Salinity-driven size variability in *Cyprideis torosa* (Ostracoda, Crustacea)

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Abstract: The living ostracod *Cyprideis torosa* (Jones, 1850) is geographically widespread, often abundant, occurring in modern and late Quaternary marginal-marine and athalassic environments world-wide. The species is capable of withstanding varying salinity over short (diurnal) timescales as well as adjusting to longer-term changes. Much attention has been paid in the past to the development of eco-phenotypic nodes and the shape of sieve-type pores on the external, lateral surfaces as indicators of particular salinity levels. In this paper we demonstrate a bimodal distribution between shell size (which can be determined directly from optical microscopy) and the salinity of the water in which the carapace formed. Between almost 'freshwater' salinity of about 1‰ up to about 8‰ the length of *C. torosa* increases linearly by about 10%, after this point there is a sharp break in the size–salinity relationship with carapace length reverting to values at or below those of freshwater and gradually declining in size by about 5% through the observed range (a maximum salinity of almost 40‰ in this study). This switch in size–salinity relationship coincides with a physiologically important switch between hypo- and hyper-osmotic regulation at about 8‰ known for *C. torosa*.

Keywords: *Cyprideis torosa*; salinity; size; variability

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Quaternary ostracods have been shown to have great utility as archives of changing environmental conditions (De Deckker *et al.* 1988; Boomer *et al.* 2003; Horne *et al.* 2012) and these approaches generally fall into one of three categories: (1) indicator taxa/assemblages, (2) trace element and isotope chemistry and (3) intra-specific morphological variability. There is one particular aspect of this latter category – carapace size – that we examine in the present study.

Within any population of metazoans one may expect to see a normal distribution in the 'size' of what are considered to be adults. Size may be determined from length, height or any other measurable physical dimension, but is deemed to reflect natural variability within a single species. Populations from different localities may exhibit differences in the mean values of those distributions; those differences we usually consider to reflect environmental controls (in the broadest sense) and result from a number of biotic and abiotic interactions. Where observations can unequivocally relate physical characteristics to one particular environmental parameter, this can provide a valuable means of reconstructing an environmental signal from the fossil assemblages.

Intra-specific size variability within ostracod species has been observed by a number of authors. Evidence from both field-based and experimental observations have shown conclusive evidence for temperature-controlled size variability within individual species of ostracods. Kamiya (1988) and Cronin *et al.* (2005) observed seasonal differences in the size of *Loxoconcha matagordensis* Swain from the eastern seaboard of North America, with cooler seasons resulting in larger valves and vice versa. Majoran *et al.* (2000) cultured the marine genus *Krithe* at different temperatures and noted that the average size of individual adult and juvenile stages was greater in cooler waters. This broadly reflects the Temperature Size Rule (TSR) or Bergmann's rule (Bergmann 1847) *sensu lato* whereby individuals of a species grow more slowly in colder environments but result in larger adults; see Angilletta &

Dunham (2003) for an overview and Aguilar-Alberola & Mesquita-Joanes (2014) for discussion relevant to Ostracoda. It has also been noted that certain groups of brackish-water organisms show a direct relationship between size and salinity with decreasing size and thinner carbonate shells in salinity ranges that diverge from the ecological optima of those species (Remane 1958).

Both temperature and salinity are primary controls on the distribution of all aquatic organisms, but it is more common for organisms to accommodate temperature variability more easily than salinity changes, true euryhaline taxa being relatively rare. Reconstructing past salinity levels (and variability) plays a fundamental role when studying the evolution of marginal-marine and estuarine ecosystems, particularly where isolated water bodies such as lakes and lagoons are subjected to climatically driven salinity change. Barker (1963) discussed the possible relationship between ostracod carapace size and salinity based on a study from the Tamar Estuary in SW England; subsequent analyses of those data suggest that the failure to identify adults and juveniles correctly had largely led to this erroneous conclusion, with the smaller juveniles having been transported post mortem.

Of greatest significance here is the work by Van Harten (1975) in which he showed evidence of a probable relationship between *Cyprideis torosa* shell size and salinity (later revisited in Van Harten 1996); the species is known to have a wide salinity tolerance but is rare in truly freshwater settings. The main summary of his work is reproduced here as Figure 1, it shows what appears to be a negative correlation between ostracod carapace length and recorded salinity (in the range of about 1–24‰) at a number of coastal, inland water bodies around North Holland and the West Frisian Islands, though Van Harten did record limited variation in salinity at some of these sites.

