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Research Article

COMPARATIVE MORPHOLOGICAL ANALYSIS OF *NEOMYSIS INTEGER* (LEACH, 1815) IN 2006–2007 PERIOD

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ABSTRACT
This paper presents the results of a detailed comparative description of the morphological characters of *N. integer* obtained during eight research seasons in 2006–2007 period from the region stretching from Świnoujście to Darłowo. An attempt was made to assess the suitability of the studied characters for establishing to which populations individuals belong, to identify secondary sex traits and to detect differences in the body shape of these shrimp-like crustaceans. Most of the measurable characters in all of the samples discrimination analysis indicated the characters which differentiated the compared groups were telson length, lower abdomen width, exopodite uropod length, cephalothorax width below the carapace, cephalothorax width above the carapace and the height of fourth and fifth abdominal segment connections.

Key words: *Neomysis integer*, Pomeranian Bay, Baltic Sea, morphometry.

INTRODUCTION

The order Mysidacea (shrimp-like crustaceans) is prevalent in surface waters from the latitudes of 80° N to 80° S, in fresh, brackish, saline, coastal, oceanic, and estuarine waters [Mauchline 1980, Tattersall and Tattersall 1951]. In 1977 Mauchline and Murano [1977] published a list in which they described 765 species from the family Mysidae. Meland [2002] wrote about over 1,000 species which belonged to approximately 160 genera; this number is expected to grow as sampling and taxonomic identification techniques improve and new habitats are discovered. One of the most commonly occurring representatives of Mysidacea of the genus *Neomysis* (Czerniawski) is *Neomysis integer* (Leach, 1815). Chojnacki [1991], Rudstam et al. [1986], and Wiktor [1961] investigated many aspects of this species in the coastal waters of the Baltic.

In 1873 Möbius was the first to observe and describe the characteristics of this species in the Pomeranian Bay [Jażdżewski et al. 2005]. *N. integer* is a euryhaline and eurythermal species [Mess et al. 1994] that usually occurs in high abundance in the waters of estuarine zones [Tattersall and Tattersall 1951, Mess et al. 1994]. It undertakes daily feeding migrations. At night its stomach contents is dominated by detritus, which is the evidence that it feeds near the bottom, while the daytime food composition is dominated by phytoplankton, which indicates it feeds in the water column [Węsławski 1981]. *N. integer* is an omnivorous crustacean that feeds on phytoplankton, zooplankton, and dead organic matter [Fockedey and Mees 1999, Jerling and Wooldridge 1995, Mauchline 1980, Odum and Heald 1972, Tattersall and Tattersall 1951]. Particles of potential food are filtered during swimming, but this species also actively hunts them. It is an important link in the food chain as a consumer of secondary production, but it is also a component of the food base for benthic and pelagic fish as well as of epibenthic crustaceans [Mauchline 1980]. In the coastal waters of the Baltic Sea, this species is a food component of juvenile Platichthyidae, Gobiidae, sea trout, herring, sprats, perch and pikeperch [Lindén et al. 2003, Mauchline 1971, Shvetsova et al. 1992, Szypuła et al. 1997, Węsławski 1981, Wiktor 1961].

The aim of the study was to compile a comprehensive, comparative description of the morphological characters of *N. integer* obtained during eight research seasons in the 2006–2007 period from the coastal zone specifically in estuarine waters from Świnoujścia to Darłowo. Additionally, an attempt was made to assess the suitability of the studied morphological characters of *N. integer* to determine the populations individuals belong to, in order to identify secondary sex characteristics and to detect differences in the body shape of *N integer* from different seasons and regions of southern Baltic.

MATERIALS AND METHODS

Twelve permanent sampling sites distributed in the coastal zone of the Pomeranian Bay in the southern Baltic were designated for the qualitative study of *N. integer* populations. The sites were located adjacent to the estuaries of more important West Pomeranian rivers or, as in the case of the town of Międzyzdroje, in the vicinity of significant submerged constructions, which in this instance was the pier. One site was located near the town of Niechorze when the canal that links Lake Liwia Łuża with the sea was not blocked by sand; if it was blocked there would be two sites – one in the west and one in the east. The locations of the sampling sites are presented in Figure 1.

The study material was collected from the water column at depths of 0.5 to 1 m with a specially constructed drag. The drag netting mesh size was 1 mm. The material collected was rinsed on a sieve with a mesh bar length of 0.5 mm, and then preserved in a 30 % solution of ethyl alcohol denatured with pyridine. The material was sorted, classified into systematic groups, and then identified to the genus, and, when possible, to the species using a Nikon C-DSS230 stereo microscope. Depending on density, the samples containing Mysidacea were diluted in a Folsom splitter [Mc Even et al. 1954] two to four times to obtain a representative sub-sample containing at least 100 individuals. All of the *N. integer* individuals caught were identified using keys compiled by Köhn et al. [1992] and Mańkowski [1955], in both of which the principle qualifying criteria for given species were the build of the telsons and scales.

The *N. integer* material used for further morphological study and analysis numbered 2894 individuals. The sampling sites and sampling seasons were assigned acronyms for statistical analysis and are presented in Tables 1 and 2.

