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Thermo-haline tolerance of *Ciona intestinalis* (L., 1767) at different developmental stages

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Abstract : The tolerance of a population of C. *intestinalis* from the Venetian Lagoon to various combinations of temperature and salinity has been tested at different stages of its biological cycle. Mortality rate, growth and a "life quality-index" have been evaluated. Young animals mainly differ from adults in the interaction between temperature and salinity : this is of high-high type in the former and of high-low type in the latter. Young animals are more resistant than adults to low temperatures; this fact could explain the presence of the species in coastal environments with winter temperatures under 10° C. Developing eggs are less tolerant to temperature and exhibit a high-low thermo-haline interraction, as it is for adults. The optimality centres at the different stages lie at a salinity of about 35 ‰ and at a temperature between 15° C and 20° C. We can conclude that our population does not present any noticeable adaptation to the lagoon environment ; on the contrary it is very well adapted to the average conditions of the Mediterranean Sea. Our résults are finally compared with those obtained by other Authors on different populations of the same species.

Résumé : Dans ce travail sont exposés les résultats d'une série d'expériences en vue d'étudier à différentes étapes du cycle biologique, la réponse d'une population de *Ciana intestinalis* soumise à plusieurs combinaisons thermo-halines. On évalue le taux de mortalité, la croissance et la qualité de vie. Les "jeunes" diffèrent des "adultes" principalement dans le type d'interaction entre température et salinité : interaction "croissante - croissante" chez les jeunes et "croissante - décroissante" chez les adultes. Les jeunes sont plus résistants que les adultes à basses températures, ce qui peut bien expliquer la présence de cette espèce tout près de la côte en hiver à des températures au-dessous de 10° C. Les embryons ne sont pas si tolérants à la température et révèlent une interaction thermo-haline du type "croissant - décroissant" comme les adultes. Les "centres d'optimalité", dans les différentes phases du cycle biologique, sont situés à une salinité d'environ 35 ‰ tandis que leur température varie entre 15° C et 20° C. Nous pouvons en conclure que notre population s'adapte mieux aux conditions moyennes de la Méditerranée plutôt qu'au milieu lagunaire, les résultats sont enfin comparés avec ceux que d'autres auteurs ont obtenu dans différentes populations de la même espèce.

INTRODUCTION

Ciona intestinalis (L., 1767) is probably the best known species of ascidians (see the monographic description of Millar, 1953) ; it is widely distributed and has been found in areas characterized by very different thermo-haline conditions (Dybern, 1965, 1967). Even in the Venetian Lagoon it is largerly present (Sabbadin, 1957 ; Brunetti & Menin, 1977).

In this paper we examine the combined effects of temperature and salinity on the species, as evidenced by a laboratory investigation. A review of the problems related to this experimental approach can be found in Alderdice (1972).

MATERIALS AND METHODS

The animals were obtained by artificial breeding of gametes from individuals collected in the Venetian Lagoon. The fertilized eggs were transferred into filtered sea water, and the resulting larvae allowed to settle on small glass slides. After metamorphosis, a first series of samples, which we labelled as "young animals", were brought to experimental steady state conditions by gradually adjusting temperature and salt content over a period of 24 hours. During the experiment the animals were placed in thermostatic containers filled with 3 liters of sea water, kept in constant movement by an aerator and daily replaced ; food consisted of a mixture of *Dunaliella* sp. and *Clorella* sp.. Salinity was adjusted by addition of artificial hyperaline sea water or of distilled water. This experiment lasted 15 days.

A second series of samples were placed in aquaria with a continuous water flow at 19° C ± 1° C temperature and 30 ‰ - 3 ‰ salinity for one month; thereafter the samples were treated according to the same procedure described above. We labelled this second series of ascidians, having vertical stigmata, as "adult animals".

For the investigation on larval development, gametes were directly placed in small bowls filled with 40 ml of filtered sea water with an assigned salt content and kept in thermostatic cells. In the absence of water movement eggs would tend to aggregate, thus favoring bacterial development. In order to avoid that, the bowls were sealed with a plastic film on which two little holes were made : through one of them a Pasteur pipe was introduced and connected to an air compressor keeping water in constant movement, while the second hole provided for the outflow of air. In this way no change in salt content by evaporation was allowed. Water renewal was performed every 24 hours. No antibiotics were used.

