Seasonal variation in populations of *Entzia macrescens* (Brady) from a salt marsh in Transylvania, Romania

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Abstract

Sediment samples were collected from a small salt marsh near Turda, Romania, in order to assess the seasonal variability and interpret the life cycle of the agglutinated foraminiferan *Entzia macrescens*. Rose Bengal stained samples were collected on a monthly basis to document variability in the population. Population dynamics, test diameter, proloculus diameter, number of chambers, and the ratio between live and dead specimens were determined. The largest specimens were found in December when the megalospheric generation is assumed to be present; smallest specimens appeared in April-May when sexual reproduction (the microspheric generation) is presumed. The proportion of living specimens is highest in January and August. The studied site is until now the only place in Romania where a living *Entzia* population has been found, and the area is under threat from development.

INTRODUCTION

Daday (1884) first described the species *Entzia tetrastomella* from a salt marsh in the city of Deva in Transylvania. Today the marsh at Deva no longer exists because a health clinic has been built on the locality. Filipescu & Kaminski (2011) visited a number of salt marshes in Transylvania (Ocna Sibiului, Cojocna, and Turda) in an attempt to find living specimens of *Entzia*, but most localities have been altered by human development. The sources of salt water that naturally occur in the Transylvanian Basin are commercially exploited in many places, including in Turda, where a salt-water swimming pool has been built just opposite our sample locality. The marsh in Turda is the only place in Transylvania where a living population of *Entzia* has been found since Daday's time.

The majority of the foraminiferal workers currently use the name *Jadammina macrescens* to describe specimens from saline lakes and salt marshes around the world (e.g., Wilson *et al.,* 2008). Filipescu & Kaminski (2011) regarded *Jadammina* to be a junior synonym of *Entzia*, based on morphological grounds and priority. A molecular investigation of *Entzia* based on partial SSU rDNA sequences by Holzmann *et al.* (2012) revealed that specimens of *Entzia* are genetically more or less identical to *Jadammina macrescens* sampled from Dovey Estuary in England, which is near the type locality of *Trochammina macrescens* Brady, 1870. The molecular results therefore confirm previous morphological investigations suggesting synonymy of the two genera. Therefore, the correct combination of the scientific name of this species is *"Entzia macrescens* (Brady, 1870)".

The main propose of this study is to document the life cycle of *Entzia* in the Turda salt marsh. The salt marsh was sampled monthly over the period of one year in order to document the seasonal changes in the *Entzia* population and better understand its reproductive cycle.

Sample Locality

The Transylvanian Basin contains numerous salty springs and ponds that are associated with the Miocene salt deposits. One of the popular tourist attractions is the Maria Theresa Salt Mine in the town of Turda, situated about 30 km from Cluj-Napoca. The samples collected for this study are from a small salt marsh on the outskirts of Turda, located 3 km from the Turda Salt Mine and opposite the public salt-water swimming pool (Fig. 1). At present, the living salt marsh at this locality in Turda is about two square meters in area (Fig. 2). The marsh is populated by a specific flora composed by halophyte species such as *Salicornia her-*

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Figure 1. Location of the salt-water swimming pool (Băile Sărate) in Turda, with arrow pointing to the location of the salt marsh opposite.

Figure 2. View of the sample locality from the adjoining road.

Festuca sulcata, Carex humilis and *Stipa* spp. *Entzia macrescens* specimens were found among the plant roots and in the upper 2 cm of mud. It is the only foraminiferal species present at this locality.

METHODS

The Turda salt marsh was sampled on a monthly basis for the period of one year beginning with October 2010 until September 2011. The samples were collected with a tablespoon; the surficial layer of mud to a depth of 1–2 cm was sampled. The total volume of sediment was measured using a small metal box (50 cm^3). Samples were preserved in a mixture of Vodka and Rose Bengal in order to distinguish the living and the dead specimens. Vodka was chosen because it is cheap and readily available at the local shop. The samples were kept at the room temperature for 24 hours and washed through a 63 µm sieve. The volume of sediment examined was not standardised, and a total of 350 specimens from each monthly sample were counted. *Entzia* specimens from each sample were picked and glued into cardboard slides, and these were used for measuring the test diameter, counting the number of chambers, and determining the proportion of live and dead specimens. The proloculus diameter was measured using an optical micrometer on 100 randomly-selected specimens in each sample.

