

Effect of water temperature on the motility of *Pelagia noctiluca* (Forsk.)¹

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Summary. Laboratory tests have been performed, on the behavior of the jellyfish *Pelagia noctiluca* as a function of the water temperature. It has been found that the usual contractions of the umbrella are almost completely missing at 6 °C; they begin to appear at about 7–8 °C and they reach frequencies of about 10 and 40 per min at 11 and 15 °C respectively. An ambient temperature of about 11 °C appears to be a threshold value below which this kind of medusa ceases to move actively and sinks, while at higher temperatures it gradually begins to shift, showing a positive thermotropism in the presence of temperature gradients greater than about 0.01 °C/cm.

The swarming^{2,3} of *Pelagia noctiluca* in the Adriatic sea was first observed on a large scale in 1977; such an occurrence, once unusual, seems to have happened regularly since that year in this area. The young forms of *Pelagia noctiluca* gather in the surface layer during summer; the characteristic circulation of the Adriatic and the local winds move them, as an average, northwards and towards the coast. Large swarms of this jellyfish have been observed mainly along the rocky and rugged Istrian coast and in the Gulf of Trieste^{4–6}.

In the Gulf of Trieste, the young forms of *Pelagia noctiluca* disappear from the surface layer at the beginning of the cold season; the following spring some mature large-sized specimens are observed there again. The opinion that the spring specimens must belong to the same generation observed during the preceding summer is supported by the fact that in many plankton samples collected during the autumn and the winter since September 1979 every phase of the larval development of *Pelagia noctiluca* has been found, from planula to young medusa⁶.

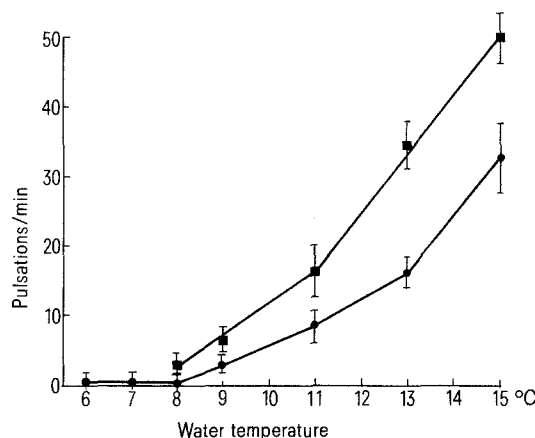
Since the seasonal sea water temperature variations play such an important role in the annual cycle of *Pelagia noctiluca*, some laboratory tests have been performed in order to study the behavior of these animals in the critical temperature range of 6–15 °C.

Materials and methods. During November 1980 10 young individuals of *Pelagia noctiluca* were collected in the sub-surface layer in the Gulf of Trieste (sea water temperature 11.2 °C). This sample was characterized by the following parameters: extended diameter of the umbrella 45–50 mm, length of the manubrium 75–80 mm, stretched tentacle length 250–350 mm; and immature gonads in all the specimens. These animals were then introduced (5 and 5)

into 2 aquaria (A and B), 80×40 cm, 30 cm high, with their bottom partially covered with pebbles. Both aquaria were filled with sea water directly pumped from the sea, and equipped with 2 oxygenators, 1 shielded heater and 3 thermometers placed diagonally in the tank at equal intervals from a bottom corner (point 1) to the opposite heater on the surface (point 3). The room was not heated; a diffuse faint light was used, so that the behavior of the animals could not be influenced by phototropic effects. The purpose of the experiment was to see how a slowly increasing water temperature, starting from 'winter' conditions, would affect the 'activity' of *Pelagia noctiluca*, i.e. the frequency of the contractions of its umbrella, and its 'motility', i.e. the active movements of the animal from site to site. The water temperature in the tanks was smoothly raised from 6 to 8 °C to a maximum of 15 °C in 24 h, in such a way that convection currents could not appreciably move the specimens from their position; these were initially confined at the maximum distance from the heat source (point 1).

