study, the season variable was replaced by the temperature variable. This replacement did not cause a substantial drop in explanatory power of the model. The effect of spatial origin (station) on the RNA–EPR relationship was never significant. This implies that it may be possible to use this model on the entire Baltic population of A. bifilosa as long as all variables are within the range of assayed variables in paper I.
Comparing usefulness of different biomarkers (paper II)

Although many studies have suggested, developed and refined biochemical methods for assessment of growth and physiological condition, the results are often contradictory and intercalibrations are much needed (Runge and Roff 2000). In paper II, using A. bifilosa as a model species, we evaluated the functional response of EPR, egg viability, ingestion, and respiration rates and four different commonly used biochemical proxies related to protein synthesis [individual RNA content, RNA:DNA ratio, RNA:protein ratio, and protein specific AARS activity (spAARS)], in response to a broad spectrum of food concentrations. Then, a correlative approach was used to evaluate the linkages between the biochemical indices and physiological rates and thus the predictive capacity of these indices for the physiological variables. There were three main findings in this paper: First, although all variables (except egg viability) increased with increasing food concentration either linearly (spAARS) or hyperbolically (all other variables), there were substantial differences in saturating food concentrations among the variables. Therefore, applicability of biomarkers as proxies of physiological rates should be restricted to the non-saturated phase of the functional response of either variable, unless both variables saturate at similar food concentrations. This restriction may have implications for assessing copepod physiological rates at high food availability, as during algal blooms. Second, RNA content and RNA:protein ratio were the best proxies of egg production and ingestion rates, while RNA:DNA ratio and spAARS activity were less suitable. The superior predictability of RNA content and RNA:protein ratio is in line with previous findings in this species (Gorokhova 2003). Overall, the best correlation was observed for ingestion rate and RNA content, a relationship not previously described for this species. Earlier, egg production was suggested to be a more sensitive indicator of nutritional quality than RNA:DNA ratios in Acartia tonsa (Speekmann et al. 2006). The strong relationships between RNA content and ingested food quantity (II) and between EPR and ingested food quality (Speekmann et al. 2006) confirms the importance of food on the EPR–RNA relationship as proposed above (page 17). Third, RNA:DNA ratio and spAARS activity were non-correlating variables, which implies that they could be used jointly as co-variables in multiple regression models to predict ingestion rate. In theory, joint use of non-correlating biochemical indices could be beneficial to improve the predictive ability of models for growth and physiological condition, but this needs to be explored further. Therefore, by investigating other biochemical indices theoretically related to growth and metabolic pathways, but uncorrelated to protein synthesis (e.g., compounds or rates related to ATP or DNA synthesis), we may find other co-variables that can be used in such combined models.
Applicability of RNA-based models for \textit{in situ} growth assessments in the Baltic Sea

To better understand production processes in the Baltic Sea, knowledge of factors governing zooplankton production is a prerequisite. Therefore, development of robust models for growth assessment in zooplankton is an important issue for many zooplanktologists. The findings of paper I and II provide good evidence that individual RNA content can be used to assess \textit{in situ} EPR in the common Baltic copepod \textit{A. bifilosa}, but much work still needs to be done before we can use this approach to assess growth of the entire zooplankton community. The EPR-method assumes that growth rates are the same for all copepod developmental stages, an assumption proved to be incorrect when available food is not equally suitable for juveniles and adults (Richardson and Verheye 1999, Calbet \textit{et al}. 2000). In the calanoid copepod \textit{Centropages typicus}, this was especially pronounced when copepods were feeding on particles larger than 5 \textmu m (Calbet \textit{et al}. 2000). For production assessment of the entire zooplankton community, the growth–RNA relationship for each species and each developmental stage needs to be defined. Nevertheless, the model(s) proposed in paper I can be used “as is” to give a rough measure of the productivity status of the zooplankton community. Moreover, our findings reported in papers I and II suggest that RNA-based models for growth assessment of \textit{A. bifilosa} could be used over larger geographical areas, as long as abiotic (e.g., temperature and salinity) and biotic (e.g., body size, food availability, and food quality) factors are accounted for. Therefore, robust models of this kind may be implemented in diverse monitoring programs, for construction of productivity maps of fish prey. However, further area of concern with respect to copepod population recruitment is that the EPR model described in paper I does not discriminate between viable or total EPR, which has crucial implications for estimating population recruitment. Yet, as shown in paper II, the proportion of viable eggs was generally high and not related to total EPR or RNA levels. Indeed, in our further study, that evaluated how egg viability is reflected in individual RNA content of field collected \textit{Acartia tonsa} females, no significant effect of egg viability on the EPR–RNA relationship was observed (Hogfors \textit{et al}.., in prep.). On the other hand, as egg viability decrease has often been observed in copepods feeding on diatoms (Ianora \textit{et al}. 2003), while our copepods were fed with a green algae \textit{(Tetraselmis suecica, paper II)} or ambient summer plankton (Hogfors \textit{et al}.., in prep.), this needs further experimental studies with other prey. In the Baltic Sea, diatoms are very abundant during spring blooms and therefore, caution should be taken when inferring recruitment from biochemical proxies during diatom blooms, in line with the recommendation to restrict use of biochemical assays for growth assessment to unsaturated feeding conditions (paper II).
QUANTITY – QUALITY

