

Morphometric variables and individual volume of *Eurytemora americana* and *Acartia tonsa* females (Copepoda, Calanoida) from the Bahía Blanca estuary, Argentina.

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ABSTRACT

Eurytemora americana and *Acartia tonsa* are two of the most important copepods from the Bahía Blanca estuary plankton. In this study 30 females from each species were sorted from seasonal and recently preserved zooplankton samples. Prosome length (PL), width (PW) and height (PH) and urosome length (UL) and width (UW) were measured and subsequently the following ratios determined: PL:PW, PL:PH, PW:PH and PL:UL. Individual volumes for *E. americana* and *A. tonsa* females were estimated following the morphometric method in order to obtain individual biomass values for rapid application in the future. The formula for individual volume was: $V \text{ (mm}^3\text{)} = \pi (PL \text{ PW PH})/6 + \pi (UL \text{ UW}^2)/4$. Regression tests were conducted for both species utilizing individual volume vs prosome length or width as independent variables and fitting data to a power model. Covariance analysis and single comparison between regression lines were used in order to evaluate the temporal behavior of these relationships. PL:UL was the best ratio for separating one species from the other, being 1.46 for *E. americana* and 3.53 for *A. tonsa*. Average individual volume and std. error estimated for *E. americana* and *A. tonsa* were $0.0749 (\pm 0.0097) \text{ mm}^3$ and $0.0399 (\pm 0.0049) \text{ mm}^3$, respectively. Despite their similar size, strong differences between

both copepods morphometric variable and rate values were observed. Seasonal variability of body dimensions, ratios and individual volumes in both species were observed, finding differences between dates. A size and volume decrease was detected associated with higher temperatures in the environment. Determination coefficient (R^2) values of regression lines demonstrated that while prosome width and prosome length were good volume predictors on different dates for *E. americana*, prosome width was the best predictor on the majority of dates for *A. tonsa*. Differences in slopes and means from regression lines demonstrating body seasonality in both species were observed.

INTRODUCTION

Eurytemora americana Williams, 1906 and *Acartia tonsa* Dana, 1849 are the most abundant copepods in the Bahía Blanca estuary plankton and coexist from June-July to October of each year. *E. americana* lives in plankton during this period only, and according to Marcus (1984b), it is likely to remain as diapause eggs in the bottom sediments during the rest of the year. Although this copepod was cited in some previous papers as *E. affinis* (De Aracama, 1987, Hoffmeyer, 1994; Hoffmeyer & Prado Figueroa, 1997), its identity was only recently clarified (Hoffmeyer *et al.*, 2000). *E. americana* inhabits in the innermost estuarine area (Hoffmeyer & Tumini, unpubl.), while *A. tonsa* has a broader distribution in the entire estuary. *A. tonsa* occurs all year around in the plankton, being abundant during spring, summer, and autumn, but scant during a short period in winter (June-July) (Hoffmeyer, 1983) when it is likely to produce diapause eggs as it happens in another environments (Uye & Fleminger, 1976). Temperature and salinity are the main environmental factors that regulate both species populations in the estuary. In addition, food availability and predation represent other control factors for them. Up to date, there are no records about predation on *E. americana* in this estuary. However, it has been largely reported on *A. tonsa* mainly by the ctenophore *Mnemiopsis mccradyi* Mayer (Mianzán & Sabatini, 1985), and fishes: Atherinidae larvae (Hoffmeyer, 1986; 1990) and *Ramnogaster arcuata* (i.e. fam Clupeidae) (López Cazorla, 1987; unpubl.).

E. americana and *A. tonsa* have similar size, but body shape in each one is clearly different as far as morphometry is concerned. These differences may contribute to create also differences in biomass values between both copepods. However, how much important in different seasons or dates of year will this difference be? And also, from all morphometric variables, which will the best specific individual volume predictor be?

Seasonal variation of size in both species has been observed in this estuary (Hoffmeyer, 1987; Sabatini, 1989) and for *A. tonsa* in another environments as well (Durbin *et al.*, 1983). In addition, variability of both, prosome length and body carbon content inversely correlating with temperature and directly with food availability was observed for other copepod species (e.g. Durbin & Durbin, 1978; 1992; Kankaala & Johansson, 1986; Tanskanen, 1994). Besides this seasonal variation, spatial geographic variation of size must be taken into account when length- weight regressions are used to estimate biomass.

The aim of present study was to obtain volume estimates of *E. americana* and *A. tonsa* females from the Bahía Blanca estuary, evaluate the power of prosome length and width as volume

predictors and discuss any seasonal variations of morphometric variables, ratios and individual volumes of both copepods.

MATERIAL AND METHODS

Specimens used in this study came from zooplankton samples collected in Cuatrerros Port, situated in the innermost area of the Bahía Blanca estuary, Argentina. Sampling was done with a 0.30 m mouth diameter and 200 μ m mesh open net, in several vertical tows from 5 m depth to the surface. In order to obtain data of different dates within the planktonic pulse of the species (i.e. end of June-middle of October), *E. americana* specimens were isolated from two samples collected in August 5 and September 21, 1993. *A. tonsa* specimens from four samples collected in October 10, 1990, and January 8, May 22 and August 8, 1991 respectively. Thirty adult females of each species and date were used. Whenever possible, females were studied within a month after collection, in order to avoid size differences caused by preservation time (Omori, 1978).

