Effects of sample fixation on body shape of *Capitella capitata* (Polychaeta, Capitellidae)

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SUMMARY

Formaldehyde fixed specimens of *Capitella capitata* from the littoral zone of Barcelona showed several body forms : "contracted *C. capitata*" (CC) - pointed prostomium, contracted thorax, anterior setigers distinct; "elongated *C. capitata*" (EC) - triangular prostomium/peristomium, elongated thorax, anterior setigers indistinct; "intermediate *C. capitata*" (IC) - intermediate form between CC and EC. Five fixation tests were performed using different formaldehyde concentrations, sieving or unsieving before fixation, and different periods between sample collection and fixation. By these tests it was shown that CC is the regular form, whereas EC and IC are caused by deficiencies in sample fixation. Recommendations are given for avoiding the fixation aberrant fixation forms which might impair studies in taxonomy and population dynamics of this species.

RÉSUMÉ

Effets de la fixation des échantillons sur la forme du corps de *Capitella capitata* (Polychaeta, Capitellidae)

Des spécimens préservés de *Capitella capitata* du littoral de Barcelone montrent plusieurs formes du corps : "*C. capitata* contractée" (CC) - prostomium pointu, thorax contracté, sétigères antérieurs bien differenciés ; "*C. capitata* allongée (EC) - prostomium/peristomium triangulaire, thorax allongé, sétigères antérieurs pas différenciés ; "*C. capitata* intermédiaire" (IC) - forme intermédiaire entre CC et EC. Cinq expériences de fixation ont été effectuées avec différentes concentrations de formol, tamisage et pas de tamisage des échantillons avant la fixation et différentes périodes de temps entre le prélèvement et la fixation. Les résultats indiquent que CC est la forme régulière, tandis que EC et IC sont le résultat des imperfections de la fixation des échantillons. On propose plusieurs recommandations pour éviter les formes aberrantes comme conséquence du procédé de fixation qui peut contrarier les études de systématique et de dynamique des populations de cette espèce.

INTRODUCTION

Traditional procedures for treating soft-bottom samples to obtain benthic macrofauna were given, among

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others, by BANSE & HOBSON (1974), FAUCHALD (1977), and ELEFTHERIOU & HOLME (1984). Those techniques include screening and fixation of samples which may cause alterations to the organisms such as contraction or damage to soft-bodied animals. Degrees of contraction may vary in some organisms and therefore, changes are unpredictable and possibly resulting confusions in identifications.

In previous studies of benthic macrofauna from the littoral zone of Barcelona several body forms of preserved specimens of *Capitella capitata* (FABRICIUS, 1780) were observed (ROS *et al.*, 1990; ROS & CARDELL, 1991; FLOS & SERRA, 1992). The different body shapes are found in the same preserved sample and which have not been distinguished in live specimens. This suggests that treatment of samples may modify the body form of this species thus impairing taxonomy and population dynamics research. In view of future studies it is of interest to determine which sample treatments may produce changes in the body shape of *C. capitata*.

MATERIAL AND METHODS

Bottom samples were collected by SCUBA diving from the upper 10 cm of sediment near the Olympic Harbour of Barcelona (15 m depth) between February and May 1992. The sediment in this site was fine sand (0.125 - 0.250 mm).

Five different tests were designed to evaluate single and combined effects of the following variables of fixation: formaldehyde concentration, screening of samples and time between collection and fixation of samples.

Test 1. — Formaldehyde concentration.

Live specimens were sorted from the sediment and submerged in 11 different solutions of formaldehyde in sea water (from 0.1 to 4%).

Test 2. — Formaldehyde concentration and screening of samples.

Twenty samples of sediment were collected. The combined effect of the following two variables was tested: sieving (through a 0.5 mm mesh) or unsieving samples before fixation and formaldehyde concentration (1 and 4%). Five replicates from each combination were obtained.

Test 3. — Time.

Sixteen samples of sediment were collected. The combined effect of the following three variables was tested: sieving (0.5 mm) or unsieving samples before fixation, formaldehyde concentration (1 and 10%) and time between collection and fixation (0, 2, 4 and 6 hours).

In both test 2 and test 3, sieved samples consisted of 125 ml of sediment and 62.5 ml of seawater, while unsieved samples consisted of 250 ml of sediment and 125 ml of seawater. The different formaldehyde concentrations were calculated according to the volume of the overlying water by the addition of concentrated formaldehyde.

Test 4. — Presence of sediment.

