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Induction and regulation of metamorphosis in planktonic larvae: *Phoronis mülleri* (Tentaculata) as archetype

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ABSTRACT: The larvae of *Phoronis mülleri* are comprised of many diverse behavioural forms that can be manipulated experimentally to facilitate precise assertions about the induction of metamorphosis. Various parameters for inducing metamorphosis as exemplified in *Phoronis*, such as species-specific substrate, bacteria, the cations Rb^+, Cs^+ and Hg^{2+} and tensides, are considered, and their ecologic relevance to natural factors in the sea is demonstrated. Findings on metamorphosis in other marine larvae are summarized. The function of marine bacteria as "ecological ushers" is particularly emphasized.

INTRODUCTION

The planktonic-benthic life cycle is of immense importance for many sessile or hemisessile benthic invertebrates of the sea. Drifting in the surface layers of the water, the larvae are able to colonize new ecologic niches, and the abundant phytoplankton there provides ample nourishment. The critical phase of this survival strategy is finding and recognizing the substrate that is appropriate for the species. Since their sensory inventory is modest, it was formerly thought that the larvae reach the species-specific substrate by chance according to the "hit or miss" principle (Colman, 1933) and either survive or perish. More recently, ecologic studies and experiments have demonstrated that, despite the paucity of sensory apparatus, marine larvae are indeed able to recognize their species-specific substrate (Wilson, 1932, 1937, 1952; Cole & Knight-Jones, 1949; Knight-Jones, 1951; Crisp & Meadows, 1963; Gray, 1966; Chia & Rice, 1978). The larva perceives external signals which thus trigger reactions according to the lock-and-key principle (Müller, 1969; Herrmann, 1976, 1979). In the following work, recognition of the substrate and induction of metamorphosis is illustrated, essentially on *Phoronis mülleri* as a model, although the system is valid for very many other marine larvae of sessile and hemisessile benthic invertebrates.

MATERIALS AND METHODS

Larval material

During the summer months, larvae of sessile invertebrates can be pipetted out of plankton from the waters of Helgoland in sufficient quantities. The stage of maturity of the larvae of *Phoronis mülleri* can be determined exactly on the basis of particular features (Fig. 1), such as the number of larval tentacles, the presence of centres in which red blood cells are produced and the existence of the secondary nerve complex (Herrmann, 1976).

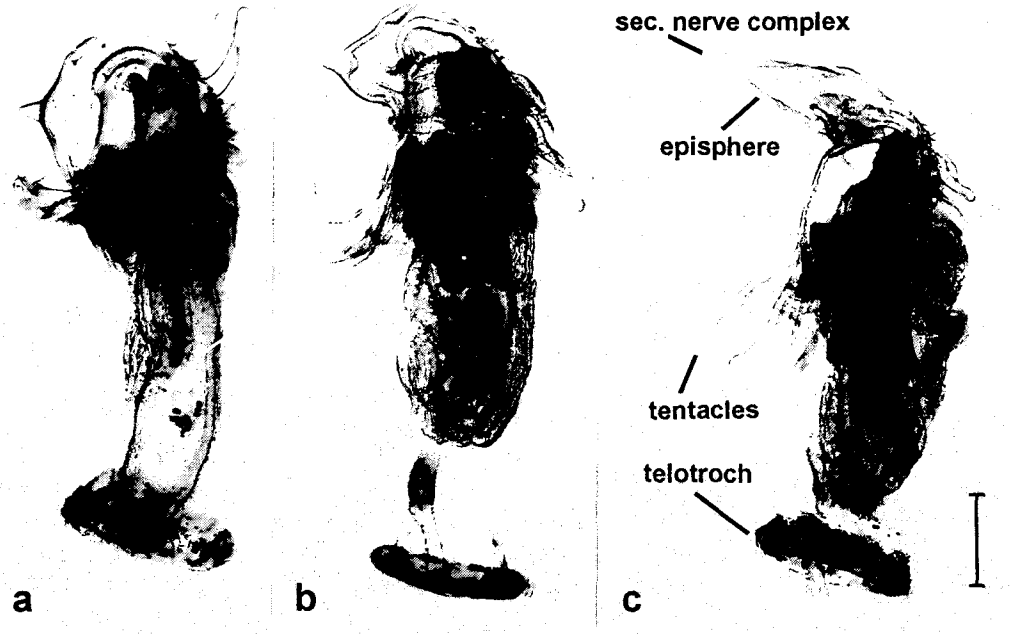


Fig. 1. Larva (Actinotrocha) of *Phoronis mülleri*, not activated (1a), slightly activated (1b) and highly activated by bacteria or cations (1c). Scale bar 500 µm

Mature larvae can be maintained in good condition in sterile seawater at 15 °C for 6–7 days by regular feeding with phytoplankton or cultured *Amphidinium carteri*, *Scrippsiella faerörense* or *Coccolithus* sp. (Hagmeier, 1978). Substantial contamination by bacteria must be avoided by changing the seawater frequently.