Twenty-three linear measurements were taken of *N. integer* female individuals and 24 of *N. integer* male individuals in each sample according to the methodology presented below, which is a modification and extension of the method by Mauchline [1971]. The measurements were

Fig. 1. Sampling sites in the Pomeranian Bay

Table 1. Sampling site acronyms assigned for tests and statistical analysis

Station	Symbol
Świnoujście west.	Ś.w.
Świnoujście east.	Ś.e.
Międzyzdroje	M.
Dziwnów east.	D.e.
Niechorze	N.
Mrzeżyno west.	M.w.
Mrzeżyno east.	M.e.
Dźwirzyno west.	Dź.w.
Dźwirzyno east.	Dź.e.
Kołobrzeg east.	K.e.
Darłowo west.	Da.w.
Darłowo east.	Da.e.

performed on the crustaceans which had been straightened in a Petri dish containing a small volume of water under a Nikon stereo microscope coupled with a Nikon DS-U2 microscope camera controller. The images were sent from the camera to the computer coupled with it, and then processed using image analysis NIS 32. The scheme of the linear measurements is presented in Figure 2, and the symbols of the studied characters are presented in Table 3.

STATISTICAL ANALYSIS OF RESULTS

All of the measurable characters studied were not only analyzed in terms of differences stemming from sampling site and the sex of individuals, but also with regard to the season in which the samples were collected. Initially, one of the methodological concepts of this study was also to compare individuals according to length classes. However, after an introductory analysis it was determined that samples from each subsequent study season comprised individuals from new generations of *N. integer*. In this case, the further division of samples into length classes would have excessively complicated the resulting data sets later used in the statistical tests.

The criteria described above for dividing results permitted designating 13 comparative groups linked to sampling sites with sub-divisions by sex, and 50 groups according to sampling season. The analyzed material did not always include at least 31 individuals, which would permit it to

Table 3. Symbols assigned for the studied measurable characters and their descriptions

Symbol	Name
X_1	Total length
X_2	Telson length
X_3	Upper teslon width
X_4	Lower telson width
X_5	Lower abdomen width
X_6	Exopodite uropod length
X_7	Endopodite uropod length
X_8	Carapace length
X_9	Carapace width
X_{10}	Cephalothorax width below the carapace
X_{11}	Cephalothorax width above the carapace
X_{12}	Scale length
X_{13}	Distance between eyes
X_{14}	Eye diameter (at the base)
X_{15}	Height of the connection of the last abdominal segment with the telson
X_{16}	Height of the sixth and seventh abdominal segment connection
X_{17}	Height of the sixth and fifth abdominal segment connection
X_{18}	Height of the fifth and fourth abdominal segment connection
X_{19}	Height of the fourth and third abdominal segment connection
X_{20}	Height of the third and second abdominal segment connection
X_{21}	Height of the second and first abdominal segment connection
X_{22}	Anterior carapace height
X_{23}	Posterior carapace height
X_{24}	Length of male copulatory organs

Table 2. Sampling season acronyms assigned for tests and statistical analyses

Season	Symbol	Season	Symbol
Spring 2006 (12–13.05.2006)	Sp2006	Winter – spring 2007 (06–07.03.2007)	WSp2007
Summer 2006 (28–29.06.2006)	S2006	Spring 2007 (24–25.04.2007)	Sp2007
Autumn 2006 (16–17.09.2006)	A2006	Summer 2007 (20–21.06.2007)	S2007
Late autumn 2006 (19–20.10.2006)	LA2006	Autumn 2007 (14–15.09.2007)	A2007