Van Harten's work appeared at around the same time as a number of other papers that discussed the relationship between salinity and noding (Kilenyi 1972; Vesper 1972a) and sieve pore shape (Vesper
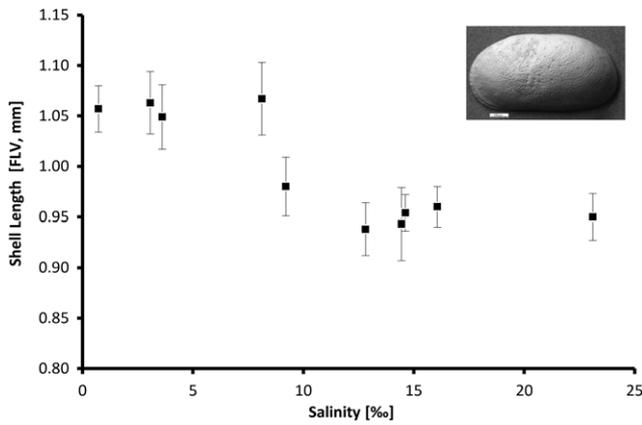
Fig. 1. Summary chart of *Cyprideis torosa* length (female left valve, FLV) v. salinity, based on Van Harten's (1975) original dataset. Note that the original dataset was recorded as chlorinity, but in this diagram has been converted to salinity (ppt). Data provided in Table 1.

Table 1. *Original data on chlorinity and length of female left valve from Van Harten (1975)*

	Chlorinity (‰)	Salinity (‰)	Length (mm)
BOL-1	7.1	12.8	0.938
BOL-2	8.1	14.6	0.954
HBV-1	4.5	8.1	1.067
HBV-2	2	3.6	1.049
MUY	0.4	0.7	1.057
PET-1	12.8	23.1	0.950
PET-2	8.9	16.1	0.960
PUT-1	8	14.4	0.943
PUT-2	1.7	3.1	1.063
VLD	5.1	9.2	0.980

1972*b*; Rosenfeld & Vesper 1977) in the same species. The work relating to noding and sieve pores was at best semi-quantitative, yet Van Harten's (1975) paper contained a dataset that could form the basis to begin establishing a quantitative model (Fig. 1 and Table 1). Despite this, and observations on salinity-dependent noding in cultured *C. torosa* (Frenzel *et al.* 2012), no further investigations were published and the relationship was not investigated beyond the salinity range in Van Harten's paper. In the following sections, we describe the relationship between shell size and host water salinity from a range of environments and cultured populations adding more data points to the Van Harten dataset and extending the recorded salinity range.

It must be noted that the change in converting Van Harten's data from chlorinity to salinity has led to a change in the distribution of the data, particularly at the higher salinities (salinity = chlorinity × 1.805). This has the effect of extending the higher salinity data points to form a more horizontal line, rather than a sharper decrease in the valve-size/salinity relationship.

Sample material and methodology

The two senior authors have spent much time studying assemblages containing *C. torosa* from around Europe, the Mediterranean and western Asia and it is clear that the 'size' (this could be length, height, width, volume, etc.) of adult specimens varies depending on locality. From these collections, samples were selected from modern localities where we had a record of salinity at the time of collection and from settings where salinity was not considered to vary significantly over the short term, i.e. not from estuaries (Table 2). Samples were selected from as wide a range of salinities as possible using our own collections, and we thank Francesc Mesquita-Joanes (University of Valencia) for an additional sample from Santa Pola, eastern Spain. From each sample, initially at least 10 specimens were measured. Most of the specimens were dead at the time of collection but samples were generally surface sediment

