

Do rotifer jaws grow after hatching?

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Abstract

The hard articulated jaws of some pseudocoelomate metazoans were recently used in reconstructing their phylogenetic relationships, but we still do not know if these structures could change in size and shape during the life of individuals, and experimental data are lacking on their post-embryonic development. Rotifers are one of the groups in which hard articulated jaws, called trophi, are well known, and are widely used taxonomically. Here we report on SEM study of trophi of rotifers of different ages, to determine if the trophi structures change in shape and/or in size during post-embryonic development. We used linear measurements and geometric morphometrics analyses from scanning electron microscopic pictures of trophi of *Cupelopagis vorax*, *Dicranophorus forcipatus*, *Macrotrachela quadricornifera*, *Notommata glyphura*, *Rotaria macrura*, *R. neptunoida*, and *R. tardigrada*. Results for these species show that trophi do not change after hatching, either in size or in shape. In contrast, data on *Asplanchna priodonta* reveal trophi growth after hatching.

Introduction

Hard articulated jaws are present in various groups of pseudocoelomate metazoans as, for instance, Gnathostomulida, Micrognathozoa and Rotifera, which recently have been grouped (together with Acanthocephala) in the monophylum Gnathifera (Ahlrichs, 1997). Using scanning electron microscopy, analyses of fine morphology of jaws were carried out for gnathostomulids (Sørensen, 2000, 2002b; Sørensen & Sterrer, 2002), for micrognathozoans (Kristensen & Funch, 2000; De Smet, 2002; Sørensen, 2003) and among rotifers, mostly monogononts (Nogrady et al., 1995; Segers, 1995; De Smet & Pourriot, 1997, Segers, 1997; Melone, 2001; Segers & Wallace, 2001) but also seasonids (Segers & Melone, 1998) and bdelloids (Melone et al., 1998a). Shape and organization of rotifer jaws (trophi) was considered an important trait in taxonomy and phylogeny (Markevich, 1989; Segers & Melone, 1998; Melone et al., 1998b; Sørensen, 2002a).

Nine types of trophi were attributed to rotifers (Gosse, 1856; Nogrady et al., 1993). The ramate trophi of bdellooids hardly ever have traits that can be considered species-specific (Melone et al., 1998a; Ricci et al., 2001; Fontaneto & Melone, 2003; Fontaneto et al., 2004). Trophi usually consist of 7 different pieces: a single fulcrum, connected to 2 rami, on which lie 2 unci, articulated to 2 manubria (Nogrady et al., 1993). The ramate trophi typical of bdellooids (Fig. 1a–h) show a rather uniform morphology, no fulcrum, rami articulated together and connected with the apical parts of unci teeth, and with curved manubria at the base of the unci teeth (Melone et al., 1998a).

Jaws of rotifers are made of chitin and scleroproteins (Klusemann et al., 1990), the trophi development during embryogenesis is not well known and the only studies dealing with this point are dated (Zelinka, 1891; Tannreuther, 1920). Data regarding post-embryonic growth are available for some gnathostomulids only, in which jaws seem not to grow after hatching (Sørensen &