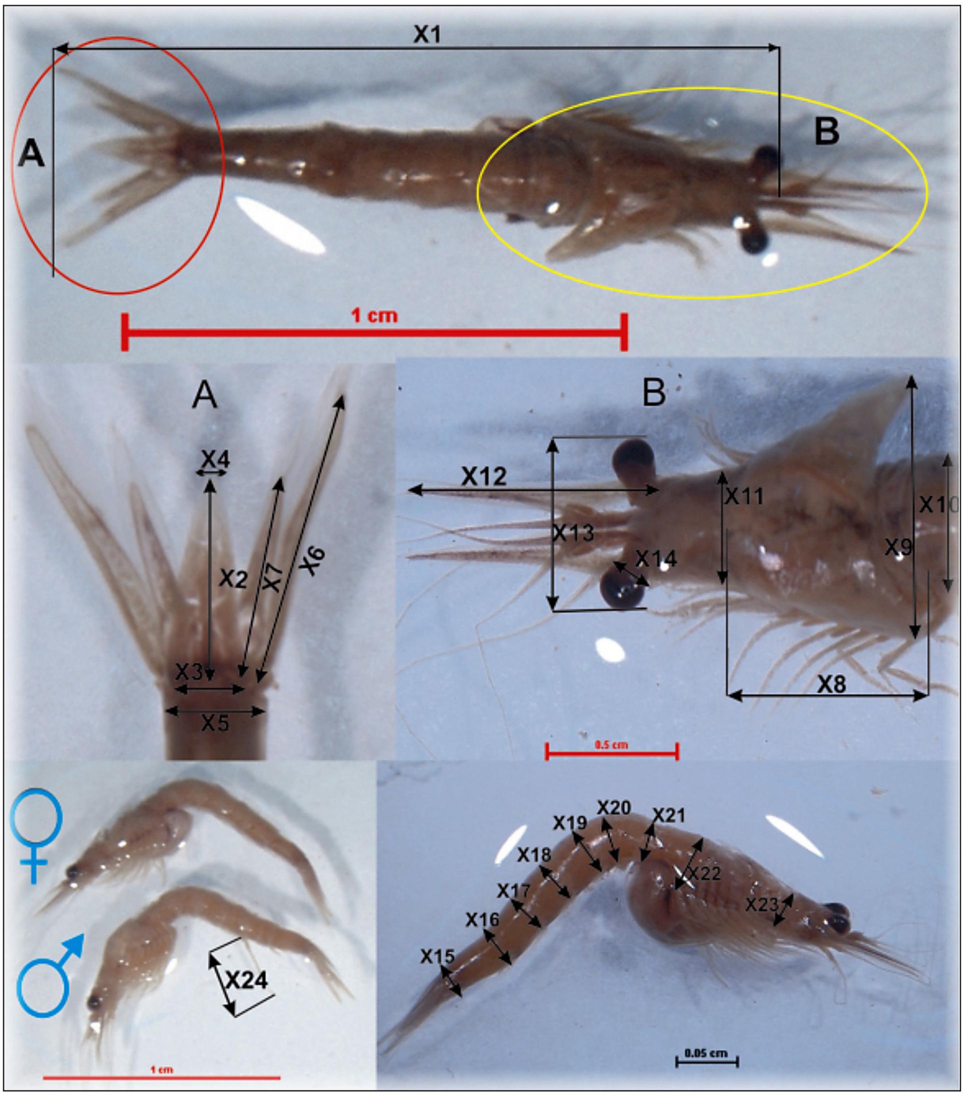

Fig. 2. Scheme of linear measurements taken for the current study

be a statistically large enough sample (n > 30). Thus, the samples were redivided, what permitted obtaining more optimal statistical analysis. Sampling sites where and seasons in which there were fewer than 30 individuals were ignored in further analysis, thus, two additional data sets were created on which all of the statistical analyses were conducted.

The results of the measurable values were processed statistically with Excel 2000, and statistical analysis was done with STATISTICA 5.1. G by StatSoft. The raw measurement results were expressed in absolute values with the aim of using them in further analysis transformed to relative lengths and described as percentage coefficients calculated relative to the total length (x1).

The following were applied in the primary analysis of the results: average value ($\bar{x}$), standard deviation (S), standard error (m), and the coefficient of variation (V). The correlation between total length and body weight was examined. Several different statistical tests were applied to determine if statistically significant differences existed among the *N. integer* groups examined, including the differences between males and females.

The variation coefficient, which is the relative measure of the distribution of a number set, was calculated with the following formula [Ruszczyc 1981]:

$$V = \frac{S \times 100}{\bar{x}} \ (\%)$$

where: V – coefficient of variation,
$\bar{x}$ – arithmetical average,
S – standard deviation,

Ruszczyc [1981] reports the variation coefficient is important for comparisons when its value exceeds 10 %. It is very important that characters with high variation coefficients are not suitable for comparisons aimed at demonstrating the significance of differences. Since there is a lack of studies focusing on the morphometrics of the *N. integer* species, the statistical analysis methodology applied in the present study was based on authors' judgments. The results obtained indicated that *N. integer* body parts grow allometrically, which is a typical of body growth among crustaceans [Kossakowski 1967, De Giosa and Czerniejewski 2010]. This meant that the value of the variation coefficient for the individual characters studied ranged from several to even 70%. This is why the significance of coefficient V for *N. integer* was 20%, which is twice as that in the analysis by Ruszczyc [1981].

The description of variation in the group of individuals based on the analysis of just one or two characters can be incomplete. Some characters in the population can be stable, while others can vary within a wide range depending on the sizes of the individuals examined. It is possible that the characters examined separately do not exhibit significant dependencies, which is why they are usually omitted in a univariate analysis, and only when viewed multi-dimentionally do they acquire significant informational power. A richer picture of variation is obtained when many characters are analyzed simultaneously. This method is known as multivariate analysis, a statistical technique that permits determining the type and range of variation in a set.

In order to confirm whether statistically significant differences occurred among the various characters analyzed in the data sets (all individuals in the samples, from sampling sites where at least 30 individuals had been collected, in seasons in which at least 30 individuals had been collected, and divided according to sex), the results were subjected to analysis of variance (ANOVA/MANOVA), described by the following formula [Sokal and Rohlf 1998]:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where: μ – mean population value
α, β – impact of appropriate factor
$\alpha\beta$ – impact of the interaction of appropriate factors
ε_{ijk} – impact of random factors specific to the k element in a sub-group (i, j)

$$(i = 1, 2, ..., p; j = 1, 2, ..., q; k = 1, 2, ..., m)$$

If the analysis of variance did not detect statistical significance between or among the characters examined, no further tests were applied. However, when the zero hypothesis regarding the equality of means in groups was rejected by analysis of variance, the question arises about which of the compared populations is responsible for the rejection of the zero hypothesis? It is also worthwhile discovering which of the n means differ between one another and which are equal. This is why special post-hoc tests, known as multiple comparison tests, were applied to examine the differences in the means of the various groups more precisely. In this instance Tukey's test was used.