In order to find out the area of optimal living conditions, we measured mortality rate and growth level (expressed by the number of rows of stigmata at the end of treatment) of "young animals" at different combinations of temperature and salinity. On "adults" we measured the mortality rate and the "life quality index" (LQI), a parameter obtained through a quantification of qualitative observations according to a procedure that will be described below. Finally we measured the "rate of development" of eggs (per cent of developed larvae over the total number of eggs).

All these parameters were processed according to the "response surfaces" method (Box, 1954 ; Box & Youle, 1955 ; Alderdice, 1972).

The "life quality index" (LQI) of adults was obtained taking into consideration 4 characters (attributes of the animals), namely aspect, feeding, cardiac contraction and reactivity (Tab. I). To each one of these characters several levels (mutually exclusive categories) were associated, according to the possible ways of manifesting themselves (normal or swollen aspect ; abundant, scarse or absent feeding ; normal, slow or absent cardiac contraction ; normal, scarse or absent reactivity) and to the j-th category of the i-th character, with $i = 1$ to 4 and $j = 1$ to $k(i)$, a definite score

Table I. Categories referring to quality of life

CHARACTERS	Categories of caracters	Scores	Absolutes scores
aspect of animal	normal	7	14
	lightly swollen	5	12
	swollen	-1	6
	very swollen or contract	-2	5
feeding	abundant	4	11
	present	3	10
	scarce	2	9
	absent	-3	4
cardiac contraction	normal	0	7
	slow	-6	1
	absent	-7	0
reactivity	normal	1	8
	scarce	4	3
	absent	-5	2

w(i,j) was assigned, ranging from a minimal value w_{min} to a maximal value w_{max} (in our case $w_{min} = -7$, $w_{max} = +7$); a not-negative value was attributed to a "healthy" living condition, as for instance "normal reactivity" or "abundant feeding", a negative value to a pathological one, as "swollen aspect" or "scarse reactivity". The maximal positive score (+ 7) was assigned to the category (in our case "normal aspect"), disappearing first under the influence of unfavorable environmental conditions and the least negative one (—7) to the category (in our case "absent cardiac contraction") manifesting itself only in the presence of worst environmental conditions. The scores were chosen according to a scale based on our research experience.

Let us call "absolute scores" the variables

$$w_{abs}(i,j) = w(i,j) - w_{min}$$

Indicating by $w_{max}(i)$ the maximal score attributed to the i-th category (i = 1 to 4), the LQI associated to a given set of categories (j (i), j(2), j(3), j(4)) was then defined according to the formula :

$$LQI = \sum_{1}^{4}{}_{i} w_{abs}(i,j(i)) \Big/ \sum_{1}^{4}{}_{i} [w_{max}(i) - w_{min}]$$

The value of LQI ranges from 0 to 1 : the closer to 1 the LQI value, the better the life quality of the animal ; the closer to zero, the worse the living condition. It should be noticed that this parameter is invariant under a linear change of scale

i.e. under a change of scale preserving the ratios w_{abs} (ij)/ [w_{max} (i)-w_{mins}], which makes it a "robust" estimator of life quality.

This way of defining LQI was introduced by us in a previous paper (Brunetti *et al,* 1980), according to a general remark due to Feinstein (1975).

RESULTS

YOUNG ANIMALS (Tabs. II, III ; Fig. 1 : A, B)

Table II • Mortality of young animals under different experimental conditions

	Temperature (°C)						Salinity
	7	10	15	20	25	30	(‰)
N° of animals		37	35	35			13
Mortality (%)		100	100	100			
N° of animals	-	30	31	31	32	-	17
Mortality (%)	-	100	100	100	100		
N° of animals	40	33	34	33	35	38	21
Mortality (%)	100	93.9	47.1	30.3	94.3	100	
N° of animals	35	33	69	37	36	37	29
Mortality (%)	5.7	0	43.5	0	2.8	100	
N° of animals	35	38	73	31	46	37	37
Mortality (%)	0	0	19.1	0	0	100	
No of animals	36	35	71	69	78	37	45
Mortality (%)	19.4	28.6	9.9	7.2	33.3	100	
N° of animals	-	38	36	26	38	36	50
Mortality (%)	-	100	91.6	30.8	44.7	100	

The distribution of mortality rates exhibits two minima corresponding to temperatures of 10° C and 20° C. This curious pattern (confirmed by other experiments not quoted here) is due, according to us, to the fact that the length of treatment was the same for all samples. At low temperatures, indeed, the reduced metabolism causes a delayed manifestation of the effects of the unfavorable environmental conditions and consequently a reduced mortality in the short term. This fact is confirmed by field observations where adult animals at temperatures <10° C exibit very reduced activity and remain in these conditions for many days before dying.

