The Ir_2 complex with $A^+ = 2,4,6\text{-}Me_3py^+$ (Me = methyl) provides a clear example of this effect (Table 2). The ^1ET rate in this molecule is more than 300 times the ET^b rate, although the latter process has a 1.3 eV greater driving force. Maximizing both the ^1ET rate and the $^1ET/ET^b$ ratio are the primary requirements for the efficient generation and maintenance of photoinduced hole:electron separation. The Ir_2 ET data clearly demonstrate that the driving force–dependent Franck-Condon barrier to ET can be exploited to achieve this goal. If coupled with $^1ET/ET^b$ discrimination in the electronic coupling term, $^1ET/ET^b$ ratios comparable to those found in the photosynthetic reaction center could be achieved in synthetic photochemical energy storage systems.

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Direct Coupling of Marine Invertebrate Spawning with Phytoplankton Blooms

MICHEL STARR, JOHN H. HIMMELMAN, JEAN-CLAUDE THERRIAULT

Spawning of green sea urchins and blue mussels may be triggered by a heat-stable metabolite released by various species of phytoplankton. Mussels require a higher phytoplankton density for a maximum response than urchins, perhaps because mussels are exposed to higher concentrations of phytoplankton as a result of their filtering activity. Phytoplankton as a spawning cue appears to integrate numerous physical and biotic factors indicating favorable conditions for larval growth and survival. Evolution of similar direct coupling of the larval phase with phytoplankton blooms may be common among marine invertebrates.

FOR HALF A CENTURY BIOLOGISTS have observed the close timing between the production of pelagic larvae by benthic invertebrates and phytoplankton blooms (*1*). This synchrony provides the obvious advantage of ensuring an abundant food supply for the larvae. However, the mechanisms accounting for this coupling are poorly understood. Most workers favor the hypothesis that gamete and larval release are controlled by physical environmental variables such as changes in temperature, salinity, photoperiod, and turbulence. However, only a few studies have shown a relation with a particular factor that could be confirmed in the laboratory (*2*).

An alternative hypothesis is that phytoplankton induces spawning. This possibility, although briefly discussed many years ago by Thorson (*1*), has received little attention. Himmelman (*3*) showed that spawning of the urchin, *Strongylocentrotus droebachiensis*, and of two species of chitons is synchronous with the spring phytoplankton outburst and that natural plankton collected with a 50-μm mesh net stimulates spawning in the laboratory. Subsequently, it was noted in mariculture work that high concentrations of phytoplankton induce spawning in *Mytilus californianus* (*4*). An analogous situation exists for species such as barnacles, which brood their embryos and later release pelagic larvae when food becomes abundant (*5*).

We examined the mechanisms that couple spawning of invertebrates and the spring blooming of phytoplankton (*6*). We tested the following hypotheses: (i) that phytoplankton stimulates spawning, (ii) that spawning can be caused by numerous species of phytoplankton, (iii) that phytoplankton at concentrations found in nature can stimulate spawning, and (iv) that spawning results from direct contact with phytoplankton cells (either chemical or nutritional) or from the detection of substances released by them.

The spawning experiments were performed with green sea urchins (*Strongylocentrotus droebachiensis*, 35 to 45 mm in diameter) and blue mussels (*Mytilus edulis*, 35 to 45 mm in length) collected in the St. Lawrence estuary shortly before natural spawning. They were kept in holding tanks, supplied with recirculated seawater containing no phytoplankton, at a temperature of 5° to 7°C and a salinity of 21 to 27 parts per mil.

M. Starr and J. H. Himmelman, GIROQ (Groupe Interuniversitaire de Recherche Océanographique du Québec) and Département de Biologie, Université Laval, Québec, Canada G1K 7P4.
J.-C. Therriault, Institut Maurice-Lamontagne, Ministère des Pêches et des Océans, C.P. 1000, Mont-Joli, Québec, Canada G5H 3Z4.

The spawning response to various treatments was quantified for individual animals held in glass bowls containing 500 and 400 ml of test medium (*p*H 7.7 to 8.1) for urchins and mussels, respectively. Animals were acclimated in the bowls for 1 day before testing at the experimental temperature of 7° ± 1°C. Experiments ran for 7 to 9 days. Each day a visual examination was made for the presence of gametes, and the test medium was changed (*7*).