RESULTS

Environmental Changes in the salt marsh in Turda

The most important environmental factor is the salinity, which varied between 3.3‰ and 43.3‰ over the span of a year (Table 1). During the summer the salinity shows moderate values (17.5‰ and 35.5‰). The winter season begins with low salinity values of 8.6‰ and 6.3‰, but in January when the marsh was frozen, the values display a marked change, with the highest value recorded during the year (43.3‰). Other parameters such as pH, percent carbonate and humus do not display major variations during the year.

Living/dead ratio

The monthly frequency of live and dead specimens is shown in Figure 3. Living *Entzia macrescens* has a test that stains deep red with Rose Bengal, whereas specimens that are recorded as dead usually have white tests. *Entzia macrescens* constructs a thin test that quickly becomes fragile and disintegrates upon death of the individual. The early chambers of the test have a naked test wall, with agglutinated grains appearing only along the stutures – these chambers are delicate and collapse upon drying (Plate 1). In January and August the highest discrepancy between the proportions of live and dead specimens is observed, suggesting that taphonomic processes may be accelerated during winter when the marsh is frozen, as well as during the summer. The dead specimens are more abundant in spring, from April to June. Our results suggest that the live individuals present in January and August die rapidly, since a greater proportion of dead tests is observed the following month, i.e., February and September.

Table 1. Environmental parameters measured in the Turda salt marsh.

Month	pH	TDS (g/l)	S (‰)	EC (ms/cm)	Carbonate (%)	Humus (%)
October	8.58	3.25	3.5	6.5	3–5	4
November	7.8	7.3	8.6	14.6	3–5	4
December	8.2	5.5	6.3	11.02	3–5	4
January	7.6	31	43.3	63.7	>5	4
February	8.02	3.05	3.3	6.1	<1	4
March	8.5	6.5	9	12.45	3–5	4
April	9.78	9.56	11	18.39	3–5	4
May	9.66	10.23	11.8	19.68	<1	4
June	7.7	1.33	17.5	25.7	3–5	3
July	7.85	1.21	14.6	24.2	>5	3
August	7.28	2.64	35.3	5.34	<1	3.5
September	7.52	1.72	21.5	3.44	3–5	3.5