This situation has been noticed in our experiments only in young animals : they should be considered therefore more resistant than adults to low temperatures.

Samples referring to temperatures <10° C have not been taken into account in the evaluation of the surface responces because otherwise the contour lines (ellipses) would have been exagerately extended up to biologically meaningless regions as far as temperature is concerned. As it becomes apparent from fig. 1, A, the optimality centre lies at 20° C of temperature and 37 ‰ of salinity, and at a mortality rate of 0 %, temperature ranges from 17° C to 24° C and salinity from 31 ‰ to 43 ‰.

The dominant factor is temperature, and a positive (high-high) interaction with salinity is evidenced.

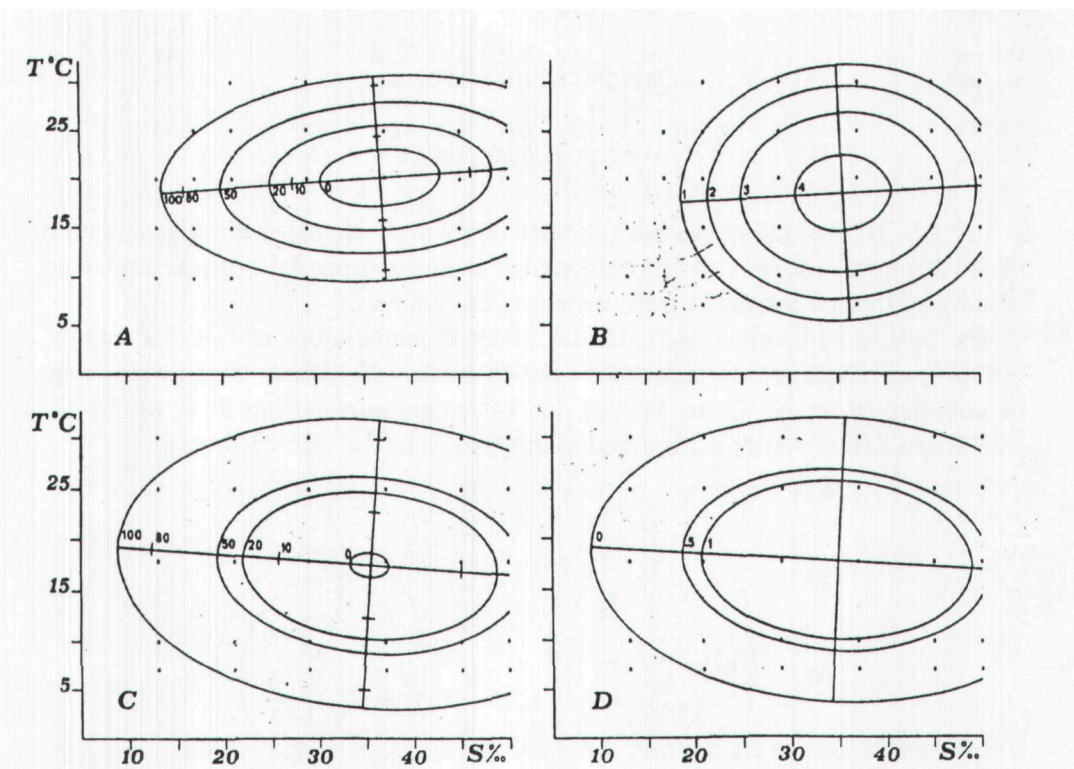

Fig. 1 - Response surfaces at various contour levels. *Young animals : A* = percent mortality rate (Y = 584 805 - 35 343 T - 12 979 S + 0.975 T^2 - 0.107 TS + 0.207 S^2 ; F(5/1018) = 5 823 ; r^2 = 0.853). *B* = growth (Y = - 16 035 + 0.667 T + 0.798 S - 1.888E-02 T^2 + 9.613E-04 TS - 1.151E-02 S^2 ; F (5/1338) = 1.108 ; r^2 = 0.526).
Adults : *C* = percent mortality rate (Y = 300.493 - 14.652 T - 8.615 S + 0.383 T^2 + 3.661E-02 TS + 0.113 S^2 ; F(5/740) = 2.096 ; r^2 = 0.677). *D* = Quality of life index (Y = - 1.863 + 0.135 T + 7.972E-02 S - 3.536E-03 T^2 - 2.678E-04 TS - 1.077E-03 S^2 ; F(5/740) = 0.606 ; r^2 = 0.377).
Dots indicate the experimental points. For some contour lines only their intercepts with the axes have been drawn.

