Chapter 5

Effect of food quality on the growth of the brackish water mysid *Neomysis integer* (Crustacea: Mysidacea)

ABSTRACT

The growth of the brackish water mysid *Neomysis integer* can be described in three alternative ways: (1) by the increase in standard length (SL), (2) by the intermoult period (IMP) and growth factor (GF), or (3) by the intermoult growth rate (GR). Individual variation of these growth parameters in growth experiments is small. These endpoints can thus be used to evaluate the effects of environmental variables, food quality and quantity, and toxic substances on the growth of the mysid species.

The present study evaluates to what extent diets of *Artemia salina* (nauplii), *Eurytemora affinis* (copepodites and adults), laboratory-made estuarine flocs and macrophytal detritus (*Scirpus maritimus* and *Spartina anglica*), all administered *ad libitum*, influence the survival and somatic growth of subadult *Neomysis integer*.

Detritus originating from non-leached *Spartina anglica* was toxic to *Neomysis integer*, leading to morphologic aberrations and a high mortality. The growth of *N. integer* individuals was slower on a diet of *Scirpus maritimus* detritus than on a diet of animal food items or laboratory-made flocs. *Artemia* nauplii were the highest quality food for *N. integer*: a relatively smaller IMP and higher GF and GR resulted in a significantly higher SL at the end of this experiment. When fed with laboratory-made flocs, *N. integer* moulted as frequently as when fed *Artemia*, but GR decreased over the course of the experiment. A *Eurytemora affinis* diet resulted in a significantly elongated IMP from the first moult onwards as compared to mysids fed *Artemia* or flocs. The mean associated growth rate however, was comparable with the flocs treatment and significantly lower than fed *Artemia*.

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INTRODUCTION

*Neomysis integer* is a typical inhabitant of the oligohaline, turbid reaches of European estuaries (Mees et al., 1995). It plays a key role in the local food web as food for fish and macrocrustaceans (e.g. Hostens and Mees, 1999). In this part of the estuary, the food web is based on detritus, and heterotrophic processes dominate (Hamerlynck et al., 1993; Soetaert and Herman, 1995b; Muylaert et al., 2000b). Due to a shallow euphotic zone in the highly turbid water column, the local primary production is limited, despite the high nutrient concentrations (Hummel et al., 1988; Relexans et al., 1988). Except for a dense diatom bloom in summer (Muylaert et al., 1997), autotrophic phytoplankton is not considered to play a substantial role in the food web in this part of the estuary (Bernát et al., 1994; Irigoien and Castel, 1997). Still the upper estuary is highly productive and characterised by high abundances of zooplankton, hyperbenthos, epibenthic macrocrustaceans and fish (Hummel et al., 1988; Soetaert and Van Rijswijk, 1993; Hamerlynck et al., 1993; Mees et al., 1995; Hostens and Mees, 1999; Maes et al., 2003).

Suspended sediment and particulate organic matter flocculate in the presence of specific dissolved organic compounds (e.g. polysaccharides, humic acids and fluvic acids) to estuarine aggregates (Eisma et al., 1991). Due to the estuarine circulation, these aggregates accumulate at the head of the estuary (at the freshwater-brackish water interface), and form a so called maximum turbidity zone (MTZ) or estuarine turbidity maximum (ETM) (Eisma, 1986). Biota can also contribute to the turbidity in this zone through active re-suspension of sediments or increased sediment erosion due to burrowing and feeding behaviour (Widdows and Brinsley, 2002; Roast et al., 2004). Because of the entrapment and associated increased residence time in the MTZ (Siegfried et al., 1979; David et al., 2005), the reactive organic detritus (in suspension or aggregated in the flocs) is quickly remineralised by bacterial activity and/or is directly grazed upon by higher trophic levels (Heinle and Flemer, 1975; Hummel et al., 1988; Fockedey et al., submitted c – Chapter 4). As a result, little material is exported to the downstream reaches of the estuary and the coastal zone and it consists of a large refractory fraction (Soetaert and Herman, 1995b).