Table 2. *Localities sampled for living* Cyprideis torosa *used in this paper*

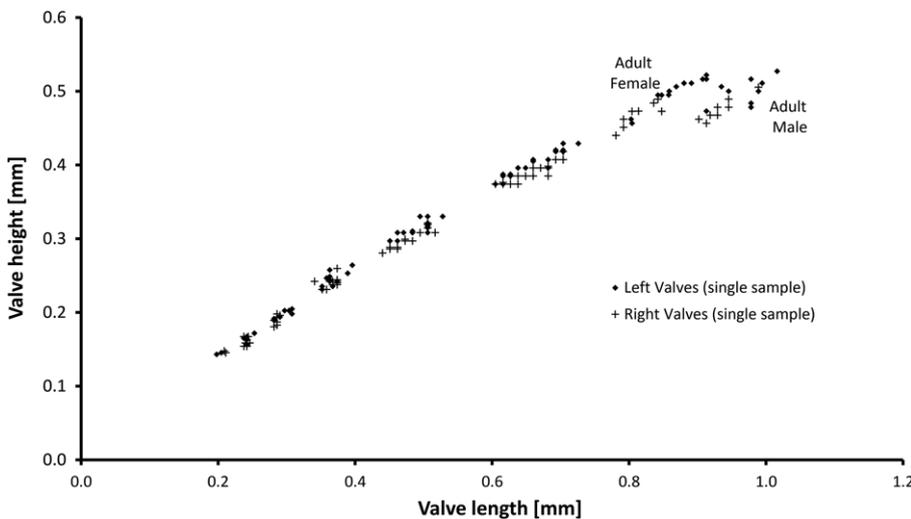| Locality	Coordinates	Water and habitat type	Mean salinity (‰)	Salinity range (‰)	Year of sampling
Oderhaff, NE Germany	53° 48′ N; 13° 58′ E	Mud ground in large lagoon	1.5	0.5–2.5	1986
Caspian Sea	38° 18′ N; 53° 05′ E	Core top, 61 m water depth	13	13 (little to no variability)	1998
Breydon Water, East Anglia, UK	52° 36′ N; 1° 41′ E	Artificial ditch bordering Breydon Water	18	18 (some small seasonal variability possible)	1990
Aral Sea	45° 35′ N; 60° 04′ E	Nearshore sample, hand-collected 2 m water depth	24	24	1991
Santa Pola, Spain	38° 11′ N; 0° 37′ E	Salt pan	38	38	2000

Fig. 2. Length–height relationship between different instars of *Cyprideis torosa* from a single subfossil sample (last millennium) from the Aral Sea (sample AR01–3, 12–22 cm below surface). Each instar is discretely separated from the preceding and succeeding instars with later instars illustrating the greatest degree of variability. The left valves of each instar, being the larger, tend to plot above and to the right of the right valves of the same instar on this plot. Note that the size variability within adults in this sample probably reflects environmental variability during deposition of this material. Data provided in Table 3.
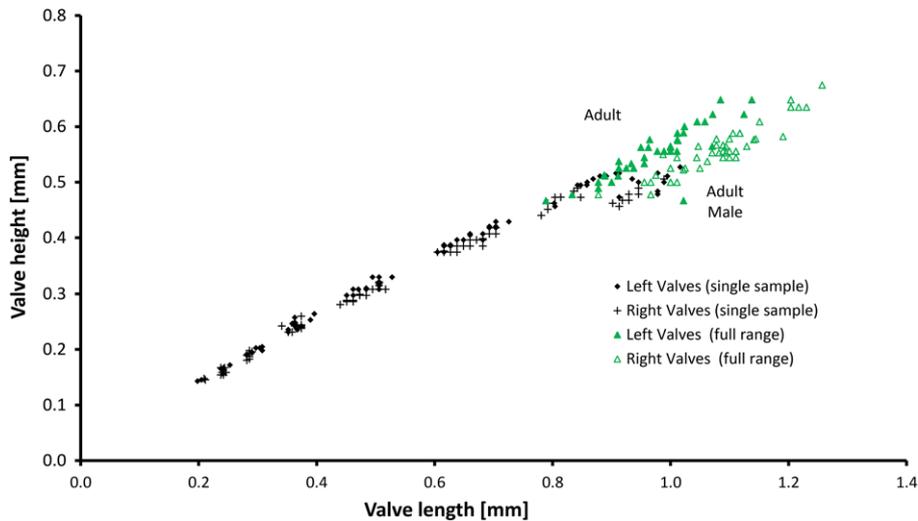
Fig. 3. Dataset shown in Figure 2 is here supplemented by additional data points (in green) to show the full size range of adults valves encountered in this study from a range of salinities (1.5–38‰); the data are provided in Table 4. These data suggest that the assemblage from sample AR01–3, 12–22 cm below surface, sit at the lower size range of *Cyprideis torosa*.

scrapes/collections (<1 cm sediment depth) designed to represent an average over recent years. Our initial field samples are currently being augmented by cultured material from ongoing microcosm experiments undertaken by PF and his research team; details of the experimental set-up are given by Frenzel *et al.* (2012).

