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Temperature and salinity effects on post-marsupial growth of *Neomysis integer* (Crustacea: Mysidacea)

Nancy Fockedey[a,*], Jan Mees[a,b], Marnix Vangheluwe[c,1], Tim Verslycke[d], Colin R. Janssen[c], Magda Vincx[a]

[a]*Ghent University, Biology Department, Marine Biology Section, Krijgslaan 281/S8, B9000 Gent, Belgium*
[b]*Flanders Marine Institute, Wandelaarskaai 7, B8400 Oostende, Belgium*
[c]*Ghent University, Laboratory of Environmental Toxicology and Aquatic Ecology, J. Plateaustraat 22, B9000 Gent, Belgium*
[d]*Woods Hole Oceanographic Institution, Biology Department, MS#32, Woods Hole, MA 02543, USA*

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Abstract

There has been an increasing interest in using the brackish water mysid *Neomysis integer* as a toxicological test species for Western European estuarine systems. In this respect, more data on growth, moulting and development in this species is needed. The influence of prevailing environmental variables (e.g. temperature, salinity) and age on these processes as well as their optimal range have to be known in order to develop optimal laboratory cultures and to differentiate between chemically induced variability and natural variability in toxicity testing. Individual post-marsupial growth (size, intermoult period, growth factor) was studied from first day neonates until adulthood at eight environmentally relevant temperature–salinity conditions. Three salinities (5, 15 and 30 psu) were tested at 15 and 20 °C, and two more extreme temperatures (8 and 25 °C) were tested at a salinity of 5 psu.

Survival and growth of *N. integer* were detected within the whole range tested, but sexual maturation was only possible in the narrower range of 15–25 °C and 5–15 psu. The size at maturity of *N. integer* increased with decreasing temperature and increasing salinity. Salinity seems to have a stronger effect than temperature on the duration of maturation. The sigmoid von Bertalanffy growth model was fitted to the individual and pooled data, except for the 8 °C experiment where growth was linear. Estimates from pooled data were comparable with individually based estimates, but generally underestimated the asymptotic length. Temperature was negatively correlated with the asymptotic length and positively correlated with the growth constant *K*. Higher temperatures caused smaller intermoult periods but had no effect on the growth increment, while salinity effects were less straightforward and dependent on the water temperature. A tool is provided to estimate the age, moult number, intermoult period, growth factor and growth rate from the body standard length of *N. integer*. Experimentally derived von Bertalanffy parameter estimates resulted in a

* Corresponding author. Tel.: +32 9 264 85 18; fax: +32 9 264 85 98.
E-mail address: Nancy.Fockedey@UGent.be (N. Fockedey).
[1] Current address: EURAS, Rijvisschestraat 118-Box 3, B9052 Zwijnaarde, Belgium.

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higher growth performance index compared with field-based estimates for the Schelde estuary and Galgenweel populations of *N. integer*.
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Keywords: Growth; Growth factor; Intermoult period; Maturation; Mysidacea; Sexual differentiation; von Bertalanffy growth model

1. Introduction

Recently, there has been increasing interest in using the brackish water mysid *Neomysis integer* (Leach, 1840) as a toxicological test species for estuarine systems (Roast et al., 1998a; Verslycke et al., 2004). It is an alternative to *Americamysis bahia* (formerly *Mysidopsis bahia*) for use in European water quality testing (Roast et al., 1998a; Verslycke et al., 2004). Due to its relatively high temperature requirements, the subtropical species *A. bahia* can not be used in temperate regions and its low tolerance for low salinities of 0.1 to 5 psu makes it an inappropriate test species to use in the turbid upper reaches of estuaries or oligohaline inland water bodies (Roast et al., 2000, 2001).

Ideally, chemical risk should be assessed by standardized endpoints that cover the molecular, individual and population level. For mysids, this implies that, in addition to evaluating mortality, acute, chronic and multigenerational bio-assays have to be developed for testing chemical effects on growth and moulting, reproduction, biochemical composition, metabolism and physiological processes, behaviour and morphologic aberrations (as reviewed by Verslycke et al., 2004). In this respect, baseline data on growth, development and reproduction, and more specifically on intermoult period, size increment at moulting, age/size at maturity, time to the first brood release, brood size, fecundity, etc., of mysids is invaluable. The influence of prevailing environmental variables (e.g. temperature, salinity, food quality and quantity) on these endpoints and their optimal range have to be known in order to develop optimal laboratory cultures and to differentiate between chemically induced variability and natural variability in toxicity testing (a.o. De Lisle and Roberts, 1988; Verslycke et al., 2004).

The estuarine environment is characterized by strong fluctuating conditions of salinity and temperature. Both are considered dominant 'ecological abiotic master factors', which may act either singly or in concert to modify the population dynamics and distribution of estuarine organisms (McKenney and Celestial, 1995). As a typical estuarine species, the brackish water mysid *N. integer* must be able to functionally adapt to this dynamic environment. The species is euryhaline and tolerates salinities of 1 to 40 psu (Vlasblom and Elgershuizen, 1977; Roast et al., 2001). In the field it can be found at salinities between 0.1 and 38 psu, although it is rare in waters of more than 18 psu (Tattersall and Tattersall, 1951; Vlasblom and Elgershuizen, 1977). The isosmotic point of *N. integer* is described to vary between populations from 16 psu to higher than 20 psu (Ralph, 1965; McLusky and Heard, 1971; Moffat, 1996). *N. integer* is highly tolerant to large, acute salinity fluctuations between 1 and 30 psu (Moffat and Jones, 1992; Roast et al., 1998b). In the Schelde estuary, *N. integer* was recorded at salinities ranging from 8 to 25 psu with a maximal abundance at around 15 psu (Mees et al., 1994). Ongoing studies suggest that the population is shifting towards the more oligohaline zone of the estuary as a consequence of improved oxygen conditions in the upstream reaches (Fockedey, personal communication). In other, more oxygenated Western European estuaries such as the Guadalquivir (Spain), Gironde (France), Elbe (Germany) and Ems (The Netherlands) the abundance peak is typically found around 5 psu (Mees et al., 1995; Drake et al., 2002; Fockedey, personal communication).

N. integer is a eurythermic species that occurs in brackish waters along the Western European coast at longitudes between 36°N and 68°N and in the Baltic Sea (Deprez et al., 2004; http://intramar.ugent.be/NeMys). Its temperature tolerance measured under laboratory conditions ranges from 0 to 30 °C (Arndt and Jansen, 1986; Mauchline, 1980). Within the range Elbe–Guadalquivir, the summer water temperature of the brackish estuarine zone varies from 25 °C in the North to 29 °C in the South, while winter water temperatures range from 1 °C in the North to 10 °C in the South (Drake et al., 2002, Zimmermann, 1997). Temperature is generally thought to overshadow sa-

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linity in its effects on growth and reproduction in crustaceans. Still, temperature can influence the salinity tolerance of a species (Vlasblom and Elgershuizen, 1977; Arndt and Jansen, 1986) and interaction effects of both on the survival and growth of a species can change with age (Kinne, 1955; McKenney, 1994; McKenney and Celestial, 1995).

Most studies on the population dynamics of *N. integer* are exclusively based on field data (see Mees et al., 1994 and the references herein). Generally, length frequency distributions are obtained through regular (once or twice per month) sampling of the population for at least 1 year. Cohorts can then be segregated by modal progression analysis, but this is often complicated by the occurrence of overlapping generations and prolonged reproductive periods (Astthorsson and Ralph, 1984; Mauchline, 1985; Irvine et al., 1995). Growth curves have been derived from field data for several populations of *N. integer*, e.g. Mauchline, 1977; Bremer and Vijverberg, 1982; Astthorsson and Ralph, 1984; Mauchline, 1985; Mees et al., 1994. To date, these growth parameters have rarely been validated with laboratory observations. Schrotenboer (1980), Astthorsson and Ralph (1984), Irvine et al. (1995) and Winkler and Greve (2002) all performed growth experiments with *N. integer*, but only at very few temperature–salinity combinations. Kuhlmann (1984) studied the short-term effects of 16 temperature–salinity combinations on the daily growth rate of juvenile *N. integer*, but post-juvenile growth, mortality, intermoult period and growth factor were not reported.

The general objectives of the present study are (1) to describe the growth of *N. integer* under laboratory conditions and (2) to investigate the effects of salinity and temperature on growth in *N. integer*. For this purpose, mysid growth (size, intermoult period, growth factor) was recorded over a whole life span in individually based experiments at 8 environmentally relevant temperature–salinity conditions.

2. Material and methods

2.1. Field sampling and stock cultures

N. integer was collected from the brackish pond Galgenweel (salinity ± 5 psu), which is situated on the left bank of the Schelde estuary close to Antwerpen, Belgium. A handnet ($L \times W$: 0.3×0.2; mesh size 1 mm) was pushed over the bottom during short hauls of 2–3 min. Mysids were transported to the laboratory within 2 h after sampling in 15-L bins containing environmental water.

Stock cultures were maintained as reported by Verslycke et al. (2003). In short, mysids were kept in a static system in 200-L glass aquaria equipped with a circulating under-gravel filter. The culture medium was filtered (1.2 μm) seawater diluted with aerated tap water until a final salinity of 5 psu. Every 2 weeks, 50% of the culture medium was renewed. A 12 h:12 h light–dark photoperiod was used and water temperature was kept at 20 ± 2 °C. Cultures were fed twice a day with 24–48 h old *Artemia* nauplii at a feeding rate of 150 nauplii mysid^{-1} day^{-1}. Mysid culture density was 20 organisms per litre. The under-gravel filter was replaced every 6 months.