The stepwise discriminant analysis module in STATISTICA was applied to explain morphological differences by detecting the most differentiating characters in the populations studied and to discriminate between the sexes and with regard to seasonal variability among the various groups of *N. integer*. The main aim of these analyses was to determine which variables, or characters, discriminate between two or more naturally emerging groups. In other words, this refers to determining whether groups differ in terms of the mean of a given character, and then utilizing that variable to predict inclusion in that group. The aim of this analysis was to identify characters that are of diagnostic significance, and those that are not important to the emergence of groups, such as secondary sex traits, or changes in body shape that result from the size of the examined individual or the season of the year [Reist 1986].

Hierarchical agglomeration methods (cluster analysis) were used to illustrate possible differences between the mean values of the characters analyzed between sampling sites and seasons and in consideration of gender. This is the geometric distance in multi-dimensional space expressed in dendrograms in which the measure of distance among the analyzed objects is the Euclidean distance. It is calculated as follows:

$$\text{Distance}(x,y) = [\sum_{and} (x_i - y_i)^2]^{½}$$

where: x, y – objects for which distance is calculated

RESULTS

Linear measurements were taken from a total sample of 2894 *N. integer* specimens from eight research seasons. These measurements yielded over 65000 pieces of working data that were used to prepare the overall mean lengths of the analyzed characters at various sites in 2006 and 2007

(Table 4). All of the measurable values were analyzed statistically.

Test of the significance of differences among measurable characters among groups

Comparing the results of two-factor analysis of variance (ANOVA) at particular levels of division of the studied individuals (all individuals in the samples, sites where at least 30 individuals were collected, in seasons in which at least 30 individuals were collected in the samples) led to the observation that the number of characters differentiating the groups lessened with more precise divisions. The smallest number of differences was noted in study seasons divided by sex. This division system confirmed the method for comparing characters which was based on identifying precisely which segment of the studied population is responsible for the noted differences.

The results of the two-factor analysis of variance indicated there was no character that did not differ significantly statistically from all the individuals from the 13 sites; however, one of these characters, namely lower abdomen width (x5), had a very low coefficient of variation value. The post-hoc test also indicated that scale length (x12) differed decidedly among the following sites: Ś.w., Ś.e., M. and M.w., M.e., Dź.w., Dź.e., K.e., while at site N.e. there was no difference in the values of this character in relation to other sites. The values of the exopodite uropod length (x6) were similar at virtually all sites, and they differed decidedly only between sites D.e. and M.w.

After excluding sites with abundances of fewer than 30 individuals, it was noted that all of the characters, with the exception of distance between the eyes (x13), still differed significantly statistically, including character x5, which had a very low coefficient of variation value. Considering the results of the post-hoc test for scale length (x12), differences among the Ś.w., Ś.e., M and Dź.e., K.e, Da.e. sites were noted as well as among the M., D.e. and M.w., M.e., Dź.w. sites.

Table 4. Mean lengths (mm) of the studied characters of *N. integer* at various sampling sites in 2006 and 2007

Character	Station												
	Ś.w.	Ś.e.	M.	D.e.	N.e.	N.	M.w.	M.e.	Dź.w.	Dź.e.	K.e.	Da.w.	Da.e.
x_1	9.80	8.49	6.95	8.06	9.91	8.72	10.89	8.84	11.47	11.80	10.82	6.30	13.81
x_2	1.21	1.04	0.84	0.98	1.29	1.03	1.30	1.09	1.40	1.40	1.30	0.83	1.69
x_3	0.46	0.42	0.35	0.41	0.38	0.39	0.53	0.43	0.55	0.60	0.49	0.34	0.75
x_4	0.12	0.09	0.07	0.09	0.11	0.09	0.12	0.09	0.12	0.12	0.11	0.06	0.14
x_5	0.66	0.55	0.46	0.53	0.58	0.57	0.70	0.58	0.74	0.78	0.72	0.43	0.89
x_6	1.88	1.59	1.34	1.55	1.79	1.68	2.00	1.66	2.25	2.26	2.19	1.24	2.63
x_7	1.36	1.16	0.99	1.13	1.45	1.22	1.45	1.20	1.67	1.61	1.59	0.93	1.89
x_8	1.73	1.53	1.26	1.49	1.83	1.54	1.85	1.62	1.96	1.95	1.90	1.19	2.44
x_9	1.44	1.30	1.04	1.25	1.35	1.25	1.48	1.36	1.46	1.61	1.40	1.06	2.08
x_{10}	1.05	0.88	0.75	0.85	1.08	0.93	1.05	0.93	1.12	1.22	1.08	0.71	1.44
x_{11}	0.93	0.82	0.69	0.79	0.84	0.82	0.97	0.83	1.08	1.14	0.98	0.66	1.36
x_{12}	1.03	1.10	1.20	1.12	0.75	1.12	1.31	0.96	1.49	1.92	1.65	1.07	2.41
x_{13}	1.15	1.01	0.81	0.98	1.05	1.04	1.24	1.03	1.25	1.27	1.14	0.66	1.64
x_{14}	0.35	0.32	0.27	0.32	0.37	0.33	0.39	0.32	0.39	0.44	0.37	0.25	0.52
x_{15}	0.55	0.45	0.38	0.43	0.58	0.49	0.56	0.46	0.63	0.64	0.63	0.36	0.74
x_{16}	0.69	0.57	0.50	0.56	0.70	0.62	0.71	0.60	0.75	0.83	0.75	0.44	0.95
x_{17}	0.77	0.64	0.55	0.62	0.71	0.68	0.79	0.67	0.83	0.93	0.82	0.52	1.06
x_{18}	0.82	0.69	0.58	0.66	0.82	0.71	0.85	0.72	0.88	1.00	0.85	0.55	1.13
x_{19}	0.87	0.74	0.62	0.70	0.87	0.77	0.90	0.76	0.95	1.06	0.92	0.58	1.19
x_{20}	0.87	0.74	0.62	0.70	0.85	0.74	0.93	0.76	0.96	1.07	0.90	0.59	1.20
x_{21}	0.78	0.65	0.54	0.62	0.65	0.65	0.82	0.68	0.85	0.92	0.68	0.51	1.06
x_{22}	0.92	0.87	0.77	0.85	0.74	0.84	1.10	0.86	0.99	1.13	1.02	0.71	1.44
x_{23}	0.63	0.55	0.46	0.50	0.50	0.52	0.73	0.54	0.63	0.72	0.66	0.43	0.89
x_{24}	1.89	1.51	1.20	1.49	1.97	1.55	1.96	1.63	2.05	2.07	1.91	1.61	2.23