Results and discussion. The results of the experiments are presented in the table and in the figure. The activity of *Pelagia noctiluca* is expressed by the number of contractions per min of its umbrella; mean values and SD observed on the 5 individuals samples in aquarium A and B are given. The motility is expressed according to a conventional scale: 0 indicates that not any translational movement is observed, +, ++, +++ indicate an increasing active displacement.

The pulsating rate of the animals in the sea before their capture could not be observed. Both thermal shock and shock due to capture were inevitably produced by their arrival in the aquaria; the pulsating rate of the umbrella



Pulsating rate of the umbrella of *Pelagia noctiluca* at increasing water temperature. Mean value and SD of samples in aquarium A ($t_0 = 8$ °C, squares) and in aquarium B ($t_0 = 6$ °C, circles).

Pulsating rate of the umbrella and motility of *Pelagia noctiluca* at increasing water temperature. Heat source at point 3

Time	0	2 min	4 h	6 h	7 h	9 h	24 h
Aquarium A							
t/°C 1	8.0	8.0	9.0	11.0	13.0	—	15.0
t/°C 2	8.0	8.0	9.5	11.5	14.0	—	15.0
t/°C 3	8.0	8.0	10.0	12.5	14.0	—	15.0
contractions/min	7.0	3.0	7.2	16.8	34.8	—	48.8
SD	1.0	1.2	1.3	3.4	3.3	—	3.6
Motility	0	0	0	+	++	—	+++
Direction	—	—	—	1→2	2→3	—	Random
Aquarium B							
t/°C 1	6.0	6.0	7.0	9.0	11.0	13.0	15.0
t/°C 2	6.0	6.0	7.5	10.0	11.5	13.5	15.0
t/°C 3	6.0	6.0	10.0	12.5	13.0	15.0	15.0
contractions/min	0.6	0.0	1.0	3.4	9.2	16.2	33.8
SD	0.5	0.0	1.0	1.1	2.3	2.3	5.6
Motility	0	0	0	0	+	+	++
Direction	—	—	—	—	1→2	2→3	Random

decreased with in 2 min to about 3 contractions/min at 8 °C in aquarium A, and stopped altogether in aquarium B (6 °C). In both cases the jellyfish sank immediately and stayed near the bottom without any further displacement (table). Some motility began when the temperature exceeded about 11 °C: it was clearly observed at that point that the animals were moving following the temperature gradient towards the surface and towards the heat source (point 3). Once in the warmer corner of the tank, they did not depart from its surroundings until the temperature in the aquarium reached a uniform value of 15 °C: only then did the animals begin to swim randomly throughout the basin.

The inverse experiment, i.e. to observe the effects of lowering the temperature, has not been performed completely owing to practical difficulties and to the lack of fresh specimens. We could, however, observe that some specimens of *Pelagia noctiluca*, left unattended in a small tank filled with sea water at the external temperature (11 °C), corresponding to the conditions of their capture, reduced their activity from about 54 to 4–6 contractions/min after 12 h at 8 °C, when they were found to be staying at the bottom.

The effect of thermal shock is evident from the figure: specimens in the aquarium B ($t_0 = 6$ °C) present a reduced activity with respect to those in aquarium A ($t_0 = 8$ °C) at all temperatures. In both cases, however, 11 °C seems to be the temperature (table) at which *Pelagia noctiluca* starts to move showing a positive thermotaxis for temperature gradients greater than about 0.01 °C/cm.

A correlation between water temperature and the pulsating rate of the umbrella of *Pelagia noctiluca* was found by Skramlik in the Gulf of Napoli⁷; in that case, frequencies in

the range from 24 contractions/min at 5 °C to 66 contractions/min at 20 °C were observed. Our experimental data confirm the existence of such a correlation, but show that the contractions in the young specimens collected in the Gulf of Trieste are almost completely inhibited at 6 °C. Furthermore, according to our experience, *Pelagia noctiluca* can be reasonably considered as having a positive thermotaxis.