Prosoma length, width and height, and urosoma length and width were measured in each specimen, with a Wild M 5 stereoscopic microscope (10 X ocular and 50 X objective). Measurements were made to the nearest 10 μ . Individual volume of each female was estimated following the morphometric method proposed by Chojnacki & Hussein (1983). Their formula (i.e. which assumes the same urosoma width and height) with Fernández Aráoz's modifications (in which antenna and leg volume is not considered) (1991) was used:

$$V = \pi (PL \cdot PW \cdot PH)/6 + \pi (UL \cdot UW^2)/4$$

Where: V is volume (mm^3), PL, PW and PH, prosoma length, width and height (mm), and, UL and UW, urosoma length and width (mm) respectively. Volumes obtained may be converted to wet weight assuming the relation: $1 \text{ mm}^3 = 1 \text{ mg}$ wet weight, as the copepod density is close to 1 (Omori & Ikeda, 1984). Ratios between different morphometric variables: PL: PW, PL: PH, PW: PH, and PL: UL, were also calculated. *E. americana* and *A. tonsa* prosoma length (PL) - width (PW) vs individual volume (V) relationships for each data set were described through regression tests applying the power model as this was the best in fitting data. The determination coefficient (R^2) was used to explore the independent variable power to predict volume. The comparison among dates of the regression lines was made through a covariance analysis determining general equations for each species.

RESULTS

Morphometric variables and individual volume

Mean *E. americana* female morphometric variable values, except the urosoma width value, were smaller on September 21, 1993 than in August 5, 1993. Ratios between all but one morphometric variable were similar on both dates; UL/ UW (Table 1) decreased markedly in September. Mean *E. americana* individual volume values decreased from 0.0975 mm^3 in August to 0.0522 mm^3 in September (Table 1). Female individual volume average was $0.0749 \text{ mm}^3 (\pm 0.0097)$ for both dates. Scatter plots from Fig. 1 show volume and prosoma length and width changes that occurred from one date to the other.

Mean *A. tonsa* female morphometric variable values varied seasonally showing the lowest values in January, 1991, highest values in August, 1991 and intermediate values in October, 1990 and May, 1991 (Table 1). Generally, ratios found between variables were similar along dates, with an increase in UL/UW during May and August (Table 2) being the most important variation. The lowest PL/PW and highest PL/UL values were detected in January. Mean *A. tonsa* individual volume values ranged from 0.0307 in January to 0.0465 in August and intermediate values were detected in October and May (Table 1). Female individual volume average from four dates during the year was $0.0399 \text{ mm}^3 (\pm 0.0049)$. Scatter plots from Fig. 2 show seasonal changes of volume - prosoma length and width.

In both copepods, taking into account the variation coefficient (VC) as a percentage of the mean, the prosome length had a smaller variability than prosome width, so we consider it as the most stable measure. With regard to different dates, *E. americana* individual volume data showed a higher VC in August than in September, while *A. tonsa* individual volume showed a higher VC in May than in August, 1991 (Table 1).

Regression tests for prosome length (PL) – width (PW) vs individual volume (V)

Regression tests for *E. americana* PL and PW vs V relationships conducted using data from two dates within the species planktonic pulse delivered significant results (Table 3). For August the determination coefficient (R^2) was higher in the PW vs V relationship (70.82 %) than that in the PL vs V relationship (58.53 %). For September the R^2 was higher in the PL vs V relationship (78.05 %) than that in the PW vs V relationship (52.79 %). The latter means that PW was the best predictor in August, explaining nearly 71% from total variance while PL was the best in September, explaining 78% from variance. Similarly, regression tests between the same independent variables for *A. tonsa* females conducted using data from four dates along its annual cycle delivered significant results (Table 3). For October, 1990, a R^2 (88.48%) higher than that in the PL vs V relationship (39.46 %) was obtained in the PW vs V relationship, meaning that PW explained more than 88% from total variance. For January, 1991, the R^2 in both regression tests was similar although it was slightly higher in the PL vs V relationship (65.51 %) than in the PW vs V relationship (58.39 %). In this case, the scant difference between percentages demonstrates that total variance was only slightly better explained by PL than by PW. For May and August 1991, R^2 values were higher in the PW vs V relationships (72.91 and 59.57 %) than those in the PL vs V relationships (53.70 and 50.59 %). This is demonstrating that in May PW was a better predictor than PL explaining near 73% from total variance. However, similar R^2 values were obtained for August, and this does not allow for any conclusion on which of them explains more about the total variance than the other.

Regression lines comparison

Covariance analysis results for both copepods regression lines are showed in Table 4. The comparison between regression lines of *E. americana* PL vs V and PW vs V relationships from August and September '93 demonstrated that no significant differences were found either between slopes ($p = 0.36$ and 0.59 , respectively) or between adjusted volume means ($p = 0.05$). Thus, unique regression equations were obtained for this species females: $Y (\ln V) = -2.4 + 2.7 X (\ln PL)$ and $Y (\ln V) = -0.86 + 1.87 X (\ln PW)$, respectively (Fig. 3, A- B).