The lack of differences for character x13 confirmed during the analysis of variance is an excellent illustration of the post-hoc test.

In groups only divided by season with more than 30 individuals in the sample, statistically significant differences were again noted for all of the characters examined. The post-hoc test indicated there were no differences in scale length (character x12) among sites M., D.e. and Dź.e., K.e, Da.e. However, differences in carapace height (x23) occurred between site M.w. and sites M.e., Dź.w., Dź.e.

The ANOVA test conducted between sexes, excluding juvenile stages, for individuals from all the sites permitted concluding that among all the characters compared only five did not exhibit statistically significant differences (x8, x10, x18, x20, x23).

Test for significant differences of measurable characters among groups – females

The results of two-factor analysis of variance indicated a lack of statistically significant characters among females from the 13 sites. Only one character, lower abdomen width (x5), had a very low coefficient of variation value, which was identical for all the individuals in the sample.

After excluding the sampling sites with fewer than 30 individuals per sample, all of the characters still differed significantly statistically, including character x5, which had a very low coefficient of variation value.

In groups that excluded season and with samples numbering more than 30 *N. integer* individuals, again statistically significant differences were noted for all characters examined. The post-hoc test indicated a lack of differences in carapace width (character x9) among sites Ś.w., Ś.e., M. and a high similarity in the values of this character at sites Ś.w. and Da.e. in comparison with the other sites. No regularities describing the differences in the values of character x10 (cephalothorax width below the carapace) at the various sites were noted.

Test for significant differences of measurable characters among groups – males

The results of two-factor analysis of variance indicated that when only males were considered, as was the case with females, no characters were confirmed to be statistically significantly different in the 13 sampling sites. Three characters (x2, x5, x10) had very low coefficient of variation values. The distribution of the values of the characters examined at the various sites exhibited considerable similarity to other sites for character x12 (scale length), particularly for N.e. and N and, to a lesser degree for Da.w. The value of character x13 (distance between the eyes) was virtually similar at all of the sites studied.

After excluding the sampling sites with fewer than 30 individuals per sample, it was noted that among nine sites only two characters (x13 and x15) did not differ statistically significantly. Similarly to the previous case, for character x12 (scale length) highlighted sites Ś.e. and M.w., while the values of character x13 (distance between the eyes) were similar at all sites.

After excluding season from groups with more than 30 *N. integer* individuals in the sample it was observed that, with the exception of characters x7 (endopodite uropod length) and x23 (posterior carapace height), all of the remaining characters exhibited significant differences. In this data group, as many as five characters had very low coefficient of variation values. With regard to the post-hoc test for character x12 (scale length), two sites were similar – Ś.w. and Ś.e. and M. and D.e. Character x23 is a typical example of values that were very similar in all the sampling sites.

Test for significant differences of measurable characters among seasons

The results of two-factor analysis of variance indicated that among the eight study seasons compared only one character (x21 – height of the first and second abdominal segment connection) did not differ significantly statistically. The post-hoc test, however, indicated that the values for individual characters were highly variable depending on season. In the case of character x11 (cephalothorax width above the carapace) the values were practically unchanged among seasons Sp2006, S2007, A2007, LA2006, while the values from WSp and Sp 2007 were decidedly different from those in the other seasons. The heights of the first and second abdominal segment connection (x21) were virtually unchanged in all seasons.

Conducting the ANOVA test between the sexes, excluding juvenile stages, for all individuals in all seasons permitted determining that of all the characters compared only six did not exhibit statistically significant differences.

The ANOVA test performed in the group of males for all seasons indicated a lack of characters that did not exhibit statistically significant differences. However, after analyzing these same dependencies, but only for males, two characters (x7 and x21) did not differ significantly statistically. The post-hoc test performed for this group indicated high variation in the values of the studied characters in different seasons. A typical example of this variation was character x3 (upper teslon width). Even character x7, which did not exhibit high variability, differed substantially among some seasons.

