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Influence of temperature and salinity on embryonic development of *Paracentrotus lividus* (Lmk, 1816)

M. Bressan, M. Marin & R. Brunetti
Department of Biology, University of Padova, Padova (Italy)

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Abstract

The combined effects of temperature and salinity on early development of the sea urchin *Paracentrotus lividus* (Lmk, 1816) are reported. The optimal temperature-salinity combinations for development are 18 °–20 °C and 34–35‰; there is a significant temperature-salinity interaction. The optimal conditions found in the experiments are above the mean yearly values for the sampled population's environment (North Adriatic Sea), being more similar to those of the Tyrrhenian Sea. These results suggest that embryonic tolerances to temperature and salinity are under genetic and not environmental control.

Introduction

Temperature and salinity are two of the most important abiotic factors which affect the activity and distribution of marine organisms. They should be considered together, since they are known to interact (Kinne, 1970).

Several studies demonstrate that earlier embryonic stages are more sensitive than adults to these abiotic factors (Runnstrom, 1927; Lönning, 1959; Calabrese, 1969; Gorodilov, 1969; Roller & Stickle, 1985, 1993). Furthermore, the tolerance limits of embryos are probably responsible for the relatively fixed thermo-saline range of reproduction (Andronikov, 1975; Fujisawa, 1989). Instead, adults do not necessarily have the same environmental requirements as embryos, and the distribution of a species may be in relation to the adults' tolerance, which may be very different from that of the embryos: for example, the geographical distribution of *Hemicentrotus pulcherrimus* and *Strongylocentrotus nudus* does not fit the optimal temperature ranges for their embryonic development (Fujisawa & Shigei, 1990).

With respect to salinity, Roller & Stickle (1993), testing various salinities on the embryonic development of *Lytechinus variegatus*, found that adults and embryos do not really have the same requirements.

Paracentrotus lividus (Lmk, 1816) is widespread along the Mediterranean and European Atlantic coasts; its reproductive cycle has been studied by Rose (1926), Runnström (1936), Lubet (1953), Keckes (1966), Fenaux (1968), Hörstadius (1973, 1975), Régis (1979), Byrne (1990) and Fernandez & Caltacirone (1992). From these studies, temperature seems to be one of the most important factors acting on the distribution of this species. However, the effects of temperature and salinity on the embryonic development of *P. lividus* have been poorly investigated.

The present study analyses the combined effects of temperature and salinity on the early developmental stages of this organism.

Materials and methods

Sea urchins were collected in the littoral zone outside the Lagoon of Venice, kept in the laboratory (Hydrobiological Station of Chioggia, University of Padova) in running sea water at salinity of 34±1‰ and at a temperature of 18±1 °C, and fed with *Ulva rigida*. Experiments were performed with artificial sea water (Marin *et al.*, 1987). For each experiment only one pair of sea urchins was used as recommended by Bougis (1967). Gametes, obtained by KCl coelomic injection (Tyler,

Table 1. Embryonic growth: centres of the response surfaces of figure 3 and, in brackets, maximum and minimum extents of contour levels at 95% of maximum length of somatic rod. Mean values and standard errors are reported for temperature and salinity for the first and the second set of experiments.

1st set of experiments		
Exp.	Temp. °C	Sal. ‰
A	20.46 (18.1–22.9)	34.19 (31.4–37.0)
B	18.94 (16.0–21.9)	36.09 (33.1–39.1)
C	19.10 (16.0–22.2)	35.12 (32.2–38.1)
Mean	19.50 (16.7–22.3)	35.13 (33.2–38.1)
S.E.	0.49	0.55
2nd set of experiments		
Exp.	Temp. °C	Sal. ‰
D	18.09 (15.2–21.0)	34.92 (32.0–37.9)
E	18.12 (15.2–21.0)	34.76 (31.8–37.7)
F	18.08 (15.2–20.5)	34.88 (31.9–37.8)
G	18.11 (15.2–21.0)	34.79 (31.9–37.7)
Mean	18.10 (15.2–20.9)	34.84 (31.9–37.8)
S.E.	0.01	0.04

Table 2. Mean percentages ± standard errors of survival at the end of the experiments E, F and G, and results of two-way ANOVA. *** = $P < 0.001$; ** = $0.01 > P > 0.001$; * = $0.05 > P > 0.01$; n.s. not significant.