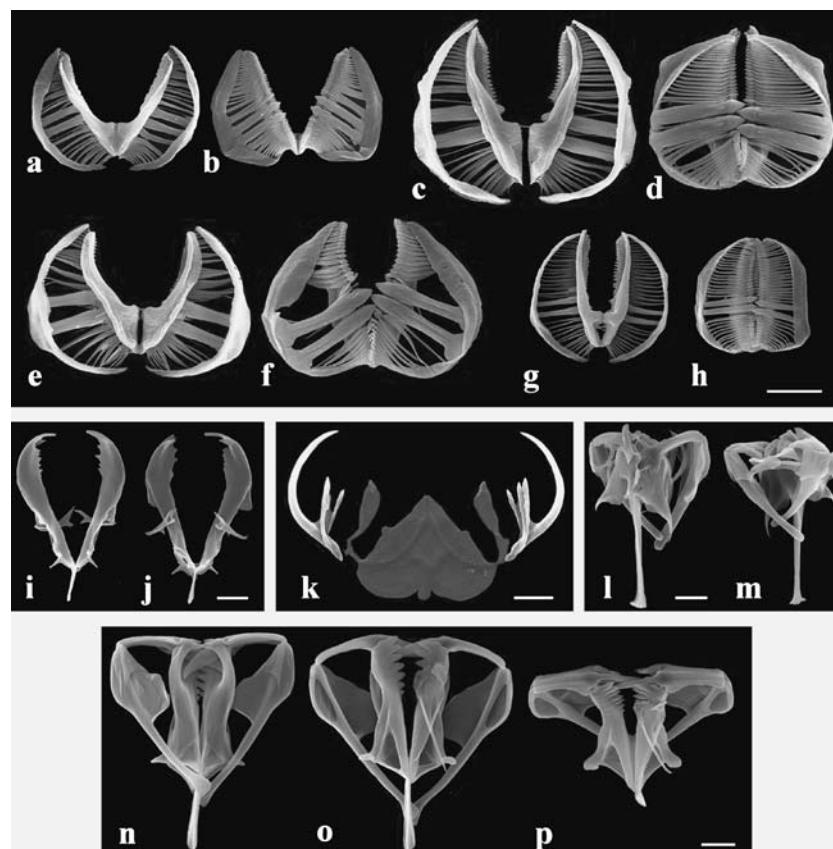


Figure 1. SEM pictures of trophi: (a) *Macrotrachela quadricornifera*, caudal view; (b) idem, cephalic view; (c) *Rotaria tardigrada*, caudal view; (d) idem, cephalic view; (e) *Rotaria macrura*, caudal view; (f) idem, cephalic view; (g) *Rotaria neptunoida*, caudal view; (h) idem, cephalic view; (i) *Asplanchna priodonta*, ventral view; (j) idem, dorsal view; (k) *Cupelopagis vorax*, ventral view; (l) *Notommata glyphura*, ventral view; (m) idem, dorsal view; (n) *Dicranophorus forcipatus*, dorsal view; (o) idem, ventral view; (p) idem, frontal view, Scale bar = 10 μ m.

Sterrer, 2002). Because of the taxonomic and phylogenetic relevance of shape and size of trophi in rotifers, assessing if and how their forms change during growth is of paramount importance.

The present study was aimed at detecting possible post-embryonic changes in size and shape of trophi, both of bdelloids and of monogononts. We focused mainly on bdelloids, characterized by obligatory parthenogenesis and absence of meiosis (Mark Welch & Meselson, 2000). Taxonomy and systematics of the taxon are not well resolved (Donner, 1965; Ricci & Melone, 2000). Thus, morphological analyses of trophi could help in finer discrimination among bdelloids, as has been recently applied to *Rotaria* (Fontaneto et al., 2004).

Materials and methods

Analysed species

We used four bdelloid species of the family Philodinidae: *Macrotrachela quadricornifera* (Milne, 1886), *Rotaria macrura* (Schrank, 1803), *Rotaria neptunoida* Harring, 1913, and *Rotaria tardigrada* (Ehrenberg, 1832), and four monogonont species: *Asplanchna priodonta* (Gosse, 1850), *Cupelopagis vorax* (Leidy, 1857), *Dicranophorus forcipatus* (O.F. Müller, 1786), and *Notommata glyphura* Wulfert, 1938.

All bdelloids have ramate trophi. *Macrotrachela quadricornifera* is a benthic filter-feeder which reproduces via ovipary; its life-cycle lasts about

30 days and somatic growth occurs mainly during the first 10 days of life (Ricci & Fascio, 1995). We isolated eggs from the population cultivated in the laboratory and selected animals at 1st, 5th and 10th day of age to extract trophi.

Species of the genus *Rotaria* are also benthic filter-feeders, but they are viviparous and the developing embryos can be easily seen inside the maternal body (Donner, 1965). We isolated individuals with mature embryos inside and could get trophi of a number of mother–daughter pairs for all three species. We used specimens directly collected in the field in different freshwater environments in the Piedmont region of northern Italy.

Asplanchna priodonta (Asplanchnidae) is a planktonic predator with incudate trophi, showing a wide trophic spectrum, feeding on rotifers, protists, and various unicellular algae (Josè de Paggi, 2002). We collected this species in Endine Lake (Bergamo). This species is viviparous and the developing embryos can be seen inside the maternal body; we isolated individuals with mature embryos and extracted the trophi of mother–daughter pairs.

Cupelopagis vorax (Atrochidae) is a sessile, viviparous predator with uncinate trophi. This species came from Robert L. Wallace's laboratory cultures at Ripon, U.S.A. We compared trophi from mature embryos and large adult specimens, all from fixed material.