Each of 100 live specimens was transferred to separate jars with 2 ml of a 4% formaldehyde solution, and each of 100 live specimens was covered by a 3 mm layer of fine sand and fixed by the addition of 2 ml of a 4% formaldehyde solution.

Test 5. — Volume of sediment.

Five samples of different volumes of sediment were placed and fixed with a 4% formaldehyde solution inside cylindrical sample jars of several sizes wich were turned several times. After 24 hours of sedimentation, each sample was divided in three horizontal layers and each level was analyzed separately.

Individuals of the different body shapes were counted in all the tests. A two way ANOVA test and Pearson's correlations were performed to assess the effect of these variables on the body shape of *C. capitata*.

RESULTS

Three different body forms of *C. capitata* were distinguished according to their thorax shape. They were called "contracted *C. capitata*" (CC),"enlarged *C. capitata*" (EC) and "intermediate *C. capitata*" (IC). CC has a rather pointed prostomium partially withdrawn back into the contracted first setiger, indistinct peristomium and thoracic setigers are strongly distinct (wider than they are long). EC presents a prostomium and peristomium with a broadly rounded triangular shape, thoracic setigers indistinct (slightly broader than they are long) and a slender thorax. IC presents an intermediate thorax shape between CC and EC and has a rather pointed prostomium partially withdrawn