The brackish water mysid *Neomysis integer* is known to be omnivorous. Populations living in the MTZ of Western European estuaries predominantly feed on the calanoid copepod *Eurytemora affinis*, estuarine aggregates and macrophytal detritus (Fockedey and Mees, 1999 – Chapter 2). Also, aggregations of sediment collected at the substratum surface have been demonstrated to be a relevant food item in the shallow estuarine areas (Roast et al., 2000b). In laboratory experiments, *Eurytemora affinis* is an adequate prey item to fulfil the species’ energy requirements (Irvine et al., 1993; Aaser et al., 1995; Winkler and Greve, 2004), but the impact of this mono-specific diet on growth and reproduction of *N. integer* is not known. Macrophytal detritus, imported from the fluvial part of the estuary and/or from the local tidal marshes, is also hypothesized to be an important food source in the oligohaline food web, especially in periods when mesozooplankton is scarce (Sorbe, 1980; Irvine et al., 1993; Fockedey, unpublished). The nutritional and energetic value of this material for *N. integer* is still uncertain, but the species possibly represents a trophic link between salt marsh macrophyte production and higher trophic levels (Zagursky and Feller, 1985; Cattrijsse et al., 1994). Estuarine macro-aggregates seemed to be an adequate food item for *N. integer* as demonstrated by laboratory growth experiments (Fockedey et al., submitted c – Chapter 4).
Studies with euphausiids, gammarid amphipods and copepods demonstrate that the growth and reproduction rates are determined by environmental variables (mainly temperature and salinity), food quantity, food quality and genotype (e.g. Willoughby and Sutcliffe, 1976; Heinele et al., 1977; Koski et al., 1998; Haywood and Burns, 2003). The relationship between an animal’s production and the food concentration in the field is often obscured by variations in the food quality (Koski et al., 1998). Until now, few studies have examined the survival and growth rates of mysids in relation to food quality (Lehtiniemi et al., 2002) and little information is available on the impact of food quality on their reproduction (Domingues et al., 2002). For Neomysis integer, only Ferguson (1973) performed experiments comparing growth efficiencies of selected size classes of mysids feeding them an animal diet and two detrital diets.

In the present study it was evaluated to what extent diets of Eurytemora affinis (copepodites and adults) and macrophytal detritus, administered ad libitum, are able to support the survival and somatic growth of subadult Neomysis integer. The growth is described by the increase in standard length over time, and by the intermoult period, the growth factor and the intermoult growth rate (Fockedey et al., in press – Chapter 3). The obtained values of the growth parameters are compared with results from (simultaneously performed) experiments on N. integer growth performance when feeding on Artemia salina nauplii and laboratory-generated estuarine aggregates (Fockedey et al., submitted c – Chapter 4). In addition, a starvation experiment was conducted to observe the effect on the survival and the growth parameters of N. integer when deprived from food.

MATERIAL AND METHODS

Neomysis integer

Specimens of Neomysis integer were collected with a handnet (opening: 29.0 x 18.5 cm; mesh size of 1 x 1 mm) in the brackish water pond Galgenweel (4 psu; 5 °C) situated at the left bank of the Schelde estuary. Short hauls were taken using a handnet. The animals were transported to the laboratory in environmental water within 2 hours, where they were gradually adapted to a water temperature of 15 °C (over 2 days) under continuous aeration. Before the start of the experiments they were kept for 2 days in artificial seawater (Instant Ocean®, Aquarium Systems, France) of 5 psu and given the food that they would be exposed to in the growth experiments (see later).

The standard length of Neomysis integer individuals was determined as the distance from the tip of the rostrum to the end of the last abdominal segment measured laterally on individuals using a drawing mirror mounted on a stereomicroscope. For each treatment, 15 individuals (10 in the case of estuarine flocs) with a standard length between 4 and 6 mm were selected. During the experiments the animals were kept individually in 400 ml glass jars with 350 ml aerated artificial seawater of 5 psu, except for the experiments with estuarine aggregates where the rolling experimental containers have a volume of 845 ml. Fockedey et al. (submitted c – Chapter 4) demonstrated that the growth performance of individual N. integer was comparable in the two experimental setups. All experiments were performed in a climate-controlled room at 15 °C with a light regime of 12h light:12h dark. Each day the mysids were gently transferred to a new jar using a conical measuring spoon and they were offered daily fresh food ad libitum.
Table 1: Food items and ration administered to subadult Neomysis integer individuals.