Dicranophorus forcipatus (Dicranophoridae) is a benthic predator with forcipate trophi (De Smet & Pourriot, 1997). We collected this species in Piedmont. We compared trophi from eggs with mature embryos, newly hatched juveniles and adult specimens, obtained from laboratory cultures, feeding them various small monogononts and bdelloids.

Notommata glyphura (Notommatidae) is a benthic predator with virgate trophi (Nogrady et al., 1995). We collected it in Piedmont. We compared trophi from mature embryos and from adult specimens, obtained from laboratory cultures. This species can be cultivated provided it is fed live food such as small monogononts and bdelloids.

Trophi images

Trophi were isolated following Segers' (1993) and De Smet's (1998) method with preparation

on a circular cover slip by sequentially dissolving tissues in a 5% NaOCl solution, and rinsing with distilled water. Dried trophi were then coated with gold and observed with a LEO 1430 scanning electron microscope.

Rotifer trophi prepared for SEM observation, depending on the preparation, can be seen in caudal or cephalic view, in dorsal or ventral or lateral view (Fig. 1). This situation can affect the trophi measurements. We decided to measure rami length, as reference of the size for comparisons, in ramate, incudate, uncinate, and forcipate trophi. Virgate trophi have a more complex three-dimensional shape, and only fulcrum length was available for comparisons.

In bdelloids, measures of rami length from SEM images of trophi in caudal or cephalic view were not directly comparable because they lay on different points; so caudal and cephalic lengths were analysed separately. For the mother–daughter pairs of the genus *Rotaria*, we could not distinguish which SEM picture was of the mother's trophi, and which belonged to the daughter, except for those in which embryos were not so mature, and in that case trophi of the daughters were not completely formed and so they could not be used in this study.

Images were processed with Adobe Photoshop 5.0.

Statistical analyses

We used various measurements for analysis of size and we carried out statistical comparisons using *t*-test for paired data to analyse left/right symmetries in trophi of bdelloids. After checking for symmetries, we used the mean value of each pair of rami in bdelloids and different single measurements in monogononts, to compare lengths at different ages. We used Mann–Whitney *U*-test, *t*-test, or ANOVA for comparison of the size data (Sokal & Rohlf, 1995). We gave mean values \pm standard deviations for all species.

Geometric morphometrics

Geometric morphometric techniques are generally used to assess sexual dimorphism, growth and allometry in skeletal elements of vertebrates (e.g., Rohlf, 1998, Cardini & Tongiorgi, 2003) or in hard

chitinous parts of arthropods (e.g. Adams & Funk, 1997; Klingenberg et al., 1998). These techniques were recently applied also in the analysis of SEM pictures of hard elements of microinvertebrates (Fontaneto et al., 2004).

In the present study, trophi of *M. quadricornifera* of different ages were compared in a geometric morphometric analysis (Rohlf & Marcus, 1993), which employs the Cartesian coordinates of a set of topographically corresponding landmarks to capture the morphological information of the specimens under study. Six landmarks were digitized on SEM pictures of the cephalic view of trophi (Fig. 2). As trophi used were symmetrical structures, landmarks were digitized on one half only to avoid redundant information. However, because of the way in which uncini are fitted when trophi are closed, the disposition of the major teeth is asymmetrical: the major tooth can be closer to the proximal articulation of the rami in either the left or the right half depending on the specimen. We chose pictures with the first proximal major tooth in the left half; pictures of trophi with that tooth in the right half were electronically minor-reflected. This reflection allowed us to obtain comparable specimens always having major teeth in the same half as shown in Figure 2.

Landmark (L) description (uncus in the right half, after reflection of the picture, when necessary): L1, point where the first proximal minor tooth is connected with the ramus; L2, tip of first major tooth; L3, tip of second major tooth; L4, point where the most distal minor tooth is

connected with the ramus; L5, distal point of the base of the second major tooth; L6, proximal point of the base of the first major tooth (Fig. 2).

Generalized Procrustes analysis (GPA) removes differences due to the specimen position during data collection, and separates size and shape components of form by scaling the landmark configurations to the same size, centring them at their origin and rotating them to minimize the distances among corresponding landmarks. After the GPA, each landmark configuration corresponds to a point in a curved shape space, which is then projected in a tangent Euclidean space to perform statistical analyses (Rohlf, 1998). The landmark coordinates are transformed into a set of shape variables (linear combinations of the original coordinates) describing those morphological features that do not change with scale, translation and orientation (Bookstein, 2000). Centroid size (CS) measures the overall size of the landmark configuration (before GPA), and it is computed as the square root of the sum of squared distances from the landmarks to their centroid.

