Induction of metamorphosis in *Paracentrotus lividus* larvae (Echinodermata, Echinoidea)

Echinoidea
Metamorphosis
Conspecifics
Algae
Detrital particles

Echinidea
Métamorphose
Conspécifiques
Algues
Particules détritiques

Pol GOSSELIN [a] and Michel JANGOUX [b]

[a] Laboratoire de Biologie Marine, Université de Mons-Hainaut (U.M.H.), 19, avenue Maistriau, 7000 Mons, Belgium.

[b] Laboratoire de Biologie Marine, Université de Mons-Hainaut (U.M.H.), and Laboratoire de Biologie Marine, Université Libre de Bruxelles (U.L.B.), Belgium.

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ABSTRACT

Various experiments were performed to investigate the inductive effect of some biotic agents – adult conspecifics, macroalgae of the genera *Laminaria* and *Enteromorpha* and detrital particles – on the onset of metamorphosis in competent larvae of the echinoid *Paracentrotus lividus*. Adult conspecifics and laminarians did not induce larvae to metamorphose. Detrital particles always had a high inductive power, provided that contact occurred between larvae and particles. The inductive effect of enteromorph algae was seasonal, and metamorphosis was induced either through direct contact with the alga or with a surface previously conditioned by the alga, as well by placing larvae in water previously conditioned by the alga.

RÉSUMÉ

Induction de la métamorphose des larves de *Paracentrotus lividus* (Echinodermata, Echinoidea).

Diverses séries expérimentales ont été réalisées pour tester l'effet inducteur de certains agents biotiques – adultes conspécifiques, macroalgues des genres *Enteromorpha* et *Laminaria*, particules détritiques – sur l'entrée en métamorphose des larves compétentes de l'oursin *Paracentrotus lividus*. Ni les adultes conspécifiques ni les laminaires n'ont d'effet sur l'induction de la métamorphose. Les particules détritiques induisent des taux élevés de métamorphose à condition que les larves entrent en contact direct avec elles. Le pouvoir inducteur des entéromorphes est saisonnier. La métamorphose est induite ici au contact direct de l'algue ou d'une surface conditionnée en présence de l'algue autant que par immersion des larves dans de l'eau préalablement conditionnée par l'algue.

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INTRODUCTION

It has been demonstrated that metamorphosis of competent echinoid larvae can be induced by the biofilm covering marine substrata (Hinegardner, 1969; Cameron and Hinegardner, 1974), by conspecifics (Highsmith, 1982; Pearce and Scheibling, 1990 *a*), or by macroalgae (Pearce and Scheibling, 1990 *b*, 1991). Empirical observations made in the (adult) echinoid cultivation system of the marine laboratory of Luc-sur-Mer (Legall, 1989) showed that competent *Paracentrotus lividus* larvae metamorphose massively when they are placed in the above-mentioned cultivation system, where detrital particles, adult conspecifics and macroalgae occur. The present paper seeks to determine

which is (are) the biotic agent(s) responsible for the massive metamorphosis observed.

MATERIAL AND METHODS

Adult cultivation system

Adult specimens of *Paracentrotus lividus* measuring some 40 mm in diameter collected intertidally at Morgat (Brittany, France) were maintained in the cultivation system of the marine laboratory of Luc-sur-Mer (Normandy, France). This system consists of superposed toboggans in which water, pumped into a reserve tank, circulates by gravity from one toboggan to the other (Legall, 1989). Water renewal is regulated at rates between 50 and 600 % per day (depending on the echinoid population density in the system) using filtered (15 µm mesh) natural sea water. Water depth in the toboggans varies from 5 to 10 cm. The whole system is thermo-regulated (18 °C ± 1 °C) and placed under natural dim light. The biomass of echinoids maintained in the cultivation system ranged from 5 to 15 kg per m^2. Echinoids were fed *ad libitum* with algae (*Enteromorpha linza* and *Laminaria digitata*).

Larval rearing

Gametes were obtained by inducing adult echinoids to spawn by injecting into the body cavity, between 0.5 and 2 ml of 0.5 M KCl, depending upon body size. At the gastrula stage, embryos were transferred into large cultivation jars containing 200 litres of natural filtered (0.2 µm mesh) sea water (final concentration: 250 embryos per litre). Larval cultures were stirred and aerated continuously, and maintained at 20 °C ± 1 °C. Larvae were fed daily with *Phaeodactylum tricornutum* algae. The water was not renewed during the period of premetamorphic life, which take 18 days in our rearing conditions (competence appears generally on the 18 th day after fertilization).