Ostracod size data can be obtained relatively rapidly using standard microscopy with individuals aligned under an optical graticule (or reticle), usually held within one of the eyepieces of a standard binocular microscope. The graticule must first be calibrated by measuring a test scale of known size. Once set up, 4–5 specimens can be measured in a minute, fewer if the specimens are measured for more than one dimension. Care should be taken to ensure that the measurement is consistent between specimens; it is often simplest to start with the measurement of greatest length in lateral view. The exact line that this measurement may take will depend on the lateral outline of the species in question; this is relatively straightforward for species such as *C. torosa*. Disarticulated single valves are preferred as they provide a naturally flat surface to rest on; carapaces may be difficult to align in lateral view to ensure that fore-shortening of the viewed image does not occur. Similarly, there may also be a difference between left and

right valves which might not be easily resolved from carapaces. Measurement resolution depends on the quality of the microscope optics, number of divisions in the graticule and magnification but, with practice, is generally considered to be better than 10 μm.

Length–height plots for a typical assemblage of *Cyprideis torosa* are shown in Figure 2. From such plots, individual instars can be discerned as discrete groups (A-6 to adult in this case), the centroids of which progress linearly towards the penultimate instar (A-1) when sexual dimorphism begins to show some effect and, finally, the adult valves have two distinct groupings, one for the longer male dimorph and one for the shorter female. Within this species the left valve is always greater in length than the right and is also usually greater in height so the left valves can be distinguished by their relative positions in the upper right hand of each instar cluster.

To standardize records between samples, we focus on the female left valves (FLV) for three reasons. First, we must ensure that the populations have reached adulthood, i.e. that environmental conditions are not inimical to their survival. Second, female valves always outnumber males within a sample by anything from 5:1 through to 10:1 and are therefore more abundant. Third, the left valve of a *C. torosa* carapace is always the larger.
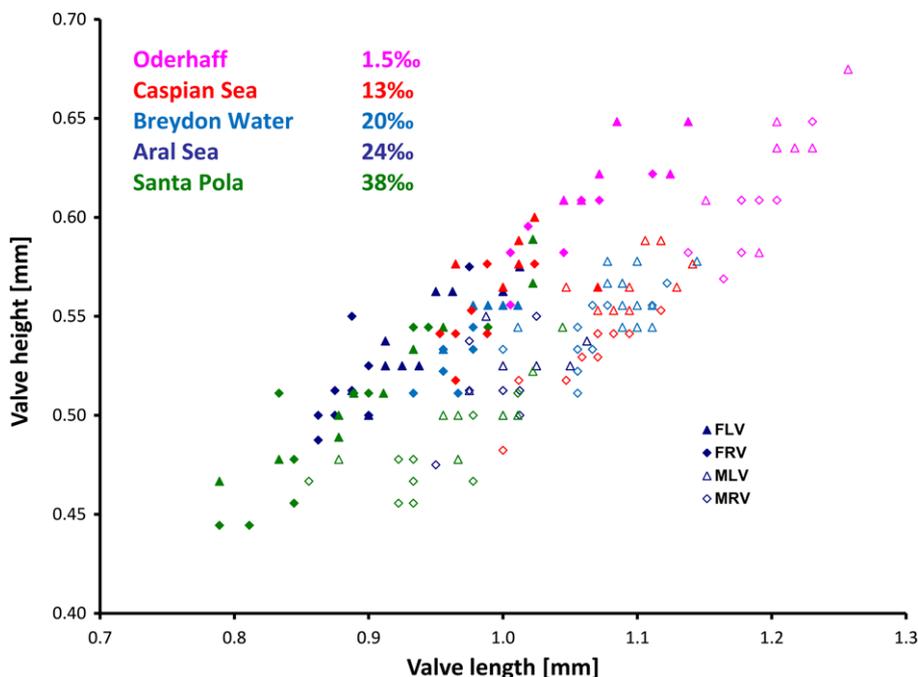

Fig. 4. Length–height relationship for adult male and female valves of *Cyprideis torosa* (both left and right valves) from a suite of samples that span the salinity range 1–38‰. This is a detailed breakdown of the dataset shown in green in Figure 3. Data provided in Table 4.