In the comparison among regression lines for *A. tonsa* PL vs V relationships from four dates of 1990-1991 year, no significant differences among slopes ($p = 0.20$) were detected. However, significant differences among mean adjusted volume values ($p < 0.05$) were found. Pair comparisons allowed to separate all data set into two groups: January-May and August- October, the resulting equations being for Jan-May: $Y (\ln V) = -3.01 + 2.51 X (\ln PL)$, and for Aug-Oct: $Y (\ln V) = -2.94 + 2.51 X (\ln PW)$ (Fig. 4, A). In the comparison among regression lines for *A. tonsa* PW vs V relationships from those dates, differences among slopes ($p < 0.01$) were observed and therefore it was necessary to separate the May data set. According to the results from a new covariance analysis performed with three remaining data sets, even though very significant differences among adjusted volume means ($p < 0.01$) were found, differences among slopes ($p > 0.7$) were not. Pair comparisons allowed to separate January from August-October data, the resulting equations being for May: $Y (\ln V) = -0.53 + 2.32 X (\ln PW)$, for January: $Y (\ln V) = -1.66 + 1.48 X (\ln PW)$ and for Aug-Oct: $Y (\ln V) = -1.41 + 1.48 X (\ln PW)$ (Fig. 4, B).

DISCUSSION

Differences observed between morphometric variable values found in *E. americana* and *A. tonsa* females obviously reflect the different body shapes of both copepods, particularly different prosome and urosome lengths. In *E. americana* prosome and urosome lengths are similar, while in *A. tonsa* prosome length is much greater than urosome length.

With regard to ratios between pairs of morphometric variables, mean prosome width to height (PW: PH), was slightly larger than 1 in both copepods, though in *E. americana* it would represent a nearly circular section (1.08) while in *A. tonsa* a flatter one (1.2). PL:PW ratio was larger in *A. tonsa* (2.92) than in *E. americana* and PL:PH and PL:UL ratios were manifestly larger in the former as well. It was evident that PL:UL is the best ratio to morphologically separate both species, with the mean values being 1.46 in *E. americana* and 3.53 in *A. tonsa*. Fernández Aráoz (1994) found for *A. tonsa* females from Buenos Aires coastal waters in October, 1982 mean values of 4.17 for the PL:UL ratio and 3.26 for the PL:PW ratio, while for females from San Jorge Gulf this author reported for January 1985 values of 3.61 and 3.11 for the PL:UL and PL:PW ratios. The ratio values obtained for our specimens are smaller than those. Seasonal and geographic causes could be reflected in the observed differences with Fernández Aráoz's specimens.

Size of both species evidenced an important seasonal variation. All morphometric variables analyzed in the two copepods showed a variability of around 20%. *E. americana* became smaller to the end of the phytoplankton pulse in the estuary (September). These results agree with the observation of an inverse relationship detected between temperature and *E. americana* size made in this environment during 1998 (Hoffmeyer, unpubl.), which was interpreted as an seasonal effect. Katona (1971) and Hirche (1992) had already observed this type of effect in *E. affinis*. Heron (1964) reported for *E. americana* females from the Washington coast collected during the summer (July-August 1958 and 1960) a mean total length of 1.43 mm, with a range of 1.35 - 1.58 mm. If we take into consideration what this author mentioned, that a 59% of the total body length would corresponded to PL, the derivative mean value would be of 0.844 mm within a 0.796 - 0.932 mm range. In comparison, mean PL and range values obtained for our specimens in the Bahía Blanca estuary from August to September 1993 were lower than Heron's data: 0.929 and 0.760 - 0.980 mm, respectively. In fact, this situation could be explained as a consequence of different environmental conditions on growth and development through consecutive generations of copepods in a different geographic location (Viñas, 1985) and due to a seasonal effect as well.

On the other hand, *A. tonsa* became smaller during summer, recording the largest female sizes during winter. Fernández Aráoz (1994) observed in *A. tonsa* females collected from Buenos Aires coast, with similar latitude to that of the Bahía Blanca estuary, during October, mean prosome length values of 0.968 mm and individual volumes of 0.0462 mm³. These values are similar to those obtained for females in October, 1990 from the Bahía Blanca estuary. Prosome length variation in adults of *A. tonsa* was reported by Sabatini (1989) in this estuary for the 1982-1983 annual period. Mean monthly values from females were lower than the values presented in this paper, ranging from 0.750 mm in January to 0.850 mm in June. Prosome length variations and differences in maxilla 2 size of *A. tonsa* from this estuary were also observed for the 1985-1986 period (Hoffmeyer, 1987). Mean monthly values in female prosome length shown in that paper ranged from 0.835 in summer to 0.910 mm in winter and were slightly lower than values found in the present study: 0.904 to 0.946 mm. In both papers mentioned above, copepod size variability was related to differences in food and temperature conditions throughout the year. The smallest *A. tonsa* generations (Sabatini, 1989) were linked to increased temperature values and low food availability levels during spring and summer, after the winter-spring phytoplankton blooming conditions.