Readiness for metamorphosis of *Ph. mülleri* larvae can be ascertained by testing with subthreshold amounts of metamorphosis inducers. Changes in behaviour indicate impending metamorphosis. Using these means, it is possible to obtain homogeneous larval material for experiments on induction from heterogeneous plankton samples.

Inducers of metamorphosis

Experiments on the induction of metamorphosis employed both natural (various substrates, bacteria) and artificial (cations, tensides) inducers.

The species-specific substrate ("tiefe Rinne", Helgoland) was removed using a punch from the Van-Veen sampler, transported in a vertical position with the water above and used immediately for experimentation. The uppermost parts of the substrate are most important. Bacteria were grown in liquid culture using yeast extract (0.3–0.4 g/l seawater) as the medium. Bacterial concentrations were determined using a Neubauer chamber or nephelometry with an Eppendorf photometer. Isolated strains of bacteria were obtained by plating from dilution series onto agar surfaces for marine bacteria ("2216 E", Gunkel, BAH, pers. comm.) and further grown in liquid culture (Herrmann, 1976).

Cations as inductors were applied in the form of chlorides (analysed reagents, purity 99.5 %; Merck, Darmstadt). The respective 0.572 mol stock solutions represent approximate ionic content of artificial seawater (Dietrich & Kalle, 1965). The molar concentrations given are corrected for dilution and thus are final concentrations.

Tensides in pure form were obtained from the analytic laboratory of Hüls (Marl, Germany). Experiments were carried out with an ionic tenside, Marlon^R A (linear C_{10}-C_{13}-alkylbenzol sulfonate, LAS, ABS) and a non-ionic tenside, Marlophen^R 810 (Nonylphenol oxethylate, 10 mol ethylene oxide). During experimentation, the actual concentration was determined using the ring-shear method with a Krüss tensiometer. All experiments were carried out in the glass vessel prescribed for the tensiometer type. Tenside residues were removed by washing with acetone.

Experimental procedure

During the experiments, a specific amount of the inducing agent was added to a constant amount of sterile seawater obtained by membrane filtration (0.22 µm pore size). The experiments were performed at room temperature or in constant temperature rooms in evaporation dishes (Jena glass, 50- or 100-ml) or Boveri vessels. In each of a total of over 3000 experiments 10 mature larvae were used that had been previously tested for readiness to undergo metamorphosis and held for at least half a day in sterile seawater. Compared to the total volume, the amount of water carried over with the larva (max. approx. 0.1 ml) was negligible. The results were corroborated by means of parallel and control experiments and by many duplicate experiments carried out in various years. Larvae that did not undergo metamorphosis under the given experimental conditions were tested subsequently for inducibility.

The behavioural form, the onset of metamorphosis, time of exposure of the larva to the inducer (i.e. from the introduction of the larva to the time at which the metasome diverticulum is evaginated), duration of metamorphosis and course of metamorphosis were recorded and registered photographically or cinematographically (Herrmann, 1973, 1975b). The larva of *Phoronis mülleri* proved to be an ideal experimental object in all experiments. The rapid reaction of the larva, its wide behavioural spectrum (test of the capacity for metamorphosis, phases of activation), its adaptability, the short triggering time (1–9 min), the limited duration of transformation (max. 15 min) and finally, the high

concentration of individuals (20 000 m^{-3}) in the plankton around Helgoland, have made the experiments possible.

RESULTS

The causal relationships in the metamorphosis of marine benthic invertebrates are demonstrated using *Phoronis mülleri* as a model, because many different inducers and their ecologic relevance have been experimentally tested on this species.