The phytoplankton for these experiments came from unialgal cultures (axenic cultures supplied by Institut National de la Recherche Scientifique-Océanologie, Québec) maintained in exponential growth (1×10^9 to 4×10^9 cells per liter) in batch culture (20 liters), with the use of natural seawater (filtered at 0.2 μm) enriched with f/2 medium (*8*) without vitamins. The cultures were grown at 12° to 16°C under a 16-hour light: 8-hour dark cycle (fluorescent cool white lighting). Air filtered at 0.2 μm was continuously bubbled through the cultures.

Treatments of urchins with various phytoplankton species showed that phytoplankton stimulates spawning (Table 1) (*9*). Concentrations of 5×10^7 to 10×10^7 cells per liter of the diatoms *Skeletonema costatum* and *Phaeodactylum tricornutum* provoked ≈50% spawning, and a concentration of 10×10^7 cells per liter of *Dunaliella tertiolecta* (a green flagellate) and *Thalassiosira nordenskioldii* (a diatom) caused 31 to 33% spawning. The spawning response to the different phytoplankton species did not vary significantly ($\chi^2 = 2.70$, df = 3, $P = 0.44$). Similarly, several phytoplankton species at concentrations between 40×10^7 to 80×10^7 cells per liter stimulated spawning in the mussel (Table 2). Thus, the spawning response of urchins and mussels is not specific to a single phytoplankton species, or even to spring species, as *P. tricornutum* and *D. tertiolecta* are usually absent during spring blooms.

The spawning response depended on phytoplankton concentration (Fig. 1). A low percentage of spawning occurred when urchins were exposed to *Skeletonema costatum* at a cell concentration of 0.7×10^7 to 1.3×10^7 cells per liter (equivalent to 2 to 4 mg of chlorophyll a per cubic meter; SD = 1.8) (*10*), and a maximum response was obtained at or above $\approx 5 \times 10^7$ cells per liter (equivalent to 15.4 mg of chlorophyll a per cubic meter; SD = 1.8). A dose-response curve was also obtained for mussels tested with *P. tricornutum*, although a much higher phytoplankton concentration was required for maximum spawning ($\approx 40 \times 10^7$ cells per liter, equivalent to 97.6 mg of chlorophyll a per cubic meter; SD = 2.2). Less than 5% spawning occurred in control experiments, in which mature urchins or mussels were exposed to f/2 culture medium (without phytoplankton) at the concentrations used in preparing the corresponding diatom culture (n = 25 to 60 for each control test). Whereas the phytoplankton concentrations that stimulate spawning in urchins (10 to 15 mg of chlorophyll a per cubic meter) are usually attained during spring blooms, those required to stimulate mussel spawning rarely, if ever, occur in nature (*10*). A possible explanation for this difference lies in the fact that mussels filter large volumes of seawater, thus concentrating phytoplankton. When mussels were placed in different volumes of seawater at a constant phytoplankton concentration, 10×10^7 cells per liter, the spawning response increased from 20% at a volume of 0.5 liter to 50 to 60% at volumes of 1 to 2 liters (Fig. 2A). The mussels were actively filtering in these tests and thus were exposed to more *P. tricornutum* cells as the volume increased. The absolute number of *P. tricornutum* in tests with the 1 to 2 liter volumes was similar to that of experiment 3 (Table 2) where the mussels were placed in a 0.4-liter volume at 40×10^7 cells per liter (1.0×10^8 to 2.0×10^8 and 1.6×10^8 cells, respectively). Given that mussels filter >10 liters daily (*11*), the natural phytoplankton concentration necessary to induce heavy spawning would be relatively low (mussels would be exposed to the quantity of chlorophyll a in 400 ml at a concentration of 97.6 mg of chlorophyll a per cubic meter after filtering 10 liters at 3.9 mg of chlorophyll a per cubic meter). Therefore, we conclude that natural phytoplankton levels