Gravid females were transferred at regular intervals to 10-L aerated static incubators, in which the culture medium was renewed every day for 50%. In these incubators, animals were fed twice a day with 24–48 h old *Artemia* nauplii ad libitum and were checked daily for the release of juveniles from the marsupium. These juveniles were separated from the adult females using a netted brood chamber to prevent the adults from cannibalizing their young.

2.2. Growth experiment procedures

Neonates (<24 h old, standard lengths of 2.18 to 2.86 mm) were individually placed in a glass container of 400 mL filled with 350 mL of artificial seawater (different experimental treatments of salinity and temperature, see below) and reared to the late adult stage or until mortality occurred. These experiments lasted between 2 months for the higher temperature experiments and 4 months for the lower temperature experiments. No gradual adaptation from stock to experimental salinity and temperature was done, since it is known that estuarine mysids adapt within a 1.5–3 h to changes in salinity and temperature (De Lisle and Roberts, 1987; Dormaar and Corey, 1973). The artificial seawater (Instant Ocean®, Aquarium Systems, France) was diluted with distilled water to the respective test salinity and aerated for at least 24 h prior to being used. The experimental containers were

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not aerated, but at least half of the content was renewed daily. The four experimental temperatures (8, 15, 20 and 25 °C) were kept constant by using warm-water baths in temperature-controlled climate rooms at 4 and 15 °C. Salinity and temperature were monitored daily with an YSI salinity meter, but variations were small, i.e. 8.5 ± 0.2 °C; 15.0 ± 0.4 °C; 20.1 ± 0.8 °C; 25.0 ± 0.6 °C and 5.1 ± 0.4 psu; 15.2 ± 0.5 psu and 30.2 ± 0.7 psu.

The containers were checked daily for exuvia (moults). These were carefully harvested with a wide-mouthed glass pipette and transferred to a 4% formaldehyde solution. Since mysids moult at night, this was preferably done early in the morning to reduce the risk of disintegration or scavenging of the moults. At the same time freshly hatched nauplii of *Artemia* were added. The number of <24 h nauplii were counted in a 0.2 mL subsample to calculate food concentration. Juveniles were fed approximately 250 nauplii mysid^{-1} day^{-1}, subadults 500 to 750 nauplii mysid^{-1} day^{-1}, and adults 1000 nauplii mysid^{-1} day^{-1}. This corresponded to an ad libitum feeding regime, without excessive accumulation of left-over food in the containers. Every 4 to 5 days the individuals were transferred to new, clean jars. The mysids were handled and transferred using a conical plastic measuring spoon to avoid physical stress.

2.3. Experimental design

Eight temperature–salinity combinations were selected based on their relevance to European estuarine mysid populations. For the core experiment, temperatures of 15 and 20 °C and salinities of 5, 15 and 30 psu were tested. These temperatures correspond to spring and summer temperatures in mid-European estuaries (Mees et al., 1994). The salinities correspond to the upper, middle and lower reaches of an estuary. Originally, experiments included a 1 psu treatment, however, mortality was extremely high (80% before fifth moult) in this treatment. An additional experiment was set up to test the more extreme lower and higher temperatures of 8 and 25 °C at a salinity of 5 psu. These temperatures correspond to winter temperature in the Schelde and summer temperature in the Gironde estuary, respectively.

Only mysids that survived at least 5 moults were used for further analyses; mysids dying at an earlier stage were replaced by new <24 h juveniles. An overview of the total number of introduced and successful (i.e. those surviving for more than 5 moults) individuals per treatment is shown in Table 1.

2.4. Measurements

Measurement of standard length of exuvia is impossible because of the elasticity of the moult. Furthermore, the collected moults were generally broken into two or more parts. Therefore, well-defined rigid parts of the moults were measured using a microscope with drawing tube (magnification 110×), with the moult mounted temporally (in water) under a glass cover slip (Fig. 1). Preferably, the lengths of the left and right exopodites of the uropod were used. The standard length (SL) of the mysid was calculated from the mean length of the exopodites using a linear regression

Table 1
Mortality statistics (n.d.: not sexually differentiated)

Temperature (°C)	Salinity (psu)	Max. # of days	Max. # of moults	Total started	Died before 5th moult/injured	# of individuals in analysis	Gender		
							♀	♂	n.d.
15	5	123	18	28	3	25	9	16	0
15	15	123	18	30	6	24	11	13	0
15	30	122	19	29	8	21	13	8	0
20	5	62	14	36	10	26	12	14	0
20	15	98	17	34	12	22	12	10	0
20	30	98	19	34	14	20	10	7	3
8	5	122	12	43	19	24	8	16	0
15	5	123	18	28	3	25	9	16	0
20	5	62	14	36	10	26	12	14	0
25	5	61	15	46	27	19	8	10	1

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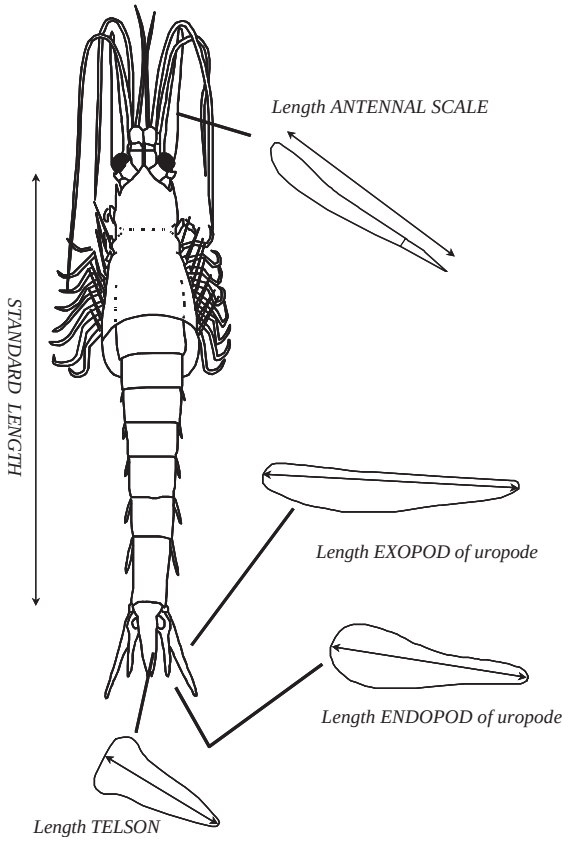

Fig. 1. Schematic representation of *Neomysis integer* with indication of the parts of the moults that were measured: length of antennal scale, lengths of the endopod and exopod of the uropod and telson length. Standard length (from the rostrum in between the eye stalks to the end of the last abdominal segment) and mean uropodal exopod length were measured on 100 individuals collected in the field for the linear regression.

(Table 2), based on measurements of 100 randomly selected individuals from the source population (in spring and autumn). If the exopodites were absent or broken, other body parts were used (length of the endopodite of the uropod, length of the antennal scale or length of the telson) and exopodite length was derived using morphometric linear regressions (Table 2). These were derived from measurements made on all moults collected within the experiment.

2.5. Description of growth

Growth is described as the increase in body length over time. For each experimental treatment, the generalized version of the von Bertalanffy growth curve was fitted to the pooled data points within each treatment. The model was also used to describe the growth of each individual.

$$L_t = L_{\inf}\left(1 - e^{-K(t-t_0)}\right)$$

where L_t is the predicted standard body length (in mm) at age t (in fractions of the year), $L_{\inf}$ is the asymptotic length, K is a growth constant and t_0 is the (theoretical) age at a standard length zero. The fitting was done with the non-linear estimation module in Statistica™, using the least squares loss function and the Levenberg–Marquardt estimation method. The individually based estimates of the growth parameters were tested between the sexes with a Mann–Whitney U-test. Comparison of the growth parameters between treatments and available field data (Schelde: Mees et al., 1994; Galgenweel: Fockedey, personal communication) was approached from a multivariate perspective in which both K and $L_{\inf}$ were taken into consideration. The growth performance index (Φ') was estimated by applying the equation derived by Munro and Pauly (1983) in the form of $\Phi' = \log_{10}(K) + 2\log_{10}(L_{\inf})$, with K in year$_{-1}$ and $L_{\inf}$ in cm.

Growth in crustaceans is a discontinuous process, i.e. the succession of moults (=exuvia, ecdyses) is separated by intermoult periods. Each time an individual moults, the old integument is shed and a rapid, extensive growth occurs during the short period before the new integument hardens (Hartnoll, 1982). The standard length at subsequent moults was tested in function of both temperature and salinity using a repeated measures analysis of variance (ANOVA). In addition, a repeated measure ANOVA with temperature as independent variable was applied to the more extended temperature experiment at 5 psu. Both tests were performed with a complete design until the 11th moult. Standard length was linearized by a logarithmic transformation.