Behaviour of the larva before and during metamorphosis

The larva of *Phoronis mülleri* cannot undergo metamorphosis unless activated by external inducers. This activation (Fig. 1) becomes manifest by more rapid motility and by muscle contractions in the body (dorsal bending) and in the episphere (change in form). Three stages of activation can be discerned (Fig. 2):

Slight activation begins with undirected exploratory behaviour. The larva swims in curves and turns frequently about its centre of gravity (tumbling). It shows a so-called "seismic behaviour". When vibration occurs in the laboratory or wave action in the

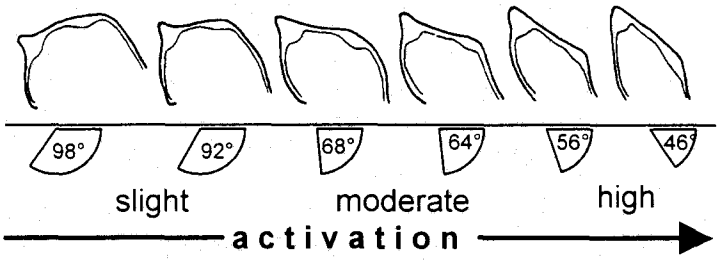


Fig. 2. Shape of the episphere of the larva of *Phoronis mülleri* drawn from living material. Changes in form due to contraction of the episphere muscles caused by activation by bacteria or cations. The angle data denote the angle subtended by the front end of the larva with the secondary nerve complex at its tip

open water, ciliary beating on the telotroch ceases periodically and the larva sinks. The length of the pauses is directly correlated with the degree of activation or with the strength of the inducer. When the larva touches the "substrate", the long axis of the body forms an acute angle with it. The secondary nerve complex thereby takes up contact with the substrate. This is the first phase prior to successful metamorphosis. In experiments with substrates of low inducing power, the seeking movements of the larva make circular arena-like tracks in the light mud without the initiation of metamorphosis (Herrmann, 1976).

Moderate activation of a *Ph. mülleri* larva ready for metamorphosis initiates with the behaviour just described, followed by jerky elevation of the episphere (Fig. 1b), strong distension of the larval tentacles and further protrusion of the episphere resulting from contraction of the ring muscle fibres in the episphere. The episphere loses its umbrella-like shape and becomes conical in form. The angle of the cone is a direct indicator of the extent of activation.

A high degree of activation is evident when further changes in shape of the episphere into a sharp cone occur (Fig. 1c). Brief contractions of the muscle fibrils at intervals of 3–15 sec press the secondary nerve complex as a proboscis far forward, even when no substrate is present in the vessel. Increasing activation shortens the intervals between muscle contractions. The beginning of metamorphosis, characterized by the evagination of the metasome diverticulum, takes place at the point of highest activation.

These behavioural forms and particularly the changes in the shape of the episphere are suitable for testing the maturity of the larva. Using subthreshold concentrations of bacteria, for example, every larva can be tested for its readiness to undergo metamorphosis without actually causing metamorphosis to begin. The activation can be reversed fully by placing the larva so tested in sterile seawater. Previous slight or moderate activations have no effect either on time for induction or on the strength of induction.

A successful induction of metamorphosis is a cumulative process. When induction is optimal, all stages of activation are undergone when the larva is placed into the experimental vessel. The duration of the individual phases depends upon the quality and quantity of the inducer. The phases take place more regularly when cations are used in place of bacteria. At optimal cation concentrations the minimum duration is nine minutes. When optimal concentrations and compositions of bacteria are employed, the time can be reduced to one minute, whereas when other bacterial compositions are used, metamorphosis can be extended to 10–15 min.

This is also valid for the *Phoronis*-specific substrate. In general, the substrate particles accelerate the process of metamorphosis by stimulating the secondary nerve complex such that 5–10 min of induction time is enough.

Inducers of metamorphosis in the larva of *Phoronis mülleri*

Various inducers can be identified experimentally as causes for triggering metamorphosis in *Ph. mülleri*. Every inducer requires different parameters and is functional by itself. The strength of an inducer can be given as the necessary time of exposure (induction time) until metamorphosis begins (see above).

Substrate as inducer: The most logical inducer is the species-specific substrate for *Phoronis*. Only the upper layer of the substrate, a mixture of mud and sand with an organic content of about 4% and a grain size of 0.2–0.63 mm for the most, exhibits an inductory effect. The inductory effect lasts only a few days, probably because the nutritive content of the substrate is used up by bacteria. Experiments with sterilized *Phoronis*-substrate showed no successful induction of metamorphosis.