Table 3. *Dataset of length and height for 155 individual valves of* C. torosa *(both left and right valves) covering the range of instars from Adult down to A-6*

Left valves (mm)		Right valves (mm)	
Length	Height	Length	Height
1.02	0.53	0.99	0.51
0.99	0.51	0.95	0.48
0.99	0.50	0.95	0.49
0.98	0.48	0.93	0.47
0.98	0.48	0.93	0.48
0.98	0.52	0.92	0.47
0.95	0.50	0.91	0.46
0.94	0.51	0.90	0.46
0.91	0.52	0.85	0.47
0.91	0.52	0.84	0.49
0.91	0.47	0.84	0.48
0.91	0.52	0.81	0.47
0.89	0.51	0.80	0.47
0.88	0.51	0.79	0.46
0.87	0.51	0.79	0.45
0.86	0.50	0.78	0.44
0.86	0.50	0.70	0.41
0.85	0.49	0.70	0.42
0.84	0.49	0.69	0.41
0.80	0.46	0.68	0.39
0.80	0.46	0.68	0.40
0.73	0.43	0.68	0.40
0.70	0.43	0.67	0.40
0.70	0.42	0.66	0.39
0.70	0.42	0.66	0.40
0.70	0.42	0.65	0.39
0.69	0.42	0.64	0.39
0.69	0.42	0.64	0.37
0.68	0.41	0.63	0.37
0.68	0.40	0.63	0.39
0.66	0.41	0.62	0.37
0.66	0.40	0.62	0.38
0.65	0.40	0.61	0.37
0.64	0.40	0.52	0.31
0.63	0.39	0.51	0.32
0.63	0.39	0.51	0.31
0.62	0.39	0.51	0.32
0.62	0.39	0.50	0.31
0.62	0.37	0.48	0.30
0.61	0.37	0.47	0.30
0.53	0.33	0.47	0.30
0.51	0.32	0.46	0.29
0.51	0.32	0.46	0.29
0.51	0.32	0.45	0.29
0.51	0.33	0.45	0.29
0.51	0.31	0.44	0.28
0.51	0.31	0.37	0.24
0.50	0.33	0.37	0.24
0.48	0.31	0.37	0.24
0.48	0.31	0.37	0.24
0.47	0.31	0.37	0.26
0.46	0.31	0.37	0.24
0.46	0.30	0.37	0.24
0.45	0.30	0.36	0.24

(continued)

Table 3. (Continued)

Left valves (mm)		Right valves (mm)	
Length	Height	Length	Height
0.40	0.26	0.36	0.23
0.39	0.25	0.35	0.23
0.37	0.24	0.34	0.24
0.37	0.24	0.29	0.20
0.36	0.24	0.29	0.19
0.36	0.24	0.29	0.19
0.36	0.25	0.29	0.20
0.36	0.26	0.29	0.18
0.36	0.25	0.28	0.18
0.35	0.24	0.28	0.19
0.35	0.23	0.25	0.16
0.31	0.20	0.24	0.17
0.31	0.20	0.24	0.15
0.30	0.20	0.24	0.16
0.30	0.20	0.24	0.16
0.29	0.19	0.24	0.16
0.28	0.19	0.24	0.15
0.28	0.19	0.24	0.17
0.25	0.17	0.21	0.15
0.24	0.17	0.21	0.15
0.24	0.17		
0.24	0.16		
0.24	0.16		
0.24	0.17		
0.24	0.17		
0.20	0.15		
0.20	0.14		

Sample comes from a subfossil sample from the Aral Sea (12–22 cm below surface), probably mid-twentieth century date.

variability that is observed in Figure 2. The data also show that the male and female valves continue to be clearly distinguished and that the size variability remains linear, i.e. for any change in length, there is a concomitant change in height. We have not attempted to study variability within juvenile instar stages in relation to salinity variation.

The additional samples (shown as green in Fig. 3) are reproduced as a separate dataset in Figure 4 and are colour-coded according to locality/salinity, this illustrates a clear salinity control on the size of valves. The five additional sites span the salinity range 1–38‰, illustrating the potential range in size variation caused by
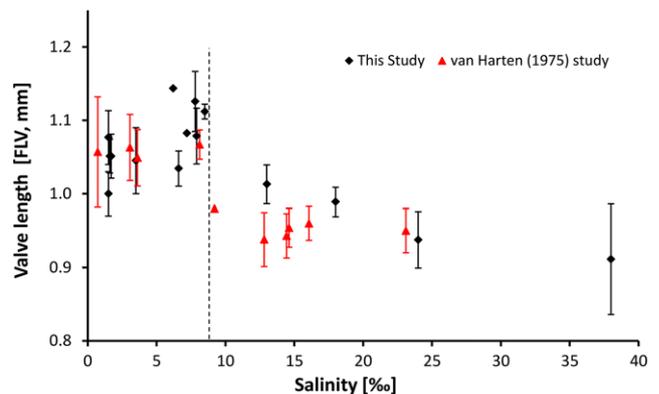

Fig. 5. Relationship between mean valve length (female left valve, FLV) in *Cyprideis torosa* within the salinity range 1–38‰. The original Van Harten data are shown in red. Vertical solid lines indicate 1 standard deviation for each assemblage. The dashed vertical line marks the proposed boundary separating two distinct trends within the data. Original data provided in Table 5.