<table>
<thead>
<tr>
<th>Food type</th>
<th>Food concentration (l⁻¹)</th>
<th>Administered daily mysid†</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DW (mg)</td>
<td>C (mg)</td>
<td></td>
</tr>
<tr>
<td>Artemia nauplii (&lt;24h)</td>
<td>5700</td>
<td>2.4 †</td>
<td>1*</td>
</tr>
<tr>
<td>Eurytemora affinis (copepodes + adults; &gt; 250μm)</td>
<td>143</td>
<td>2.1</td>
<td>1 **</td>
</tr>
<tr>
<td>Macrophytal detritus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scirpus maritimus (&lt;400μm) – fresh</td>
<td>70 mg DW</td>
<td>25</td>
<td>29 ***</td>
</tr>
<tr>
<td>Spartina anglica (&lt;400μm) – fresh</td>
<td>115 mg DW</td>
<td>40</td>
<td>48 °</td>
</tr>
<tr>
<td>Laboratory-made estuarine aggregates *</td>
<td>16000 ± 2000</td>
<td>30 – 175</td>
<td>2 – 11 ***</td>
</tr>
</tbody>
</table>

†: conversion factor according to Paffenhöfer (1967); * conversion according to Evjemo and Olsen (1999); ** conversion according to Parsons et al. (1984); *** conversions according to Fockedey et al., submitted d – Appendix 2; ° conversion according to De Mesel (personal communication)

**Food quality**

To study the effect of the food quality on the growth of Neomysis integer, 5 different food items were supplied at an excess concentration (Table 1): *Artemia salina* nauplii, adults and copepodite stages of the calanoid copepod *Eurytemora affinis*, laboratory-made estuarine flocs, and detritus of two species of estuarine macrophytes (*Spartina anglica* and *Scirpus maritimus*). An additional treatment was set up where *N. integer* was starved (no food added). Except for the *Artemia* nauplii, the food items chosen are relevant in the diet of *N. integer*, as demonstrated by Fockedey and Mees (1999 – Chapter 2) by stomach content analyses and by Gorokhova and Hansson (1999) by isotopic composition analysis. No attempt was made to estimate feeding rates on the different food items. The food was offered *ad libitum* and the growth performance (see later) was monitored for 32 days (4.5 weeks).

*Artemia salina* cysts (San Fransisco Bay) were hatched daily in a 2 litre conical glass container (25 psu; 28 – 32 °C) under continuous aeration. Less than 36 hours old *Artemia* nauplii (ART) were supplied to the mysids in a concentration of 2000 ± 200 *Artemia* ind⁻¹ d⁻¹ (± 2.4 mg DW). Adult and large copepodite stages of *Eurytemora affinis* (Copepoda, Calanoidea) were collected in the pond Galgenweel by filtering surface water through a 250 μm sieve. The copepods were transferred to the laboratory in environmental water and kept in the laboratory without additional feeding for maximally 3 days. We assumed that the environmental water contained enough phytoplankton to keep the copepods in optimal condition in this period. Every 3 days new copepods were sampled in the field. Daily, 50 copepods (EURY) were supplied to the mysids (± 2.1 mg DW). They were counted according to the spotting technique of Reeve (1970).

Macrophyte detritus was artificially made in the laboratory using two plant species that are abundant in the marshes of the brackish water zone of the Schelde. *Spartina anglica* (living plant) and *Scirpus maritimus* (died-off plant) were collected from the banks of the Schelde river at Doel. After washing off the sand and epiphytes, the plants were oven dried (60 °C) for 2 days and ground to 400 μm particles. Each detritus type was supplied daily as a constant volume corresponding to 40.1 ± 2.6 mg DW for *S. anglica* (SP-0) and 24.6 ± 2.4 mg DW of *S. maritimus* (SC-0).