The substrate of *Phoronis mülleri* lies in the transition area between sand and mud. *Ph. mülleri* cannot survive in pure sand or mud. Substrates with increased proportions of sand can be colonized by juvenile animals, but longer tubes and older animals are never found there. *Phoronis* tubes over 10 cm in length are occasionally found in pure mud but they do not contain animals.

The spotty distribution of *Phoronis mülleri* is attributable to the need for a balanced composition of substrate with appropriate proportions of sand and mud. The *Phoronis* substrate is characterized by a slight but constant increase in organic material during the summer months. This is the prerequisite which continually maintains the bacterial population in the surface mud in the growth phase (Gunkel, 1964). In appropriate

experiments the possibility that the structure of the substrate itself, such as grain size, surface texture or chemical composition, induces metamorphosis in *Phoronis mülleri* can be excluded.

Bacteria as inducers: The real inducers in the natural substrate are bacteria. This was shown by testing cultured bacteria. Moreover, bacteria from decomposing plankton from the littoral region and bacterial strains from the National Collection of Marine Bacteria (NCMB, Aberdeen) were used. In the experiments on the induction of metamorphosis, two conditions had to be fullfilled: (1) the bacterial culture used had to be in the exponential growth phase (Fig. 3) and (2) the bacterial concentration in the experimental set-up had to exceed a certain concentration.

The bacterial concentration necessary depends upon whether a pure or mixed culture is used. Mixed cultures are more efficient; for lower concentrations of bacteria, 5×10^6 ml^{-1} suffice, on the average. When pure cultures are used, the necessary bacterial concentration ranges from 15–55 $\times$ 10^6 ml^{-1} for cultures in the logarithmic growth phase and can rise to 90 $\times$ 10^6 ml^{-1} at the end of the growth phase. The advantage of using pure bacterial cultures lies in the shorter times for induction to take place; mixed cultures do not trigger metamorphosis until after 10–15 min.

Bacteria that induce metamorphosis have the following characteristics: they are facultatively aerobic and the majority are motile; they are common gram-positive and gram-negative bacteria of the families Micrococcaceae, Pseudomonadeaceae and Spiril-

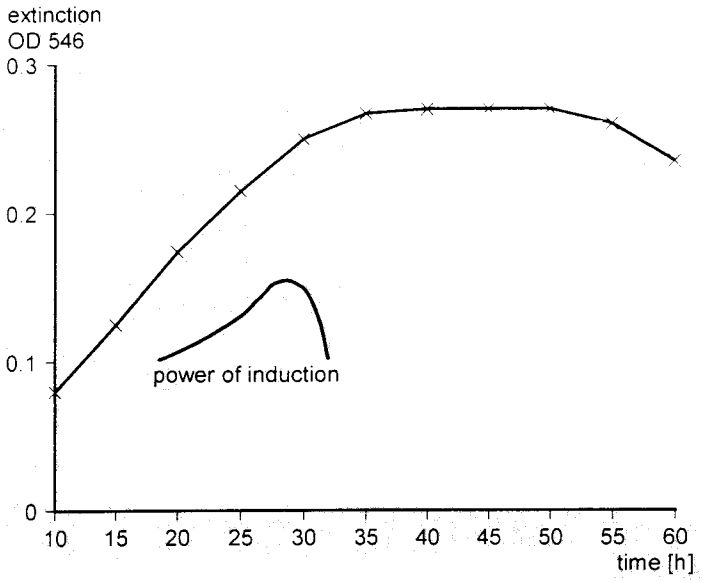


Fig. 3. Growth curve of a bacterial population in liquid medium (yeast extract in seawater, 0.3 g $\times$ l^{-1}) based on extinction measurements with a photometer (Eppendorf, filtre OD 546). The small curve represents the inductive power of the bacterial culture relative to growth. The inductive strength becomes gradually manifest at a certain bacterial concentration, then increases rapidly and finally decreases quickly at the end of the exponential phase

laceae; flagellated bacteria that show pigmented colonies on agar are more effective (Herrmann, 1976).