Exp.E	13°C	16°C	19°C	22°C
30‰	74.5 ± 5.3	66.0 ± 2.9	79.5 ± 5.8	67.2 ± 4.4
33‰	63.7 ± 2.3	86.8 ± 2.2	70.2 ± 12.5	55.6 ± 3.6
36‰	75.6 ± 7.4	86.1 ± 3.9	77.2 ± 4.6	52.5 ± 4.6
39‰	76.8 ± 5.3	73.3 ± 3.8	76.8 ± 2.0	44.4 ± 4.0
ANOVA table		$n = 6$		
Source of variation		df	MS	F
Among temperature		3	499	22.7***
Among salinities		3	24	1.1 ns
Interaction		9	83	3.8***
Error		80	22	
Exp.F	13°C	16°C	19°C	22°C
30‰	99.4 ± 7.0	92.4 ± 4.6	97.6 ± 5.3	88.5 ± 4.3
33‰	89.0 ± 5.6	90.7 ± 3.8	100.0	90.7 ± 6.2
36‰	100.0	96.7 ± 5.4	100.0	100.0
39‰	95.0 ± 4.6	79.4 ± 3.6	88.5 ± 5.5	77.7 ± 4.3
ANOVA table		$n = 12$		
Source of variation		df	MS	F
Among temperature		3	29	2.6*
Among salinities		3	78	7.0***
Interaction		9	10	0.9 ns
Error		176	11	
Exp.G	13°C	16°C	19°C	22°C
30‰	66.9 ± 5.1	58.9 ± 3.6	70.1 ± 6.7	66.3 ± 3.8
33‰	72.7 ± 3.9	71.4 ± 5.4	73.3 ± 5.8	88.4 ± 7.8
36‰	76.8 ± 4.7	72.3 ± 5.9	76.2 ± 5.4	89.0 ± 6.7
39‰	76.2 ± 3.9	74.6 ± 4.1	77.1 ± 4.0	79.4 ± 4.3
ANOVA table		$n = 12$		
Source of variation		df	MS	F
Among temperature		3	74	3.3*
Among salinities		3	115	5.2**
Interaction		9	14	0.6 ns
Error		176	22	

1949), were mixed in filtered (0.45 μm) sea water at 18 °C and 35‰ salinity, at a 20,000:1 sperms:egg ratio. After the raising of the fertilization envelopes, the eggs were washed and distributed in glass beakers at the experimental salinities, so as to have a suspension of 50 eggs ml^{-1}. The beakers were then placed in temperature-controlled chambers, at different experimental temperatures.

Four larval cultures for each temperature-salinity combination were set up and fixed with neutralized formalin 10% at the end of the experiments. One culture was used to determine frequencies of the various developmental stages, including plutei with skeletal anomalies. The other ones were filtered through a 25-mm diameter Millipore filter (1.2 μm); the filters were then washed with deionized water, dried at 60 °C and mounted on slides with cedar oil, according to the technique described by Bougis (1967), in order to measure the length of the somatic rods of 80 4-armed plutei. Each four radiate spicule includes one somatic rod, one post-oral rod, one antero-lateral rod and one anal rod (Pressoir, 1959); the somatic rod length is reported as a very sensitive index of the effects of different environmental conditions on the endotrophic growth of the plutei (Bougis, 1967). In order to avoid effects related to the feeding of the embryos, our experiments were performed during the endotrophic phase. In *P. lividus* this phase ends up when the length (somatic rod + post-oral rod) of the four-armed plutei reaches a mean value between 390 and 500 μm (Fenaux *et al.*, 1985). The relation between temperature and duration of the endotrophic phase is expressed by a linear equation

Table 3. Mean percentages (n = 10) and, in brackets, standard errors of different embryonic stages in the experiments H, I, J, K, L; g = gastrulae; pr = prisms; pl = plutei.