The estuarine aggregates (FLOC) administered to *Neomysis integer* in the experiment were re-assembled in the laboratory, out of filtered (250 μm) water that was collected from the Schelde river at Antwerpen, by rotation on a roller table (for a detailed description see Fockedey et al., submitted c – Chapter 4). The mysids were kept individually in the rolling tanks (10 – 11 rpm). The water was changed daily. The floc formation process was in equilibrium after three to four hours.
Effect of food quality on growth

Measuring the growth performance

Survival and growth of the *Neomysis integer* individuals were followed as described by Fockedey *et al.* (in press – Chapter 3; submitted c – Chapter 4). The containers were checked daily at the start of the light period. Moults were collected and the length of the uropodal exopods (EXO) measured. Standard body length (SL) was calculated using the regression

\[ SL = 1.085566 + 4.081793 \text{ EXO} \quad (p < 0.0001; \ R^2 = 0.9569; N = 97). \]

The overall growth of *Neomysis integer* can be described by the von Bertalanffy growth curve (Fockedey *et al.*, in press – Chapter 3), but the growth of individuals of the size class 4 – 6 mm SL over a period of 4.5 weeks is linear. Thus, linear regression analysis was applied to the results. The slopes of the linear regression equations were tested between treatments using an ANCOVA and subsequent multiple comparison with a Tukey test (Zar, 1996). Growth was expressed as intermoult period (days), growth factor (%) and intermoult growth rate (mm d$^{-1}$) according to Fockey *et al.* (in press – Chapter 3). Differences between experimental treatments were tested at each consecutive moult event by using a Kruskal-Wallis test and multiple comparisons (Conover, 1980).

RESULTS

Survival

All *Neomysis integer* survived the experiment when feeding on *Eurytemora affinis* (Figure 1). Survival on *Artemia* nauplii, estuarine flocs and on *Scirpus* detritus ranged from 73 – 80 % at the end of the experiment. When feeding on *Spartina* detritus, and when administered no food (STARV), mortality was 100 % after 26 and 20 days respectively.

The animals fed *Spartina* detritus moulted 0 to 2 times during the experiments, but morphological deformations of the uropods and the presence of external spherical tumours could be observed. The muscles became white coloured and mysids had a copious growth of an epizoootic protozoan on their exoskeleton. Occasionally, the starved individuals moulted twice (13 %), but most of the individuals died after 15 days after moultmg only once (40 %) or without having moulted (46 %). The individuals surviving the 32 day time span of the experiment in the other treatments moulted 3 – 5 times when feeding on *Scirpus* detritus, 4 – 5 times on *Artemia* or *Eurytemora*, and 5 – 6 times on laboratory-made flocs.

Figure 1: Survival functions of all treatments over the course of the experiment (ART: *Artemia salina* nauplii; EURY: *Eurytemora affinis*; FLOC: laboratory-made estuarine flocs; SC-0: *Scirpus maritimus* detritus; SP-0: fresh *Spartina anglica* detritus; STARV: starved).
Chapter 5

Table 2: Linear regression analyses performed with all measurement points within each treatment.

<table>
<thead>
<tr>
<th>Food type</th>
<th>Regression</th>
<th>#moults</th>
<th>R²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artemia nauplii</td>
<td>SL = 4.8127 + 0.1162 day</td>
<td>61</td>
<td>0.87</td>
<td>p &lt; 0.00001</td>
</tr>
<tr>
<td>Eurytemora</td>
<td>SL = 4.9035 + 0.0615 day</td>
<td>52</td>
<td>0.76</td>
<td>p &lt; 0.000001</td>
</tr>
<tr>
<td>Laboratory-made flocs</td>
<td>FLOC: SL = 4.4778 + 0.0623 day</td>
<td>36</td>
<td>0.79</td>
<td>p &lt; 0.00001</td>
</tr>
<tr>
<td>Scirpus detritus</td>
<td>SC-0: SL = 5.2472 + 0.0216 day</td>
<td>41</td>
<td>0.19</td>
<td>p &lt; 0.00473</td>
</tr>
<tr>
<td>Spartina detritus</td>
<td>SP-0: SL = 5.7156 + 0.0224 day</td>
<td>31</td>
<td>0.18</td>
<td>p &lt; 0.01627</td>
</tr>
<tr>
<td>Starvation</td>
<td>STARV</td>
<td>17</td>
<td>0.08</td>
<td>p &lt; 0.28618</td>
</tr>
</tbody>
</table>

Increase in SL over time

All individuals within one treatment grew linearly and a linear regression analysis was applied to all data points for each treatment (Figure 2; Table 2). A significant regression could be fitted to all treatments, except for STARV (p < 0.05). The regressions for Neomysis integer feeding on both macrophyte detritus types gave a relatively low R² value (0.18 – 0.40). In the other three regression equations, the R² ranged between 0.76 and 0.87. The data on the growth of N. integer feeding on Spartina detritus and when starved are not considered further.