Among the strains from the National Collection of Marine Bacteria, Aberdeen, NCMB 129 (*Pseudomonas fluorescens*), NCMB 308 (Moraxell-like *Coccobacillus*). NCMB 1495 (*Planococcus citreus*) and NCMB 407 (*Vibrio anguillarum*) induced metamorphosis. The latter did not produce pigmented colonies on agar, but the vibriones and spirillochetes induced metamorphosis particularly readily. The bacteria from the Schlei river used by Rieper (1976), *Agrobacterium* sp. and *Brevibacterium* sp., likewise induced metamorphosis. More precise microbiological experiments on the real causes of bacterial induction and on the common physiological and genetic features of inducing and non-inducing bacteria are planned in connection with the BAH.

Cations as inducers: All the commonly available chlorides were tested for their inductive effect on metamorphosis in *Phoronis mülleri*. Experiments with bromides and iodides invariably resulted in damage to the larvae (histolysis of the episphere or of the telotroch) and not in sucessful induction. The only cations that induced metamorphosis in *Phoronis mülleri* were RbCl, CsCl and $HgCl_2$.

The onset of metamorphosis, an important parameter for the inductive capacity of the cations, depends on (1) the concentration, (2) the temperature (normally room temperature) and (3) the total ion composition of the experimental set-up. The list of chemical substances with inductive effect can be extended, when the nervous system of the larva is experimentally modified (Herrmann, in prep.).

RbCl (Fig. 4a): Rubidium chloride in a very narrow range of concentration induces metamorphosis. A clear relationship between the concentration used and the minimal time necessary for induction can be determined. The induction time for 10^{-2} mol RbCl in seawater is 36 min, decreasing with increasing RbCl concentration. The optimal concentration is 1.71×10^{-2} mol and at this concentration the minimal time for induction is 9 min.

At increasing concentrations the time necessary for inducing metamorphosis rises to 25 min and at concentrations higher than 0.03 mol aberrant forms (y-anomalies) are encountered in the course of metamorphosis. In the latter case, the metasome diverticulum becomes only half evaginated and a portion of the larval tentacle is cast off. Metamorphosis is aborted.

CsCl (Fig. 4b): Cesium chloride exhibits a very wide spectrum with respect to the molar concentration needed for inducing metamorphosis. The concentration ranges from 0.57×10^{-2} mol in seawater with an induction time of 16 h to a concentration of 7.4×10^{-2} mol with an induction time of 23 min. As with RbCl, a further increase in concentration causes anomalies, although in altered form. In this case, the metasome diverticulum becomes half evaginated and metamorphosis aborted (h-metamorphosis anomaly, Fig. 7A).

The minimal induction is, as is the case with RbCl, 9 min at a concentration of 5.7×10^{-2} mol.

$HgCl_2$ (Fig. 4c): Mercury-II-chloride is a toxin with low solubility in water (6.68 g in 100 ml at 20°C). Nevertheless, $HgCl_2$ induces metamorphosis in a comparatively minimal concentration of 10^{-5} to 2×10^{-4} mol. As for RbCl or CsCl, at the optimal concentration (1.8×10^{-4} mol) the time for induction is 9 min. When the optimum is exceeded, as is the case for RbCl, anomalies (y-anomalies) arise.