	18°C			25°C		
	g	pr	pl	g	pr	pl
30‰	10.6	40.2	49.2	6.4	15.6	78.0
	(10.1)	(10.9)	(15.8)	(6.1)	(13.1)	(28.8)
35‰	18.8	22.7	58.5	19.1	20.3	60.6
	(17.7)	(7.2)	(15.0)	(18.0)	(7.5)	(15.7)
40‰	91.5	8.5	0	68.6	21.9	9.5
	(6.3)	(6.3)	(0)	(19.2)	(13.9)	(5.6)

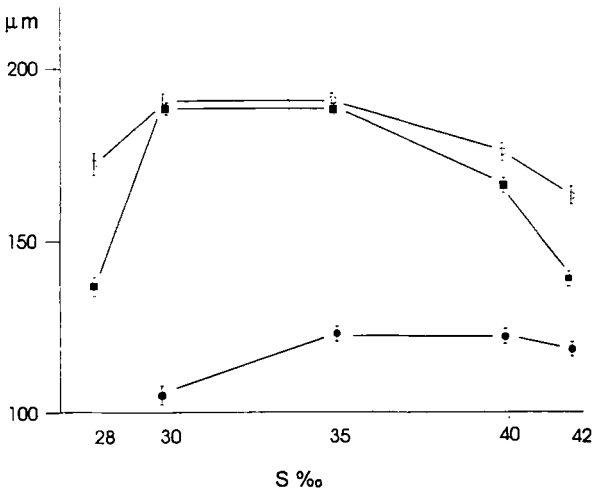

Fig. 1. Mean length (μm) of somatic rods of 80 4-armed echinoplutei, with confidence limits of the mean ($\alpha = 0.05$), in the first set of experiments (A, B, C); • = 11 °C, o = 18 °C, ■ = 25 °C.

(Bougis, 1971); therefore, in order to make possible the comparison among embryos developed at different temperatures, the durations of the experiments were calculated according to this equation.

Three sets of experiments were planned. The first set was performed to determine preliminarily the thermo-saline conditions optimal for embryonic growth. The specimens used in this set were collected on October 1990 and kept in the laboratory in controlled conditions (18 °C, 35‰) for one month, before inducing experimental spawning. In each experiment (indicated as A, B, C) fifteen thermo-saline combinations were tested, using three temperatures (11, 18 and 25 °C) and five salinities (28, 30, 35, 40 and 42‰). Embryonic growth (as mean length of somatic rod), and the frequencies of developmental stages

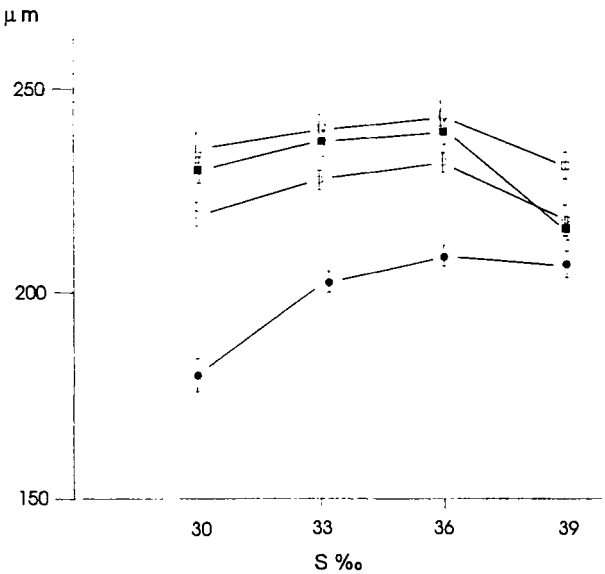

Fig. 2. Mean length (μm) of somatic rods of 80 4-armed echinoplutei, with confidence limits of the mean ($\alpha = 0.05$), in the second set of experiments (D, E, F, G); • = 13 °C, o = 16 °C, □ = 19 °C, ■ = 22 °C.

were determined. These preliminary experiments lasted 80% of the time required by the embryos to reach maximum somatic rod length, according to the equation of Bougis, that is 150 hours at 11 °C, 69 hours at 18 °C and 41 hours at 25 °C.