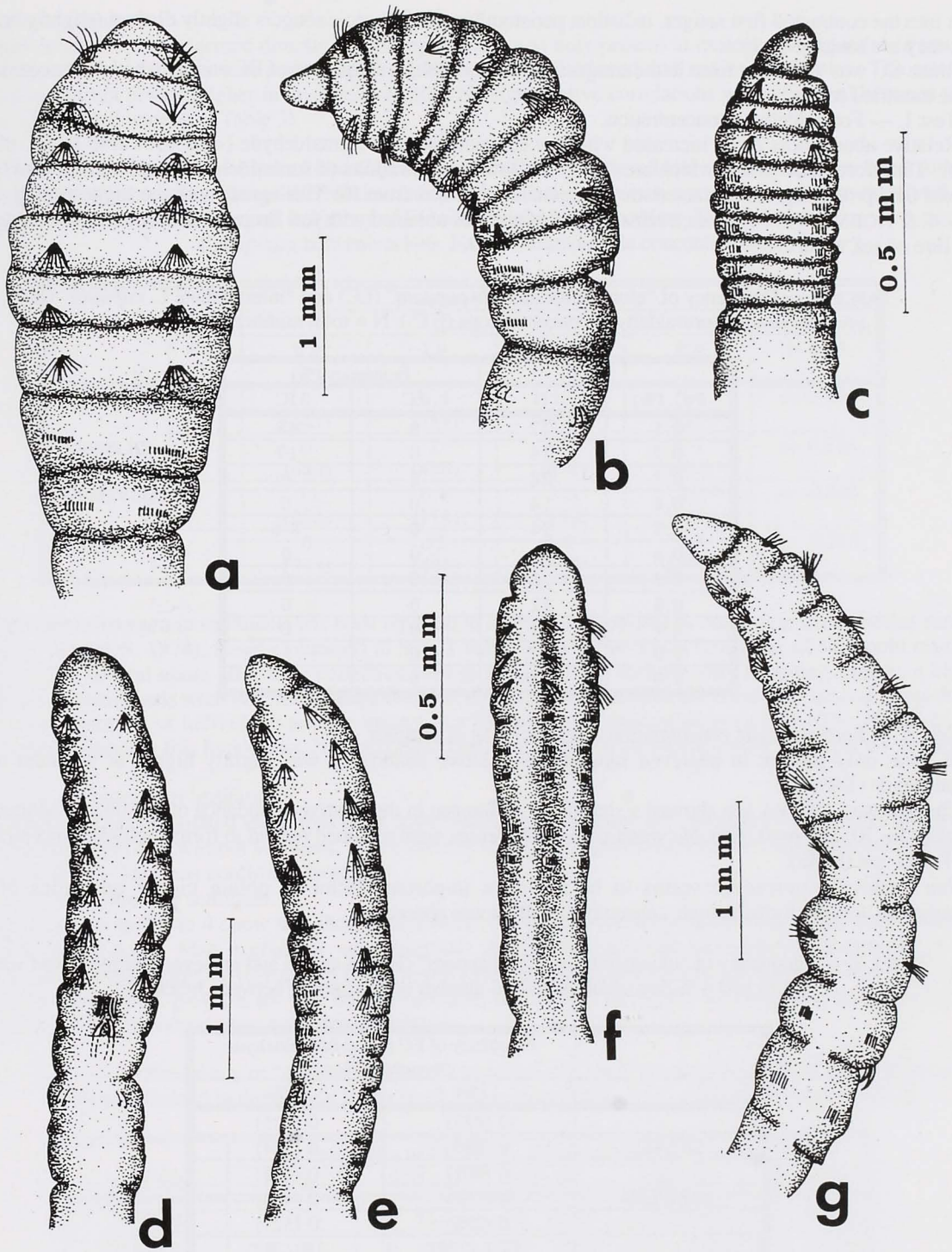

FIG. 1. — Anterior end of "contracted *Capitella capitata*" (CC): **a**, dorsal view (female). **b**, lateral view (male). **c**, lateral view (juvenile). Anterior end of "elongated *C. capitata*" (EC): **d**, dorsal view (male). **e**, lateral view (female). **f**, lateral view (juvenile). Anterior end of "intermediate *C. capitata*" (IC): **g**, lateral view (male).

back into the contracted first setiger, indistinct peristomium, and thoracic setigers slightly distinct (slightly broader than they are long) (Fig. 1).

Since CC was dominant form in the samples, results only show frequency of EC and IC which were considered as the modified forms.

Test 1. — Formaldehyde concentration.

Relative abundances of IC increased with low concentrations of formaldehyde (<0.2%) (Table 1). EC did not occur. Therefore, individuals which are fixed with low concentrations of formaldehyde probably die slowly and relaxed thus producing the characteristic enlargement of thorax from IC. This agrees with the suggestion given by BANSE & HOBSON (1974) about a relaxed state of animals obtained with few drops of concentrated formaldehyde in 1 litre of sea water.

TABLE 1. — Frequency of "elongated *Capitella capitata*" (EC) and "intermediate *C. capitata*" (IC) at various formaldehyde concentrations (F.C.). N = total number of *C. capitata*.

		Frequency (%)	
F.C. (%)	N	EC	IC
0.1	27	0	48
0.2	24	0	17
0.3	28	0	0
0.4	14	0	0
0.5	34	0	8.8
0.6	16	0	0
0.7	20	0	0
0.8	22	0	0
0.9	12	0	0
1.0	281	0	1
4.0	130	0	0

Test 2. — Formaldehyde concentrations and screening of samples.

EC was only present in unsieved samples. Its relative abundance was slightly higher at 1% than at 4% formaldehyde (Table 2).

The two way ANOVA test showed a significant difference in the relative abundance of EC on sieved/unsieved samples ($F= 10.206$, $p= 0.006$). No significative differences were obtained related to formaldehyde concentrations ($F= 1.506$, $p= 0.238$).

The unsieving procedure seems to be the most important factor to obtain higher quantities of EC. Nevertheless, a slight formaldehyde concentration effect was observed.

TABLE 2. — Frequency of "elongated *Capitella capitata*" (EC) in sieved and unsieved samples fixed with 1 % and 4 % formaldehyde. Total number of *C. capitata* between brackets.

	Frequency of EC (%) at formaldehyde concentration	
	1 (%)	4 (%)
	0 (423)	0 (705)
SIEVED	0 (280)	0 (584)
	0 (401)	0 (455)
SAMPLES	0 (382)	0 (488)
	0 (295)	0 (527)
	13.7 (270)	3.9 (282)
UNSIEVED	6.4 (328)	6.3 (269)
	0.5 (381)	2.2 (273)
SAMPLES	9.8 (265)	1.4 (286)
	0.5 (210)	0.7 (286)

Test 3. — Time.

When fixation was performed directly after sampling, EC was only present in unsieved samples (as in test 2). Nevertheless, when fixation was performed later, EC appeared in sieved and unsieved samples. The relative abundance was not always higher in unsieved samples. No significative correlations were observed between time and the other tested variables (Table 3).

TABLE. 3. — Frequency of "elongated *Capitella capitata*" (EC) in sieved and unsieved samples fixed with 1% and 4% formaldehyde at different times after their collection. Pearson's correlations (r) are calculated between time and frequency of EC obtained for each procedure. Total number of *C. capitata* betwenbrackets. F.C. = formaldehyde concentration.