Table III - Growth of young animals (as mean number of rows of stigmata : for sample size see Table 2)

	Temperature(°C)						Salinity
	7	10	15	20	25	30	‰
Growth		0	0	0	0	-	**13**
Growth		0	0	0	0	-	**17**
Growth	0	0.12	1.11	2.12	0.11	0	**21**
St. dev.		0.48	1.12	1.65	0.47		
Growth	1.88	2.61	2.11	4.17	5.0	0	**29**
St. dev.	0.46	0.93	1.94	0.39	1.09		
Growth	2.0	2.26	3.45	4.56	5.8	0	**37**
St. dev.	0.0	0.68	1.76	0.51	0.48		
Growth	1.61	1.43	3.55	3.94	2.68	0	**45**
St. dev.	0.79	0.92	1.23	1.33	2.12		
Growth		0	0.17	1.38	1.10	0	**50**
St. dev.			0.56	0.94	1.01		

In table III the growth values (number of rows of stigmata) are given : values under 2 (number of rows of "protostigmata" at metamorphosis) must be explained by the fact that we assigned a zero value to dead animals.

The optimality centre (Fig. 1, B) lies at 18° C temperature and 36 *‰* salinity, but the area where growth is possible (growth index > 2) extends over a wide range of salinities (from 22 *‰* to 50 *‰*) and of temperatures (from 7° C to 30° C). Interaction between temperature and salinity is still of the high-high type.

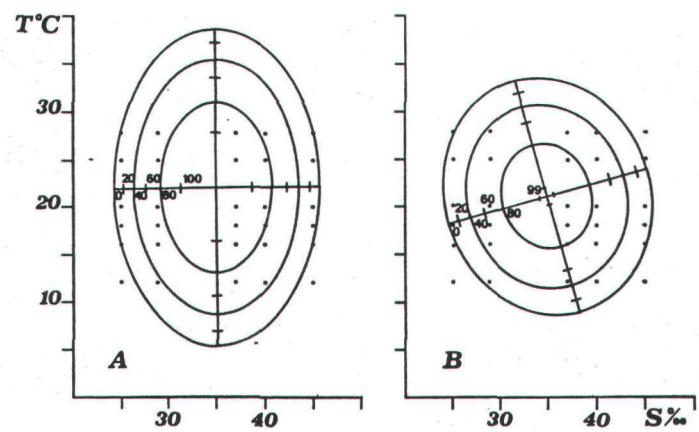

Fig. 2 - Response surfaces of embryonic development at various contour levels. *A* = percent of obtained larvae ($Y = -1310.786 + 18.469\ T + 70.015\ S + 0.409\ T^2 - 0.014\ TS - 0.999\ S^2$; $F(5/4374) = 10.349$; $r^2 = 0/912$). *B* = percent or normal obtained larvae ($Y = -1\,298.716 + 31.249\ T + 61.478\ S - 0.638\ T^2 - 0.127\ TS - 0.845\ S^2$; $F(5/4374) = 4\,580$; $r^2 = 0.821$).
Dots indicate the experimental points.

ADULT ANIMALS. (Tabs. IV, V ; Fig. 1 : C, D)

A comparison between the mortality rates of adult and young animals shows a reduced resistance power of the former to unfavorable environmental conditions and, more noticeably, a shifting of the optimally centre toward lower temperatures together with a temperature-salinity interaction of opposite (high-low) sign.

The life quality index confirms the results obtained from the analysis of mortality rate in what refers to the shape and size of contour curves and to the interaction type and degree. Nonetheless it should be observed that the optimal area ($LQI \geq 1$) almost coincides with a mortality range $\leq 20\%$ which allows us to assume that adult's mortality depends also on our experimental conditions, probably not well suited for aged animals, owing to an insufficient variety in food supply.

Table IV • Mortality (%) of adult animals under different experimental conditions : sample size = 25.