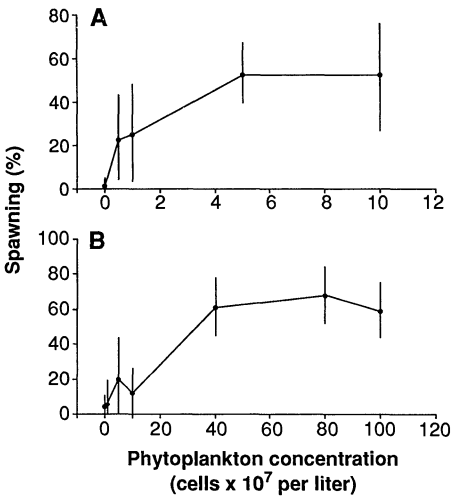

Fig. 1. Percentage of urchins and mussels that spawned. (**A**) The urchin *Strongylocentrotus droebachiensis* exposed to various concentrations of the diatom *Skeletonema costatum* and (**B**) the mussel *M. edulis* exposed to various concentrations of the diatom *P. tricornutum*. The number of animals tested at each concentration was 21 to 70. Vertical bars indicate 95% confidence intervals with correction of continuity.

Table 1. The percentage of urchins, *Strongylocentrotus droebachiensis*, that spawned in response to various treatments.

Experiment number	Treatment	Phytoplankton concentration ($\times 10^7$ cells per liter)	Spawning	
			Percentage	Number tested
	Skeletonema costatum			
1	Culture	5	52.5*	40
2	Culture	10	52.4*	21
3	Filtrate of culture	5	17.6	17
4	Filtrate of culture	10	30.1*	30
	Phaeodactylum tricornutum			
5	Culture	5	48.8*	43
6	Culture	10	50.1*	24
7	Filtrate of culture	10	43.5*	23
8	Filtrate boiled for 15 min	10	31.3*	16
	Thalassiosira nordenskioldii			
9	Culture	10	33.3*	15
	Dunaliella tertiolecta			
10	Culture	10	31.3*	16
	Controls			
11	Seawater without phytoplankton		1.4	70
12	Culture medium; concentration equivalent to 5×10^7 cells per liter		5.1	20
13	Culture medium; concentration equivalent to 10×10^7 cells per liter		1.4	70
	Sperm			
14	Sperm suspended in seawater		39.2*	51

*Significantly different (Fisher's exact test with Bonferroni correction to the probabilities; $P < 0.05$) from the response of urchins exposed to seawater and to culture medium at the concentration used in preparing the corresponding diatom culture.

during the spring bloom should be sufficient to stimulate spawning in mussels as well as urchins.

The saturation curve shown in Fig. 1B was obtained from tests that ran for 9 days. Examination of the spawning response over time revealed that its speed varied with phytoplankton concentration. The mussels spawned progressively earlier as the phytoplankton concentration increased (Fig. 2B). A similar spawning response over time was also observed for the urchin (*12*). This suggests that a rapid increase in phytoplankton enhances the synchrony of spawning in nature.

Although the spawning mechanism of *M. edulis* involves its filtering activity, it does not involve nutritional gain resulting from feeding, as there was no significant spawning when mussels were treated with *P. tricornutum* cells that had been filtered from the culture, lyophylized, and then resuspended in seawater (Table 2, experiment 5). By contrast, statistically significant spawning was obtained when mussels (Table 2, experiments 2 and 6) and urchins (Table 1, experiments 4 and 7) were exposed to the filtrate of phytoplankton cultures. These responses were lower than for the cultures themselves, but nevertheless support the hypothesis that phytoplankton releases a substance that stimulates spawning (*13*).

The reduced spawning response to the filtrates compared to the cultures is possibly due to an instability of the spawning inducer. In most of the experiments on urchins and mussels the filtrate showed between 6 and 22% less activity than the cultures (Tables 1 and 2). However, a >45% decrease occurred in experiments 6, 7, and 8 when mussels were treated with filtrates from *P. tricornutum*. Filtrates were held for 6 to 8 hours before use in experiments 6 and 7 and for 24 hours in experiment 8, compared to 1 to 3 hours in other experiments with filtrates. The relatively slight decrease in activity between experiments 6 and 8 suggests that most of the loss occurs during the first 6 to 8 hours.

Experiments with both urchins and mussels showed no statistically significant difference between tests in which boiled and unboiled filtrates were used (Table 1, experiments 7 and 8; Table 2, experiments 6 and 7). This indicates that the spawning inducer is heat stable.

Although there were no statistically significant differences between the sexes after 24 hours, we often noted that male urchins and mussels spawned more rapidly than females. Sperm suspension from conspecifics strongly stimulates spawning in the two species (Table 1, experiment 14; Table 2, experiment 13), and this should enhance the synchrony of spawning. In nature, phytoplankton probably induces the most receptive males to spawn first, and their gametes together with phytoplankton stimulate subsequent massive spawning.