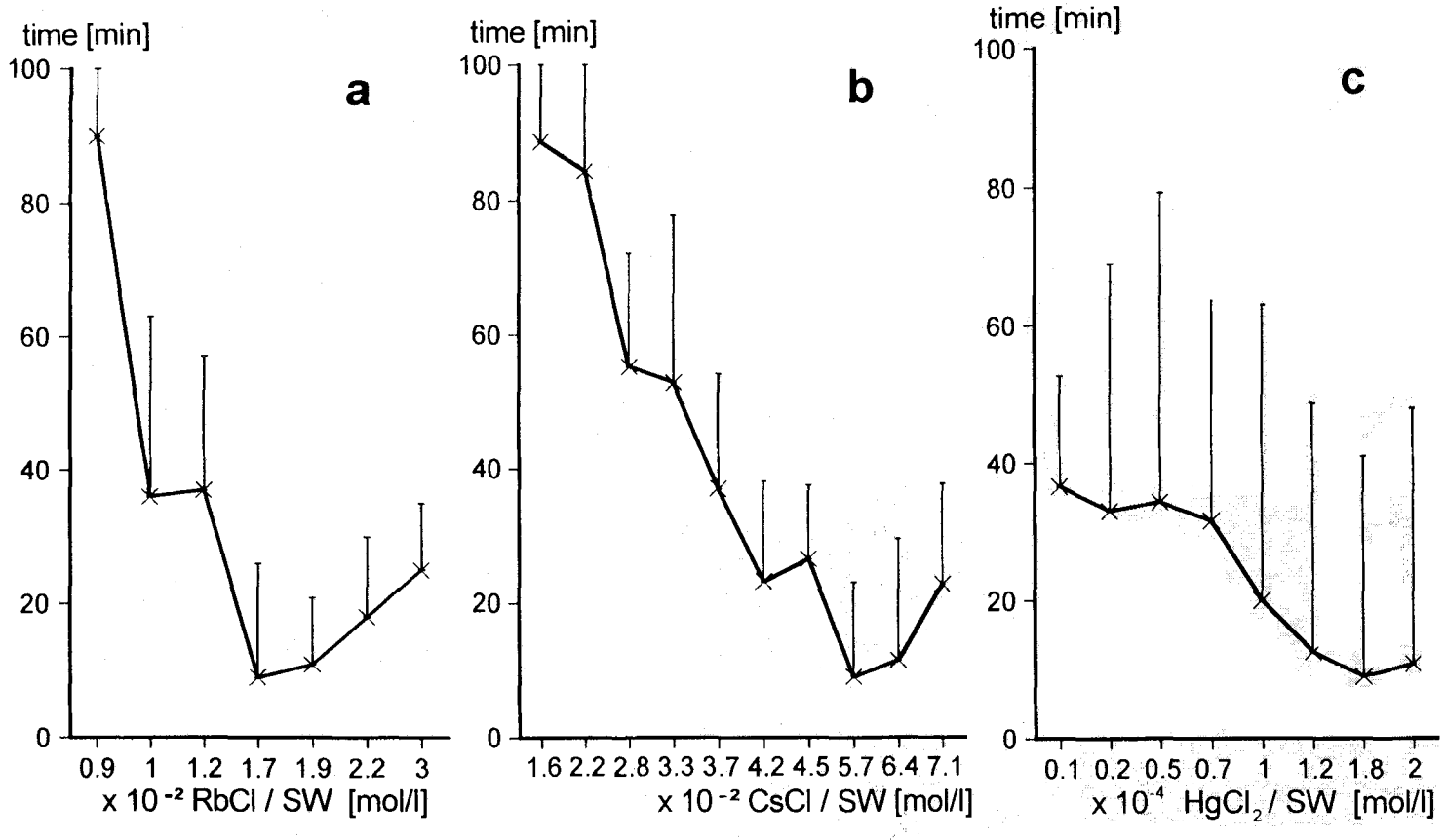


Fig. 4. Induction of metamorphosis in the larva of *Phoronis mülleri* by cations. The curves represent the shortest times of induction as parameter for inductive power. The vertical lines give the standard deviations. Aberrant metamorphoses occur at higher concentrations than those given in the figures. Rubidium chloride has the narrowest effective range (4a), cesium chloride the widest (4b), and mercuric-II-chloride is effective at very low concentrations (4c)

Tensides as inducers: The addition of tensides lowers the surface tension of seawater. How much, depends on (1) the kind of chemical additive and (2) the proportion of tenside. Marlon^R A reduces the surface tension less than Marlophen^R 810. The behaviour exhibited by *Phoronis mülleri* larvae depends upon the tenside used and its concentration.

The effect of Marlophen^R 810 in the range of concentration from 0.001 ppm to 10 ppm on metamorphosis was studied. Concentrations higher than 0.7 ppm for 2 h, caused histolysis of the body epithelium. At concentrations lower than 0.7 ppm, the larvae showed distention of the tentacles, arrest of ciliary movement on the telotroch and on the tentacles.

At concentrations below 0.4 ppm, the larva begins exploratory movements and elevates the metasome diverticulum; further dilution amplifies the "seismic behaviour" (see below). The induction of metamorphosis begins at approximately 0.5 ppm (43 dyne $\times$ cm^{-1}).

The anionic tenside Marlon^R A forms an insoluble complex with the Ca^{++} ions of seawater. The few precipitates that arise adhere, on contact, to the tentacles of the larva and cause local histolysis, especially on the edge of the episphere and on the tips of the tentacles. Nevertheless, concentrations of 10 ppm are tolerated for short intervals by the larva, whereby the tentacles are annexed to the body and the volume of the coelom changes, causing the procoelom to shrink and the mesocoel to expand, whereas the metacoelom remains unchanged.