Not only the quantity of zooplankton (addressed in papers I and II), but also their nutritional quality are essential for determining feeding conditions for higher trophic levels, such as zooplanktivorous fish and, consecutively, predatory fish, humans, and seabirds in the Baltic Sea. An altered nutritional quality for zooplanktivorous fish can result from an altered zooplankton community composition, as different species may have different macro- and micronutrient contents (Persson and Vrede 2006). However, it may also reflect alterations in nutritional quality of certain taxa. Effects of nutritional deficiencies in fish diet have been addressed earlier by many studies in different aquatic systems, including the Baltic Sea. For example, changes in the diet composition, and thus altered nutritional value, have been suggested to be responsible for the decrease in the weight-at-age of Baltic sprat and herring (e.g., Raid and Lankov 1995, Rönkkönen et al. 2004). Although it is not clear why some prey species seem to enhance fish growth, it may be related to species specific variations in C:N:P ratio (Walve and Larsson 1999), their ability to store fatty acids (Persson and Vrede 2006) or other biochemical differences.

Carotenoids in the food web

There are many aspects of food quality, besides the energetic value, and fatty acid and amino acid composition; these aspects include quantities and composition of other essential macromolecules, trace elements, and minerals. During the last decades, carotenoid pigments have been recognized to play an important part of the food quality in aquatic food webs (Pettersson and Lignell 1999, Liñán-Cabello et al. 2002, Vuori and Nikinmaa 2007). Apart from being pigments necessary for photoprotection, carotenoids have also showed characteristics of being antioxidants and vitamin A precursors (Schiedt et al. 1985, Liñán-Cabello et al. 2002). The carotenoid astaxanthin is acknowledged to be one of the strongest naturally existing antioxidants (Edge et al. 1997), and has proved to be an efficient protector against UVA and UVB radiation (Davenport et al. 2004). It is abundant in many marine animals, but seems to be particularly important for crustaceans and salmonids (Matsuno 1989). In the Baltic Sea, failed reproduction in salmon (Salmo salar) has, at least partly, been associated with an early mortality syndrome (M74). This disease seems to be connected with low levels of astaxanthin (Amcoff et al. 1998) and thiamine (Pettersson and Lignell 1999) in the eggs and fry of affected females. Low thiamine levels in Baltic salmon have been suggested to indicate an oxidative stress (Vuori and Nikinmaa 2007), a condition that was possible to cure with an astaxanthin enriched diet (Nakano et al. 1995). The diet of Baltic Salmon was, however, very similar before any signs of M74 was seen and during the period when this symptom peaked (Hansson et al. 2001). This suggests that any dietary
difference may be related to the quality of the salmon prey (primarily sprat and herring).

In nature, astaxanthin is almost exclusively synthesized by animals from carotenoid precursors, mainly β-carotene and zetaxanthin (Katayama et al. 1973, Foss et al. 1987) that must be supplied to the animals. In the Baltic Sea pelagic ecosystem, as in most marine pelagic systems, copepods are likely to be the major producers of astaxanthin. The astaxanthin produced by copepods is transferred to the higher trophic levels. Of particular interest, therefore, are factors affecting astaxanthin content in copepods.