		Frequency of EC (%) for fixation after :				Pearson's correlations
	F.C. (%)	0 h	2 h	4 h	6 h	(n= 4)
SIEVED SAMPLES		0 (443)	0.4 (789)	38 (490)	0 (552)	r= 0.256
		0 (680)	2 (438)	0.1 (628)	0.7 (404)	r= 0.028
UNSIEVED SAMPLES		72 (254)	0.8 (118)	7.5 (132)	25 (394)	r= -0.540
		0 (344)	52 (406)	10 (312)	0.4 (502)	r= -0.213

Depletion of oxygen in sediments has been reported to cause the organisms to crawl near the sediment surface (BANSE & HOBSON, 1974). Results obtained in tests 1 and 2 suggest that lower quantities of EC could result in samples fixed several hours after their collection than in samples fixed directly after sampling. Hence, a higher percentage of individuals wich have a CC shape could be expected when samples are fixed after several hours due to the upward migration of individuals and the impact of a formaldehyde concentration near to 40 %. However, our results did not confirm this hypothesis. It is suggested that time produces an unpredictable effect on the amount of EC.

Test 4. — Presence of sediment.

Fourteen percent of isolated specimens fixed under sediment belonged to EC, whereas this form was not present among individuals fixed without sediment. A thin layer of sediment, appears sufficient to change the body shape of some individuals. This was confirmed in test 5.

Test 5. — Volume of sediment.

Results given in Table 4 show that there was a direct relationship between percentage of EC and volume of sediment inside a jar. Also a gradient was observed: the lower layers had the highest percentages of EC. Correlations between cumulative volumes (from upper to lower layers) and relative abundance of EC were highly significative ($r= 0.939$, $p< 0.01$, $n= 13$). High frequencies of EC may be caused by compression increasing and formaldehyde diffusion decreasing with sample volume.

TABLE 4. — Frequency of "elongated *Capitella capitata*" (EC) in five samples of different volumes of sediment divided in three equal layers inside a jar. Total number of *C. capitata* between brackets.

	Frequency of EC (%) in different volumes of sediment				
LAYER	100 ml	200 ml	500 ml	1000 ml	1800 ml
UPPER	0 (3)	0 (27)	2.6 (190)	12.0 (241)	3.7 (106)
MEDIUM	0 (6)	0 (41)	12.6 (214)	19.1 (507)	38.3 (201)
LOWER	0 (6)	0 (173)	23.8 (231)	20.4 (401)	59.3 (221)

Results obtained in the five tests suggest that the simultaneous presence of the CC, EC and IC in a sample can be explained by the combined effect of formaldehyde concentration and sediment. Lower formaldehyde concentrations, during fixation, may induce organisms to change from a contracted (CC) to a more relaxed shape

(EC). The combined effect of sediment and formaldehyde in an unsieved sample inside a jar may be explained due to the thickness of the sediment which can delay the diffusion of formaldehyde, and prolonging death. The presence of large amounts of sediments may be responsible of an increase in the quantities of EC.

DISCUSSION

Traditional techniques used in taxonomy of polychaetes are based on collection, screening (before fixation), fixation and preservation. Nevertheless, in population dynamics studies of *C. capitata*, fixation is generally performed before screening (WARREN, 1976; TSUTSUMI & KIKUCHI, 1984; TSUTSUMI, 1987). When fixation is performed before screening, some EC and IC may be present and thus result in errors of taxonomy and populations dynamics:

- Taxonomy. The evident peristomium of EC can be confused with the achaetous first segment of the genus *Capitomastus*, although there are some other features to distinguish between both genera. This confusion has recently been studied by WARREN (1991).
- Population dynamics. Population dynamics studies of *C. capitata* are performed by different thoracic measures (WARREN, 1976; TSUTSUMI & KIKUCHI, 1984; TSUTSUMI, 1987; MARTIN, 1991), which may be strongly affected by treatment of samples, so erroneous interpretations can be obtained.

CONCLUSIONS

Treatment of soft-bottom samples during fixation may affect the shape of fixed *C. capitata*. Body shapes were related to different combinations of the tested variables. The elongated thorax of IC seems to be associated with low formaldehyde concentrations. Fixation of samples without sieving may produce an increase in the frequency of EC: compression and weight of sediment inside a sample jar affect the shape of *C. capitata*, probably because of decreased formaldehyde diffusion and mechanically prevented body contraction.

Mistakes in taxonomy and population dynamics studies can result from inappropiate fixing procedures. Therefore, to avoid fixation anomalies in *C. capitata* it is recommended:

- Sieve the sample before fixation to avoid effects by compressed sediments. If this is not possible, fix small volumes of sediment (e.g. 200 ml) in several sample jars to allow the diffusion of formaldehyde and to minimize compression.
- Ensure that fixation is done at least at 4% formaldehyde, particularly if the sample cannot be sieved before fixation.
- Turn the sample jar several times after fixation for about 15 minutes to be certain that all the individuals have died.
- Maintain the time between collection and fixation constant during the study.

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