Analysis of variance (ANOVA) and canonical variate analysis (CVA) were employed to test the significance of size and shape differences among the trophi of the different age classes.

Geometric morphometric analyses were performed with the computer programs of the TPS series (Rohlf, 2002a). Statistical analyses were done with NTSYS (Rohlf, 2002b) computer programs and with routines available in the geometric morphometric programs.

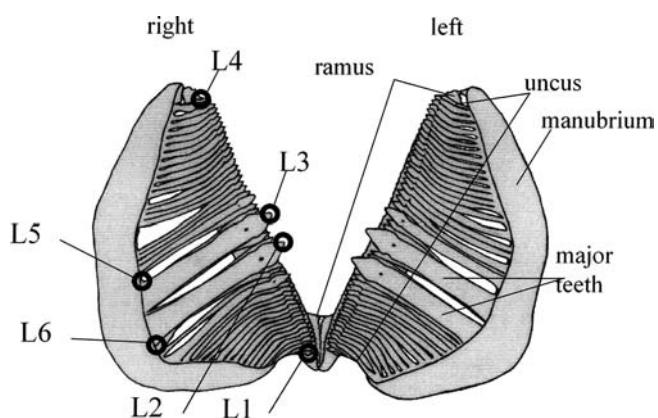


Figure 2. Landmark configuration for geometric morphometric analyses of trophi of *Macrotrachela quadricornifera* in cephalic view.

Results

Bdelloids

We obtained 23 pictures of trophi from *R. macrura*, 10 from *R. neptunoida*, 36 from *R. tardigrada*, and 55 from *M. quadricornifera* (Tables 1, 2).

Rotaria macrura: (Fig. 1e, f) rami were symmetrical ($t = 1.08$, d.f. = 21, n.s.) with maximum left-right discrepancy of $0.35 \mu\text{m}$ (1.4%). Rami lengths in caudal and cephalic view were significantly different ($t = 3.38$, d.f. = 20, $p < 0.01$). We obtained four mother-daughter pairs images in cephalic view, one in caudal view, five of mixed view. One group of one mother and two daughters had trophi in cephalic view. Mean difference in length of rami in each pair (cephalic view) was $0.52 \pm 0.15 \mu\text{m}$. The greatest observed discrepancy was $0.70 \mu\text{m}$ (2.6%), but in this case longer rami belonged to the trophi of the daughter. Caudally viewed pairs had a mean difference of $0.42 \mu\text{m}$ (1.6%), while the mixed pairs could not be used.

Rotaria neptunoida: (Fig. 1g, h) the 4 analysed mother-daughter pairs were all seen caudally; one pair was unfit for use because one specimen was in caudal view and the other in cephalic view. In the four caudally viewed pairs, rami were symmetrical ($t = 2.24$, d.f. = 7, n.s.) with maximum left-right discrepancy of $0.14 \mu\text{m}$ (0.6%). Length of rami

Table 1. Mean values of length of rami in the genus *Rotaria*

	Caudal view (μm)	Cephalic view (μm)
<i>Rotaria macrura</i>	26.50 ± 0.21	26.04 ± 0.58
<i>Rotaria neptunoida</i>	22.38 ± 0.28	
<i>Rotaria tardigrada</i>	33.06 ± 0.57	32.02 ± 1.62

Table 2. Mean values of length of rami of trophi of *Macrotrachela quadricornifera* at different ages

	Cephalic view			Caudal view		
Day of age	1st	5th	10th	1st	5th	10th
Number of trophy	8	6	7	10	14	10
Mean length of rami (μm)	21.49	22.49	22.51	22.75	22.77	22.80
Standard deviation (μm)	0.86	0.66	0.45	0.91	0.55	0.63
CV (s.d./mean)	1.34	1.09	0.47	1.33	0.56	1.04

seemed not to change: differences in each pair were from $0.02 \mu\text{m}$ (0.1%) to $0.07 \mu\text{m}$ (0.3%).