Results of discriminant function analysis

The analysis of results following the application of the discriminant function indicated that their accuracy for the classification matrix was within the range of 38–70%. It is worth considering whether accuracy of approximately 39% is sufficient for conducting further analysis; however, in this instance many of the characters had coefficients of variation above 10, and this impacted the results of the classification. Because of the program limitations of the function applied, the result obtained did not permit excluding these characters from the final analysis, and this is why they are identified as the most differentiating of various groups. The coefficient of variation value for character x12 (scale length) was in excess of 35%. After excluding those with coefficients V>20m, the greatest share in the studied groups identified were:

- comparisons of sampling sites:
  x3 – Upper teslon width
  x5 – Lower abdomen width
  x10 – Cephalothorax width below the carapace
  x18 – Height of the fifth and fourth abdominal segment connection
  x24 – Length of male copulatory
- comparisons of sampling sites by sex:
  x3 – Upper teslon width
  x7 – Endopodite uropod length
  x10 – Cephalothorax width below the carapace
- comparisons of sampling sites by sex for females:
  x8 – Carapace length
  x15 – Height of the connection of the last abdominal segment with the telson
- comparisons of sampling sites by sex for males:
  x2 – Telson length
  x18 – Height of the fifth and fourth abdominal segment connection
- comparisons of study seasons, including by sex:
  x4 – Lower telson width
  x5 – Lower abdomen width
  x10 – Cephalothorax width below the carapace
- comparisons of sex:
  x2 – Telson length
  x3 – Upper teslon width
  x10 – Cephalothorax width below the carapace
  x11 – Cephalothorax width above the carapace
  x13 – Distance between eyes
  x14 – Eye diameter (at the base)
  x21 – Height of the second and first abdominal segment connection

After the comparative analysis of differences obtained using this test, it can be concluded with high probability that width above the carapace and the distance between the eyes are secondary sex traits of *Neomysis integer*, while upper telson width and cephalothorax width below the carapace might also be secondary sex traits as well as characteristic traits of populations.

Cluster analysis

The analysis of measurable characters by sampling site, taking into consideration all of the individuals in the sample, indicated the sites that were most similar included Niechorze, eastern Dziwnów and western Świnoujście (Figure 3). After dividing the groups by season, three groups had the highest levels of similarity. The groups of eastern Kołobrzeg Sp2007, eastern Darłowo WSp2007, eastern Dźwirzyno Sp2007, eastern Dziwnów Sp2007, Międzyzdroje Sp2007, and eastern Świnoujście S2007 are characterized by similar Euclidean distances as the groups of western Mrzeżyno WSp2007, western Darłowo WSp2007, eastern Mrzeżyno Sp2007, eastern Mrzeżyno WSp2007, eastern Dziwnów Sp2007, Międzyzdroje WSp2007, and eastern Świnoujście WSp2007. The third group that was much farther away comprised western Dźwirzyno Sp2006, eastern Mrzeżyno Sp2006, eastern Świnoujście A2007, eastern Mrzeżyno F2006, eastern Dziwnów A2007, and western Świnoujście A2007, but their components were associated with the same degree of strength. The comparison of the

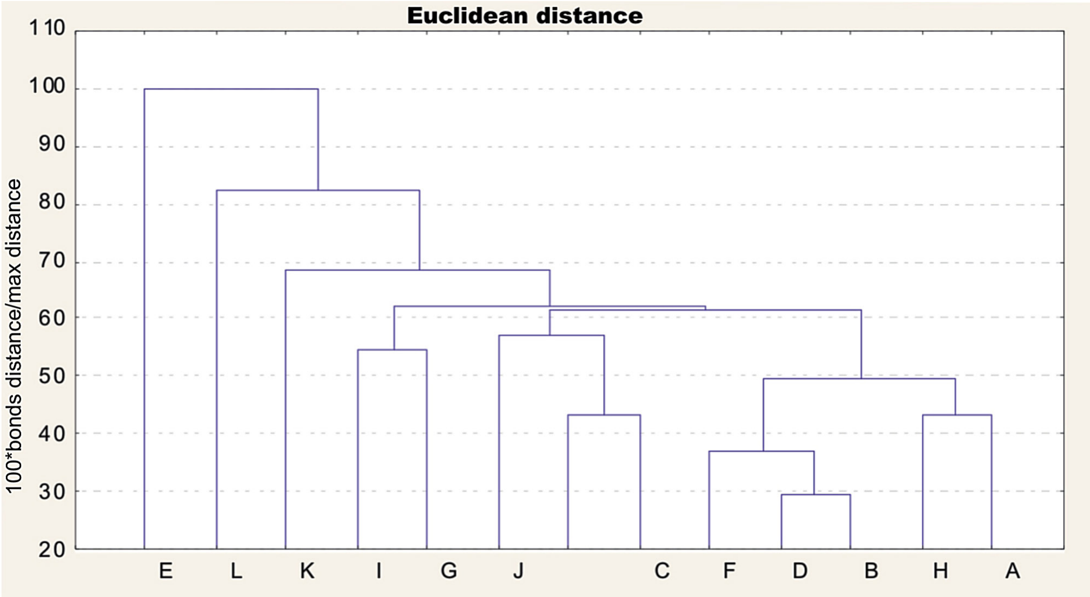

Fig. 3. Euclidean distance diagram based on relative dependencies of 23 measurable characters among different *N. integer* sampling sites

sampling sites together, with division of samples by sex, indicated that the strongest similarity between males and females occurred at eastern Dziwnów site and among males in Niechorze site. The same sites with regard to just females formed two very similar groups: eastern Darłowo and Międzyzdroje and eastern Dziwnów and eastern Świnoujście. However, males were most similar at the Niechorze, eastern Dziwnów, and eastern Mrzeżyno and eastern Świnoujście sites.