The second set (four experiments indicated as D, E, F and G) was planned within a more restricted thermo-saline range, to test if the temperature at which gonads ripened influences the thermal tolerance of the embryos. Experiments D and F were performed with gametes immediately furnished by animals collected on April 1991 and on September 1991, respectively, that is ripened in the field at 15 or less °C and at 20 or more °C. Experiments E and G were performed with gametes ripened under laboratory conditions at 15 ° and 20 °C. In order to obtain the latter situation some animals, collected on April 1991, were induced to spawn and then kept in controlled laboratory conditions, until their gonads ripened. The ripeness of the gonads of sea urchins under laboratory conditions is reported only by Yamamoto *et al.* (1988) and Sakairi *et al.* (1989) for three other species of Echinoids. Sixteen thermo-saline combinations, resulting from the previous experiments to allow embryonic development, were tested, using four temperatures (13, 16, 19 and 22 °C) and four salinities (30, 33, 36 and 39‰). Embryonic growth and the frequencies

of developmental stages were determined; in three of these experiments (E, F and G) the survival rate, evaluated as the frequency of embryos at the end of the experiment on the initial number of eggs, was also calculated. In this set the experiments lasted the time necessary to reach maximum somatic rod length, that is 144 hours at 13 °C, 104 hours at 16 °C, 79 hours at 19 °C and 63 hours at 22 °C.

At last a third set of 5 experiments (indicated as H, I, J, K and L) was performed to highlight the influence of temperature-salinity conditions (t ° 18 and 25 °C; S‰ 30, 35 and 40‰) on developmental rate. For this purpose, samples at 18 and 25 °C were fixed respectively at 34 and 22 hours from fertilization, which are the times necessary for the embryos to develop between prism and pluteus stages.

Embryonic growth and survival data were processed according to the 'response surfaces' method (Box, 1954; Box & Youle, 1955; Alderdice, 1972), because of its comprehensive pictorial representation of the optimality region and of the interaction of the variables. The response surface consists of a family of ellipses (contour levels), with the same centre and the same axis; the centre singles out the optimal temperature and salinity for the embryonic development or survival.

The 2-way ANOVA and G-test methods (Sokal & Rohlf, 1981) were performed on the embryonic growth and survival data and for comparing the frequencies of the different embryonic stages.

Results

Embryonic growth at different temperature-salinity combinations is shown in Fig. 1 for experiments A, B, C, and in Fig. 2 for experiments D, E, F, G. These data, analysed by means of a two-way ANOVA, show that temperature-salinity interactions are always highly significant ($P<0.001$). The combinations of high salinity-high temperature and low salinity-low temperature are the most negative.

The same results analysed with the 'response surfaces' methodology are shown in Fig. 3. They indicate that the optimal temperature-salinity combination falls between 18.1 and 20.5 °C and 34.2 and 36.1‰ (Table 1).

The mean percentages of the plutei living at the end of the experiments E, F and G are shown in Table 2. The results of the two-way ANOVA shows that temperature significantly affects the survival, while an effect of the salinity as well as a temperature-salinity interaction, is not always present. The response surfaces calculated from survival rates are presented in Fig. 4. Although the size of these surfaces at the same contour level (70% of maximum survival value) differs considerably in these three experiments, probably as a consequence of genetic differences among the pair of breeders, the optimality centres nearly coincide (mean values±S.E.: 17.36±0.21 °C and 34.53±0.11‰).

The mean frequencies of prisms (pr), plutei (pl), as well as those of embryos precociously stopped in development at or before gastrulation (est), detected in the first two sets of experiments, and the results of the G-test are shown in Figs 5 and 6. At 11 °C, 'est' are present at all salinities except 35‰, and this phenomenon is stronger at low salinities. The fastest developmental rate, expressed as frequencies of plutei at the end of the experiment, was registered at 18 °C at salinities of 30 and 35‰. The two-way ANOVA was also performed on the frequencies of the plutei with skeletal anomalies on the total plutei; the relations between these frequencies and the thermo-saline conditions are never statistically significant.

The results of the third set of experiments (H, I, J, K, L), are indicated in Table 3 and Fig. 7, where the comparisons according to G-test method are shown. The results clearly indicate that development is faster at 18 than at 25 °C, and at salinity of 30–35 rather than 40‰. In particular, 30‰ salinity seems to reduce the length of the gastrula stage and to prolong that of the prism.