According to Mauchline (1977), the stepwise growth of mysids can be described as the duration of the intermoult period (IMP, in days) and the increase in length at each moulting event (the growth factor (GF) in % of the pre-moult length) as illustrated in Fig. 2. The IMP and GF data were analyzed by (1) a two-way analysis of covariance (ANCOVA) for the

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Table 2
Results of the allometric regression analyses to estimate (1) the standard body length from the mean length of the exopodites of the uropods and (2) the length of the exopodite of the uropod from the mean length of the endopodites of the uropods, the mean length of the antennal scales and telson length ($X=a+b*Y$)

X	Y	a	b	R^2	p	N
Standard body length	$Length_{exopodites}$	1.0856	4.0818	0.9569	<0.0001	97
$Length_{exopodite}$	$Length_{endopodites}$	−0.2235	1.5420	0.9939	<0.0001	1798
$Length_{exopodite}$	$Length_{antennal\ scales}$	−0.0059	0.7642	0.9965	<0.0001	1660
$Length_{exopodite}$	$Length_{telson}$	−0.0210	1.3320	0.9947	<0.0001	1779
Total body length	Standard body length	−0.080	1.165	0.997	<0.001	112
Carapace length	Standard body length	0.439	0.266	0.908	<0.001	112

For comparison with other data, allometric regressions are added to calculate total body length and carapace length from the standard body length (Mees et al., 1994).

combined salinity (5, 15, 30 psu) and temperature (15, 20 °C) effect and (2) a one-way ANCOVA for the temperature effect (8, 15, 20, 25 °C) at 5 psu, both with standard length as the covariable. The intermoult period and standard length were logarithmically transformed, the growth factor was submitted to an arcsine transformation to fulfil to the ANCOVA assumptions. Using moult number as the covariable in the respective ANCOVA's yielded the same results, but these are not presented here.

2.6. Age and size at maturity

Data on the size and age at sexual differentiation and maturity was collected during all experiments. An animal was identified as a subadult male as soon as the second ramus of the fourth pleopod and the lobus masculinus could be identified on the moult. Animals were classified as adult males when the lobus masculinus became setose and/or the fourth pleopod stretched to the end of the last abdominal segment. Females gradually develop a marsupium between the thoracopods. Since the oostegites were never found attached to the moult during the experiments, the same moult number was used as for males within the same treatment to classify subadult and adult females.

3. Results

3.1. Mortality

Only mysids that survived at least 5 moults were used for further analyses; mysids dying at an earlier

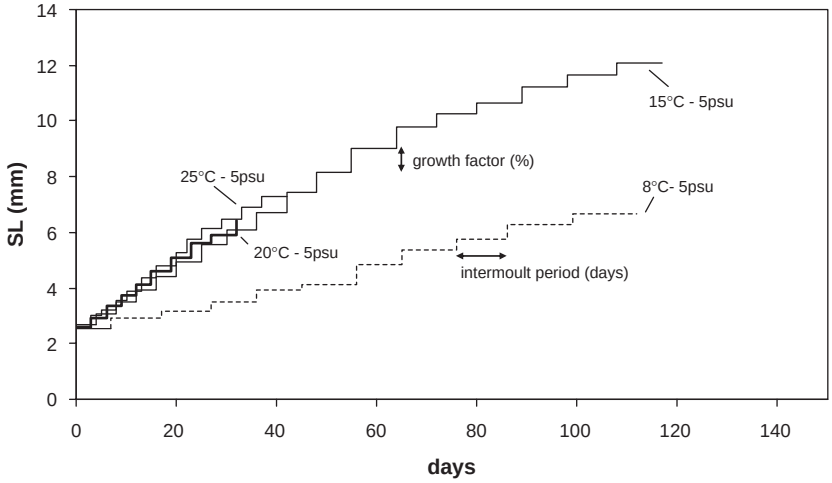

Fig. 2. The stepwise growth as observed for 'typical' long-living individuals at four experimental temperatures and a salinity of 5 psu, with indication of growth factor (growth rate) and intermoult period.

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stage were replaced by new < 24 h juveniles. Per salinity–temperature combination 28 to 46 mysids were introduced into the experiments and between 19 and 26 individuals were available for the description of the growth (Table 1).

Overall survival was markedly higher at 15 °C when compared to the other temperatures (Fig. 3). The life cycle of *N. integer* was shorter in the higher temperature treatments (20 and 25 °C), i.e. individuals reached adulthood fast and died after 61–62 days, but mortality was relatively high over the whole lifespan. At 8 °C, initial mortality was also high; a trend that stabilized after 24 days (44–56% survival).

3.2. Growth curves

Generalized von Bertalanffy growth curves were fitted to the pooled data for each treatment (Fig. 4). The growth parameters asymptotic length (L_{inf}), growth constant (K) and the theoretical age at a standard length zero (t_0) were calculated and presented in Table 3. The overall goodness of fit for the different growth curves was high (R^2: 0.92–0.98). No von Bertalanffy growth model was fitted to the 8 °C data. In this treatment, growth was slow and linear for the duration of the experiment (122 days) and was therefore described using a linear model; SL=2.259+0.042*age (N=206, R^2=0.93, p<0.00001). The asymptotic length (L_{inf}) was larger at 15 °C (L_{inf} between 14.60 and 16.81 mm) as compared to higher temperatures (10.53–12.39 mm at 20 °C and 8.55 mm at 25 °C). The growth rate increased with temperature; K of 11.95 at 25°C; K of 5.37–7.30 at 20°C and K of 3.54–4.64 at 15 °C. Salinity had a less straightforward effect on the growth parameters L_{inf} and K. At 15 °C, the highest L_{inf} was reached at 15 psu (16.81 ± 0.48 mm), whereas at 20 °C L_{inf} was highest at 5 psu (12.39 ± 0.83 mm). K values were highest at 5 psu at 15 °C (4.64 ± 0.25) and at 15 psu at 20 °C (7.30 ± 0.41).

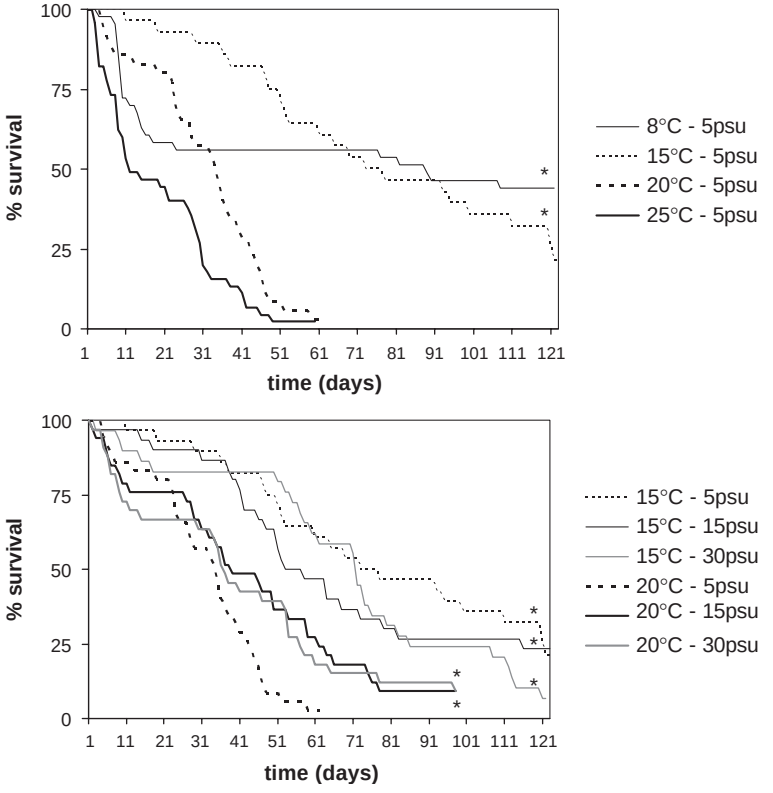

Fig. 3. Survival curves at the different salinity–temperature combinations. * indicates that the data include censored data with animals still alive at the end of the experiment.