Factors affecting astaxanthin levels in copepods
As astaxanthin production in copepods is dependent on the availability of precursors in the diet, some studies dealing with factors affecting astaxanthin content in copepods have focused on diet effects (e.g., Andersson et al. 2003, Van Nieuwburg et al. 2005). Andersson et al. (2003) found the astaxanthin content per copepod to increase at higher phytoplankton biomass and diversity, while it decreased at lower phytoplankton biomass or high phytoplankton biomass dominated by diatoms. Van Nieuwburg et al. (2005) on the other hand found a decreased astaxanthin content in copepods feeding in nutrient (NP or NPSi) enriched macrocosms as compared to the natural concentrations and proposed the lack of need for copepod photoprotection in the higher algal biomass (and therefore lower water transparency) to be a plausible explanation for the latter result. Moreover, they found no difference in astaxanthin content per copepod between the two enriched treatments, dominated by different algae. Other studies have looked at the importance of algae in starved/fed experiments and found a positive relationship between availability of algae and copepod astaxanthin content (Davenport et al. 2004, Sommer et al. 2006). Also, as astaxanthin is recognized as a strong antioxidant with photoprotective properties, the effect of light on astaxanthin production has been investigated. These studies found that light was not a prerequisite for astaxanthin production (Davenport et al. 2004, Sommer et al. 2006), although it seemed to enhance the astaxanthin content in copepods (Sommer et al. 2006). Therefore, it is proposed that the main function of astaxanthin is to improve the antioxidant protection of storage lipids, also in situations where photoprotection is not required (Sommer et al. 2006). If this is the case, astaxanthin content should be more strongly regulated by food intake than by light.

Copeland astaxanthin content in relation to food concentration (paper III)
Although no study has investigated the response curve of astaxanthin content to elevated food concentrations, it is reasonable to believe that an increased food intake will contribute to a higher supply of astaxanthin precursors, which will support
higher astaxanthin production, and thus a higher astaxanthin content, at least up to a saturation point. In paper III, we used A. bifilosa as a model species, to investigate the effect of feeding activity on individual astaxanthin levels. In adult female copepods exposed to a broad range of food concentrations, ingestion rate and EPR along with astaxanthin content were measured. While both EPR and ingestion rate increased with increasing food concentration, as expected, a different response was observed for the astaxanthin content: It increased in concert with increasing food concentrations up to 150 µg C L⁻¹, however at the two highest food concentrations (600 and 1200 µg C L⁻¹), the astaxanthin content dropped significantly and became similar to the concentrations observed in starved individuals. The decrease was significant, even after adjustment for astaxanthin deposition in eggs, and coincided with lower carbon assimilation efficiencies at these food concentrations. Although the mechanisms of this decrease need further investigation, we propose that the reduced astaxanthin accumulation resulted from reduced uptake of precursors, because of decreased assimilation efficiency at the higher feeding rates. However, irrespective of the mechanism, the ecological consequences of this phenomenon are extremely relevant to eutrophication and fish feeding conditions. Indeed, in a eutrophicated system, copepods of poor nutritional quality (with respect to astaxanthin content) may reproduce at high rates and sustain high population densities. Therefore, although this high zooplankton production may be considered positive for the fish feeding conditions, the low quality of the prey may cause astaxanthin deficiency in the predators. These findings also suggest ecological effects on a seasonal scale as copepod quality (but not quantity) may decline during algal blooms or in nutrient enriched environments with high productivity. In line with this, in previous studies (Van Nieuwburg et al. 2005), reduced astaxanthin content in copepods exposed to high food concentrations (and therefore low water transparency) was proposed to be a result of a lowered demand for photoprotection. An alternative explanation to the observed decrease could be the same physiological mechanisms as those proposed in our study (III). However, further experiments investigating these responses using other zooplankton and algal species, in mono and mixed cultures, would greatly improve our understanding of factors affecting qualitative aspects of food web interactions. Furthermore, it is plausible that other important biochemical compounds for zooplankton and their predators (such as other carotenoids, Liñán-Cabello et al. 2002, Sies and Stahl 2005; fatty acids, Kainz et al. 2004; vitamins and microelements, Guillaume et al. 2001) may exhibit the same response as seen for astaxanthin in paper III, thus exerting additional negative effects on diet quality for the higher trophic levels. Therefore, response of other essential elements and compounds to food concentration as well as ecological effect of these responses need to be carefully investigated in controlled laboratory experiments as well as in field surveys.
THE ROLE OF NON-INDIGENOUS ZOOPLANKTON IN THE BALTIC SEA PELAGIC FOOD WEB