The slopes of the 4 remaining significant linear regression equations were significantly different (p < 0.001). The Fisher post-hoc test indicated that the mean length of Neomysis integer during the experiment was significantly higher on a diet of ART than respectively EURY, FLOC and SC-0 (all pairwise comparisons with p < 0.001). The mean length in the EURY treatment was also significantly higher than on FLOC and SC-0 (all pairwise comparisons with p < 0.001), while the latter two did not significantly differ.

Figure 2: Linear regressions representing the growth of Neomysis integer in the six treatments over the course of the experiment (ART: Artemia salina nauplii; EURY: Eurytemora affinis; FLOC: laboratory-made estuarine flocs; SC-0: Scirpus maritimus detritus; SP-0: fresh Spartina anglica detritus; STARV: starved).
Effect of food quality on growth

Overall IMP, GF and GR

*Neomysis integer* moulted most frequently (Figure 3) when feeding on ART and FLOCS (mean IMP of 6.07 ± 0.10 days). The longest IMP was measured when feeding on EURY, both types of macrophyte detritus and when starved (7.71 ± 0.17 days). The mean GF was highest when given an animal diet or estuarine flocs (9.97 ± 0.42 %) and was significantly smaller when feeding on macrophyte detritus or when being starved (5.06 ± 0.60 %). When expressed as mean length increase per day within an intermoult period, the growth was highest in ART (0.106 ± 0.006 mm d⁻¹), followed by EURY and FLOC (0.070 ± 0.005 mm d⁻¹), and SC-0, SP-0 and STARV (0.031 ± 0.004 mm d⁻¹).

**Figure 3**: Mean (a) intermoult period IMP, (b) growth factor GF and (c) growth rate GR of all treatments. Different letters indicate statistically significant different groups (multiple comparison p<0.05).
Figure 4: (a) Pre-moult standard length (SL) at each moult event (1 – 5), (b) intermoult period (IMP), (c) growth factor (GF), and (d) intermoult growth rate (GR) in between successive moults in the treatments ART, EURY, FLOC and SC-0. Different letters indicate significant differences (multiple comparison p<0.05).
Effect of food quality on growth

SL at each moult event

Within a period of 32 days, subadult *Neomysis integer* grew on average 2.79 ± 0.13, 1.90 ± 0.24, 1.44 ± 0.15 and 0.65 ± 0.01 mm when feeding on *Artemia* nauplii, *Eurytemora* flocs and *Scirpus* detritus, respectively. This means a relative length increase of 51.7 ± 2.7, 38.0 ± 5.7, 30.4 ± 3.2 and 13.2 ± 0.5 %, respectively. The mean initial standard length of the individuals (Figure 4a) selected for the FLOC treatment was biased and significantly smaller (4.48 ± 0.13 mm) than for the other treatments (5.35 ± 0.05 mm).

Significant differences in SL (Figure 4a; p < 0.05) between the different diets were observed from the 3rd moult onwards, when the standard length of ART fed *Neomysis integer* became significantly larger compared to all other treatments. From the 4th moult onwards, a significant gap between the individuals feeding on animals prey (ART and EURY) and individuals feeding on FLOC and macrophyte detritus (SC-0) became apparent. At the fifth moult, a significantly lower SL was observed in the SC-0 treatment in comparison to FLOC as well.

IMP at each moult event

*Neomysis integer* moulted most frequently (Figure 4b) in the treatments ART (6.2 ± 0.2 days) and FLOC (5.9 ± 0.1 days); while the mean IMP was subsequently longer in EURY (7.0 ± 0.1 days) and SC-0 (7.8 ± 0.2 days). The intermoult period generally increased during the course of the experiment in ART (+34 %), FLOC (+18 %), and SC-0 (+24 %), but it remained more or less constant in EURY (+2 %). From the first IMP onwards, the frequency in mouling is significantly different (p < 0.05) between the diets although these differences disappeared after the 4th moult.