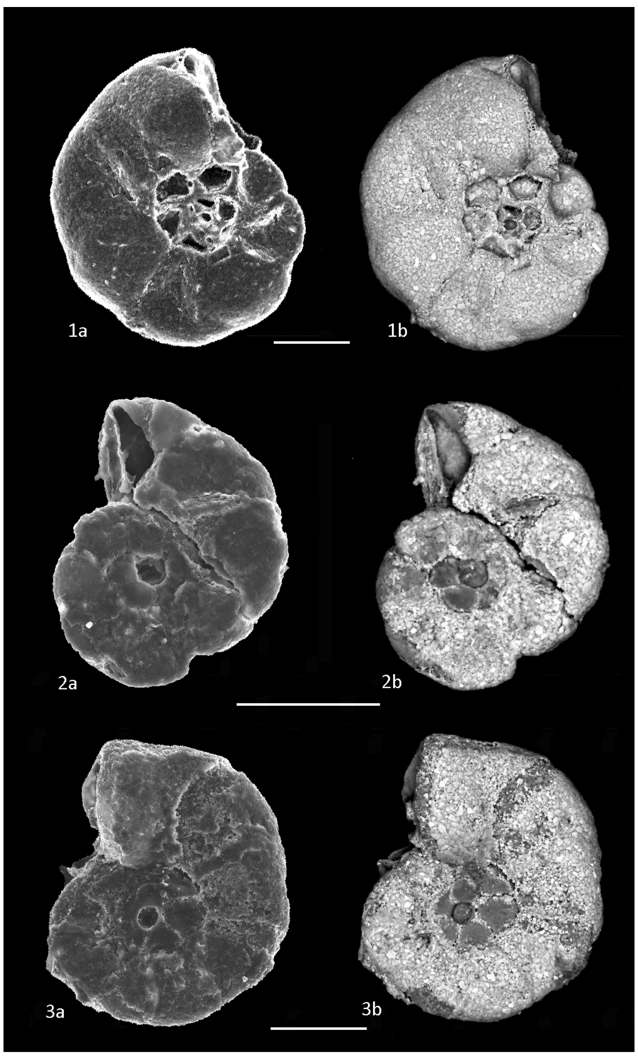

Plate 1. Representative specimens of *Entzia macrescens* from the Turda salt marsh. (a) SEM images (b) backscattered electron images, showing the presence of the agglutinated grains only on the younger chambers. Early chambers of the test have a naked chamber wall with few agglutinated grains appearing only along the sutures.

Test diameter and the number of chambers

The monthly change of the test diameter of *Entzia macrescens* is shown in Fig. 4 in the form of frequency distributions. In the living population, the frequency of adult individuals (>300 µm) is highest in late autumn and early winter (November–February) and again in during the summer (July–September). The mean test diameter of the living population varies from a minimum of about 275 µm in October to a maximum of about 340 µm in May–July. The frequencies of juvenile specimens were relatively high in early autumn (October), when the frequency distribution of living individuals is skewed toward smaller size classes. Later in the autumn the size measurements appear to be more normally distributed. The average diameter of the living individuals is lower in January and in April at about 310 µm. In February, the mean test diameter of the living population is lower than that of the dead assemblage. During the spring period of growth, few small specimens appeared and the mean test diameter grew larger, about 340 µm. This period can be considered to be the growing season.

A similar pattern is observed with the number of chambers (Fig. 5). The population in March–April contains a high number of individuals possessing fewer than 15 chambers (with a mean value of about 9 chambers). From May to June the average number of chambers increased to >10. Juveniles again dominate the population during the month of September, when the average value is again below 10. During the autumn an increase is observed in the average number of chambers among the living population. The highest numbers of living individuals with >20 chambers were found in November–January. In February most of largest individuals (>20 chambers) were found dead.

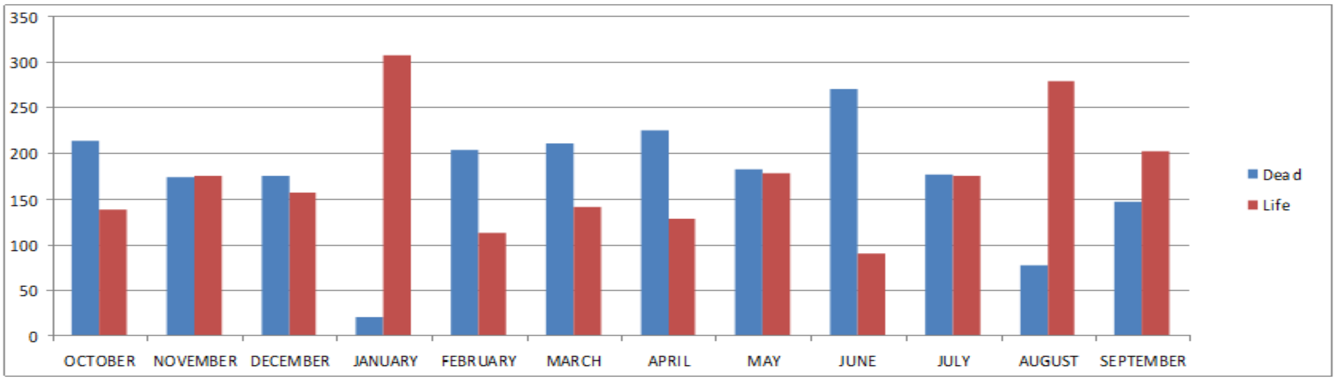

Figure 3. Monthly variability in the proportions of living (red) vs. dead (blue) specimens.