The analysis of the results obtained among study seasons for all individuals confirmed interesting similarities among seasons S2006 and A2007 and A2006, and also between seasons S2007 and LA2006. After dividing the samples by sex, it was unequivocally confirmed high degrees of similarity among individuals of the different sex in the same season with a simultaneously strong association with the seasons of WSp, Sp, and S in 2007. Given the similarity of the particular body measurements only among females, the most distinguished seasons were WSp and Sp in 2007, and among males the summers of 2006 and 2007.

DISCUSSION

As has already been noted several times, all of the comparative analyses among the populations examined in the present study were performed not only with consideration to the catch group as a whole, but also with sub-divisions by season and sex. The intention for the comparative analysis criteria chosen for the present work was to identify differences that could stem from the length of the specimens in the studied samples as well as those influenced by geographic and environmental variations. It appeared that the majority of results indicated the existence of differences among the groups compared in virtually every analysis.

The best diagnostic characters in systematic are those that are most stable throughout growth. In the case of the results presented in this paper, large quantity of statistically different characters was impacted by high variation coefficient, which was in turn impacted by two factors: small number of individuals in the samples and wide size range of individuals in numerous samples. This is why characters with high coefficients of variation (V) were omitted from further considerations and comparisons. The data obtained in this manner were analyzed thoroughly.

As mentioned previously, the results obtained through analysis of variance indicated a vast number of differences in characters for sex, season, and among sites. Not until performing discrimination analysis among the studied groups was it possible to establish that the characters which were decidedly different among the groups compared were as follows: x2, x5, x6, x10, x11, x18 (telson length, lower abdomen width, exopodite uropod length, cephalothorax width below the carapace, cephalothorax width above the carapace, height of the fourth and fifth abdominal segment connections). The causes of these differences depended on several unrelated factors; however, it was not possible to detect or demonstrate decided differences among populations. Thus, the hypothesis proposed for this study was not confirmed.

Comparative data regarding variation in the measurable characters of different *N. integer* populations are not, in principal, available in the literature, and those data that do exist were obtained based on studies of small sample numbers or on those comprising exclusively females with full brood pouches [Remerie et al. 2005]; therefore, the analyses of the causes and effects of the resulting differences were based largely on the results obtained from the authors' own research.

Studies to date on the species *N. integer* have not discussed differences in body structure between the sexes, which is why the finding from the current authors' research of the difference in the height of the segment in males from which the copulatory organ emerges (x18) is very interesting. In sexually mature male *N. integer* individuals this segment is larger, and this is probably because of the copulatory organ that, as an additional, highly mobile morphological element of structure of this segment, automatically increases it volume.

Differences in character x10, cephalothorax width below the carapace, is indirectly linked to sex. In large, sexually mature females measuring more than 11 mm, this character assumes much higher values than those noted in males and juvenile females. The probable cause of this dependence is the occurrence of the brood pouch, or marsupium, in this location during maturation, which means that this element is heavier in comparison to those of other individuals. Simultaneously, it is also a character that indicates differences between the sexes, but it also serves to identify to which generation of a year the individual belongs.

Another character, the value of which is linked strictly with size of the examined individual, and, thus, also of the generation to which the animal belongs, is the cephalothorax width above the carapace (x11). The result of analysis suggested that a juvenile *N. integer* individual does not yet have a fully formed cephalothorax, which means that it has not yet assumed the characteristic teardrop shape, but still remains rectangular. Because of this, the percentage of cephalothorax width above the carapace is higher in juvenile individuals, but the value of this character decreases along with body growth and subsequent values are lower.