The Baltic Sea ecosystem, like any other aquatic ecosystem of low salinity, low biodiversity and few specialized niches, is very vulnerable to introductions of non-indigenous species, NIS (Carlton and Geller 1993). Today, about 30 NIS are recorded in the Swedish waters of the Baltic Sea (Anon. 2009), including the mesozooplankton species: *Acartia tonsa* (Copepoda), *Cercopagis pengoi* (Cladocera), and *Eudne anonyx* (Cladocera) (Anon. 2009). In the eastern parts of the Baltic Sea, the cladoceran *Cornigerius maeoticus maeoticus*, has also been recently recorded (Rodionova *et al.* 2005). Since the biology of this species resembles that of *E. anonyx* and *C. pengoi* (Rivier 1998), a further spread of *C. m. maeoticus* in the Baltic Sea is likely.

The magnitude and mechanisms and thus the consequences of these introductions on the native Baltic Sea food web are generally poorly understood. However, it could be hypothesized that introduced predatory zooplankton species (such as *C. pengoi*, *E. anonyx* and *C. m. maeoticus*; Rivier 1998) will alter the feeding conditions for zooplanktivorous fish, either by competing with fish for food or by becoming an additional prey for fish. In the Baltic Sea, most studies investigating the ecological impact of these planktonic NIS have so far focused almost exclusively on *C. pengoi*, as it was introduced much earlier than the other two cladocerans, and as it is still the only planktonic NIS present in different sea areas every summer in substantial quantities. However, it cannot be ruled out that the other newcomers will increase their abundance with time and that already today their food web effects, albeit perhaps less pronounced, are similar to those of *C. pengoi*, because of the similarities in feeding biology of these species (Rivier 1998).

*C. pengoi* as a prey (paper IV)

In the Gulf of Riga, Ojaveer and Lumberg (1995) have found herring (but not young-of-the-year, YOY, herring) to feed on *C. pengoi* to a great extent. This has also been reported from the northern Gulf of Finland, where *C. pengoi* became a main food source from August to mid October for larger herring (Antsulevich and Välipakka 2000). In the Gulf of Riga, other fish such as three-spined stickleback, nine-spined stickleback and smelt were found to feed somewhat on *C. pengoi*, while sprat, white bream and bleak were not (Ojaveer and Lumberg 1995, Ojaveer *et al.* 2004).

In paper IV, we investigated feeding of herring and sprat, the two most common zooplanktivorous fish in the Baltic Sea, on *C. pengoi*. Contribution of *C. pengoi* to
fish diet was estimated by examining stomach content of fish from a coastal area of the northern Baltic proper (Himmerfjärden Bay). The stomach content was then compared to the ambient zooplankton community abundance and structure to estimate prey selectivity. We found that both herring (5.2 to 25.2 cm) and sprat (5.7 to 11.6 cm) feed on *C. pengoi*. Overall, the proportion of fish with *C. pengoi* in the stomach was 70 % and 61 % for sprat and herring, respectively. Moreover, herring proved to become more selective for *C. pengoi* with increasing size. The fact that YOY of both herring and sprat feed on *C. pengoi*, indicates that this prey is not too large for fish as small as 5 cm long, as suggested earlier (Ojaveer and Lumberg 1995, Bushnoe et al. 2003). These results imply that trophic position of zooplanktivorous fish has shifted up as a result of the invasion, a conclusion later supported by Gorokhova et al. (2005). They used stable isotopes of C and N to elucidate food web interactions between *C. pengoi* and potential prey and predators and found that since the introduction of *C. pengoi* into the food web, the trophic position of YOY herring has shifted from 2.6 to 3.4. Also, even smaller fish such as the sand goby (1.4 cm; Ehrenberg 2008), sticklebacks (3.2 cm; Ojaveer et al. 2004), and herring (4.1 cm; Ojaveer et al. 2004) have now been reported to feed on *C. pengoi*. Therefore, it seems reasonable to believe that many other fish species at most ages can utilize *C. pengoi* as a prey. Moreover, in addition to the impact that *C. pengoi* may have on the diet of zooplanktivorous fish, it may also influence the diet of other zooplanktivores, such as mysids. Indeed, Baltic mysids (*Neomysis integer, Mysis mixta*, and *M. relicta*) were reported to feed on *C. pengoi* (Gorokhova 2006, Gorokhova and Lehtiniemi 2007). This variety of predators indicates that *C. pengoi* is well integrated into the Baltic Sea pelagic food web. However, whether this actually contributes positively to the overall availability of prey for e.g. fish is dependent on whether *C. pengoi* competes with fish for the same prey or not.