Female *E. americana* individual volume was 87.72% on average larger than that of female *A. tonsa*. Previous estimates of *E. americana* individual volume were not found in the literature. Seasonally, from the beginning to the end of its plankton pulse and due probably to increasing temperature and less food availability, *E. americana* volume varied from 0.0975 mm³ in August to 0.0522 mm³ in September. Female *A. tonsa* individual volume displayed a similar behavior, from

0.0307 mm³ in January to 0.0465 mm³ in August, most likely due to size and temperature-food condition changes already discussed. These observations agree with those reported by Deevey *et al.* (1960b), who pointed out that conditions prevailing during development regulate copepods size and weight. They also agree with Mc Laren (1963), Miller *et al.* (1977) and Klein Breteler & Gonzalez (1982) observations that temperature and food availability are the main factors acting in the seasonal control. Females of *A. tonsa* collected during January 1985 in the North coast of San Jorge Gulf studied by Fernández Aráoz (1991) had a mean individual volume of 0.0573 mm³ while those from October had a volume of 0.0739 mm³. In the present study, the mean volume was 0.0307 mm³ for January females and 0.0435 mm³ for October females. The lowest size and biomass of our specimens as compared with those values reported from San Jorge Gulf was most likely due to different geographic conditions, as it was the *E. americana* case reported earlier. With regard to *E. americana* size-volume regressions, the prosome width was the best individual biomass predictor for the August data, while the prosome length was for the September data. Resulting general equations of individual volume vs prosome length or prosome width of this species female show a different behavior of each variable against volume. These equations may be useful, though the contagious behavior of data in two groups clearly reflect the existence of two size-distinct populations (from August and September months). In the case of *A. tonsa*, prosome width had better predictive power than prosome length, except for January 1991 data. This agrees with what Fernández Aráoz (1991) observed in San Jorge Gulf for *A. tonsa* females collected in October 1985, in that prosome width was more suitable than prosome length to predict volume. General resulting equations of *A. tonsa* female individual volume vs prosome length (Jan-May and Aug-Oct) showing equal slopes (volume increments) but different volume means, denote the seasonal change of this variable and its proportional effect on volume. On the other hand, general equations of *A. tonsa* female individual volume vs prosome width (May, Jan and Aug-Oct) show differences in means and variances between May, Jan and Aug-Oct sets and equal slopes for Jan and Aug/Oct lines. The higher slope for May data indicates a distinct volume increment respect to the prosome width seasonal variation probably linked to particular population state or environment conditions.

Taking into consideration results obtained during this study about the variability of body dimensions and volume of *E. americana* and *A. tonsa* females, it would be advisable to avoid the use of biomass estimation extrapolations from one area to another or from one season to another. Further studies should be carried out on individual volume estimates for males and copepodites and their seasonal variability to complete the information about this subject for the two copepods at the Bahía Blanca estuary.

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CAPTIONS OF FIGURES AND TABLES

Fig. 1. Scatterplots of *Eurytemora americana* females prosome length (mm) vs volume (mm³)(left) and prosome width (mm) vs volume (mm³) (right) during August 5, 1993 (top) and September 21, 1993 (bottom).

Fig. 2. Scatterplots of *Acartia tonsa* females prosome length (mm) vs volume (mm³)(left) and prosome width (mm) vs volume (mm³) (right) during October 10, 1990 (top), January 8, May 22 (middle) and August 8, 1991(bottom).

Fig. 3. Resulting general regression lines and equations of *E. americana* prosome length –prosome width vs volume for August and September, 1993 data. A- Prosome length (PL) vs volume (V). B- Prosome width (PW) vs volume (V).

Fig. 4. Resulting general regression lines and equations of *A. tonsa* prosome length –prosome width vs volume for May, 1990, January, August and October, 1991 data. A- Prosome length (PL) vs volume (V). B- Prosome width (PW) vs volume (V).