Discussion and conclusions

Our data suggest that in *P. lividus* the three considered biological parameters (embryonic endotrophic growth, survival and developmental rate) show the same optimality ranges: 18–20 °C for temperature and 34–35‰ for salinity. Nevertheless from the comparison between the response surfaces calculated on the embryonic growth (Fig. 3) and on the survival rates (Fig. 4) it is clear that survival is more sensitive than embryonic growth to the effects of thermo-saline variations.

However, the temperature-salinity combinations which, on the basis of the present study, turn out to be optimal do not fit the mean hydrological conditions of the area where the sampled population lives (Table 4). Thus, the problem arises as to whether optimal thermal-salinity conditions for the development of

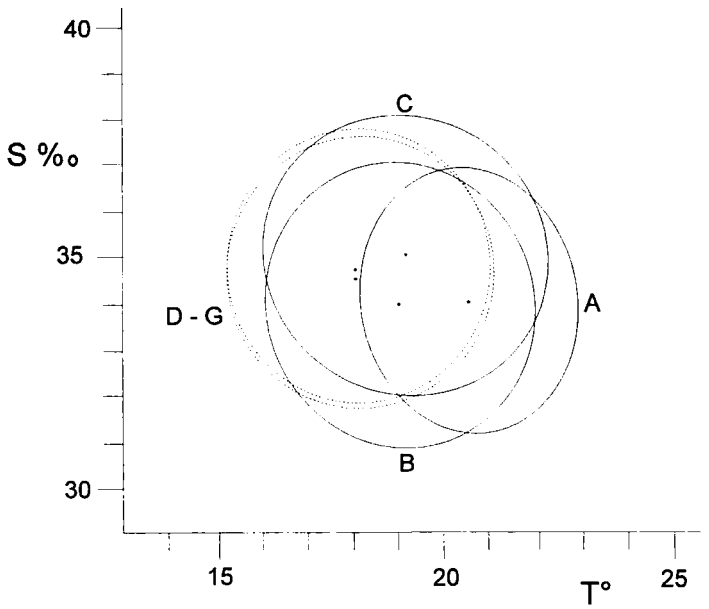

Fig. 3. Response surfaces: optimality centres and contour levels at 95% of maximum length of somatic rods from experiments A, B, C, D, E, F, G. Dashed contour lines delimit the surface in which contour levels for experiments D, E, F and G superpose.

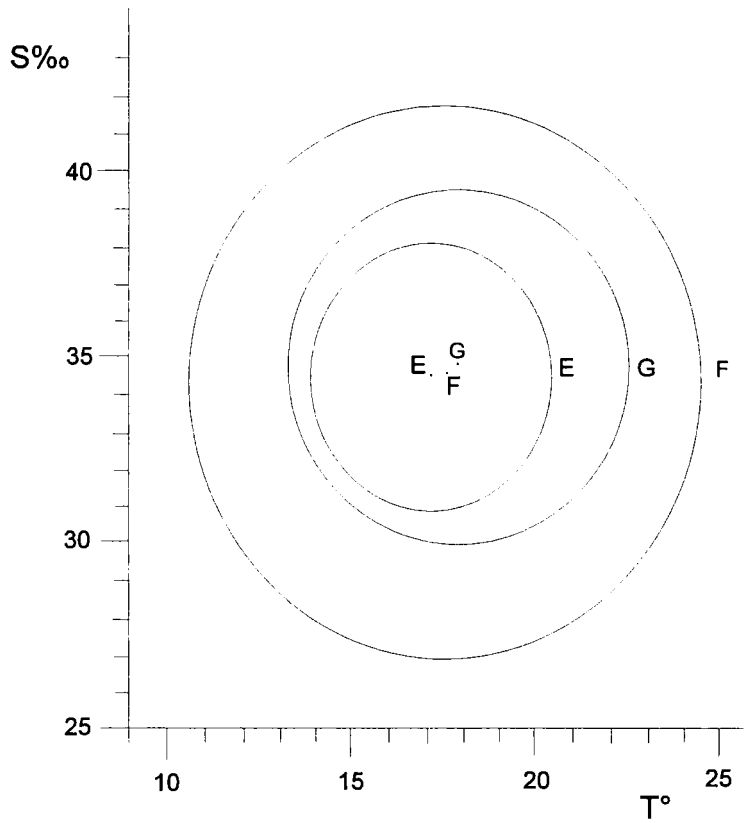

Fig. 4. Response surfaces: optimality centres and contour levels at 70% of maximum survival from experiments E, F, G.