GF at each moult event

Over the course of the experiment, the GF (Figure 4c) increased in EURY and SC-0 by 35 % and 65 %, respectively. In the ART treatment, the GF increased over the first 3 moults (+71 %) and then decreased again (-44 %). In *Neomysis integer* feeding on FLOC, the GF generally decreased by 31 % according to the initial GF.

From the first moult onwards, the GF was different between ART, EURY and FLOC on the one hand and in SC-0 on the other hand. After the second moult, the GF of ART-fed mysids increased to 13.38 %, while the other 2 groups remained at a value similar to the first moult (respectively 9.33 and 3.67 % for ART – FLOC and SC-0). After the 3rd moult, the GF of all treatments became more and more alike.

GR at each moult event

Over the course of the experiment, the mean growth rate during each intermoult period (Figure 4d) generally increased in ART (from 0.078 to 0.130 mm d⁻¹), EURY (from 0.056 to 0.091 mm d⁻¹) and SC-0 (from 0.023 to 0.033 mm d⁻¹). Only in the FLOC treatment the GR decreased over time (from 0.095 mm d⁻¹ to 0.064 mm d⁻¹), mainly due to the short IMP in this treatment.

From the first moult onwards the GR differed between ART, EURY and FLOC on the one hand and a lower valued GR in SC-0 on the other hand. After the 3rd moult the GR was significantly larger in ART and significantly lower in SC-0 than EURY or FLOC. After the 4th moult the GR on an animal diet was significantly higher than on the detrital diets.
DISCUSSION

Starvation

The capacity to withstand starvation in *Neomysis integer* is dependent on salinity, temperature and size (Vlasblom and Elgershuizen, 1977; Winkler, 2000). At 9 °C, juvenile and subadult *Neomysis integer* survived starvation for maximally 7 weeks (Gorokhova and Hansson, 1999). Armitage et al. (1978) starved *N. integer* on kaolin and obtained a mortality of 60 % after 6 to 8 days (8 psu at 15 °C). In the present experiment, the subadult *N. integer* survived starvation for maximally 20 days at 15 °C (5 psu), with 50 % mortality occurring after 14 days. In experiments performed under identical conditions as the ones presented here, subadult *N. integer* could survive for at least 4.5 weeks when fed only 10 *Artemia* nauplii per day (Fockedey, unpublished).

The mysids produced faecal pellets until the last day of their survival. The water was renewed daily, so animals were prevented from eating their cast exuvia and faecal pellets. However, *Neomysis integer* has been described to feed directly on its own faecal string (Molloy, 1958; Ferguson, 1973; Parker and West, 1979) and this could have provided a source of energy for some time. Faecal pellets derived from organically rich food sources still have a carbon content of 40 – 60 % (Ferguson, 1973).

Starvation in *Neomysis integer* causes a decrease in dry weight and a reduction in total body carbon (Gorokhova and Hansson, 1999), indicating a substantial loss of organic reserves. The actual amount of sugars in *N. integer* is small, and allows the mysid to live for only a few hours (Raymont et al., 1968). The low amount of lipid reserves of *N. integer* is enough to maintain respiration rates and activity levels for at least up to 48 h (Weisse and Rudstam, 1989). When starved for a longer period, the species can deaminate body proteins (Raymont et al., 1968). In their natural estuarine habitat, *N. integer* probably never have to overcome periods of food shortage, as the omnivorous mysids can easily take advantage of a wide range of food items in the estuary (Winkler, 2000; Fockedey and Mees, 1999 – Chapter 2).

Excess food concentration

The *Neomysis integer* individuals were offered food *ad libitum*, so the mysids were assumed to feed at a maximum feeding rate in all treatments. Food quality, as measured in the present experiment by the ability of *N. integer* to grow and moult on a certain food item, actually reflect the ability of *N. integer* to assimilate that food item and thus, its nutritional value to the mysid.

The maximum feeding rate of subadult and adult *Neomysis integer* on *Artemia* nauplii, is respectively 200 and 600 – 800 nauplii d⁻¹ (Astthorsson, 1980; Fockedey, unpublished). Thus, *N. integer* was fed *Artemia* nauplii well in excess in the current experiment (2000 d⁻¹).