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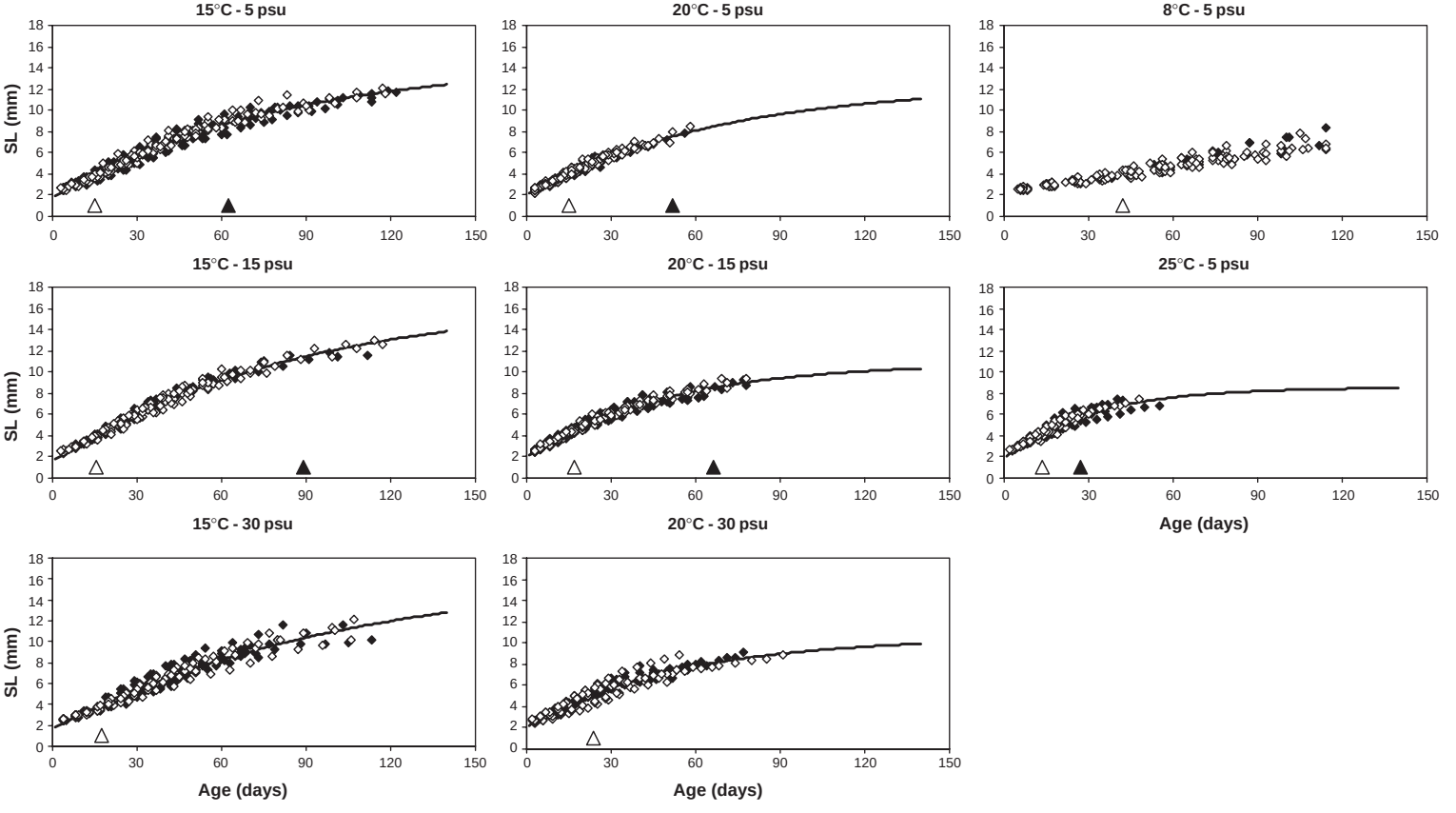

Fig. 4. The generalised von Bertalanffy growth curve (—) fitted to the pooled data of females (♦) and males (◇) of each salinity–temperature combination; (△) indicate the age at sexual differentiation and (▲) the age at sexual maturity.

Table 3
Pooled and individually based von Bertalanffy growth parameter estimations (±standard error) and performance index Φ' (–: linear growth)

Temperature (°C)	Salinity (psu)		# of individuals	# of moults	L_{inf} (mm)	K (day 1)	t_0 (fraction of the year)	t_0 (days)	p	R^2	Φ'
15	5	Pooled	24	310	14.60 ± 0.39	4.64 ± 0.25	0.027 ± 0.002	10 ± 1	<0.001	0.974	1.558
		Individually	15	8–18	15.93 ± 0.85 (ns)	4.55 ± 0.47 (ns)	0.029 ± 0.002 (ns)	11 ± 1 (ns)	<0.0001	0.988–0.999	1.625
15	15	Pooled	24	258	16.81 ± 0.48	4.25 ± 0.21	0.023 ± 0.001	8 ± 1	<0.001	0.984	1.642
		Individually	10	10–18	18.12 ± 0.98 (ns)	4.07 ± 0.32 (ns)	0.024 ± 0.002 (ns)	9 ± 1 (ns)	<0.0001	0.992–0.998	1.688
15	30	Pooled	21	254	16.66 ± 1.07	3.54 ± 0.38	0.030 ± 0.003	11 ± 1	<0.001	0.952	1.555
		Individually	5	8–18	15.75 ± 1.65 (°)	4.89 ± 0.49 (°)	0.019 ± 0.003 (°)	7 ± 1 (°)	<0.0001	0.994–0.998	1.646
20	5	Pooled	27	223	12.39 ± 0.83	5.37 ± 0.59	0.033 ± 0.002	12 ± 1	<0.001	0.974	1.478
		Individually	11	7–13	12.85 ± 0.91 (ns)	5.63 ± 0.49 (ns)	0.033 ± 0.002 (ns)	12 ± 1 (ns)	<0.0001	0.998–0.999	1.531
20	15	Pooled	22	261	10.88 ± 0.28	7.30 ± 0.41	0.028 ± 0.002	10 ± 1	<0.001	0.976	1.499
		Individually	14	7–16	10.55 ± 0.35 (*)	7.82 ± 0.52 (ns)	0.029 ± 0.002 (ns)	11 ± 1 (ns)	<0.0001	0.989–0.999	1.502
20	30	Pooled	20	228	10.53 ± 0.52	6.85 ± 0.73	0.032 ± 0.003	12 ± 1	<0.001	0.922	1.443
		Individually	9	10–18	10.68 ± 0.72 (ns)	7.98 ± 0.66 (ns)	0.029 ± 0.002 (ns)	11 ± 1 (ns)	<0.0001	0.996–0.999	1.521
8	5	Pooled	–	–	–	–	–	–	–	–	–
		Individually	–	–	–	–	–	–	–	–	–
15	5	Pooled	24	310	14.60 ± 0.39	4.64 ± 0.25	0.027 ± 0.002	10 ± 1	<0.001	0.974	1.558
		Individually	15	8–18	15.93 ± 0.85 (ns)	4.55 ± 0.47 (ns)	0.029 ± 0.002 (ns)	11 ± 1 (ns)	<0.0001	0.988–0.999	1.625
20	5	Pooled	27	223	12.39 ± 0.83	5.37 ± 0.59	0.033 ± 0.002	12 ± 1	<0.001	0.974	1.478
		Individually	11	7–13	12.85 ± 0.91 (ns)	5.63 ± 0.49 (ns)	0.033 ± 0.002 (ns)	12 ± 1 (ns)	<0.0001	0.998–0.999	1.531
25	5	Pooled	19	162	8.55 ± 0.43	11.95 ± 1.48	0.021 ± 0.003	8 ± 1	<0.001	0.920	1.504
		Individually	7	7–13	10.01 ± 0.52 (ns)	10.25 ± 1.25 (ns)	0.023 ± 0.004 (ns)	8 ± 1 (ns)	<0.001	0.995–0.999	1.574

Gender differences using the Mann–Whitney U-test are shown in between brackets (ns: not significant; *: significant; °: test not allowed).

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The growth performance index Φ' (Table 3, Fig. 5) showed analogue trends, with the highest growth performance at 15 °C in comparison with higher temperatures (Spearman Rank R: −0.65; $p=0.02$). Slightly higher values were obtained at 15 psu in comparison with other salinities at 15 and 20 °C, although no correlation could be demonstrated. The growth performance index Φ' in all our experimental treatments was significantly higher (t-test: $p=0.000064$) than in all cohorts of the field populations of the Schelde estuary and Galgenweel (Table 4, Fig. 5).

Individually based estimates of the von Bertalanffy growth parameters were only significant for the long living animals. The growth of shorter living individuals, comprising 36% to 76% of the total of all tested individuals, behaved linearly. The number of animals and moults used for the individual estimates of L_{inf}, K and t_0 are presented in Table 3. The goodness of fit was always higher (>0.99) for the individually based data than for the pooled data. The asymptotic length was generally underestimated based on the pooled data in comparison with the individually derived estimates. Overestimated K values are compensated, however, by underestimated t_0 values and vice versa. Gender had no significant effect on the estimates of the growth parameters in our experiments.

3.3. Standard length in function of moult number

Temperature, salinity and moult number were tested in a two-way repeated measures ANOVA (Fig. 6, Table 5a). Generally, subsequent moults resulted in significantly larger animals and this at least until the 11th moult. Temperature significantly affected the standard length of *N. integer* and the largest individuals were found at 15 °C. This temperature effect on standard length was significant from the 3rd moult on. In addition, salinity had a significant effect on the standard length of *N. integer*. At 15 psu animals were larger than at 5 or 30 psu, and this from the

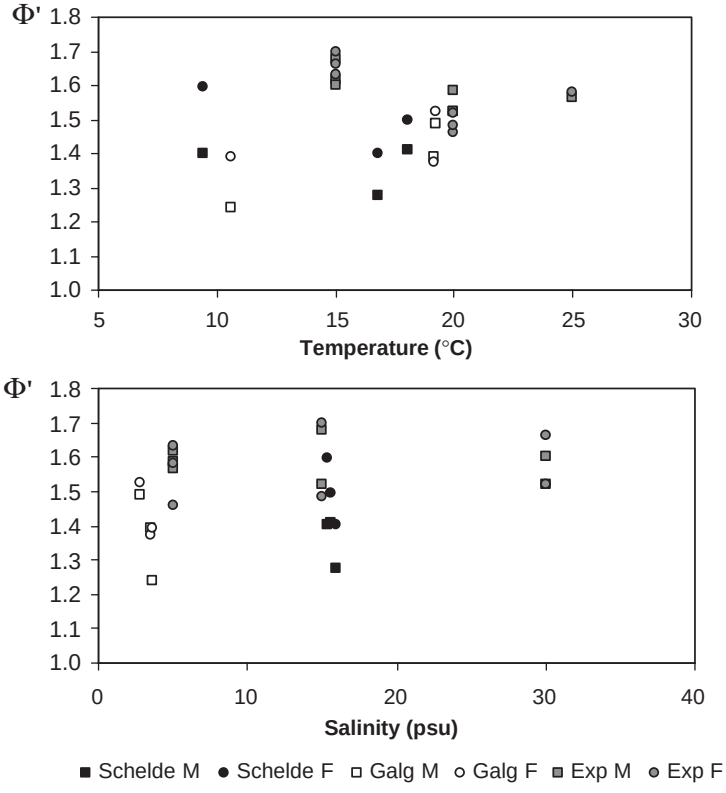

Fig. 5. Growth performance index (Φ') for field and experimentally derived growth for male and female *Neomysis integer* in function of temperature and salinity (Schelde: Schelde population, Galg: Galgenweel population, Exp: present experiments; M: males, F: females).