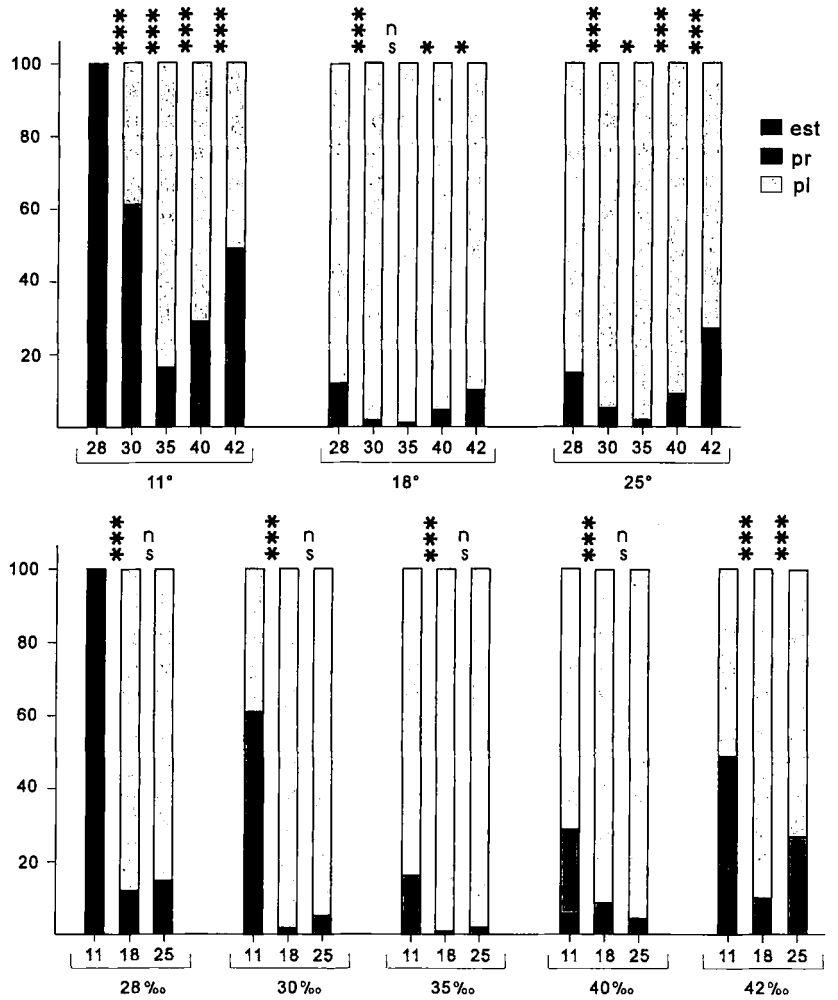

Fig. 5. Mean percentages of developmental stages at different thermo-saline combinations from experiments A, B, C. est = early stopped in development, pr = prisms, pl = plutei. Statistical comparisons (G-test) between columns are reported: $\star\star\star = P<0.001$, $\star = 0.05>P>0.01$, n.s. = not significant. Upper graph: as function of salinities; lower graph: as function of temperatures.

a species are due to acclimatization or are under genetic control.

Kinne (1970) suggests the possible influence on embryonic development of environmental conditions at the moment of gonad ripening; for salinity, this was confirmed by Davis (1958) on bivalve embryos. Giudice (1985) and Sconzo *et al.* (1986) report that unripe oocytes of sea-urchin are able to react to a temperature increase, producing heat-shock proteins which induce thermal tolerance in the eggs. However, for *Psammechinus miliaris*, Gezelius (1963) states that the cleavage rate at a given temperature does not depend on the salinity at which the gonads ripened, but on the salinity to which the adults have become adapted.