To ensure reproductive success, it is critical that a spawning cue triggers gamete or larval release when environmental conditions are favorable for the larvae. For planktotrophic larvae, such as those of sea urchins and mussels, success may be strongly influenced by food availability (*14*); because the phytoplankton bloom is often of short duration, it would be advantageous if spawning occurred at the beginning of the bloom. However, the timing of the phytoplankton bloom is not clearly predicted on the basis of climatic variables such as day length and temperature because it depends on a number of complex hydrodynamic factors (*15*). One obvious advantage of phytoplankton as the spawning signal is that it directly indicates that food is abundant.

Coupling of the pelagic larval phase with phytoplankton abundance must have other advantages because species with lecithotrophic larvae also spawn during the spring phytoplankton bloom (*3*). An essential condition leading to a phytoplankton bloom is the stratification of the water column so that the mixed surface layer does not extend below the critical depth (*16*). In most areas, this stratification is caused by warming at the surface. As a consequence of the conditions associated with the bloom, pelagic larvae produced during the bloom are likely retained in the surface layer and not transported to depths where conditions for development may be less desirable and habitats for settlement less frequent. Because tem-

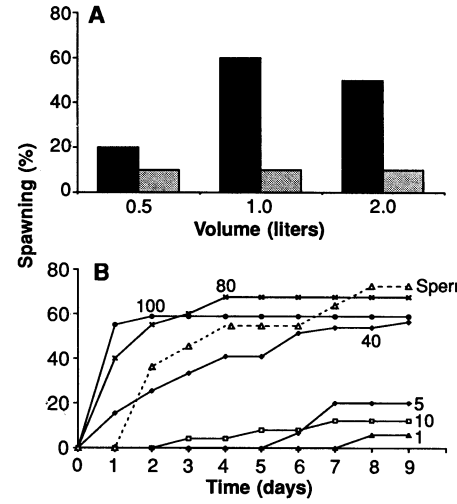

Fig. 2. (**A**) Relation between the spawning response of *M. edulis* and the volume of test medium in the experimental containers. In phytoplankton tests (solid bars), the concentration of *P. tricornutum* was maintained constant at 10×10^7 cells per liter in all test volumes. Controls (shaded bars) were exposed to f/2 medium culture without phytoplankton. In all tests, $n = 10$. (**B**) Cumulative daily spawning response of *M. edulis* at various concentrations of *P. tricornutum*. The numbers associated with each curve indicate the concentration in cells $\times 10^7$ per liter. The number of mussels tested at each concentration was 25 to 60.

Table 2. The percentage of mussels, *M. edulis*, that spawned in response to various treatments.

Experiment number	Treatment	Phytoplankton concentration ($\times 10^7$ cells per liter)	Spawning (%)	Spawning Number tested
	Skeletonema costatum			
1	Culture	80	76.7*	30
2	Filtrate of culture	80	58.3*	30
	Phaeodactylum tricornutum			
3	Culture	40	62.5*	24
4	Culture	80	73.9*	23
5	Lyophylized cells	80	10.5	19
6	Filtrate of culture	80	28.6*	21
7	Filtrate boiled for 15 min	80	28.6*	21
8	Filtrate held at 20°C for 24 hours	80	20.0	10
	Thalassiosira nordenskioldii			
9	Culture	40	88.9*	9
	Controls			
10	Seawater without phytoplankton		3.3	60
11	Culture medium; concentration equivalent to 40×10^7 cells per liter		3.3	30
12	Culture medium; concentration equivalent to 80×10^7 cells per liter		3.3	60
	Sperm			
13	Sperm suspended in seawater		72.7*	22

*Significantly different (Fisher's exact test with Bonferroni correction to the probabilities; $P < 0.05$) from the response of mussels exposed to seawater and to culture medium at the concentration used in preparing the corresponding diatom culture.

perature often varies erratically, particularly in the intertidal zone, the bloom may be a more reliable signal of impending favorable temperatures than temperatures at the time of spawning. Density of zooplankton is at an annual minimum at the onset of the spring phytoplankton outburst (*17, 18*); therefore, larval mortality due to zooplankton predators may be minimized. In addition, during the bloom when many species are spawning, larval mortality is likely reduced because feeding by predators has attained a maximum (*19*). In conclusion, a major advantage of phytoplankton as a spawning cue is that it integrates various environmental parameters indicating favorable conditions for larval success. In many species mechanisms may have evolved that directly couple their larval phase with phytoplankton abundance.