In Figure 3 the length–height data for five additional sites that span the salinity range from 1.5 to 38‰ (grouped as a single dataset in green) are appended to the data in Figure 2. From these combined data it is clear that the potential variation in the size range for adults of *C. torosa* is very much greater than the 'within-sample'

Table 4. *Length–height data for male and female, left and right valves from the five main localities detailed in Table 2*

Site	Females LV (mm)		Females RV (mm)		Males LV (mm)		Males RV (mm)	
Oderhaff 1.5‰	1.05	0.61	1.01	0.58	1.26	0.67	1.23	0.65
	1.06	0.61	1.02	0.60	1.22	0.64	1.19	0.61
	1.08	0.65	1.06	0.61	1.19	0.58	1.16	0.57
	1.14	0.65	1.11	0.62	1.23	0.64	1.20	0.61
	1.05	0.61	1.01	0.58	1.20	0.64	1.18	0.58
	1.12	0.62	1.07	0.61	1.20	0.64	1.18	0.61
	1.05	0.61	1.01	0.56	1.20	0.65	1.18	0.61
	1.07	0.62	1.05	0.58	1.15	0.61	1.14	0.58
Caspian Sea 13‰	1.01	0.59	0.95	0.54	1.12	0.59	1.09	0.54
	1.01	0.59	0.99	0.54	1.12	0.59	1.08	0.54
	1.07	0.56	0.99	0.58	1.09	0.55	1.07	0.54
	1.00	0.56	0.98	0.55	1.05	0.56	1.00	0.48
	1.00	0.56	0.98	0.55	1.07	0.55	1.05	0.52
	1.02	0.60	0.99	0.54	1.08	0.55	1.06	0.53
	0.96	0.58	0.96	0.54	1.09	0.56	1.07	0.54
	1.02	0.60	1.02	0.58	1.13	0.56	1.01	0.52
	1.01	0.58	0.99	0.54	1.14	0.58	1.12	0.55
	1.01	0.58	0.96	0.52	1.11	0.59	1.07	0.53
Breydon Water 18‰	0.98	0.56	0.96	0.52	1.11	0.54	1.06	0.52
	1.00	0.56	0.96	0.52	1.09	0.57	1.07	0.56
	0.99	0.56	0.97	0.51	1.14	0.58	1.12	0.57
	0.96	0.53	0.93	0.51	1.11	0.56	1.07	0.53
	1.00	0.56	0.96	0.53	1.10	0.54	1.06	0.53
	1.01	0.56	0.98	0.54	1.10	0.56	1.06	0.52
			0.98	0.53	1.08	0.57	1.06	0.53
					1.11	0.56	1.07	0.53
					1.09	0.54	1.06	0.51
					1.08	0.58	1.11	0.56
					1.01	0.54	1.00	0.53
					1.09	0.56	1.08	0.56
					1.10	0.58	1.06	0.54
					1.09	0.57		
Aral Sea 24‰	1.00	0.56	0.90	0.53	1.00	0.50	0.98	0.51
	0.91	0.54	0.88	0.51	0.99	0.55	1.01	0.51
	0.93	0.53	0.89	0.55	1.06	0.54	1.00	0.51
	0.94	0.53	0.89	0.51	1.00	0.53	1.01	0.50
	0.89	0.51	0.88	0.50	1.03	0.53	0.98	0.54
	1.01	0.58	0.90	0.50	1.00	0.53	1.00	0.51
	0.90	0.50	0.86	0.50	0.98	0.51	0.95	0.48
	0.95	0.56	0.90	0.50	1.05	0.53	1.03	0.55
	0.94	0.53	0.90	0.50				
	0.96	0.56	0.98	0.58				
	0.91	0.53	0.90	0.50				
	0.91	0.53	0.86	0.49				
Santa Pola 38‰	0.93	0.53	0.89	0.51	0.96	0.50	0.92	0.48
	1.02	0.59	0.94	0.54	0.88	0.48	0.86	0.47
	0.79	0.47	0.81	0.44	1.04	0.54	1.01	0.51
	0.83	0.48	0.79	0.44	1.00	0.50	0.93	0.47
	0.96	0.54	0.90	0.51	0.97	0.48	0.92	0.46
	1.02	0.47	0.99	0.54	1.02	0.52	0.98	0.50
	0.89	0.51	0.84	0.48	1.01	0.50	0.98	0.47
	0.91	0.51	0.93	0.54	0.97	0.48	0.93	0.46
	0.88	0.49	0.83	0.51	0.97	0.50	0.93	0.48
	0.88	0.50	0.84	0.46				