Proloculus diameter

The monthly variations in proloculus diameter measured on 100 specimens is given in Figure 6. The specimens display a wide range in the diameter of the proloculus, from a minimum of 11 µm, to a maximum of nearly 50 µm. The smallest mean proloculus diameter in the living population is observed in January, at 16.5 µm. These measurements show a unimodal distribution. Specimens with larger proloculus diameter begin to appear in April–May, when living individuals with a proloculus diameter around 30 µm are observed. The largest mean proloculus size is observed in July, at 19.7 µm. Throughout the summer and autumn, the distribution of size measurements appears to be distinctly bimodal, with a small number of specimens showing proloculus diameters in the 20-30 µm range. This suggests that megalospheric individuals are present in the population during the summer and autumn. The measured proloculus diameters of living and dead assemblages are very similar.

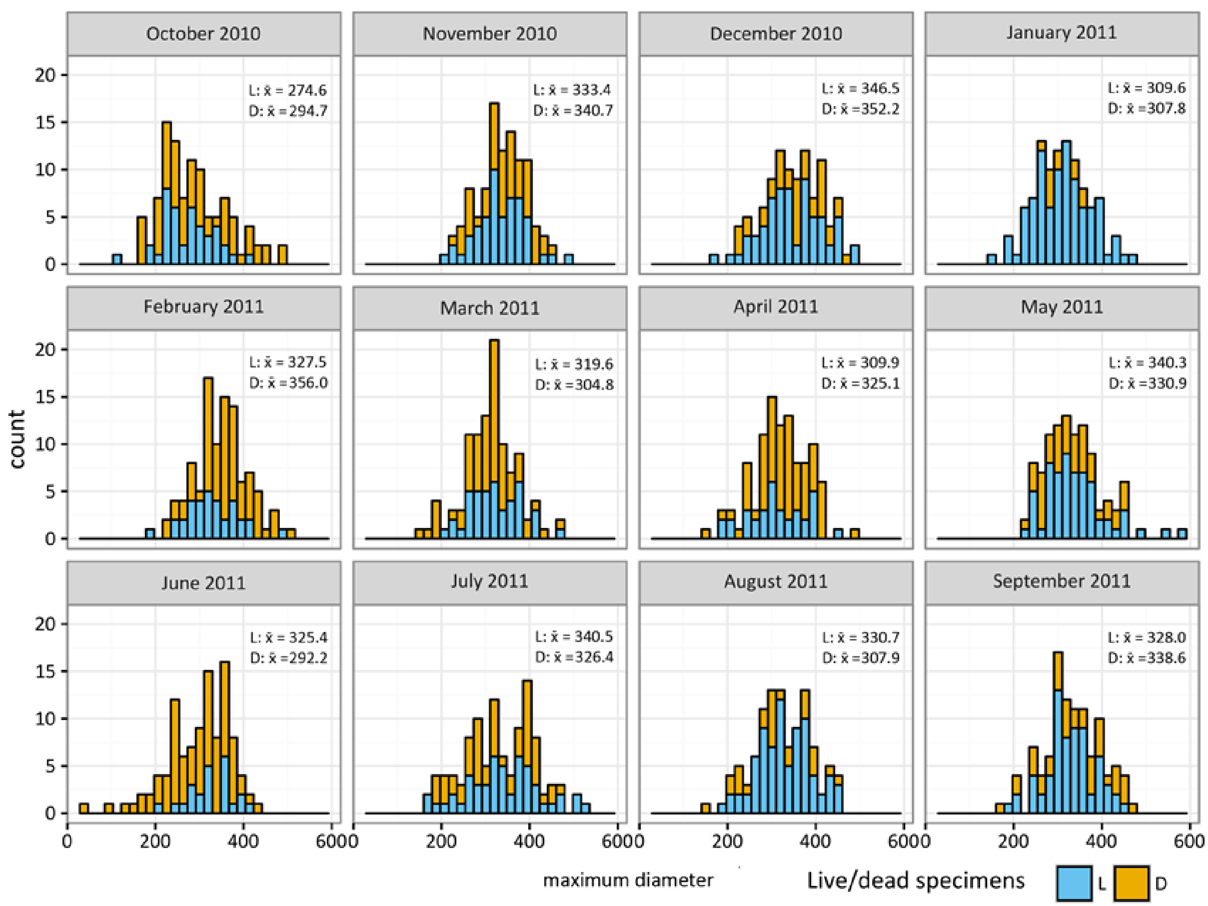

Figure 4. Seasonal variation in size classes. The mean values are shown for living and dead specimens.