*Neomysis integer* is known to prey very efficiently on *Eurytemora affinis*. Laboratory experiments in relatively small beakers and with a copepod density an order of magnitude higher than in the field resulted in a maximal daily feeding rate of 20 – 40 *E. affinis* copepodes per mysid (Winkler and Greve, 2004). When fed *E. affinis* nauplii as well (Irvine et al., 1993; Aaser et al., 1995), predation rates were even higher (up to 55 nauplii and 44 copepodites). In the present experiment we did not aim to study predation rates, however, the remaining number of copepods was counted daily.
Over the course of the experiment only 18 % of the supplied copepods were consumed on average (6 – 37 %) and therefore assumed to be administered ad libitum. Since feeding rates were substantially smaller (3 – 18 mysid$^{-1}$ d$^{-1}$) than the ones reported in literature for subadults (20 – 40 mysid$^{-1}$ d$^{-1}$), it is possible that some food limitation occurred.

Each subadult Neomysis integer received 50 copepodite per 350 ml daily (143 l$^{-1}$). This food concentration is double the maximal density in the Schelde estuary (61 ind l$^{-1}$; Soetaert and Van Rijswijk, 1993), but smaller than natural E. affinis concentrations in the Elbe estuary (220 ind l$^{-1}$; Köpcke, 2002). During the day, no replacement of eaten prey was done and prey concentration decreased gradually over time. As N. integer does not attempt to hunt at low concentrations of calanoid copepod prey (Irvine et al., 1993), this could have had an impact on the ingestion rate and subsequently on growth.

The concentration of flocs produced in the roller tanks varied according to the neap-spring tide cycle with an average of 16000 ± 2000 aggregates l$^{-1}$ (Fockedey et al., submitted c – Chapter 4). Considering a feeding rate of ± 40 flocs h$^{-1}$ ind$^{-1}$ (Fockedey et al., submitted c) and a continuous feeding intensity over 24 hours, the maximal daily ration of Neomysis integer on flocs approximates 1000 flocs. Hence, the concentration in the roller tanks provided ample food to the mysids.

In the present experiments, the mysids survived and grew when feeding on detritus from Scirpus maritimus. The macrophytal detritus was administered well in excess (25 mg DW ind$^{-1}$ d$^{-1}$) and a large fraction of the ration was left each day. Generally, detritivory is associated with low assimilation and is be partly compensated by a high ingestion rate (Marchant and Hynes, 1981; Zagursky and Feller, 1985).

**Growth performance**

Although survival was comparable, the growth of subadult Neomysis integer individuals was slower on a diet of macrophytal detritus (Scirpus maritimus) than when feeding animal food or laboratory-made flocs. This difference reflects the poor nutritional value of the macrophytal detritus. Artemia nauplii were the best quality food for subadult N. integer, as a combination of relatively small IMP and relatively high GF and GR gave rise to a significantly higher standard length at the end of the experiment. When fed laboratory-made flocs, N. integer moulted as frequently as in Artemia-fed mysids, but GR decreased relatively over the course of the experiment. A Eurytemora affinis diet resulted in a significantly elongated IMP from the first moult onwards in comparison with mysids fed Artemia or flocs, and was associated with a significantly lower growth rate in comparison with the Artemia treatment.

The individual variation on the growth parameters (SL, IMP, GF, and GR) was small, thus making it easy to distinguish the effects of food quality (Fockedey et al., submitted c – Chapter 4; present study), food quantity (Fockedey, unpublished) and toxicological effects (Ghekiere et al., submitted) on these endpoints. IMP, GR and GF were affected by the food quality from the first moult onwards. In the present experiment, the IMP increased significantly and hence growth rate (GR, GF) decreased significantly with decreasing food quality, as demonstrated for gammarid amphipods (Willoughby and Sutcliffe, 1976; Delong et al., 1993; Pöckl, 1995). At 15 °C and 5 psu (conditions of the present experiment), Neomysis integer feeding ad libitum on Artemia nauplii needs 13 moults to become mature (Fockedey et al., in press – Chapter 3). Any increase in the IMP due to poor food quality, will result in a considerable prolongation of the maturation time at this temperature and salinity. A decreased growth rate at mouling, associated with the consumption of low quality food, resulted in a
smaller size at the end of the experiment and probably a smaller size-at-maturity associated with a lower fecundity.