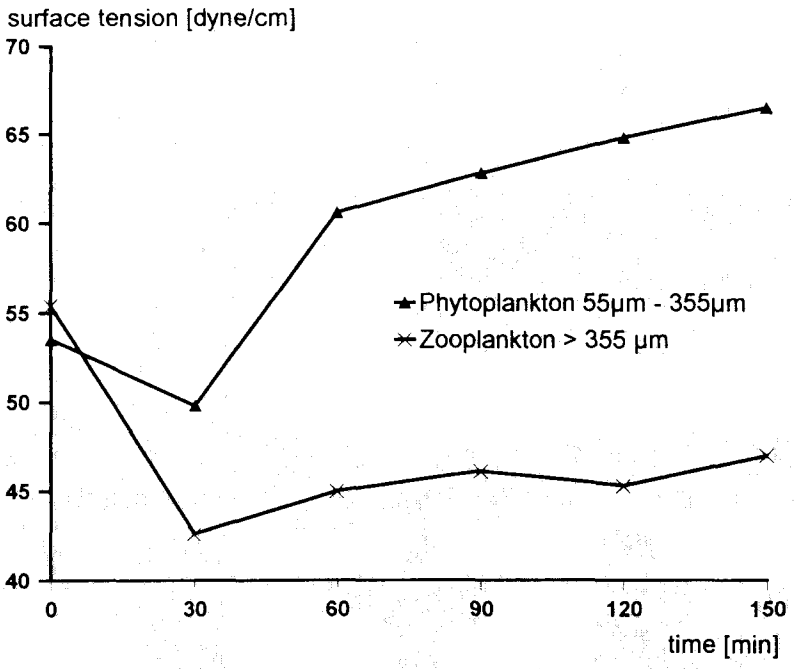


Fig. 5. Changes in surface tension in a plankton sample separated into two particle sizes, 55–355 µm (mostly phytoplankton) and >355 µm (mostly zooplankton). Phytoplankton cause a more rapid increase in surface tension

Concentrations under 1 ppm cause pronounced distension of the tentacles. At this concentration, the "seismic behaviour" extends to larvae that are not ready for metamorphosis. The induction of metamorphosis takes place at a concentration of 0.1 ppm (64–67 dyne $\times$ cm^{-1}).

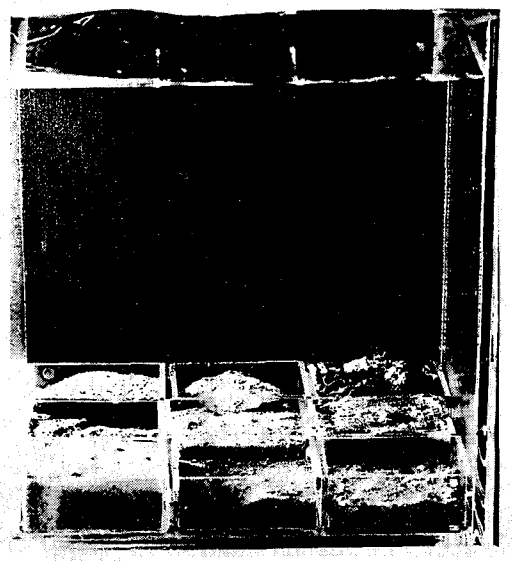
The previously described induction of metamorphosis in *Phoronis mülleri* larvae using decomposed plankton can now be explained. It is known that phytoplankton (55–355 µm) induce metamorphosis more readily than zooplankton (>355 µm).

The difference in the ability to induce metamorphosis was believed to be due to the different composition of the bacterial populations (Herrmann, 1975a). However, measurements of surface tension have shown that decomposing microplankton cause the surface tension to increase rapidly and that the surface tension rises to values (65 dyne $\times$ cm^{-1}) capable of causing induction of metamorphosis within 2 h (Fig. 5).

Thus, why freshly decomposing bacteria are suitable for reliably inducing metamorphosis can be easily explained: bacteria in the logarithmic growth phase and capable of inducing metamorphosis are present, and the surface tension rises to a value which induces metamorphosis. This double induction also explains why so many semi-mature *Phoronis* larvae in plankton samples are forced to undergo aberrant metamorphosis.

Ecologic significance of the results

The results of experimental induction of metamorphosis in the laboratory can be extended by further observations on the behaviour of the larvae in their natural habitat.