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Table 4
Comparison of field and experimentally derived von Bertalanffy growth parameter estimations and growth performance index Φ' for male and female *Neomysis integer*

	Temperature (°C)	Salinity (psu)	Generation	♂ L_{inf} (mm)	K (day 1)	Φ'	♀ L_{inf} (mm)	K (day 1)	Φ'
Schelde populatin	9 (9-22)	15 (9-21)	winter	16.0	2.7	1.40	19.0	3.0	1.60
(Mees et al., 1994)	18 (10-23)	16 (10-19)	spring	14.3	3.4	1.41	16.0	3.4	1.50
	17 (4-19)	16 (10-21)	summer	13.1	3.0	1.28	14.3	3.4	1.40
Galgenweel population	11 (4-19)	4 (3-5)	winter	16.5	3.1	1.49	17.5	3.0	1.53
(Fockedey, unpublished)	19 (17–23)	3	spring	15.5	2.8	1.39	15.5	2.7	1.37
	19 (17–23)	3 (3–5)	summer	12.6	3.0	1.24	14.5	3.2	1.39
Laboratory	8	5							
experiments	15	5		16.3	4.3	1.62	15.5	4.9	1.63
(present study)	15	15		17.7	4.2	1.68	18.7	3.9	1.70
	15	30		14.0	5.6	1.60	16.6	4.6	1.66
	20	5		13.7	5.6	1.59	11.8	5.7	1.46
	20	15		11.7	6.7	1.52	9.9	8.4	1.48
	20	30		10.4	8.5	1.52	10.8	7.7	1.52
	25	5		9.7	10.7	1.56	10.3	9.9	1.58

Mean temperature and salinity are indicated with their range within parentheses.

3rd moult onwards. The combined effects of salinity–temperature, salinity–moult number and temperature–moult number were all significant, whereas the effect of both temperature and salinity with the within-subject factor (moult number) was borderline significant ($p=0.049652$).

The effect of a larger temperature range (8, 15, 20 and 25 °C) on mysid standard length at subsequent moults was tested using a repeated measures ANOVA (Fig. 6; Table 5b). Again, temperature had a significant effect on the standard length of *N. integer* with larger individuals at 15 °C as compared to the other tested temperatures. This effect was significant from the 2nd–4th moult onwards. For example, at 5 psu an average individual at its 10th moult measured 6.64, 7.90, 6.34 and 6.26 mm at 8, 15, 20 and 25 °C, respectively.

3.4. Intermoult period

The intermoult period (IMP) is positively related to the standard length (Fig. 7) and to the moult number. The combined effect of temperature and salinity on the intermoult period at 15 and 20 °C was tested in an ANCOVA using standard length as the covariable. Temperature ($p<0.0001$) and salinity ($p<0.006$) both had a significant effect on the IMP, although the salinity effect was less important. The IMP was significantly ($p<0.001$) shorter at the highest temperatures (5.35 ± 0.02 days at 15 °C; 4.28 days $\pm$ 0.03 at 20 °C). IMP was significantly ($p=0.0016$) shorter at 15 psu (4.71 ± 0.03 days) in comparison to the other tested salinities (4.92 ± 0.03 at 5 psu and 4.81 ± 0.03 at 30 psu). The combined effect of salinity and temperature on the IMP was not significant ($p=0.365$).

A second ANCOVA, aimed at studying the effect of a larger temperature range (8–25 °C) on mysid growth confirmed that temperature has a significant effect on growth, in this case the IMP ($p<0.001$): the IMP was longest at 8 °C (10.61 ± 0.06 days) and gradually decreased at higher temperatures (5.05 ± 0.05 at 15 °C, 4.14 ± 0.06 at 20 °C and 3.42 ± 0.07 days at 25 °C). The intermoult period of immature *N. integer* was very similar (2–4 days) at 15, 20 and 25 °C, but it was markedly longer at 8 °C (7–10 days). For late subadult and adults stages, the IMP was temperature-dependent and ranged between 7–10 days at 15 °C, 5–7 days at 20 °C and 4–5 days at 25 °C.

3.5. Growth factor

The growth factor (GF) is inversely correlated with standard length (Fig. 8) and moult number. Temperature and salinity in combination had a significant effect on the growth factor of *N. integer* (ANCOVA: $p<0.001$). The GF at 15 °C is significantly ($p<0.0001$) larger ($12.31\pm0.11\%$) than at 20 °C ($8.82\pm0.12\%$). Salinity also had a significant ($p<0.0001$) effect on the GF, but this effect was different between the two tem-

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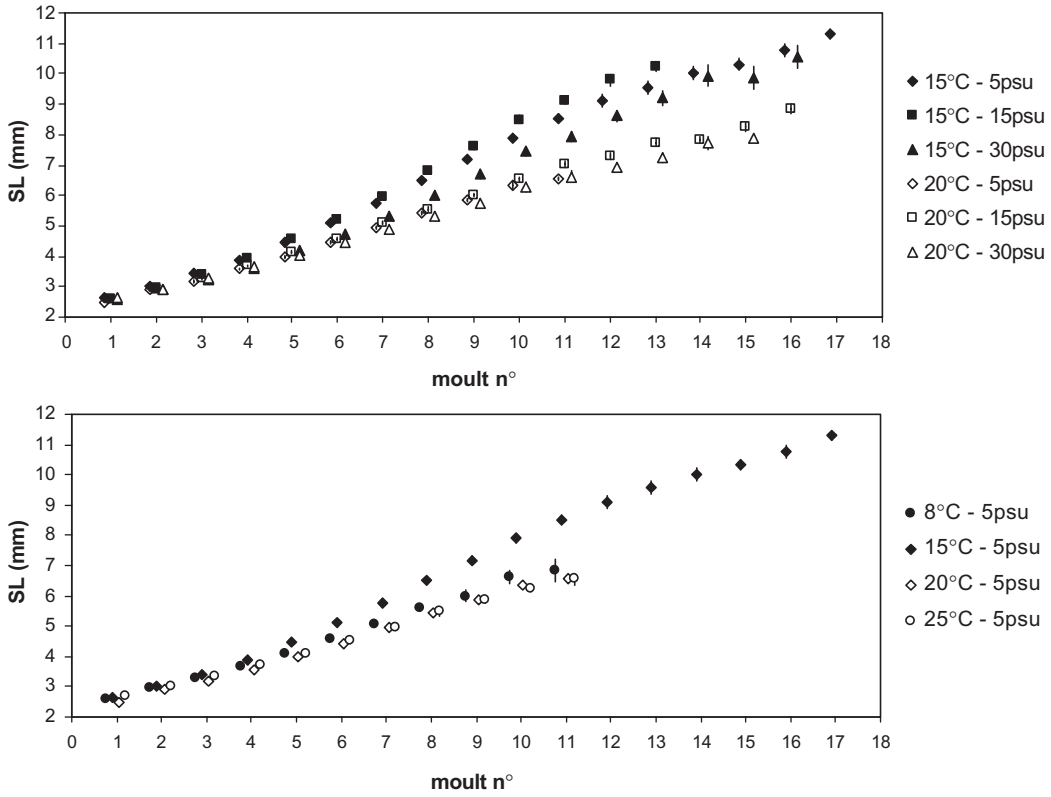

Fig. 6. Standard length (SL± standard error) in function of the moult number at the different salinity and temperature combinations.

perature treatments. At 15 °C, the GF was significantly higher at 15 psu (13.36 ± 0.19%) than at 5 and 30 psu (12.07 ± 0.17 and 11.49 ± 0.19%, respectively). At 20 °C, the highest GF was observed at 5 psu (9.34 ± 0.21%) and was subsequently lower at 15 and at 30 psu (9.08 ± 0.19 and 8.02 ± 0.21%, respectively).

To study the effects on mysid growth over the full range of temperatures observed in the field (8–25 °C), a second ANCOVA was performed using 4 temperature treatments at 5 psu ($p < 0.001$). In these experiments, no obvious temperature effect on the GF was observed, except at 25 °C where suboptimal growth was caused

Table 5a
Results of the two-way repeated measures ANOVA testing the effect of temperature and salinity on the standard length at subsequent moults (15 and 20 °C–5, 15 and 30 psu)

	F	p-value	Description of effect
Temperature	36.21	0.000002	15 °C>20 °C
Salinity	5.31	0.011091	15 psu>(30 psu=5psu)
Moult number	4133.52	< 0.0000001	log SL~moult number
Salinity × temperature	4.00	0.029589	15 °C: (5 psu=15 psu)>30 psu 20 °C: 15 psu>(5 psu=30 psu)
Temperature × moult number	52.23	< 0.0000001	From 3rd moult: 15 °C>20 °C
Salinity × moult number	4.84	< 0.0000001	From 3rd moult: 15 psu>(5 psu=30 psu)
Salinity × temperature × moult number	1.61	0.049652	–

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Table 5b
Results of the repeated measure ANOVA testing the effect of temperature on the standard length at subsequent moults (8, 15, 20 and 25 °C at 5 psu)

	F	p-value	Description of effect
Temperature	14.409	0.000083	15 °C>(8=20=25 °C)
Moult number	1086.659	<0.0000001	log SL~moult number
Temperature × moult number	9.770	<0.0000001	From 2nd–4th moult: 15 °C>(8=20=25 °C)

by a significantly lower GF. In immature *N. integer*, the GF varied between 9% and 16%, while late subadult and adult mysids increased 4–8% in size at each moult.