environmental factors. The pattern echoes the initial observations by Van Harten (1975) with smaller valves recorded at high salinities and larger valves at lower salinities. However, these samples only reflect the broad relationship. Additional data are provided from a range of intermediate localities (Table 5), the mean sizes for female left valves across all samples are reproduced in Figure 5 together with an indication of 1 standard deviation of the length measurements.

Results and observations

The results illustrated in Figure 5 indicate that, rather than the original interpretation of a single linear relationship between shell size and salinity, there are at least two separate salinity ranges with their own discrete patterns. These two subsets of the data are separated by a marked boundary at about 8.5–9.2‰ in the current dataset. At salinities below this boundary, there appears to be a

Table 5. *Summary of observed mean valve lengths (FLV) v. salinity from* Van Harten (1975) *and key field collections from the present study, plus additional assemblages from localities in northern Germany not included in* Figure 4 *and* Table 4

Site*	Salinity	Number of measured FLVs	Mean length FLV (mm)	Standard deviation[†]
Oderhaff	1.5	8	1.08	0.037
Caspian Sea	13.0	10	1.01	0.026
Breydon Water	18	6	0.99	0.020
Aral Sea	24.0	12	0.94	0.038
Santa Pola	38.0	10	0.91	0.075
Saaler Bodden	1.5	33	1.00	0.030
Oderhaff (2)	1.6		1.05	0.023
Peenestrom	1.7		1.05	0.030
Bodstedter Bodden	3.5		1.04	0.045
Großer Jasmunder Bodden	6.2	<3	1.14	na
Barther Bodden	6.6		1.03	0.024
Nordrügener Bodden	7.2	<3	1.08	na
Greifswalder Bodden	7.8		1.13	0.041
Grabow	7.9		1.08	0.038
Westrügener Bodden	8.5		1.11	0.010
BOL-1	12.8		0.94	0.026
BOL-2	14.6		0.95	0.018
HBV-1	8.1		1.07	0.036
HBV-2	3.6		1.05	0.032
MUY	0.7		1.06	0.023
PET-1	23.1		0.95	0.023
PET-2	16.1		0.96	0.020
PUT-1	14.4		0.94	0.036
PUT-2	3.1		1.06	0.031
VLD	9.2		0.98	0.029

*Bold denotes key field collections from the present study.
[†]Standard deviation of valve length within each sample.
Sample sizes vary depending on how many live adults were recovered at each site; where known, sample sizes are given. The sites from localities in northern Germany provide additional detail in the low salinity range. FLV, female left valve.

direct relationship between shell size and salinity, with the largest valves recorded in this study occurring in samples with salinity ranging 6.2–8.5‰. At salinities above that boundary there appears to be little or no relationship between salinity and shell size, if anything there is a slight decrease in size; however, there are too few data points (particularly at higher salinities) to be certain of the physical response in this part of the range. There is clearly variability within each of these samples and there is overlap between many of the samples; however, the boundary at around 8–9‰ is very distinct.

Only limited data are currently available from cultured microcosms and it is not possible to determine the relationship between carapace size and a full salinity range in these experiments (see Frenzel *et al.* (2011, 2012) for discussion of these experiments); however, we have observed that the cultured specimens were smaller than equivalent individuals from field collections at the same salinities so the field and microcosm datasets are not easily compared. Having undertaken shape analysis of a small number of cultured *C. torosa*, using the techniques outlined by Baltanas *et al.* (2003), it is confirmed that there is no relationship between salinity and the lateral valve outline.