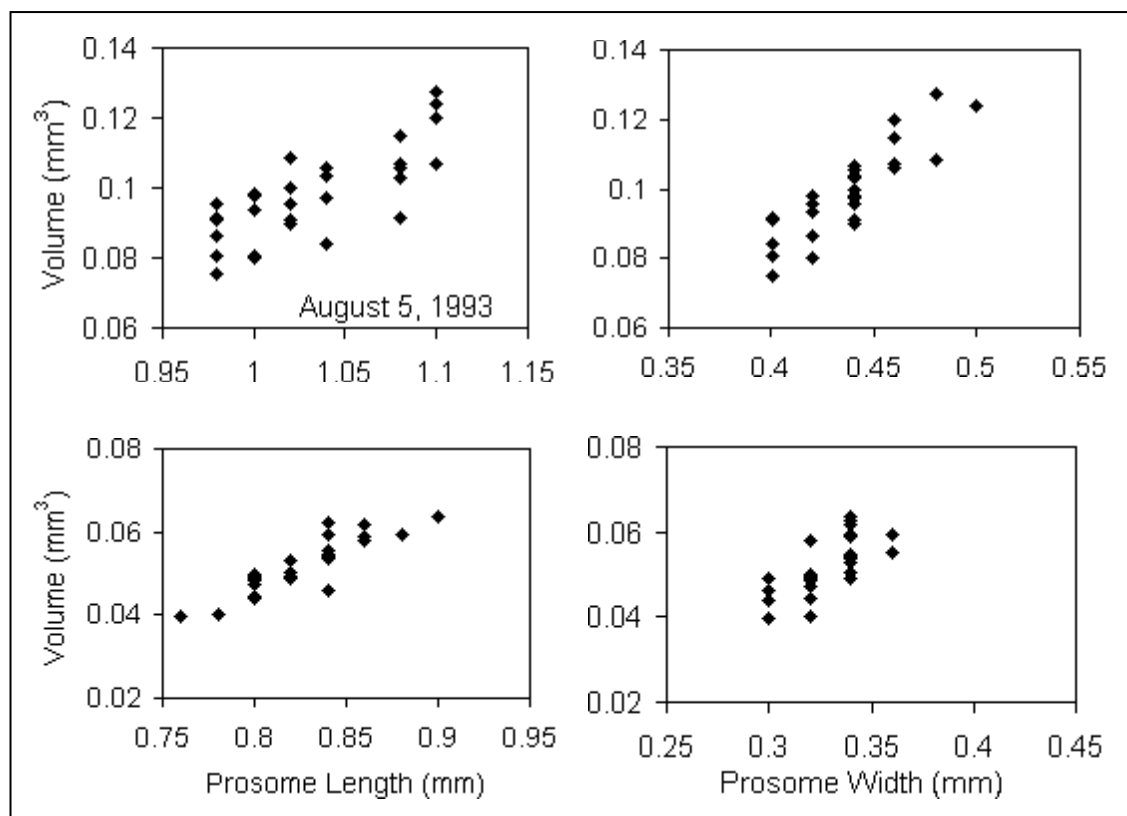


Figure 1

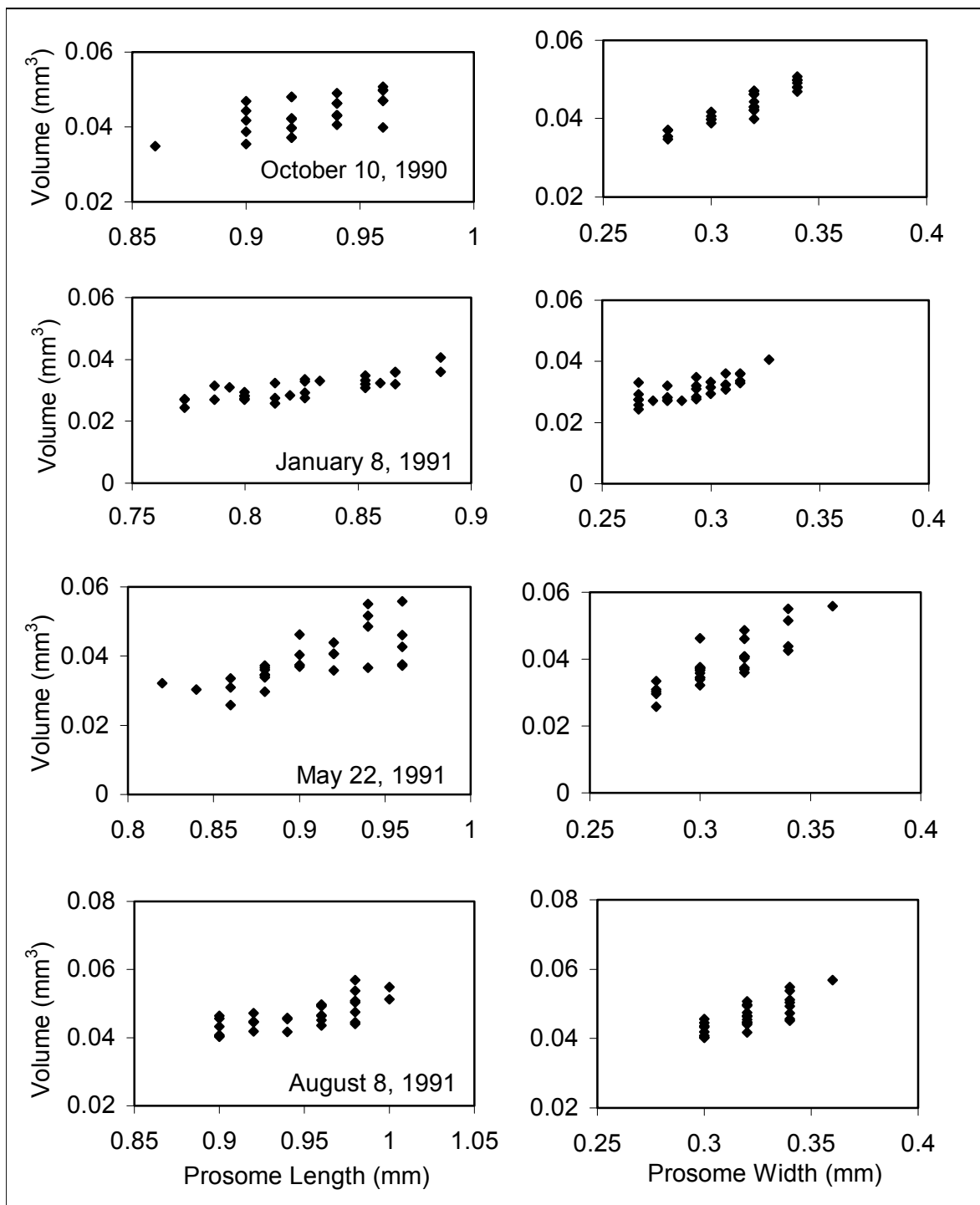