Based on the results of hybridation experiments between two morphs of *Echinaster modestus*, Watts *et al.* (1982) drew the conclusion that varying thermo-saline requirements are genetically controlled and not environmentally influenced; similar results were obtained by Roller & Stickle (1985) by means of hybrid-cross experiments between two species of *Strongylocentrotus*. Our data agree with the latter conclusions. In fact, the optimal temperature-salinity conditions observed in our experiments are higher than those present in the laboratory as well as in the collecting area (Table 4), where temperature and salinity show an annual pattern characterized by a large range with mean values below 18 °C and 35‰ respectively for most of the year. Instead, the optimal

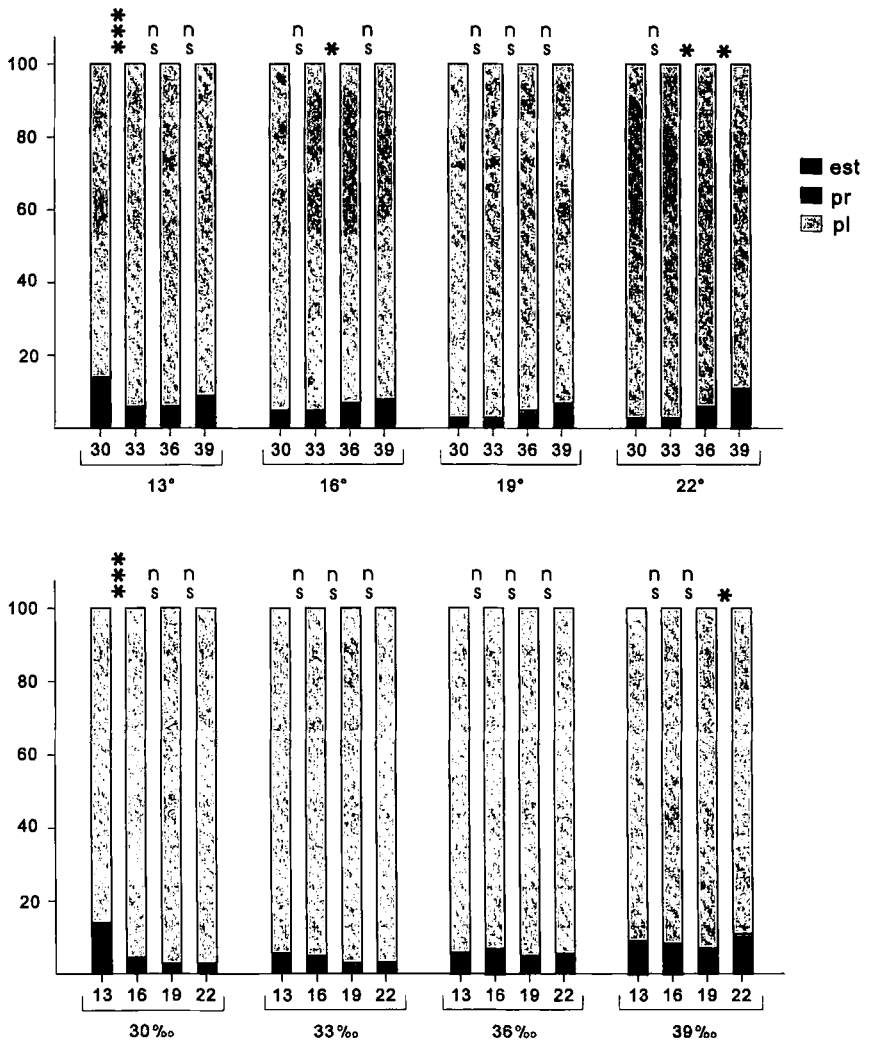

Fig. 6. Mean percentages of developmental stages at different thermo-saline combinations from experiments D, E, F, G. Abbreviations and statistical comparisons as in Fig. 5. Upper graph: as function of salinities; lower graph: as function of temperatures.

temperature and salinity for the development in our experiments are similar to the thermal-saline conditions in the Gulf of Naples, where salinity ranges from 37 to 39‰ and *P. lividus* is ripe all year round (Hörstadius, 1973). However the best periods for ripe animals are February–June and September–November (Hörstadius, 1975), when the temperature rises from 14 ° to 22 °C or decreases from 23 ° to 15 °C (Table 5). These observations are in agreement with those of Fenaux (1968) who, at Villefranche-sur-mer (Northern Tyrrhenian), found that *P. lividus* spawns in June, when the temperature rises from 19 to 21 °C, and principally in autumn when it falls from 25 to 16 °C.