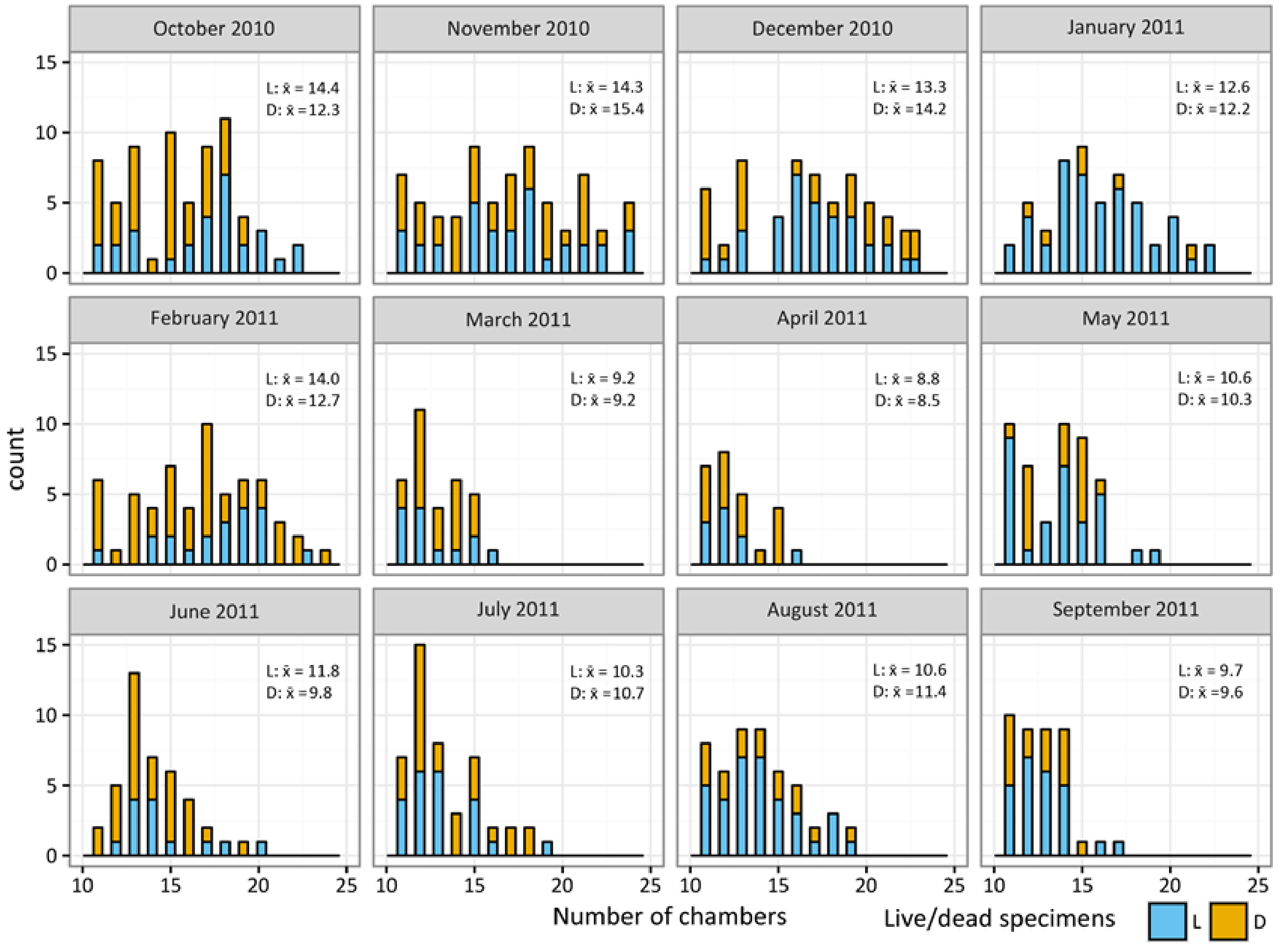

Figure 5. Seasonal variability in the number of chambers. The mean values are shown for living and dead specimens.

DISCUSSION

The Turda salt marsh is unique not only in Romania due to the presence of a living population of *Entzia macrescens*, but it is also uniquely endangered owing to its location opposite a swimming pool car park. Our observations reveal that a viable, reproducing population of *Entzia macrescens* is present in this marsh, associated with halophytic plants. The ultimate source of the foraminiferal and halophyte species in this small inland marsh is likely to be avian transport.

Based on counts of the number of chambers and measurements of the test size and proloculus diameter, it should be possible to separate the microspheric and the megalospheric generations of *Entzia macrescens*, and interpret the reproductive cycle of this species. Even though in this study we were unable to measure the proloculus diameter of every specimen as was done in the study of *Trochammina hadai* by Matsushita & Kitazato (1990), by comparing our results with their findings we can nevertheless speculate on the timing of the reproductive cycle of *Entzia macrescens* in the Turda marsh.

Accordingly, based on the measurements of proloculus diameter we speculate that the megalospheric generation of *Entzia macrescens* is present during summer and autumn to early winter. The sexual reproduction likely takes place in January, when the living population consists of forms with an average proloculus diameter of 16.5 µm. In March–April, a new cohort of small living individuals with fewer than 10 chambers is observed. This late winter to early spring population likely represents the microspheric generation. In April, individuals with larger proloculus diameter make their appearance. In July, the size distribution of proloculus diameter measurements appears to be distinctly bimodal, implying that both microspheric and megalospheric generations may be present. It is also possible that there is significant overlap in the size range of proloculus diameters of both generations. In the case of *Trochammina hadai* in Hamana Lake in Japan, Matsushita & Kitazato (1990) also