Discussion

It is most remarkable that the boundary between the two size–salinity trends coincides with the switching point between hyper- and hypo-osmotic regulation in *C. torosa*, originally recognized by Aladin (1993). Under hyper-osmotic conditions (above about 8‰) the organism experiences an excess of salt which it must excrete while at the same time taking up water, the reverse being the case under hypo-osmotic conditions. As such, this marks a key physiological boundary for living *C. torosa* which has evolved to live on both sides of this boundary but there are clearly differences

in shell-size, noding and sieve-pore shape that reflect its response in the mineralized shell, and this is also recorded in fossil assemblages. The occurrence of noding in the same species is generally considered to occur on individuals from salinities below that boundary (Frenzel *et al.* 2012). A salinity-dependent decrease in size (pauperization) was described for marine molluscs (Remane 1934; Trahms 1939) and foraminifers (Rhumbler 1940) of the Baltic Sea and may be explained by additional energy consumption during osmoregulation.

Osmoregulation as a driving factor behind size reduction is in agreement with results by Frenzel *et al.* (2012) on reproduction rates in *C. torosa* from the same microcosm experiments as cited in the present study. The number of hatched eggs along the salinity gradient suggested a salinity optimum of about 8‰ for *C. torosa*, also conforming to the threshold of size trends in the present study. These findings underline a physiological response depending on salinity of the ambient water.

Calcium ion concentration and availability affects noding frequency in *C. torosa* (Keyser 2005; Frenzel *et al.* 2012) and seems to play a similar role in the noding of *Leucocytherella sinensis* Huang (Fürstenberg *et al.* 2015). We cannot judge if this is also the case for length variation, but it must be considered a possibility. However, the threshold between the two length trends seems not to shift with proportions of ions in the ambient water.

The generally smaller size of individuals from the cultures compared to individuals from the field can be explained by two factors: (a) the microcosms were continuously kept at room temperature during the experiment causing smaller adult size than in the natural environment which would have had a lower average temperature; (b) the artificial environment and poor nutrition could have hampered ontogenetic development. This observation points to other factors besides salinity, i.e. temperature, influencing adult size in *C. torosa*.

Conclusions and remaining questions

Evidence from field collections of *Cyprideis torosa* from a range of localities and salinity levels has shown that there is a two-fold pattern in the relationship between shell size and salinity. From 1 to about 8‰ there is an increase in valve size, from about 8 to 38‰ (maximum salinity sampled in this study) there is a slight decrease in size but the data are too scattered to have confidence in this relationship. A number of additional questions and directions for future research arise from the observations detailed above.

(1) Do we have a simple quantitative model that can be used in palaeoenvironmental interpretation? No – intra-population variability combined with longer-term salinity variability in 'mixed' fossil assemblages precludes a simple quantitative model for palaeosalinity reconstruction using *C. torosa* shell size alone. However, as a rule of thumb, adult FLVs over about 1.10 mm in length are likely to represent salinities around 6–8‰ while adult FLVs under 0.95 mm in length probably represent salinities above about 15‰.

(2) How applicable is this size relationship beyond *C. torosa*? Few other modern ostracod species occur across such a wide salinity range. Other living members of the genus *Cyprideis* are recorded from North America in particular and these could provide a focus for future work.

(3) Are there specific physico-chemical conditions, concentrations of elements, cations or anions that may also influence size variability? This is beyond the scope of the current project but given the relationship between water chemistry and salinity this could be a possibility. The dilution of marine water by adding distilled water for the microcosm experiments will also lead to changes in the alkalinity of the water and the calcium ion activity, with impacts on pH and bicarbonate ion concentration. It has also been noted that shell calcification (by weight) in such culture experiments is often sub-optimal (see discussion in Dettman & Dwyer 2012) so care should be taken in determining the role of hydrochemistry through microcosm experiments. Additionally, ostracod metabolic rate will be related to the availability and concentration of dissolved oxygen which is inversely related to both water temperature and salinity; this should also be considered in any future experiments and discussion.

(4) What happens at salinities above 38‰? *Cyprideis torosa* is believed to exist in waters up to 150‰ (Neale 1988, though no details of this record are provided) but such occurrences are rare; we have not had the opportunity to sample beyond 38‰.

(5) Does the same pattern of size variability occur in juveniles? We have only examined adult valves in detail to date; we believe that the same pattern is seen in juveniles based on the growth rate rule for Crustacea.

(6) Finally, the role of osmoregulation in *C. torosa* warrants further research to understand how this species' osmoregulatory capabilities compare to those of other brackish-water species and especially of other, closely related, cytherideid taxa.

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