Rotaria tardigrada: (Fig. 1c, d) rami were symmetrical ($t = 0.49$, d.f. = 29, n.s.) with maximum left-right discrepancy of $0.21 \mu\text{m}$ (0.6%). Rami lengths in caudal and cephalic view were significantly different ($t = 2.77$, d.f. = 32, $p < 0.01$). We obtained 9 mother-daughter pairs in caudal view, 1 in cephalic view and 8 of mixed view. The difference in length of the rami in caudal view averaged $0.90 \pm 0.39 \mu\text{m}$. The greatest observed discrepancy was $1.54 \mu\text{m}$ (4.5%). Difference in the pair in cephalic view was $1.99 \mu\text{m}$ (6.2%).

Macrotrachela quadricornifera: (Fig. 1a, b) lengths of left and right rami were similar in each specimen and without statistical difference in each age class, both in caudal ($t_1 = 0.22$, d.f. = 9, n.s.; $t_5 = 0.22$, d.f. = 13, n.s.; $t_{10} = 0.31$, d.f. = 9, n.s.) and cephalic view ($t_1 = 1.53$, d.f. = 7, n.s.; $t_5 = 0.07$, d.f. = 5, n.s.; $t_{10} = 0.98$, d.f. = 6, n.s.). Nevertheless maximum discrepancy between the lengths of left and right rami, expressed as percentage of the major ramus, was 0.9% in caudal view (mean 0.4%) and 0.9% in cephalic view (mean 0.5%). When mean length of left and right ramus of each specimen was used (Table 2), size apparently did not change between trophi of individuals of the 1st and the 5th day of age, neither in caudal ($U_5 = 69$, $n_1 = 10$, $n_5 = 14$, n.s.), nor in cephalic view ($U_1 = 10$, $n_1 = 8$, $n_5 = 6$, n.s.), as between animals of the 5th and the 10th day, both in caudal ($U_{10} = 69$, $n_5 = 14$, $n_{10} = 10$, n.s.) and cephalic view ($U_{10} = 19$, $n_5 = 6$, $n_{10} = 7$, n.s.). Nine pictures in cephalic view of trophi of individuals of 1st day of age, six of individuals of 5th day and eight of 10th day were employed in geometric morphometric analyses. Either ANOVA for the size ($F_{2,20} = 0.090$, $p = 0.914$) or the CVA of the shape variables were not significant (wilks' $\Lambda = 0.44633$, $F_{16,26} = 0.80735$, $p = 0.6665$). The greatest variability in the CS was shown by specimens of the 1st day of age (Fig. 3).

Monogononts

We obtained 24 pictures of trophi from *A. priodonta*, 24 from *C. vorax*, 15 from *D. forcipatus*, and 11 from *N. glyphura*.

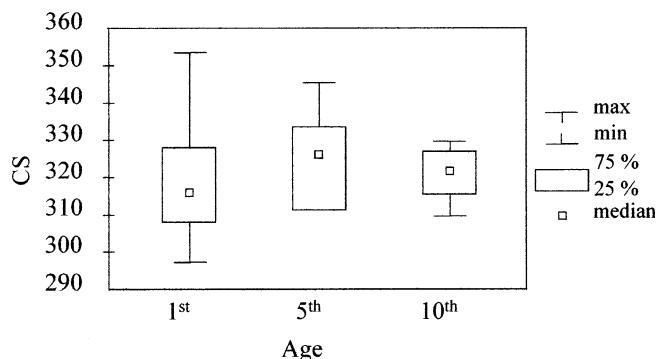


Figure 3. Box plot for the trophi centroid size (CS) in specimens of *Macrotrachela quadricornifera* of the 1st, 5th and 10th day of age.

Asplanchna priodonta: (Fig. 1i, j) significant differences were found in lengths of left ($t = 11.0$, d.f. = 11, $p < 0.001$) and right rami (11.8, d.f. = 11, $p < 0.001$) in newborns and in their mothers. Merged left-right lengths of newborns were $42.53 \pm 1.47 \mu\text{m}$, while those of their mothers were $49.44 \pm 1.99 \mu\text{m}$.

Cupelopagis vorax: (Fig. 1k) no differences ($t = 0.08$, d.f. = 22, n.s.) were present in compared rami lengths of embryos ($18.61 \pm 0.14 \mu\text{m}$) and adults ($18.57 \pm 0.20 \mu\text{m}$).

Dicranophorus forcipatus: (Fig. 1n–p) no differences were present in compared lengths of fulcrum, rami or manubria of embryos and adults (Table 3).