A logical explanation, based on the above observations, of the disappearing of *Pelagia noctiluca* from the surface layer in the northern Adriatic during winter is that these animals reduce their activity and sink when the temperature of 11 °C is approached. These animals can then survive in the bottom layer during the cold season, when the water temperature is generally vertically uniform and not less than 6–8 °C. In springtime, when the seasonal thermocline is set up and the temperature rises to about 11 °C in an intermediate-deep layer⁸, the now mature specimens of *Pelagia noctiluca* begin to move again, reach the warmer surface layer, and continue their biological cycle.

- 1 This work was supported by grant No.80.00748.88 from the National Research Council of Italy (CNR).
- 2 F.S. Russel, J. mar. biol. Ass. U.K. 47, 363 (1967).
- 3 S.M. van der Baan, Neth. J. Sea Res. 3, 600 (1967).
- 4 L. Rottini-Sandri and F. Stravisi, C.I.E.S.M.M., Cagliari 1980.
- 5 L. Rottini-Sandri, F. Stravisi and G. Pieri, Boll. Soc. adriat. Sci. 64, 77 (1980).
- 6 A. Malej, C.I.E.S.M.M., Cagliari 1980.
- 7 E. von Skramlik, Zool. Jb. allg. Zool. Physiol. 61, 296 (1948).
- 8 Annuario of Istituto Talassografico di Trieste of the National Research Council of Italy (CNR), No.591 (1979).

Restriction enzyme studies on human highly repeated DNAs¹

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Summary. Various restriction enzymes digest human highly repeated homogeneous DNA to discrete fragments, some of which are present in the male and absent in the female. The male specific 2.4 kilobase HaeIII fragment corresponds to human male satellite DNA IV.

Human satellite DNAs I, II, III and IV and an additional highly repeated fraction, called homogeneous DNA, identified within the light side of the main band DNA in an Ag^+ - Cs_2SO_4 gradient have been extensively studied³⁻⁹. Restriction enzyme digestion studies have recently been carried out on human highly repeated DNAs¹⁰⁻¹⁵. We report here on restriction patterns of human satellite and homogeneous DNAs.

DNA extraction procedures from human placenta, DNA analytical ultracentrifugation and preparative fractionation in CsCl , Ag^+ - Cs_2SO_4 and Hg^{++} - Cs_2SO_4 equilibrium density gradients were as previously described^{3,4}. All DNA fractions were dialyzed against 0.1 M ammonium carbonate and then evaporated by air insufflation at 37 °C and resuspended in a small volume of 10 mM Tris-HCl, pH 7.5, 1 mM EDTA.

DNA fractions were then digested by several restriction enzymes (commercial preparations purchased from Bethesda Research Laboratories, Bethesda, MD) at a DNA-to-enzyme ratio of 1 μg -DNA to 3 units of enzymes according to the conditions indicated by the vendor. The restriction enzymes used were the following: HaeIII, EcoRI, EcoRII,

XbaI, AluI, HindIII, HinfI, BamHI, HpaI, HpaII, BglII, and HhaI.

Assay buffers contained Tris-HCl, MgCl_2 , 2-mercaptoethanol or dithiothreitol, and NaCl at various molar concentration according to the enzyme, as indicated by the vendor's catalogue, and digestion was carried out to completion for 5–7 h at 37 °C.

After digestion sucrose was added to 6% and the samples were applied to the wells of a horizontal 17 cm \times 13 cm \times 0.3 cm agarose gel. Agarose concentration was 1% for all enzyme experiments except for HaeIII and XbaI digests in which cases 2.5% and 1.5% agarose concentrations were used respectively. The slab gels were made up in 40 mM Tris-HCl, 20 mM sodium acetate and 2 mM sodium EDTA at pH 8.3, and run at 20 mA, 40 V for 17 h. The gels were stained with ethidium bromide ($0.5 \mu\text{g} \cdot \text{ml}^{-1}$) and were photographed under UV-light with a Kodak 23A filter with a Polaroid MP-4 land camera and 665 Polaroid slides. Phage λ DNA digested with HaeIII or HindIII was used as size marker.

Figure 1A shows a fractionation experiment of high molecular weight (10 million daltons) male human placenta