Figure 2

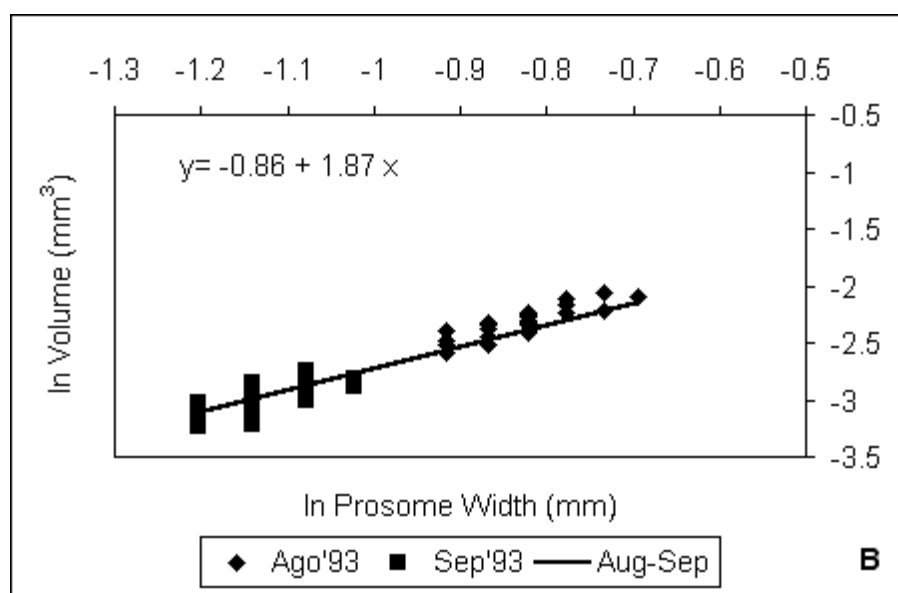
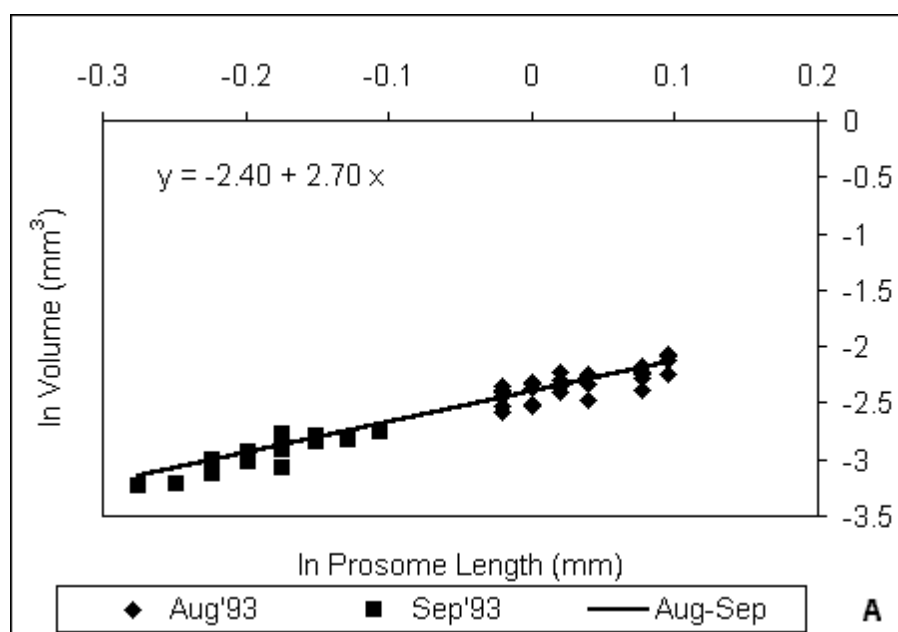