Notommata glyphura: (Fig. 1l, m) no differences ($t = 1.47$, d.f. = 9, n.s.) were present in compared fulcrum lengths of newborns (37.48 ± 0.47 , $n = 6$) and adults (37.90 ± 0.36 , $n = 5$).

Discussion

Bdelloids

From our statistical analyses we conclude that rami are symmetrical structures in bdelloids.

Possibly real, although very modest, left/right differences maybe masked/emphasized by artefacts or instrumental errors. Besides this symmetry in size, some asymmetry in structure and disposition of teeth is present (Melone & Fontaneto, this volume).

Application of geometric morphometric analyses showed that neither length of rami nor trophi size apparently changed after hatching. The only feature that may change from very young animals to adults is the behaviour of the trophi during desiccation when preparing for SEM observation. Trophi of young animals can be easily bent, indeed. In fact, in *M. quadricornifera* values of the 1st day had a greater coefficient of variation than those of the 5th and 10th day. Moreover, when trophi were seen in caudal view they lay on the unci teeth, which formed a plane structure, while when they were seen in cephalic view they lay on the rami, which could be bent. This fact explains the low mean value of rami length in cephalic view of animals of the 1st day, in which rami seemed to be shorter than those of specimens of the 5th and 10th day. Also for *R. macrura* and *R. tardigrada*

Table 3. Mean values of length of trophi pieces of *Dicranophorus forcipatus* at different life-stages, with statistical significance of observed differences

Dicranophorus forcipatus					
	<i>n</i>	Fulcrum	Ramus dx	Ramus sn	Manubrium dx
Embryo	5	24.57 ± 0.24	37.69 ± 0.93	37.77 ± 0.87	53.03 ± 2.51
Newborn	5	24.06 ± 0.31	37.80 ± 0.61	37.71 ± 0.47	53.99 ± 1.56
Adult	5	24.18 ± 0.46	37.74 ± 0.63	37.73 ± 0.53	53.81 ± 1.83
$F_{2,12}$		2.82	0.03	0.01	0.32
<i>p</i>		n.s.	n.s.	n.s.	n.s.

measurements in cephalic view showed a larger standard deviation. So we think that measurements obtained in caudal view are more accurate than those obtained in cephalic view, and we will, thus, discuss only the former.

The difference in rami length in caudal view between the 1st and 10th day in *M. quadricornifera* was $0.05 \mu\text{m}$ (0.22%), while body volume in the same laboratory population between the same ages grew up to over 5 times the starting volume (Ricci & Fascio, 1995).

Differences in rami length in mother–daughter pairs in caudal view in *R. neptunoida* were not significant (less than 0.3%), while they were larger for *R. macrura* and *R. tardigrada*. This outcome can be explained by the differently shaped major teeth of their uncini: the first species had smaller major teeth than the other two. In the latter two species also trophi lying on uncini could be bent because of the obstacle formed by great major teeth. The observation that trophi of daughters were sometimes found to be larger than those of their mothers supports this explanation likewise. We think that data from *R. neptunoida* better reflect the real size of their trophi, while those of the other *Rotaria* species are more susceptible to preparation artefacts.

Monogononts

Data from benthic predators revealed no change in size of trophi during post-embryonic development, as was previously known for the sessile filter-feeder *Floscularia ringens* (Fontaneto et al., 2003).

Only in *Asplanchna* we found that trophi of newborn were significantly smaller than those of their mothers. A great variability in trophi size in this genus is known (Josè de Paggi, 2002), mainly in *Asplanchna sieboldi*, in which trophi size was shown to be a hereditary feature (Badino & Robotti, 1975). Our results showed that trophi could grow. Only further more detailed analyses will show if this feature could be related to their food kind, to the induction of growth of spines in their brachionid preys (Gilbert, 2001), and to the presence of giant specimens with giant trophi (Koste & Shiel, 1980; Josè de Paggi, 2002).

Conclusion

Trophi of all filter-feeder rotifers, independently from their structure, apparently do not change in size after hatching; maybe dimensional invariance of their masticatory apparatus can be related to dimensional invariance of their food particles, which in filter-feeders rotifers is known to be in the range from 3 to $17 \mu\text{m}$ (Nogrady, 1982). Invariance of food prey size can be the reason of invariance of trophi of predators too. Maybe size variance in the omnivorous *Asplanchna* can be related to its great euryphagy.

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