Temperature (°C)					Salinity
7	10	18	25	30	(‰)
100	100	100	100	100	13
100	84	56	56	100	21
100	28	20	68	100	29
72	4	0	32	64	37
92	12	17.4	80	100	45
100	56	52	84	100	50

LARVAL DEVELOPMENT, (Tabs. VI, VII ; Fig. 2 : A, B)

Salinity values of 21 ‰ and 45 ‰ cause noticeable egg alterations. At 21 ‰ floating cells are destroyed within 1 h and at 45 ‰ they appear shrunk, with egg cytoplasm segregating in irregular masses. At a temperature of 12° C the development took place only at a salinity of 37 ‰, while at a salinity of 29 ‰ the development stopped at the morula stage.

At all the remaining temperatures considered, complete development took place at salinities ranging from 29 ‰ to 40 ‰.

From fig. 2, A it becomes evident that no interaction between temperature and salinity intervenes in development, while the latter acts in a negative way (high-low interaction with temperature) at the larval stage (Fig. 2, B).

Finally, in tab. VII the minimal times for hatching (i.e. time span required for the hatching of the first larvae) are given. Obviously, the development rate is mainly influenced by temperature, but a contribution is also due to salinity. The extreme salinity values act as a slowing factor : at a salinity of 16 ‰ the registered delay amounts to 10 h.

Table V - Mean value of the life quality index (LQI) in adult animals under different experimental conditions : sample size = 25.

	Temperature (°C)					Salinity
	7	**10**	**18**	**25**	30	*%c*
LQI	**0**	0	0		0	13
LQI	**0**	0.049	0.343	**0.355**	0	21
St. dev.		0.115	0.394	**0.410**		
LQI	**0**	0.665	0.777	**0.312**	0	29
St. Dev.		0.433	0.397	**0.464**		
LQI	**0.101**	0.910	0.966	**0.633**	0.285	37
St. Dev.	**0.165**	0.201	0.038	**0.447**	0.390	
LQI	**0.051**	0.772	0.733	**0.191**	0	45
St. Dev.	**0.177**	0.301	0.368	**0.392**		
LQI	0	0.271	0.330	**0.155**	0	50
St. Dev.		0.312	0.356	**0.362**		

Table VI - Embryonic development from eggs under different experimental conditions

	Salinity (‰)					Temperature
	21-25	29	37	40	45	(°C)
N. eggs	70	80	111	70	50	
Total larvae	0	0	73.9	0	0	12
Normal larvae			15.3			
N. eggs	50	120	170	50	70	
Total larvae	0	73.3	90.6	84	0	16
Normal larvae		45.8	84.1	64		
N. eggs	80	210	200	145	96	
Total larvae	0	84.4	99	96.6	0	18
Normal larvae		62.6	98	89.7		
N. eggs	65	172	236	202	110	
Total larvae	0	83.7	99.2	98.5	0	20
Normal larvae		65.7	98.7	87.6		
N. eggs	93	172	198	165	97	
Total larvae	0	86.6	99.5	90.9	0	22
Normal larvae		66.3	99	78.8		
N. eggs	110	89	184	92	50	
Total larvae	0	78.7	98.9	83.7	0	25
Normal larvae		74.2	97.8	21.7		
N. eggs	80	66	348	180	120	
Total larvae	0	77.3	96	73.3	0	28
Normal larvae		77.3	71.8	2.8		

Table VII - Minimal time to hatching (in hours)

Salinity (‰)			Temperature °C
29	37	40	
	36		12
32	22	32	16
18	16	18	18
15	15	15	20
14	14	13	22
12	12	13	25
11	11	12	28

DISCUSSION

The results of our experiments indicate that the tolerance range to salinity is wider than the variability range of this parameter in the area from which our samples originate. This environment, which has almost completely lost its estuarine character (Brunetti *et al.,* 1983), presents mean salinity values around 35 ‰, with minima rarely dropping under 30 ‰. The optimal salinity levels for the species in the different developmental stages lie between 35 ‰ and 37 ‰.

The response to temperature is quite different ; optimal temperatures lie between 17° C and 21° C, and the tolerance ranges found are narrower than the variability range of this parameter in nature. In fact, temperatures under 10° C, common in the lagoon in winter, are lethal to adult animals.