Investigations of metamorphosis

All experiments were performed with 18-day-old larvae (*i.e.* competent larvae) of the same parental origin, placed in freshly collected and filtered (0.2 µm mesh) natural sea water. In most experiments, larvae were put in polystyrene dishes 60 mm in diameter, containing 14 ml of filtered sea water. Experiments were conducted with either 15 batches of 10 competent larvae (n = 150) or 20 batches of 10 competent larvae (n = 200).

To quantify the metamorphic inductive effect (MIE) presented by the echinoid cultivation system, dishes were conditioned by immersion either in that system ("System conditioning", or Sc) or in a polystyrene jar filled with 20 l of 0.2 µm filtered sea water ("Filtered conditioning", or Fc). To take account of the possible MIE of the water itself, S and F conditioned dishes were filled either with water from the cultivation system ("System water" or Sw) or with 0.2 µm filtered sea water ("Filtered water", or Fw). Conditioning was done for 7 days at 20 °C ± 1 °C under natural dim light. The MIE produced by S and F conditioning and S and F water taken two by two were comparatively assessed by counting the number of competent larvae that had undergone metamorphosis after immersion in those environmental conditions for 24 h at 20 °C ± 1 °C.

Similar investigations were performed to quantify the MIE produced by adult conspecifics, dietary algae or the detrital particles occurring in the cultivation system. For detrital particles, surface and water conditioning was carried out by placing both particles (*i.e.* particles extracted by filtration – Wathman n° 1 filter – from 1 l of water from the cultivation system) and clean dishes in a polystyrene jar filled with 20 l of 0.2 µm filtered sea water. For dietary algae, conditioning was done by placing both algae (*i.e.* 250 g of freshly-collected intact algae – either *Laminaria digitata* or *Enteromorpha linza*) and clean dishes in similar water-filled jars. Note that algae were renewed daily, while detrital particles were not. For conspecifics, conditioning was done by placing 15 adult echinoids and clean dishes in similar water-filled jars. The MIE produced by both the wall of the dishes and the water from the jars were assessed as above, using competent larvae. Additional investigations were made to determine whether the contact between competent larvae and either detrital particles or dietary macroalgae enhance larval metamorphosis. This was done using clean polystyrene dishes 60 mm in diameter containing 50 ml of 0.2 µm filtered sea water, the bottom of which was totally covered with either detrital particles extracted from the echinoid cultivation system (see above) or freshly collected algae. Effects on larval metamorphosis was assessed as above, using competent larvae.

RESULTS

Quantification of the MIE of the adult cultivation system

The metamorphosis-inductive effect (MIE) of the echinoid cultivation system of Luc-sur-Mer was observed throughout the year without any marked seasonal variation. As an example, Figure 1 shows the results obtained in April 1993. It was determined that the higher rate of metamorphosis was obtained using both surfaces (*i.e.* dishes) conditioned in the cultivation system (Sc) and water from the

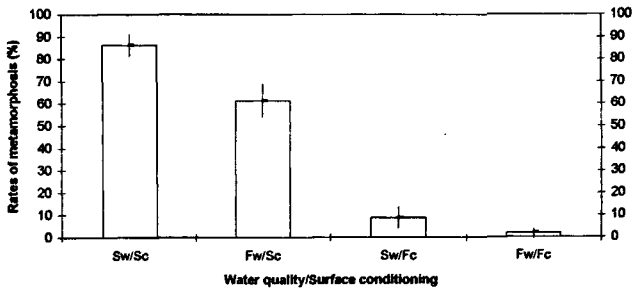

Figure 1

Mean percentage (m ± i; p = 0.05; n = 150) of metamorphosed larvae of Paracentrotus lividus *in response to either S or F water (respectively, water from the cultivation system or freshly-collected filtered sea water) associated to either S or F conditioned dishes (respectively, dishes conditioned into the cultivation system and into a polystyrene jar filled with F water).*

cultivation system (Sw), while the lowest rate was obtained using both surfaces conditioned in freshly-collected 0.2 µm filtered sea water (Fc) filled with the same water (Fw).

Quantification of the MIE produced by biotic agents

Investigations into the effect of adult conspecifics were performed in February 1993, and those using the detrital particles in July 1993 and October 1993. For the dietary macroalgae, investigations using *Laminaria digitata* were performed in October 1992 and February 1993, and those using *Enteromorpha linza* in October 1992, and February, April and July 1993.

The rates of metamorphosis obtained with water and surfaces conditioned by the above-mentioned biotic agents are presented in Table 1. Neither water nor surfaces conditioned in the presence of adult conspecifics appear to have any effect on the onset of larval metamorphosis. Surfaces conditioned in the presence of detrital particles seem to be slightly inductive. As for dietary algae, the obtained rates for *L. digitata* were always very low, although those recorded for *E. linza* were high in October 1992 and July 1993, and low in February and April 1993. Note that in both October and July conditioned water always induces more metamorphoses than conditioned surfaces.