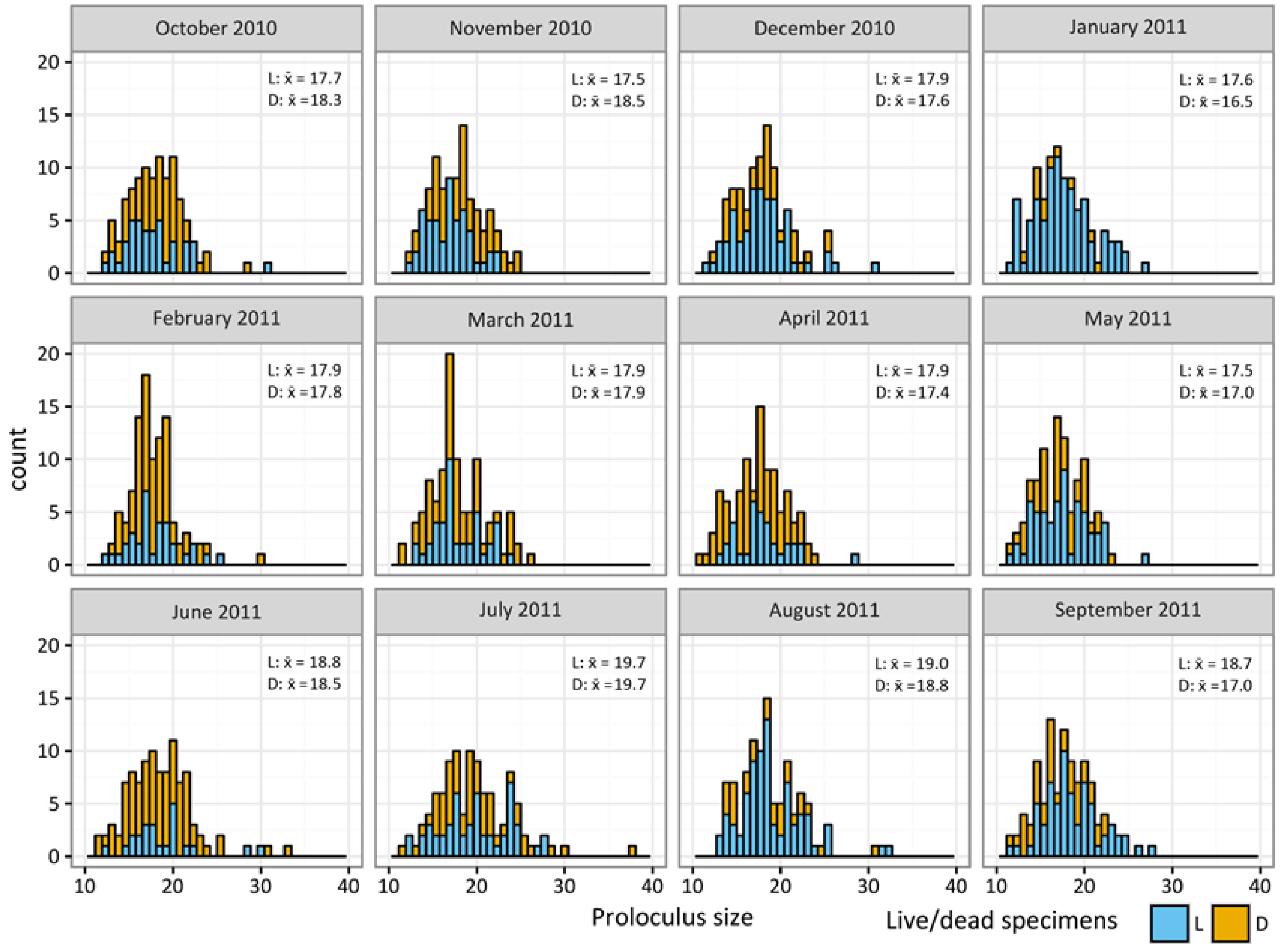

Figure 6. Seasonal variability in the proloculus diameter measured on 100 randomly-selected specimens in each sample. The mean values are shown for living and dead specimens.

recorded a biphasic reproductive cycle. The interpreted microspheric generation was present for only two months in summer in Hamana Lake, while the megalospheric forms were found during the autumn-winter months. However, the species *Trochammina inflata*, which is common in northern European marshes apparently has a more complex trimorphic life cycle, with a microspheric agamontic generation and two megalospheric phases, a schizontic phase, and finally a gamontic phase (Lehmann *et al.* 2006). We are unable to determine at present whether a schizont phase exists in *Entzia macrescens*, as our initial seasonal survey would need to be followed by laboratory culture experiments.

CONCLUSIONS

The salt marsh in Turda contains a viable reproducing population of *Entzia macrescens* (Brady). A clear reproductive event was observed in winter to early spring, when the population is dominated by juveniles with a small proloculus diameter. These are here interpreted as representing the microspheric generation. Another period during which the population contains a greater proportion of juveniles is August–September. The mean test size, number of chambers, mean proloculus diameter as well as the standard deviation of proloculus diameter increase during the autumn. We assume that the megalospheric generation is living at this time. The largest proportion of living individuals is observed in January when the marsh is frozen and salinity reaches a maximum, and once again in August.

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