This fact agrees with field observations of Sabbadin (1958) and Brunetti and Menin (1977), who noticed the almost complete absence of adult individuals at the end of the winter season. A relevant adult mortality has been observed also by Liliaci *et al.* (1977) in *Mar Piccolo* (Taranto, Southern Italy), where salinity slightly oscillates around the annual mean value of 36.4 ‰ but winter temperature (january-february) drops to 10° - 8° C due to the shallow depth of the basin. As our experiments evidence, the thermo-haline sensitivity of the species is not the same in the different developmental stages. Young animals have a higher resistance than adults (only one month older) to low temperatures. It seems therefore reasonable to assume that most of the new born survive during the winter season, even though their presence cannot be observed without a microscope. For this reason it does not seem necessary to hypothize a repopulating from the open sea, as previously suggested by Sabbadin (1958) and by Brunetti and Menin (1977) and accepted as very likely by Liaci *et al.* (1977), even if we cannot reject this assumption in the absence of planctonic data.

The physiologic state of animals determines not only their tolerance to temperature and salinity separately considered, but also the type of interaction of these

parameters. A high-low interaction in adults and a high-high one in young animals, as observed by us, tend to favour the wintering of the latter. In the Venetian Lagoon to high summer temperatures correspond the lowest salinity levels ; the opposite happens in winter (Brunetti *et al.,* 1983). This is a very common situation in coastal environments at middling latitudes.

Larval development has narrow tolerance ranges and exhibits the same type of interaction as found among adults ; the optimal values are 21° C of temperature and 35 ‰ of salinity. This is quite understandable, owing to the fact that the species reproduces in summer.

In conclusion, the data indicate that our population is well adapted to the mean environmental conditions of the Mediterranian Sea rather than to those of the Venetian Lagoon, and does not differ from the population examined by Hirai (1963) and by Yamaguchi (1975) in the japanese seas.

Finally, it is interesting to compare our data on embrionyc development with those obtained by Dybern (1967) from five populations in Scandinavian seas (Fig. 3). The sensitivity of zygotes of such populations to salinity has the character of a gradient. Considering only salinity our population could be considered as belonging to this group but taking into account temperature, we could on the contrary hypothize that our population is somewhat genetically separated.

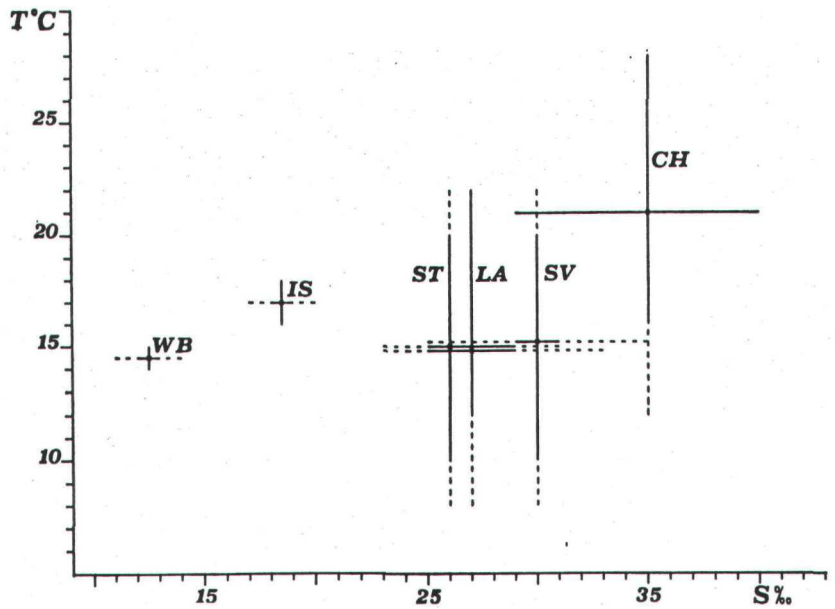

Fig. 3 - Embryonic development of *C. intestinalis* in different European populations as a function of temperature and salinity : dots indicate the optimality centres of the various populations, while orthogonal segments (solid lines) indicate the temperature and salinity ranges for a rate p of normal larvae greater than 50 %. Dashed segments indicate the thermo-haline ranges for $0 < p \leq 50$ %.
CH = Chioggia ; IS = Isefjord* ; LA=Langegap* ; ST=Strommarna* ; SV=Svenningeskar* ; WB = Wismar Bucht*.
•From Dybern (1967).

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