Figure 3

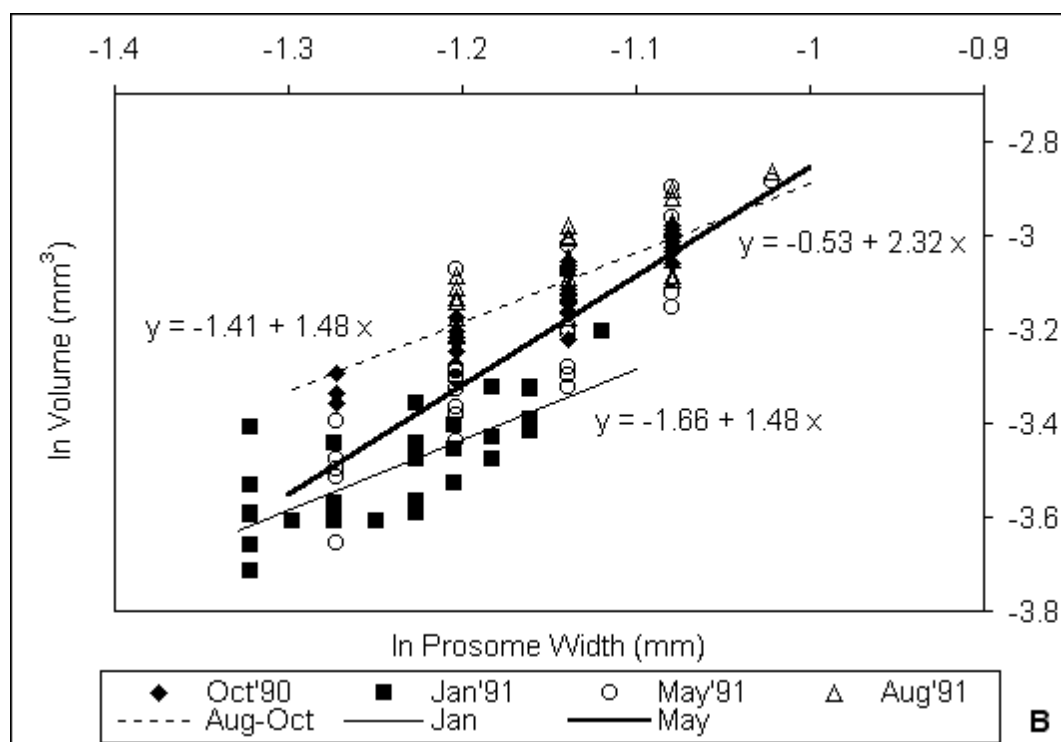
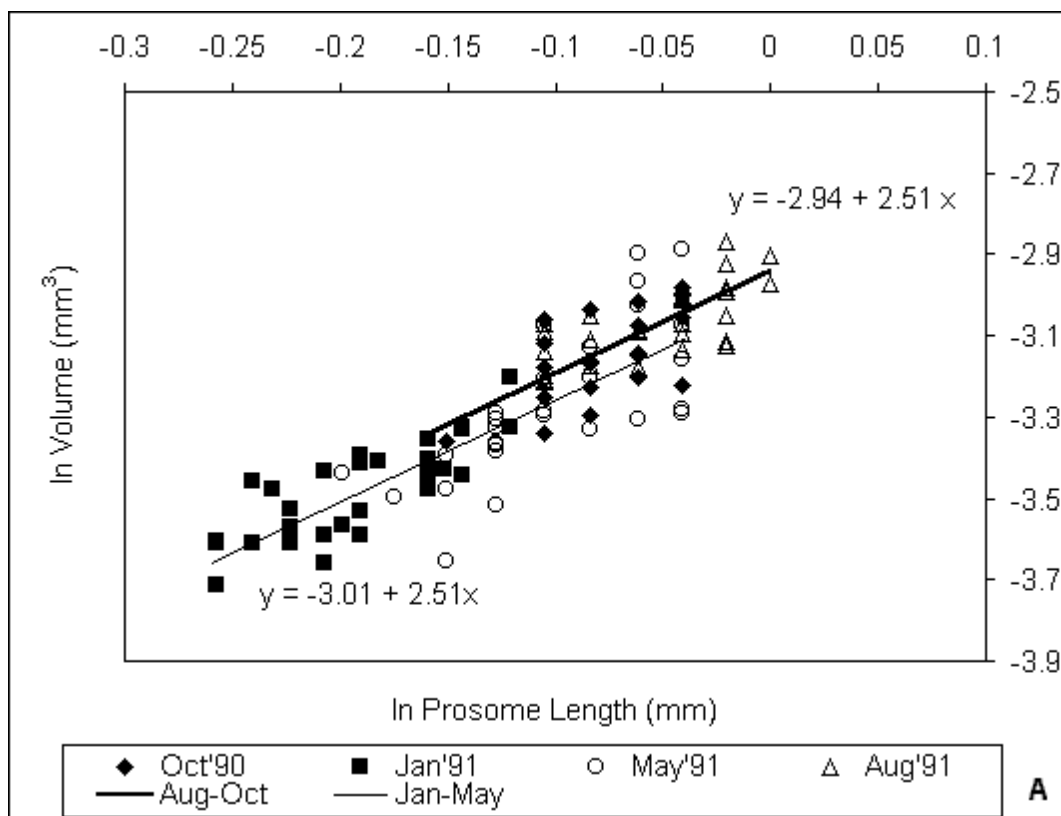


Figure 4

Table 1. Temperature, salinity, chlorophyll-a values observed at the collection dates

Date	Temp °C	Sal ppt	Chl-a ug at l ⁻¹
10-Oct-90	15.8	35.45	2.06
08-Ene-91	24.4	39.69	10.19
22-May-91	13.6	33.27	5.54
08-Ago-91	7.4	32.98	22.81
05-Ago-93	7.3	29.29	18.03
21-Sep-93	13.4	31.24	11.92

Table 2. Mean \pm standard error of *E. americana* and *A. tonsa* females morphometric variables and individual volume. General average of each species morphometric variables and volume values. Variation coefficient in percent (VC %) of PL, PW and V.