3.6. Age, moult number, intermoult period (IMP), growth factor (GF) and growth rate (GR) per 1 mm length class

Generally, animals are collected from the field or a laboratory stock culture without knowing the exact age. Table 6 can serve as a tool to estimate the age, moult number, intermoult period, growth factor and intermoult growth rate of *N. integer* based on the standard length (for the 8 temperature–salinity combinations) which is useful in ecotoxicological experiments. Variation on these values (not shown) is small as is also demonstrated by the good fit of the von Bertalanffy growth curve on the data. The maximal size (at maturity) observed was 18–35% lower than the asymptotic length L_{inf} as calculated by the von Bertalanffy model on the pooled data and 16–41% lower based on individual estimations. Therefore, additional data on 'age' was extrapolated from the respective growth models (in bold) for the largest size classes.

3.7. Sexual development and maturity

Sexual differentiation, i.e. secondary sexual characteristics appearing (△ in Fig. 4) was reached in all

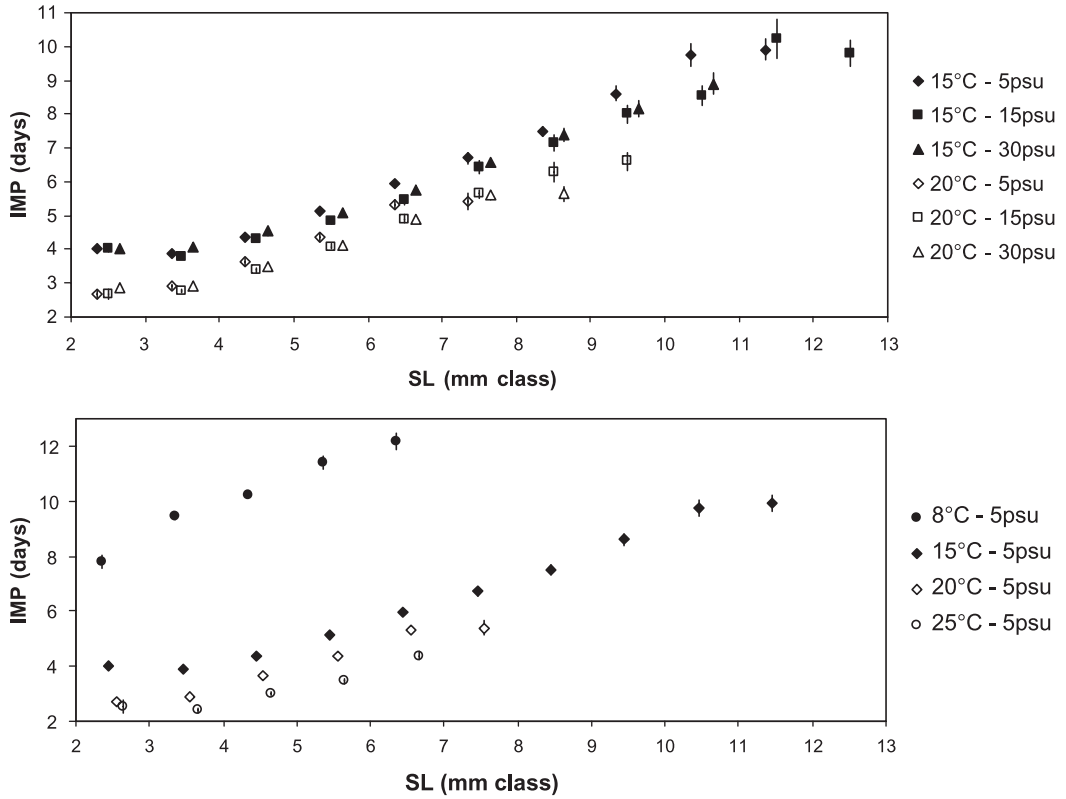

Fig. 7. Intermoult period (IMP ± standard error) in function of standard length (SL).

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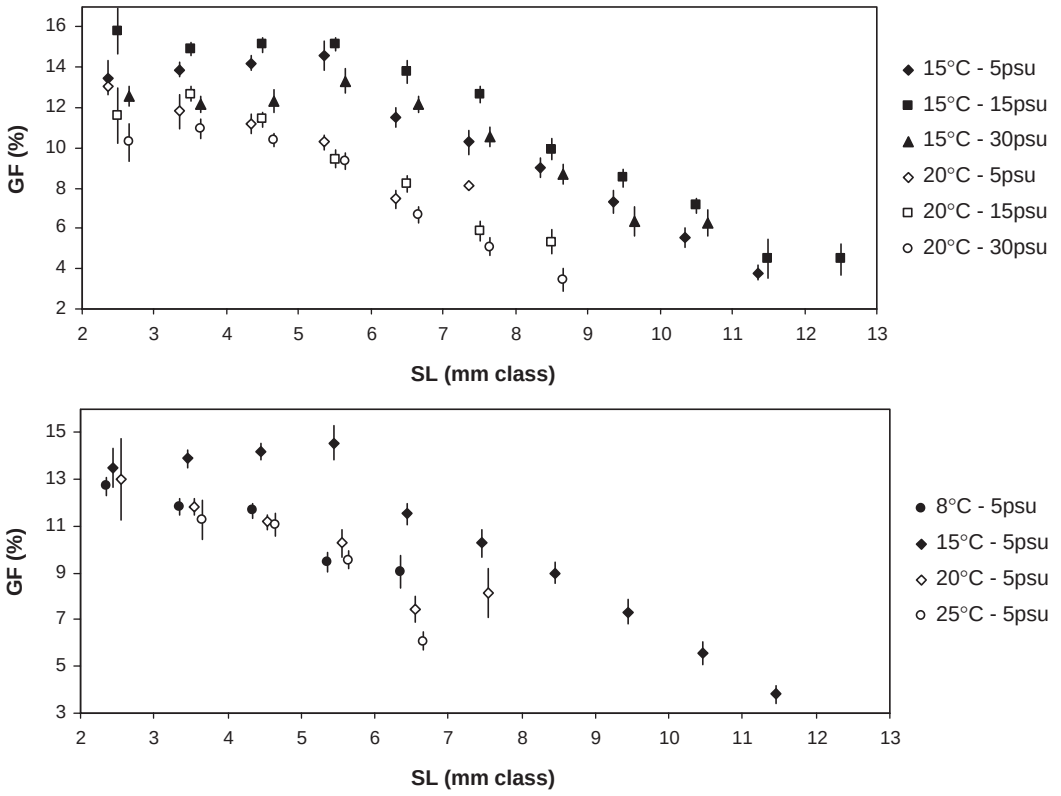

Fig. 8. Growth factor (GF ± standard error) in function of standard length (SL).

treatments, while sexual maturity, i.e. secondary sexual characteristics in adult form (▲ in Fig. 4) was only reached by mysids within the range 15–25°C and 5–15 psu (Fig. 4, Table 7).

In the different 15 and 20 °C treatments, the animals became subadult at a later point in their life with increasing experimental salinity. Sexual differentiation was observed after 15 days at 5 psu; after 15–17 days at 15 psu and after 18–24 days at 30 psu, at 15 and 20 °C respectively. The number of moults needed to reach subadulthood was 5 at 15 °C in all salinity treatments, but this number increased with salinity at 20 °C (6, 7 and 8 at 5, 15 and 30 psu, respectively). Maturity was not reached at all in the 30 psu treatments. The limited number of observations on age and size at sexual maturity in the 20 °C treatments hinders firm conclusions. However, some trends could be observed, i.e. at higher temperatures the age at maturity decreased, while at higher salinities it increased (Fig. 4).

The age where individuals became subadult at 5 psu and 8 °C was retarded (42 days) in comparison with the other temperatures tested at the same salinity (14–15 days). At 15 °C, subadults were observed after 5 moults, while at other temperatures generally one more moult was required to reach differentiation. Within 11 moults, animals at 25 °C were fully sexually developed, while at 15 and 20 °C animals required two to three more moults to reach maturity. Adulthood was not reached in any of the animals at 8 °C within the 4 months duration of these experiments. Clearly, temperature shortens the time to reach sexual maturation in mysids (62, 52 and 27 days at 15, 20 and 25 °C, respectively).