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Inhibition of Serum- and Ras-Stimulated DNA Synthesis by Antibodies to Phospholipase C

MARK R. SMITH, YA-LUN LIU, HYUN KIM, SUE GOO RHEE, HSIANG-FU KUNG

Several immunologically distinct isozymes of inositol phospholipid–specific phospholipase C (PLC) have been purified from bovine brain. Murine NIH 3T3 fibroblasts were found to express PLC-γ, but the expression of PLC-β was barely detectable by radioimmunoassay or protein immunoblot. A mixture of monoclonal antibodies was identified that neutralizes the biological activity of both endogenous and injected purified PLC-γ. When co-injected with oncogenic Ras protein or PLC-γ, this mixture of antibodies inhibited the induction of DNA synthesis that characteristically results from the injection of these proteins into quiescent 3T3 cells. However, when oncogenic Ras protein or PLC-γ was co-injected with a neutralizing monoclonal antibody to Ras, only the DNA synthesis induced by the Ras protein was inhibited—that induced by PLC was unaffected. These results suggest that the Ras protein is an upstream effector of PLC activity in phosphoinositide-specific signal transduction and that PLC-γ activity is necessary for Ras-mediated induction of DNA synthesis.

ACTIVATED *ras* GENES ARE FOUND IN a significant fraction of malignant human tumors (*1*), and it has been postulated that these genes are responsible for tumor development (*2*). Genetic mutations that transform the *ras* proto-oncogene into a gene that actively induces tumors result from amino acid substitutions at critical positions, such as Gly^{12} (*3*) and Gln^{61} (*4, 5*), in the primary sequence of the Ras protein. All members of the Ras protein family bind guanine nucleotides (*6, 7*), have intrinsic guanosine triphosphatase (GTPase) activity (*4, 8*) that is enhanced by interaction with GTPase-activating protein (GAP) (*9*), and have sequence homology to signal-transducing G proteins (*10*). These observations suggest that the biological role of Ras proteins may be to couple cell surface receptor activation to a variety of intracellular enzymes and ion channels that modulate cellular activity (*11*). Microinjection of bacterially synthesized oncogenic Ras proteins (*12, 13*) or inositol phospholipid–specific phospholipase C (PLC) purified from bovine brain (*14*) into quiescent NIH 3T3 fibroblasts induces morphologic transformation and DNA synthesis. Induction of DNA synthesis in resting fibroblast cells is also a property of serum growth factors. Thus, PLC and Ras proteins may be intermediates in the biochemical pathways used by serum growth factors to activate proliferation of cells.

Several growth factors have been shown to require a functional Ras protein for activity by microinjection experiments with neutralizing antibodies to Ras (anti-Ras) (*15*). The position of Ras participation in signal-transduction pathways was partially identified by injection of anti-Ras into various oncogene-transformed 3T3 cells (*16*). Injection of the anti-Ras monoclonal antibody Y13-259 blocked serum-induced cell division in NIH 3T3 cells (*15*) and caused the morphological phenotype of *ras*-transformed 3T3 cells to revert to that of the parental cell line (*17, 18*). These results suggested an absolute requirement for Ras proteins for 3T3 cell growth.

Bombesin (*19*) and platelet-derived growth factor (PDGF) (*20*) increase the intracellular concentration of several inositol phospholipids. PLC-γ is directly phosphorylated on tyrosine and serine residues by the PDGF receptor after PDGF treatment of quiescent 3T3 mouse fibroblasts (*21*), and further, injection of a monoclonal antibody specific for phosphatidylinositol 4,5-bis-

M. R. Smith and Y.-l. Liu, Biological Carcinogenesis and Development Program, Program Resources Inc., National Cancer Institute–Frederick Cancer Research Facility, Frederick, MD 21701.
H. Kim and S. G. Rhee, Laboratory of Biochemistry, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892.
H.-f. Kung, Laboratory of Biochemical Physiology, Biological Response Modifiers Program, Division of Cancer Treatment, National Cancer Institute—Frederick Cancer Research Facility, Frederick, MD 21701.