The analysis of the causes of differences in telson length, lower abdomen width, and exopodite uropod length in the groups compared is exceptionally interesting. It is most probable that the values assumed by these characters are strictly correlated. Observations of *N. integer* movements suggest that these three body parts are used for locomotion in this crustacean, since their various positioning results in, among other things, change in movement direction or the maintenance of holding certain positions, or equilibrium, in the water column [Grabda 1985]. Further, based on analogies with fish, it is likely that individuals are required to perform different types of swimming feats depending on water movements, thus, these body parts will develop to differing degrees accordingly. For example, individuals that are long-term inhabitants of swiftly moving waters will have muscular abdominal cores (lower abdomen width). Changes in the direction of movement can be effected by a relatively short telson with simultaneously longer exopodite uropods. Thus, these are typical differences linked to phenotype variation that is influenced by environmental conditions (density, viscosity, temperature, etc). While concrete information regarding crustacean biology is lacking in the literature, this topic is described widely as mentioned above in the ichthyological literature [Riddel and Leggett 1981, Swain and Holtby 1989]. Phenotype variation among populations can occur under the influence of various factors including genetic changes and the environment. The combined impact of these factors are responsible for differences identified among populations of this same species in various biotopes. Kinnison et al. [1998] compared two New Zealand populations of Chinook salmon, *Oncorhynchus tshawytscha*, introduced to New Zealand in 1900 from the American source population of this species in California. Presently, significant phenotypic variation is noted between the populations, and is expressed through four plastic characters: back hump depth, fin length, snout length, and caudal peduncle length. In a study of two forms of brook trout, *Salvelinus fontinalis*, Dynes et al. [1999] conclude that in comparison to the littoral form, the pelagic form has shorter pectoral and dorsal fins and a shorter, higher caudal peduncle. These morphological differences are attributed by the authors to differing sources of food exploited by these two forms of brook trout and the occurrence of specializations for different environments. Dynes and co-authors suggest that longer pre-dorsal length and shorter dorsal fin enable these fish to cruise better the pelagic waters in search of widely distributed mobile prey, while longer pectoral fins of the littoral form permit it to manoeuvre more slowly, but more precisely while searching for food and feeding in the benthic zone. Similar dependencies between pectoral fin length and food consumption have been noted in other fish species such as Arctic charr, *Salvelinus alpinus* [Malmquist 1992, cited in Dynes et al. 1999] and common sunfish, *Lepomis gibbosus* [Ehlinger 1990, cited in Dynes et al. 1999]. According to Robinson et al. [1993], environment is the main factor shaping individual forms, as is expressed in specific local adaptations, including spatial distribution and feeding habits, to various biotopes. In addition to adapting to various habitats, another possibility for the occurrence of morphological differences in fish is species phenotypic plasticity, which can modify their development depending on the environment [Swain et al. 1991]. Gąsowska [1973] studied vendace and suggests that each lake inhabited by this species has its own form of this fish. Vendace from two similar, but geographically distant, lakes exhibited greater similarity with regard to certain characters, than did the fish from closely situated lakes in which environmental conditions differed. Kozikowska [1961] concludes that plastic characters are more susceptible to environmental impact, while meristic characters are more resistant and exhibit higher degrees of stability, thus providing more information on the genotypes described by them.

The long-term studies of fish discussed above provide a good explanation of why differences were noted at the same sites in different seasons; for example, the spring generation was probably brought into the eastern Dziwnów site with river waters, while the subsequent generation at the site either swam in from the open sea zone or had already occurred at the site after the changes caused by the dynamics of the water masses. It also seems that character x5 (lower abdomen width) could have been strictly dependent on the condition of the crustaceans examined, and consequently on the environmental conditions shaping food availability.

As it has been mentioned above, unfortunately only one study found in the literature focuses on a similar topic regarding *N. integer*, but the authors, Remerie et al. [2005], conducted their research on a much smaller scale than that presented in the current paper. Following statistical analysis (i.e., discrimination analysis), the authors designated four characters that are particular to the studied population – eye diameter at the base, corneal length, upper telson width, and telson tail segment length. The results of these authors do not correspond with those of the present study even if upper telson length and eye diameter at the base (only for the sexes) were also designated by discrimination analyses in the present study. These results were omitted from further analyses because of their relatively high coefficients of variation (10-20%). Despite a lack of congruous studies, Remerie et al. [2005] underscore the facts that the specifics of body build in this species strictly depends on the environmental conditions different *N. integer* populations inhabit.

The range of minute differences in the individual populations is evidence of the high degree of plasticity in the body proportions of *N. integer*, which are even more pronounced when particular generations are compared. With such pronounced distribution of the values of various characters, it is difficult to designate those that would indicate which population we are dealing with. Cluster analysis, or dendrograms, confirm this fact indirectly. None of the dendrograms indicate unequivocally that any of the populations examined differ distinctly from others. The data obtained during the present study suggest that physiological differences are more important than morphometric differences.

CONCLUSIONS

The analysis of the material presented permitted formulating the following conclusions:

- Most of the measurable characters in all of the samples compares exhibited allometric growth, and the application of the ANOVA test indicated that most characters were statistically significantly different in all of the groups compared. The high variation coefficient automatically excluded them from further analysis.
- Modifying the results and applying discrimination analysis indicated the characters which differentiated the compared groups were telson length, lower abdomen width, exopodite uropod length, cephalothorax width below

the carapace, cephalothorax width above the carapace, and height of the fourth and fifth abdominal segment connections.

- Sexually mature males had higher fourth abdominal segments, which can be considered to be a secondary sex characteristic, which is typical of *N. integer* in the Pomeranian Bay.
- The appearance of the marsupium, or brood pouch, beneath the carapace during female sexual maturation meant that this element of the body structure was more massive than those in males or juvenile females.
- Juvenile *N. integer* individuals had partially developed cephalothoraxes, which meant that they had not yet assumed the characteristic teardrop shape, but were still rectangular. Because of this, the percentage of the width above the carapace was greater in juveniles, but it decreased as the bodies grew.
- The differences between telson length, lower abdomen width, and the exopodite uropod length in the groups compared was probably linked to the varied locomotion capabilities of the populations examined. These are typical differences in phenotype variation, upon which environmental conditions have a decisive impact.
- It is likely that the occurrence in the samples of individuals from different generations caused the problem of high and very high coefficients of differentiation among practically all of the characters analyzed. This suggests that the biometric analysis should be done in *N. integer* length classes.

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