Field relevance

The intertidal salt marshes on the margins of the Schelde estuary are massively occupied by *Neomysis integer* at flood during each tidal cycle (e.g. Mees et al., 1993a; Cattrijsse et al., 1994; Hampel et al., 2003; 2003b). The areas are favoured by the estuarine mysids for reproductive purposes (Cattrijsse et al., 1994; Mees et al., 1993a), even though residence in the marsh is coupled to a high predation pressure by fish and shrimp (Cattrijsse et al., 1994; Dean et al., 2005). The large amounts of macrophytal detritus available inside the marsh creeks are also assumed to attract the mysids (Zagursky and Feller, 1985; Cattrijsse et al., 1994).

*Spartina anglica* and *Scirpus maritimus* are abundant vascular plants in the brackish marshes of the Schelde estuary (Beeflink, 1977; Adam, 1990). Gut contents of animals living in marshes often include large quantities of vascular plant detritus, although there is little evidence that a strict diet of this material can sustain the populations of the high-order consumer-species like fish (Zagursky and Feller, 1985; Kneib, 1997). *Neomysis integer* could survive on a diet of *S. maritimus* detritus in the present experiment, although the somatic growth was low. The *S. anglica* detritus caused a high mortality and morphologic aberrations to *N. integer*. The latter detritus was made from living plants and the leaves were not leached before application. Some chemical constituents (e.g. polyphenols or tannins) released in the water by living macrophytes are toxic for *N. integer* (Lindén and Lehtiniemi, 2005).

Generally, the refractile macrophyte detritus can be digested by detritivore crustaceans through the presence of cellulolytic bacteria in the intestine (Plante et al., 1990) or the availability of specific enzymes in the gut. Cellulases have been identified in *Neomysis integer* (Molloy, 1958), but it is not known if the enzyme is produced by the mysids themselves or with the aid of an associated gut microflora (Foulds and Mann, 1978; Wainwright and Mann, 1982). Although microflora residing in the alimentary track of *N. integer* has been demonstrated (Bradshaw et al., 1989), specific cellulolytic bacteria have not yet been found for the species. Other authors (e.g. Hargrave, 1970) suggest that the main energy value of the macrophyte detritus for crustaceans lies in the associated bacteria and protozoans (trophic upgrading), while the detritus itself is egested unchanged. Observations of the faecal pellets produced when feeding on *Scirpus maritimus* detritus showed that the plant remains were digested themselves (Fockedey et al, submitted d – Addendum 2).

The detritus was supplied without aging. The extensive growth of fungi, bacteria and protozoans on decaying plant remains makes the resistant detritus acceptable to shredders by softening the leaves and by raising their protein content (Hargrave, 1970) and result in a higher growth performance in gammarid amphipods and copepods (Willoughby and Sutcliffe, 1976; Heinle et al., 1977). Preliminary results (Fockedey, unpublished), however, showed that a relatively short decay of this detritus for 3 – 5 days did not alter the growth rate of the mysid *Neomysis integer* in comparison with the unconditioned detritus.

In the subtidal reaches of the MTZ of the estuary, the availability of estuarine flocs is high in comparison with calanoid prey. *Neomysis integer* encounters at best one *Eurytemora affinis* copepodite or adult per 10 macro-flocs (Soetaert and Van Rijswijk, 1993; Zimmerman, 1997; Taocks et al., 2004). The escape reaction of the copepods makes them a less easily preyed item than flocs. The lower energetic value of the flocs is compensated for by a higher
consumption rate as demonstrated by egestion rates (Fokedey et al., submitted d – Addendum 2), but still resulted in a significantly lower growth rate in comparison with a diet of *E. affinis*.

Food selection experiments were not in the scope of the present study and nothing is known about the *in situ* preference of *N. integer*. Also we did not aim to perform growth experiments with mixed diets. It is possible that a combination of animal and plant food stimulates growth more than given one type of diet (Heinle et al., 1977; Roman, 1984). Some items may not be consumed massively, but do deliver essential nutrients like essential fatty acids and vitamins necessary for optimal growth, development and/or reproduction (Koski et al., 1998).

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