*C. pengoi* as a predator (paper V)

As *C. pengoi* is zooplanktivorous, it is important to know its diet and prey preferences, in order to evaluate its effects on the feeding conditions of zooplanktivorous fish.

In the eastern Gulf of Finland, the impact of *C. pengoi* predation on the zooplankton community has been evaluated and monitored (Telesh et al. 2001, Litvinchuk and Telesh 2006). Their results, suggest that since the introduction of *C. pengoi* to the area, its predation pressure on other zooplankton such as herbivores and suspension feeders has increased exponentially. However, the data set used in these studies is inadequate with respect to sampling frequency, and therefore modeling output is questionable. In another study performed in the Gulf of Finland, correlative statistics on field data suggested that predation by *C. pengoi* may affect seasonal dynamics and/or spatial distributions of native cladocerans and rotifers (Pöllumäe and Kotta
In particular, *Bosmina maritima* tended to stay below the thermocline when *C. pengoi* was abundant in surface waters. This was suggested by the authors to be a predator avoidance response triggered by direct predation of *C. pengoi* on *B. maritima*. Moreover, the authors suggested that a positive correlation observed between abundances of *B. maritima* and *C. pengoi* may indicate a beneficial effect of *C. pengoi* on *B. maritima*. This was proposed to be a result of *C. pengoi* preying on other species that compete with *B. maritima*, such as *Pleopsis polyphemoides* and *Eudne nordmanni* (as indicated by their negative correlations with *C. pengoi*). Moreover, in the Gulf of Riga, the abundance and phenology of *B. maritima* has changed after the introduction of *C. pengoi* (Ojaveer et al. 2004). The *B. maritima* population has decreased and disappears from the zooplankton community several weeks earlier than before the invasion (Ojaveer et al. 2004). A shift in the phenology was also apparent in the copepod *E. affinis* who started to develop about three weeks earlier following the introduction of *C. pengoi* (Ojaveer et al. 2004). Experimentally determined feeding rates on *E. affinis* support the proposed negative impact of *C. pengoi* on the *E. affinis* population (Lehtiniemi and Gorokhova 2008). As indicated by the studies above, most research on *C. pengoi* feeding in the Baltic Sea pelagic food web has employed correlative statistics on field data. When interpreting correlative statistics it is important to remember that it only verifies co-varying variables rather than expresses a dependency relationship. As there is a risk that two variables, although independent of each other, co-vary as a result of another/or several other external forces, the credibility of correlations to investigate dependency relationships is fairly weak. Nevertheless, these studies suggest plausible interactions and indicate what kinds of data are needed to verify these interactions and increase our understanding of the impact of *C. pengoi* in the Baltic Sea pelagic food web.