4. Discussion

4.1. Optimizing culture protocols for N. integer

Maintaining a laboratory stock culture of *N. integer* under standardized conditions (e.g. methodology according to Verslycke et al., 2003) has the advantage

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Table 6
Estimated age, moult number, intermoult period, growth factor and intermoult growth rate in function of the standard length of *Neomysis integer* (in between brackets: less than 5 observations; in bold: estimated age from von Bertalanffy growth curve; –: no observations)

T (°C)	*S* (psu)		Standard length (mm); 1 mm class—median														
			2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5
8	5	Age (days)	11	31	55	76	95	(103)	(114)	–	–	–	–	–	–	–	–
		Moult no.	1	4	6	8	10	(10)	(11)	–	–	–	–	–	–	–	–
		IMP (days)	8	9	10	11	12	(14)	(14)	–	–	–	–	–	–	–	–
		GF (%)	13	12	12	9	9	(8)	(11)	–	–	–	–	–	–	–	–
		GR (mm day^{-1})	0.04	0.04	0.05	0.04	0.04	(0.04)	(0.06)	–	–	–	–	–	–	–	–
15	5	Age (days)	5	12	21	28	37	46	56	70	88	**112**	**145**	**194**	**386**	–	–
		Moult no.	1	3	5	7	8	10	11	13	15	17	(18)	–	–	–	–
		IMP (days)	4	4	4	5	6	7	8	9	10	10	(9)	–	–	–	–
		GF (%)	13	14	14	15	12	10	9	7	6	4	(4)	–	–	–	–
		GR (mm day^{-1})	0.09	0.11	0.13	0.14	0.11	0.11	0.09	0.08	0.06	0.04	(0.05)	–	–	–	–
15	15	Age (days)	6	12	20	26	34	41	51	62	**76**	**91**	**108**	**131**	**162**	**210**	**335**
		Moult no.	1	3	5	6	8	9	10	12	13	15	17	–	–	–	–
		IMP (days)	4	4	4	5	5	6	7	8	9	10	10	–	–	–	–
		GF (%)	16	15	15	15	14	13	10	9	7	4	4	–	–	–	–
		GR (mm day^{-1})	0.10	0.12	0.14	0.15	0.15	0.13	0.11	0.09	0.08	0.05	0.05	–	–	–	–
15	30	Age (days)	6	14	24	32	40	50	60	75	**92**	**110**	**132**	**161**	**200**	**265**	**475**
		Moult no.	1	3	6	7	9	10	12	14	15	(16)	(18)	–	–	–	–
		IMP (days)	4	4	5	5	6	7	7	8	9	(11)	(8)	–	–	–	–
		GF (%)	13	12	12	13	12	11	9	6	6	(7)	(6)	–	–	–	–
		GR (mm day^{-1})	0.08	0.09	0.11	0.13	0.12	0.11	0.09	0.07	0.07	(0.07)	(0.09)	–	–	–	–
20	5	Age (days)	5	11	19	27	38	49	**67**	**87**	**116**	**167**	–	–	–	–	–
		Moult no.	2	4	6	8	10	12	(14)	–	–	–	–	–	–	–	–
		IMP (days)	3	3	4	4	5	5	(6)	–	–	–	–	–	–	–	–
		GF (%)	13	12	11	10	7	8	(7)	–	–	–	–	–	–	–	–
		GR (mm day^{-1})	0.14	0.13	0.13	0.12	0.08	0.10	(0.09)	–	–	–	–	–	–	–	–
20	15	Age (days)	4	9	16	25	35	48	**66**	**93**	**158**	–	–	–	–	–	–
		Moult no.	2	4	6	8	10	12	15	16	–	–	–	–	–	–	–
		IMP (days)	3	3	3	4	5	6	6	7	–	–	–	–	–	–	–
		GF (%)	12	13	11	9	8	6	5	(5)	–	–	–	–	–	–	–
		GR (mm day^{-1})	0.13	0.15	0.14	0.11	0.10	0.08	0.07	(0.07)	–	–	–	–	–	–	–
20	30	Age (days)	4	11	19	27	38	51	**76**	**112**	–	–	–	–	–	–	–
		Moult no.	1	4	6	8	11	13	16	(18)	–	–	–	–	–	–	–
		IMP (days)	3	3	4	4	5	6	6	(6)	–	–	–	–	–	–	–
		GF (%)	10	11	10	9	7	5	3	(5)	–	–	–	–	–	–	–
		GR (mm day^{-1})	0.10	0.12	0.12	0.12	0.09	0.07	0.05	(0.07)	–	–	–	–	–	–	–
25	5	Age (days)	4	9	17	23	34	**56**	–	–	–	–	–	–	–	–	–
		Moult no.	1	3	6	8	11	(13)	–	–	–	–	–	–	–	–	–
		IMP (days)	3	2	3	3	4	(5)	–	–	–	–	–	–	–	–	–
		GF (%)	(12)	11	11	10	6	(6)	–	–	–	–	–	–	–	–	–
		GR (mm day^{-1})	0.16	0.15	0.16	0.14	0.09	(0.08)	–	–	–	–	–	–	–	–	–

that all experimental specimens are born from adults living at the same temperature, salinity and food conditions. Especially when experiments run over an extended period of time (2 years in this case), variation that might be caused by different stock animals are kept at a minimum. In addition, the exact age of all individuals is known when cultures are checked daily for newly released young.

Based on the present study, some adaptations of the culture protocol for *N. integer* are suggested to enhance culture yield and/or quality. The laboratory stock culture was kept at 20 ± 2 °C. In the individually based experiments, however, this relatively high temperature resulted in a substantially higher mortality (Fig. 3). Therefore, a lower culture temperature is suggested for *N. integer*. On the other

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Table 7
Size of the juvenile *Neomysis integer* at the start of the experiment and size, age and moult number at sexual differentiation and maturity

T (°C)	*S* (psu)	Juvenile	Subadult			Adult		
		Size (mm)	Size (mm)	Age (days)	# of moults	Size (mm)	Age (days)	# of moults
15	5	2.6 ± 0.1	4.3 ± 0.1	14.9 ± 0.4	4.8 ± 0.1	9.4 ± 0.2	62.4 ± 1.3	12.8 ± 0.2
15	15	2.6 ± 0.1	4.5 ± 0.1	15.3 ± 0.5	4.9 ± 0.1	11.9 ± 0.2	89.3 ± 3.7	16.0 ± 0.4
15	30	2.6 ± 0.1	4.5 ± 0.1	17.6 ± 0.4	5.3 ± 0.1	–	–	–
20	5	2.5 ± 0.1	4.4 ± 0.1	15.0 ± 0.6	6.1 ± 0.2	8.5	52.0	14
20	15	2.6 ± 0.1	5.0 ± 0.1	16.7 ± 0.4	6.8 ± 0.1	8.9	66.3 ± 6.2	15.7 ± 0.7
20	30	2.6 ± 0.1	5.6 ± 0.2	23.6 ± 1.4	8.5 ± 0.1	–	–	–
8	5	2.6 ± 0.1	4.4 ± 0.1	42.0 ± 1.3	5.7 ± 0.1	–	–	–
15	5	2.6 ± 0.1	4.3 ± 0.1	14.9 ± 0.4	4.8 ± 0.1	9.4 ± 0.2	62.4 ± 1.3	12.8 ± 0.2
20	5	2.5 ± 0.1	4.4 ± 0.1	15.0 ± 0.6	6.1 ± 0.2	8.5	52.0	14
25	5	2.7 ± 0.1	4.6 ± 0.1	13.6 ± 0.8	6.1 ± 0.1	6.3 ± 0.2	27.0 ± 3.0	10.5 ± 0.50

hand, lower temperatures result in slower growth. Animals reach maturity in about 2 months at temperatures of 20 °C or higher. At 15 °C, maturity is only reached after twice that time (~ 4 months), but animals are significantly larger. Since size at maturity is directly linked with fecundity (e.g. Mees et al., 1994), larger females at maturity result in a substantially higher number of offspring. Alternatively, when using a temperature lower than 20 °C, it might be advisable to increase salinity to 15 psu to increase the growth performance of *N. integer*. Culturing can also be optimized by feeding mysids at a higher ration. Fockedey (personal observations) found that subadult *N. integer* (5–9 mm) show a growth limitation when fed with less than 200 nauplii mysid^{-1} day^{-1}. The rations given in the individual growth experiments were 1.7 to 6.7 times higher than given to the stock culture and assured a good growth without excessive accumulation of left-over food in the containers.

4.2. Optimizing exposure protocols for N. integer

In the present study, *N. integer* was successfully reared form the first day of release from the brood pouch till adulthood under most of the tested temperature–salinity combinations. Their growth was followed in detail using individually based experiments under steady-state conditions in relatively small vessels of 400 mL. Clutter and Theilacker (1971), Gaudy and Guerin (1979) and Cuzin-Roudy et al. (1981) concluded that it is impossible to study individual growth of mysids using static systems, as the IMP is highly variable and some individuals have indefinitely delayed moults in comparison to others. Constant renewal of the water seems to be a crucial factor for maintaining normal growth and moulting in mysids. Bulk experiments have the disadvantage that detailed information on the IMP and GF cannot be obtained. Recently, Winkler and Greve (2002) published individually based growth data of *N. integer* with a high success rate in a flow-through construction. In our experiments, water was partly (50–80%) renewed daily and the exposure jars were cleaned every 4–5 days. IMP was relatively stable for individuals of the same age within one treatment (standard error generally less than 5% of the mean value).

For a small amount of individuals (8), growth was aberrantly delayed. However, this could always be linked to an injury of the exoskeleton. These animals were regenerating the damaged part over a few moults, but this was associated with a delayed growth. The injury-induced effect on growth has previously been described for euphausiids (Murano et al., 1983; Nicol and Stolp, 1990). The aberrant individuals were not used in further analyses in the present study. Injuries were generally avoided by transferring mysids in a conic measure spoon containing a small volume of water.