The rates of metamorphosis obtained with larvae which were in contact with the different biotic agents are shown in Table 2. *L. digitata* has no effect and *E. linza* showed a clear effect only in October. Contact with detrital particles always induced very high rates of metamorphosis.

DISCUSSION AND CONCLUSION

Experiments performed with water originating from the cultivation system or using surfaces conditioned in that system clearly indicated the occurrence of a signal inducing *Paracentrotus lividus* larvae to metamorphose. That signal appears to be mostly surface-associated, although water from the cultivation system is also slightly inductive (similar observations have been reported by Cameron and Hinegardner, 1974, on *Lytechinus pictus* and *Arbacia punctulata* larvae).

Investigations to determine the origin of this signal showed that the green alga *Enteromorpha linza* and the detrital particles of the cultivation system are capable of inducing substantial rates of larval metamorphosis. In both *E. linza* and

Table 1

Mean percentage (m ± i; p = 0.05; n = 200) of metamorphosed larvae of Paracentrotus lividus *in response to water or surfaces conditioned by either adult* Paracentrotus lividus, *freshly-collected macroalgae (*Laminaria digitata *or* Enteromorpha linza*) or detrital particles.*

Tested agent	Tests performed in: Month/Year	Control experiments*		Test experiment	
		Fw/Sc	Fw/Fc	conditioned water	conditioned surface
Paracentrotus lividus	02/1993	81 ± 8.5	0	0	20 ± 20
Laminaria digitata	10/1992	79 ± 17	0	0	0
	02/1993	81 ± 8.5	0	3 ± 3	8 ± 8.5
Enteromorpha linza	10/1992	79 ± 17	0	95 ± 6.3	59.3 ± 33.7
	02/1993	81 ± 8.5	0	14.5 ± 5	15.5 ± 9.5
	04/1993	64 ± 10	0	4.6 ± 3.9	12.5 ± 10.4
	07/1993	99 ± 1.5	4 ± 2.5	78.7 ± 8.1	62.6 ± 15.6
Detrital particles	10/1993	48.3 ± 7	2.55 ± 2	1.5 ± 1.7	11.5 ± 7.8

* Rates of metamorphosis (%) obtained in standard Fw/Sc inductive and Fw/Fc non-inductive environments (*see* Fig. 1).

Table 2

Mean percentage (m ± i; p = 0.05; n = 200) of metamorphosed larvae of Paracentrotus lividus *after contact with either freshly-collected macroalgae (*Entreromorpha linza *or* Laminaria digitata*) or detrital particles.*

Tested agent	Tests performed in: Month/Year	Control experiments*		Test experiment
		Fw/Sc	Fw/Fc	
Laminaria digitata	10/1992	79 ± 17	0	20 ± 38
	02/1993	81 ± 8.5	0	1 ± 2
Enteromorpha linza	10/1992	79 ± 17	0	70 ± 19
	02/1993	81 ± 8.5	0	19 ± 14
	04/1993	64 ± 10	0	2.3 ± 3.9
	07/1993	99 ± 1.5	4 ± 2.5	14 ± 10
Detrital particles	07/1993	99 ± 1.5	4 ± 2.5	99 ± 3.2
	10/1993	48.3 ± 7	2.55 ± 2	98 ± 2

* Rates of metamorphosis (%) obtained in standard Fw/Sc inductive and Fw/Fc non-inductive environments (*see* Fig. 1).

detrital particles, metamorphosis is induced by contact. Yet *E. linza* is not always present in the cultivation system (*E. linza* is only used to feed individuals less than 1 cm in diameter), and its effect on metamorphosis is clearly seasonal. This indicates that the metamorphosis-inductive effect showed by the cultivation system is undoubtedly linked to the detrital particles it contains. The latter are composed of various biotic and abiotic components, such as echinoid faeces and spines, algal fragments, sediment grains and microorganisms, among which benthic diatoms and bacteria were shown to, or appeared to produce substances that induce metamorphosis of echinoid larvae (*e.g.* Ito, 1984; Cameron and Hinegardner, 1974; Cameron and Schroeter, 1980; Pearce and Schiebling, 1991; respectively).

It should be emphasized that, while seasonal, the metamorphosis-inductive signal produced by *E. linza* can be transferred to both the surface and the water. On the other hand, the signal produced by the detrital particles can apparently be transferred to the surface only. This indicates that substances of various origins can trigger metamorphosis in *P. lividus* larvae, and demonstrates that such signals are not necessary associated with a surface.

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