Stable isotope compositions can be used to assign trophic positions in food webs, thus contributing to understanding energy flows and ecological relationships. This approach employs relative concentrations of C and N isotopes in organisms to assess dietary connections. The ratio between different isotopes of carbon and nitrogen differs between organisms and their diets because of a slight selective retention of the heavy isotope and excretion of the lighter isotope. As a result, organisms tend to become enriched in heavy isotopes as compared with their food (Owens 1987). In the Baltic Sea, this approach was used to evaluate food web interactions after the introduction of *C. pengoi* (Gorokhova et al. 2005). It was concluded that mesozooplankton (mainly copepodites) where the main prey of larger *C. pengoi* while smaller *C. pengoi* fed equally much on microzooplankton (mainly rotifers and nauplii) and mesozooplankton.
In paper V, we further investigated prey preferences of *Cercopagis pengoi* during June–August using stable isotope analysis. The study was carried out in a coastal area of the northern Baltic proper (Himmerfjärdens Bay). Diet composition of *C. pengoi* was determined from stable isotope signatures of the predator and possible prey organisms, using two approaches: IsoSource mixing models (Phillips and Gregg 2003) and temporal tracking of prey (Melville and Connolly 2003). In parallel, zooplankton composition and abundance were analyzed to estimate selectivity for different zooplankton prey. On a seasonal basis, the main contributors to *C. pengoi* diet were copepods *Acartia spp.* and *E. affinis*, while importance of cladocerans and microzooplankton was less apparent. *C. pengoi* behaves, overall, as an opportunistic generalist predator, with a nutrition substantially overlapping that of commercially important zooplanktivorous fish.

The overall impact of cladoceran NIS in the Baltic Sea pelagic food web

Earlier analysis on stable isotopes, conducted in the same area as our study (paper V), suggested that *C. pengoi* feed, to a large extent, on copepods (i.e., the same food items as YOY herring; Gorokhova et al. 2005). We found that copepods indeed dominate in the diet of *C. pengoi* (paper V). Therefore, as not only YOY herring but also other zooplanktivorous fish (e.g. sprat) at different age feed intensely on copepods (e.g., Arrhenius 1996, Voss et al. 2003); this indicates a potential food competition between *C. pengoi* and fish. However, *C. pengoi* appears to select positively for podonids and also occasionally for microzooplankton, i.e. prey which is less preferred by fish. Thus, during periods of low copepod abundance *C. pengoi* may provide an energy link between lower trophic levels and fish.

It is clear that *C. pengoi* is well incorporated into the Baltic Sea pelagic food web, where it acts as both an opportunistic predator (paper V) and a prey for fish (paper IV) and mysids (Gorokhova 2006, Gorokhova and Lehtiniemi 2007), with many complex interactions (Fig. 4). However, the overall effect of *C. pengoi* on fish feeding conditions is still poorly understood. By integrating these results on fish (paper IV) and *C. pengoi* (paper V) diets with field data on *C. pengoi*, fish and zooplankton abundances collected in concert, the strength and outcome of the interactions between these groups could be addressed in trophic interactions models. Nevertheless, it is quite clear that *C. pengoi* alters the quantity and composition of zooplankton available for zooplanktivores. It has, however, been suggested that since *C. pengoi*, in the Baltic Sea, is only temporarily present in the water column (from mid- to late summer) its overall impact will be limited to this period (Orlova et al. 2006). Although this in principle might be true, it is important to acknowledge that this short-term impact during summer may leave traces in the food web, which
may be significant all year round. This may be the case if the presence of *C. pengoi* alters the feeding condition for fish and/or impacts the phenology of zooplankton, as suggested for *B. maritima* (Ojaveer et al. 2004).

![Figure 4. Pelagic food web interactions involving *C. pengoi* in the Baltic Sea. This schematic food web is not complete and created mainly to visualize the findings of this Thesis. Arrows represent confirmed interactions, and the number refers to the source of information: 1: Rivier 1998, 2: Gorokhova and Lehtiniemi 2007, 3: Arrhenius 1996, 4: Barz and Hirche 2009, 5: Vihervuoto and Viitasalo 2001. The Roman numerals represent papers in this Thesis.](image)