4.3. von Bertalanffy growth model

The von Bertalanffy growth model was originally used to describe fish growth, but has been applied to crustaceans and more specifically to mysids (Schnute

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and Fournier, 1980; Cuzin-Roudy et al., 1981; Mees et al., 1994; Fockedey, personal communication). It assumes an asymptotic growth and this sigmoid growth pattern has been confirmed in other studies with mysids (Astthorsson and Ralph, 1984; Winkler and Greve, 2002). Although the model is derived for a single individual, it has mostly been used to model data collected from a group of animals. The growth constant K and asymptotic length L_{inf} vary among individuals in a group, as in most populations of animals for genetic, phenotypic and behavioural reasons (Xiao, 1994). However, as supported by the findings of the present study, growth parameter estimations derived from pooled data were only moderately biased in comparison with individual estimations. More specifically, the L_{inf} was underestimated when using the pooled data (by including the short living animals in the pooled dataset).

Temperature was negatively correlated with L_{inf} and positively correlated with K. The effect of salinity on the different growth parameters was less straightforward and was dependent on the water temperature in the treatment. Highest asymptotic lengths were achieved in the 15 psu/15 °C treatment and in the 5 psu/20 °C treatment. The highest growth rate was found in the 5 psu/15 °C and 15 psu/20 °C treatments.

4.4. Standard length in function of moult number

Contrary to the findings of Astthorsson and Ralph (1984), we found the standard length to be affected by temperature (and salinity) at a specific moult number. Mysids growing at 15 °C had a larger standard length in comparison with the other temperatures from the 3rd moult on. The effect of salinity on the standard length–moult number relation was also significant, however less so than the temperature effect.

4.5. Intermoult period, growth factor and growth rate

The duration of the first intermoult period was equal for all animals in one treatment and lasted 3–4 days after leaving the marsupium in the same night. From the second moult on, there was individual variability on the stage duration and the moults were no longer synchronous between different individuals in one treatment. This effect became more obvious with increasing moult numbers which corroborates observations by Cuzin-Roudy et al. (1981) for the mysid *Siriella armata*. Within a life cycle, when animals become larger and pass through a number of moults, the intermoult period generally increases while the growth factor decreases. Astthorsson and Ralph (1984) and Mauchline (1985) described these growth parameters as logarithmically for *N. integer* and other mysids. Winkler and Greve (2002) found an initial increase and a later decrease of the growth factor from maturity on. The GF in our 8 treatments, on the contrary, was almost constant for the first 5–6 moults before gradually decreasing. The IMP behaved similarly over the first 5 moults. Both GF and IMP responses result in a general faster growth rate than would be expected from a logarithmic response (Mauchline, 1985). For *S. armata* the IMP was constant over the first 10 moults until the reproductive cycle started (Cuzin-Roudy et al., 1981). However, no relation was found in the present study between the variation in IMP/GF and sexual development.

The intermoult period in the experiments was strongly temperature dependent and became smaller at higher temperatures similar to previous studies with mysids (Astthorsson and Ralph, 1984; Winkler and Greve, 2002) and other crustaceans (Nicol and Stolp, 1990). To a lesser degree, we also found the IMP to be salinity dependent, with the shortest IMP at 15 psu. The growth increment (expressed as GF) was affected by temperature, especially at 25 °C where the GF was substantially lower (Hartnoll, 1982). Thus the fast growth at higher temperatures is caused by a higher moulting frequency and not by a higher size increment at higher temperature as reported by Astthorsson and Ralph (1984).

N. integer is described as thermophobic (Arndt and Jansen, 1986) with optimal resistance to salinities higher and lower than its isosmotic point (16–> 20 psu) in the lower temperature range. Temperatures of 20 °C and higher have a negative effect on its respiration, especially in juveniles (Arndt and Jansen, 1986). Largest animals, i.e. with maximal asymptotic length, and low mortality were indeed obtained at 15 °C in comparison with higher temperatures. At 15 °C, the highest growth rate was obtained at 15 psu, being the salinity closest to the isosmotic point. It is however difficult to explain

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why at 20 °C the highest growth rates were observed at 5 psu.

4.6. Size at sexual differentiation and maturity

Contrary to male mysids, it is difficult to determine maturity or the development of secondary sexual characteristics in females by looking at the exoskeleton. In early subadults, it remains hard to distinguish the small marsupium on the living animal while it is swimming around. Therefore, the moult number at the transition from juvenile to subadult and from subadult to adult in females was extrapolated from the information collected for males. This was possible as the von Bertalanffy growth parameters were not significantly different between genders.

Increasing temperature shortens the time to reach sexual differentiation and maturation in general. This was very obvious in animals from the lowest temperature treatment which did not sexually develop during the course of the experiment. A stop in the growth of *N. integer* during the colder winter months was reported previously based on field data (Astthorsson and Ralph, 1984; Arndt and Jansen, 1986; Mees et al., 1994). From experiments with this species at 9 °C (Astthorsson and Ralph, 1984) the duration to maturity (15 mm total length) was extrapolated to be at least 277 days.

Within the range 15–20 °C, salinity seems to have a stronger effect than temperature on sexual maturation. The highest tested salinity of 30 psu retarded the development (in age and moult number) at 20 °C. In both the 30 psu treatments (15 and 20 °C) maturity was never reached. Sexual differentiation was generally reached at a length of 4.3–4.5 mm, except at higher salinities at 20 °C (5.00–5.59 mm). Size at maturity is smaller at higher temperature and lower salinity.

4.7. Interpopulation effects

Optimal salinity–temperature conditions for optimal growth might vary between different populations of the same species for genetic and phenotypic reasons (Lee, 1999). *N. integer* used in the present experiments originated from the Galgenweel, a brackish pond with a relative constant salinity of 5 psu. Population genetic analysis (based on mitochondrial cytochrome oxidase I sequences) revealed no differentiation between *N. integer* from the experimental source population (Galgenweel) and the Schelde estuary population (Remerie, 2005). The results of the present experiments can therefore be considered representative for the *N. integer* population of the Schelde estuary. It is unknown how growth responses as related to temperature–salinity conditions vary between populations from different latitudes (temperature effect). *N. integer* of the Baltic Sea population died within 2.5 weeks when held at 20 °C and showed increased respiration rates. These animals rarely experience temperatures above 15 °C in their natural environment and never for periods exceeding a few days at a time (Laughlin and Lindén, 1983). Kuhlmann (1984) reported an optimal growth at 19–21 °C and 16–20 psu for juvenile *N. integer* from the Kiel Canal. Winkler and Greve (2002), working with *N. integer* collected from the Elbe estuary, reported a faster maturation (110 days at 10 °C; 45 days at 15 °C–20 psu) at a smaller size (standard length 8–9 mm at 10 °C and 7 mm at 15 °C) than our population. In our most comparable treatment (15 °C and 15 psu) to the Winkler and Greve study, adulthood was reached after 89 days at a length of 12 mm. *N. integer* from the Ythan estuary cultured at 16 °C and ± 10 psu (~ 30% seawater) were mature after 188 days at a standard length of 12.9 mm (Astthorsson and Ralph, 1984). This variation might indicate inter-population variation, although other factors (like food quality and quantity, flow regime in tanks, size of the recipients, etc.) might also be at the basis of the variation between experimental results.

4.8. Validation of field-derived growth parameters

To date, field-derived von Bertalanffy growth parameters have rarely been validated with laboratory observations. This can be done by means of comparing the multivariate growth performance index Φ' (Munro and Pauly, 1983). Φ' is expected to be basically equal within different populations of the same species and within different stocks of the same population, but can differ because of pollution, environmental stress or differences in habitat (Moreau et al., 1986). The growth performance index Φ'

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in all our experimental treatments was significantly higher than in all cohorts of the field populations of Schelde and Galgenweel. This is probably the effect of the ad libitum feeding with the high-energy containing *Artemia* nauplii and little energy loss by restricted swimming activity in the static experimental conditions. Abiotic stress, as reflected in the growth performance, was primarily caused by temperature and only secondary by salinity (both in the field and in the experimental treatments). In the highly dynamic estuarine habitat tidal, daily and seasonal variation of these environmental factors may have a great (adverse) effect on the growth of *N. integer*.

5. Conclusions

Based on the present study, it can be concluded that higher temperatures caused a smaller intermoult period in *N. integer*. Temperature also has an effect on the growth factor, especially at 25 °C where suboptimal growth occurred. Salinity had a secondary effect on the growth (IMP and GF) of *N. integer* in comparison to temperature, and was temperature dependent. At 8 °C, *N. integer* grew slow because of long intermoult periods and a relatively low growth factor. At 15 °C, mysids had a larger GF, but also a larger IMP in comparison with 20 °C. Consequently, they took longer to grow, but grew to a larger body length. At 25 °C, animals had the shortest IMP, but also had a significantly lower growth increment at moulting. At 15 °C, the optimal salinity for growth was 15 psu, whereas at 20 °C the shortest IMP and largest GF were found at 5 psu.

Survival and growth of *N. integer* was possible within the tested range of temperatures (8–25 °C) and salinities (5–30 psu), but maturation was only possible in a smaller range of 15–25 °C and 5–15 psu. Within this range, the size at sexual differentiation was constant, but the size at maturity increased with decreasing temperature and increasing salinity.

In comparison with field populations of *N. integer* of the Schelde estuary and Galgenweel the growth performance was higher in all the experimental treatments. Abiotic stress was primarily caused by temperature and only secondary by salinity.

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