Species/ Dates	N	PL (mm)			PW (mm)			PH (mm)		UL (mm)		UW (mm)		V (mm ³)	
<i>E. americana</i> 05-Aug-93 21-Sep-93 Total average/range	30	1.031	0.042	4.07	0.436	0.025	5.73	0.387	0.024	0.713	0.049	0.103	0.008	0.0975	0.0132
	30	0.827	0.029	3.50	0.330	0.016	4.85	0.320	0.019	0.562	0.035	0.118	0.016	0.0522	0.0067
	60	0.929	0.036	3.82	0.383	0.021	5.35	0.354	0.022	0.638	0.042	0.111	0.012	0.0749	0.0097
<i>A. tonsa</i> 10/10/1990 08-Jan-91 22-May-91 08-Aug-91 Total average/range	30	0.929	0.024	2.58	0.316	0.019	6.01	0.269	0.011	0.266	0.012	0.099	0.006	0.0435	0.0044
	30	0.824	0.033	4.00	0.291	0.017	5.84	0.230	0.013	0.225	0.014	0.097	0.005	0.0307	0.0037
	30	0.904	0.038	4.20	0.309	0.021	6.79	0.256	0.028	0.254	0.028	0.072	0.009	0.0388	0.0073
	30	0.946	0.033	3.49	0.321	0.017	5.30	0.284	0.012	0.283	0.018	0.076	0.008	0.0465	0.0042
	120	0.901	0.032	3.55	0.309	0.019	5.98	0.260	0.016	0.257	0.018	0.086	0.007	0.0399	0.0044

Table 3. Mean \pm standard error of *E. americana* and *A. tonsa* morphometric ratios: PL:PW, PL:PH, PL:UL, UL:UW, for each dates.

Species/ Dates	PL/PW	PL/PH	PL/UL	UL/UW
<i>E. americana</i> 05-Aug-93	2.37 0.1231	2.67 0.1566	1.45 0.0899	6.97 0.4979
21-Sep-93	2.51 0.1089	2.59 0.1425	1.47 0.0749	4.85 0.6192
Total average	2.44 0.1160	2.63 0.1496	1.46 0.0824	5.91 0.5586
<i>A. tonsa</i> 10-Oct-90	2.95 0.1571	3.46 0.1602	3.49 0.1894	2.69 0.1881
08-Ene-91	2.84 0.1479	3.58 0.1996	3.67 0.2051	2.30 0.1634
22-May-91	2.93 0.1480	3.57 0.3357	3.59 0.3825	3.56 0.5272
08-Ago-91	2.95 0.1375	3.34 0.1813	3.35 0.2061	3.78 0.5170
Total average	2.92 0.1476	3.49 0.2192	3.53 0.2458	3.08 0.3489

Table 4. Lineal regression test equations of *E. americana* and *A. tonsa* prosome length (PL) and prosome width (PW) vs volumen (V) for each date. N, observations number; sb, regression coefficient error; R^2 , determination coefficient; F, Fisher's statistic; p, significance level and x range, the independent variable (volume) range. R^2 Values with the higher percentages of variance explanation and the correspondent best volume predictor variables underlined.

Species/Dates	N	Equation	sb	R^2	F	p	X Range (mm)
<i>E. americana</i> Aug-93	30	$\ln V = -2.4112 + 2.4916 \ln PL$	0.3960	0.585	39.51	0.0000	0.980 1.810
	30	$\ln V = -0.7042 + 1.9625 \ln PW$	0.2380	<u>0.708</u>	67.94	0.0000	0.410 0.520
	Sep-93	30 $\ln V = -2.3956 + 2.9601 \ln PL$	0.2960	<u>0.781</u>	99.59	0.0000	0.760 0.910
		30 $\ln V = -1.0149 + 1.7512 \ln PW$	0.3130	0.528	31.31	0.0000	0.300 0.360
<i>A. tonsa</i>	Oct-90	30 $\ln V = -2.9563 + 2.4769 \ln PL$	0.5798	0.395	18.25	0.0020	0.900 0.960
		30 $\ln V = -1.3439 + 1.5554 \ln PW$	0.1245	<u>0.848</u>	156.15	0.0000	0.280 0.340
	Jan-91	30 $\ln V = -3.0294 + 2.3692 \ln PL$	0.3248	<u>0.655</u>	53.18	0.0000	0.770 0.880
		30 $\ln V = -1.6341 + 1.5009 \ln PW$	0.2394	0.584	39.29	0.0000	0.240 0.330
	May-91	30 $\ln V = -2.9496 + 3.1084 \ln PL$	0.5454	0.537	32.48	0.0000	0.820 0.960
		30 $\ln V = -0.5334 + 2.3227 \ln PW$	0.2676	<u>0.729</u>	75.36	0.0000	0.280 0.360
	Aug-91	30 $\ln V = -2.9709 + 1.8042 \ln PL$	0.3369	0.506	28.67	0.0000	0.900 1.000
		30 $\ln V = -1.5576 + 1.3305 \ln PW$	0.2073	<u>0.596</u>	412.69	0.0000	0.300 0.360