The impact of the other newly introduced cladoceran species in the Baltic Sea, *C. m. maeoticus* and *E. anonyx* remains to be investigated to see if these species will affect the food web in similar ways, or if they will have other impacts. Moreover, the unexpected (due to its lack of invasion history and previously described salinity tolerances) successful establishment of *E. anonyx* into the Baltic Sea may indicate that other common Ponto-Caspian onychopods (e.g. *Podonevadne trigona, Podonevadne camptonyx, Podonevadne angusta, Polyphemus exiguous* and *Evadne prolongata*) may exert an additional invasion threat towards the Baltic Sea (Panov et al. 2007). Furthermore, due to prevailing climatic change, the establishment and population abundance development of these species, originating from warmer climate, may be reinforced in the future.
CONCLUSIVE REMARKS AND FUTURE PERSPECTIVES

In the first two papers (I, II), we found that RNA content is a suitable biomarker for assessing growth (expressed as EPR) in adult *Acartia bifilosa* females. The proposed predictive model(s) for EPR assessment (I) could, therefore, as is, easily be implemented in monitoring programs to assess zooplankton production, i.e. food availability for zooplanktivores such as fish. However, to receive a more complete picture of zooplankton availability for zooplanktivores, similar models should be developed for other important zooplankton species and expanded to include juvenile stages. Moreover, in order to use the model on a larger geographical scale (i.e., the entire Baltic Sea), the effect of salinity on the EPR–RNA relationship needs to be tested and accounted for. This would greatly improve the usefulness of the model because it would allow for productivity comparisons between different sub-areas of the Baltic Sea. This information will help us understand variations in productivity and recruitment success between different sub-populations of Baltic fish.

In paper III, we found that high food intake in copepods may lead to decreased astaxanthin content. There may, however, be other factors, such as temperature, salinity, light, and presence of algal or man-made toxins, which may further modulate astaxanthin content in zooplankton. How, and to what extent, these factors may affect astaxanthin content in copepods needs to be further explored. Moreover, to fully understand factors affecting the quality of zooplankton as prey, the proposed studies should also focus on other essential substances in a broader spectrum of species.

Papers dealing with quantitative (I, II) and qualitative (III) aspects of copepod production emphasize the importance of food quality and food quantity available for the copepods. In the Baltic Sea, under conditions prevailing during a spring bloom (i.e., high food concentrations and dominance of diatom species), the proposed EPR–RNA model(s) for predicting *A. bifilosa* recruitment (paper I) may be less reliable, due to the high food concentration (paper II) and the possible effects of diatoms on egg viability (Ianora et al. 2003). Moreover, at high algal concentrations, a decreased quality of the copepods in terms of astaxanthin concentration might be possible (paper III). It is necessary to validate these findings in field as it will impact the feeding conditions for zooplanktivorous fish.

*Cercopagis pengoi* is well incorporated into the Baltic Sea pelagic food web, acting as both an opportunistic predator (paper V) and a prey for zooplanktivorous fish (paper IV). Although the Baltic Sea holds a relatively small number of species,
which reduces the number of possible interactions, the trophic linkages of *C. pengoi* are numerous (Fig. 4) and rather complex. Therefore, the overall effects of the *C. pengoi* introduction on fish feeding conditions are likely to vary both seasonally and regionally, depending on the food web structure. To evaluate these effects, long term data for zooplankton, *C. pengoi*, and fish abundance/biomass that cover periods before and after invasion should be integrated with the diet data (Holmborn *et al.*, in prep.).

In previous studies, correlation statistics on field data has been used extensively to investigate food web interactions involving *C. pengoi*. We used fish stomach analysis (paper IV) and stable isotope approach (paper V) to further understand these interactions. Although IsoSource mixing models, especially combined with temporal tracking of prey (as used in paper V), using stable isotope data provide stronger proof of actual interactions between species in food webs than do correlation studies, there are still some uncertainties with these methods. Therefore, stomach analysis of *C. pengoi* using genetic markers of potential prey may be an additional and even more reliable way to define and quantify these interactions.

The findings of this Thesis contribute to increase our understanding of processes important for assessment and evaluation of fish feeding conditions and trophic links between zooplankton and fish. This knowledge is crucial to make informed ecosystem based management decisions concerning the Baltic Sea fisheries; an approach that hopefully will improve and secure future harvest of Baltic sea-food. Moreover, the knowledge gained in this Thesis will also help us identify and understand future impact on the ecosystem caused by e.g. prevailing climate change, eutrophication and introductions of non-indigenous species.
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