Nevertheless, if the thermo-saline tolerances of the species are mainly under genetic control, acclimatization to mean environmental conditions might occur. This may explain the observations of Hörstadius (1925, 1973, 1975) and Runnström (1936), who report the presence of two types of eggs at Naples: winter ones which develop in the thermal range between 8–23 °C, and summer ones which develop from 16–29 °C. However, these differences may also be due to genetic make up. Our experiments, each performed with the eggs from a single animal, showed the presence of a high degree of interfemale variability in tolerance to temperature as well as to salinity. Thus, changes in environmental conditions may stimulate different members of the sea urchin population to spawn, and only when temperature salinity combinations are close to optimal values can the whole population reproduce.

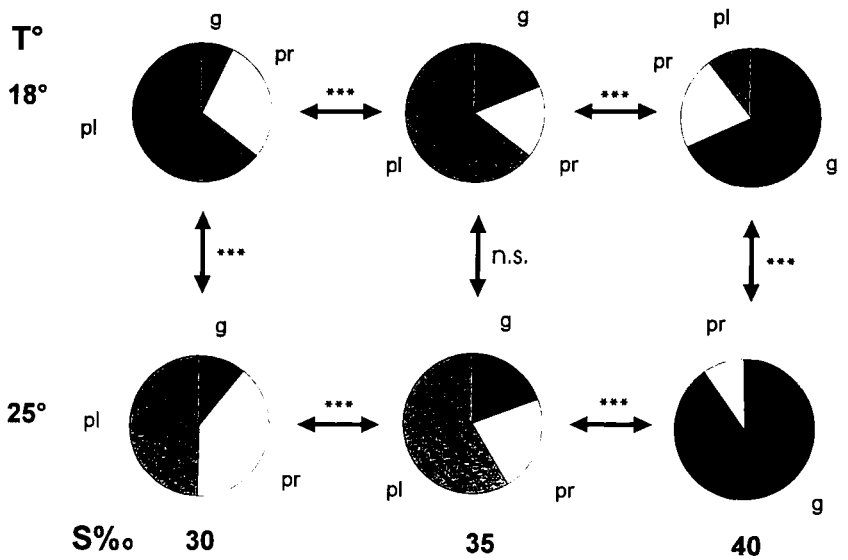

Fig. 7. Mean percentages of early gastrulae (g), prisms (pr) and plutei (pl) stages from experiments H, I, J, K, L. Statistical comparisons between thermo-saline combinations (G-test): $\star\star\star = P<0.001$, n.s. = not significant.

Table 4. Temperature and salinity conditions in collecting area (Venice-VE) (+) and at Naples (NA) (++).

	Mean monthly values											
	J	F	M	A	M	J	J	A	S	O	N	D
T°C												
VE	7.5	7.7	9.5	12.9	17.0	20.5	23.7	24.2	21.7	17.4	13.1	9.3
NA	15.5	14.3	13.7	15.4	22.7	22.5	26.0	27.5	23.4	23.2	19.2	15.6
S‰												
VE	35.4	35.2	34.9	33.2	32.9	33.1	32.3	33.4	33.8	33.6	33.8	35.2
NA	38.1	37.7	37.7	37.4	37.4	37.5	37.8	37.8	38.0	38.2	37.9	38.0

Duration (# of months) of temperature and salinity ranges in both localities.

Thermal range (°C)			Saline range (‰)		
	Venezia	Napoli		Venezia	Napoli
<10	4	0	32–33	2	0
10–15	2	2	33–34	6	0
15–20	2	4	34–35	1	0
>20	4	6	35–36	3	0
			36–37	0	0
			37–38	0	8
			38–39	0	4
<18	8	5	<36	12	0
>18	4	7	>36	0	12

\+ Brunetti *et al.* (1983) for the period 1974–1981.

++ Scotto di Carlo (pers. comm.) for the period 1984–1987.

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