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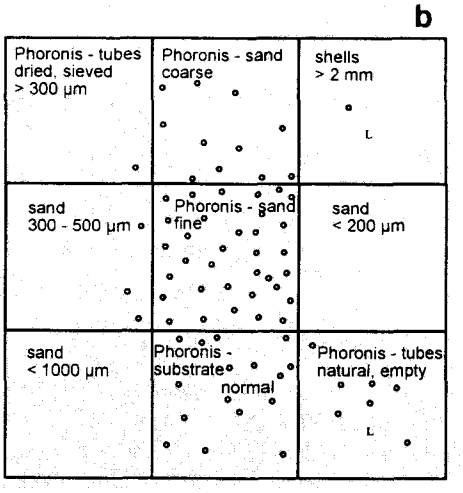

Fig. 6. Experiment to find the appropriate substrate for 100 *Phoronis mülleri* larvae ready to metamorphose. 6a: Nine plexiglas vessels 6 $\times$ 6 cm with different substrates. 6b: Results showing the locations of complete metamorphoses (circles) and larvae (L)

In experiments with a labyrinth in which *Phoronis* larvae could swim either toward the end at which a bacterial suspension capable of inducing metamorphosis had been applied or to a neutral end, 95 % of the larvae moved to the end with the bacteria. Shortly before they reached the end with the bacterial suspension, most of the larvae began metamorphosis (bacterial concentration 10.7 × 106 ml^{-1}). Light and other factors were controlled. Thus, the larvae could perceive a bacterial gradient.

A further experiment will be documented because of its ecologic relevance:
Nine plastic dishes each filled with different substrates were placed in the wells of an 18-cm large cubical container of clear plastic (Fig. 6a). Seawater was added with extreme care to prevent mixing of the substrates – especially their fine particles. Of the 100 *Phoronis mülleri* larvae ripe for metamorphosis that were added, 80 moved to the water surface and only 20 sank about 10 cm. After an hour, there were only 11 larvae at the surface.

After 48 h, only 2 larvae were free in the water and 74 young *Phoronis* and one aberrant metamorphosis were found. The remaining animals were missing, probably because they were so small that they were lost during exploration of the substrates.

The results indicated that the various *Phoronis*-specific substrates contained a total of 93 % of the metamorphoses partitioned as follows: empty *Phoronis* tubes 10 %, coarse component of sifted *Phoronis* sand 15 %, fine component of sifted *Phoronis* sand 46 %, and unmanipulated *Phoronis* sand 22 %. The other nonspecies-specific substrates contained the remaining metamorphoses (shells 1 %; sifted sand from the north beach of Helgoland, particles >200 µm: 0 %; 300–500 µm: 5 %; >1000 µm: 0 %) whereby these, especially the substrate of 300–500 µm large north beach sand, may have been induced due to their proximity to the species-specific substrates (Fig. 6b).

This result, also apparent in other similar experiments, conspicuously demonstrates the interplay between *Phoronis* larvae and their species-specific substrate.

In the sea in spring, the bacterial concentration in the *Phoronis*-specific substrate is much lower. Hickel & Gunkel (1968) found a bacterial concentration in mud with sand of 3.5–13 × 10^6 ml^{-1}. Due to successive collapses of the phytoplankton blooms during early summer, so much organic material is released in the sea such that (1) the bacterial concentration in the seawater becomes higher; (2) the surface tension of the water is elevated; and (3) enough organic matter settles on the ocean floor, likewise causing the bacterial concentration there to rise.

Thus, several essential parameters for a successful metamorphosis are present in the right substrate.

The mature larvae become sensitized by the high bacterial concentration in the free water. In laboratory experiments, the initial behavioural forms (slight and moderate activation, see above) are produced. The "seismic behaviour" of the larva plays an important role, for, in the presence of wave action, the ciliary beat on the telotroch and on the tentacles ceases and the larva sinks downward at a speed rate of ca 5 mm × sec^{-1}.

A slightly activated *Phoronis* larva reacts extremely sensitively to wave movements. In the laboratory, a single shaking of the bench is enough to cause the larva to sink down. The speed of sinking down and the depth to which the larva sinks depend upon (1) the magnitude of the vibration; (2) its duration; (3) the maturity of the larva; and (4) its degree of activation.

During an August storm at Helgoland (usually in mid-August), a completely mature

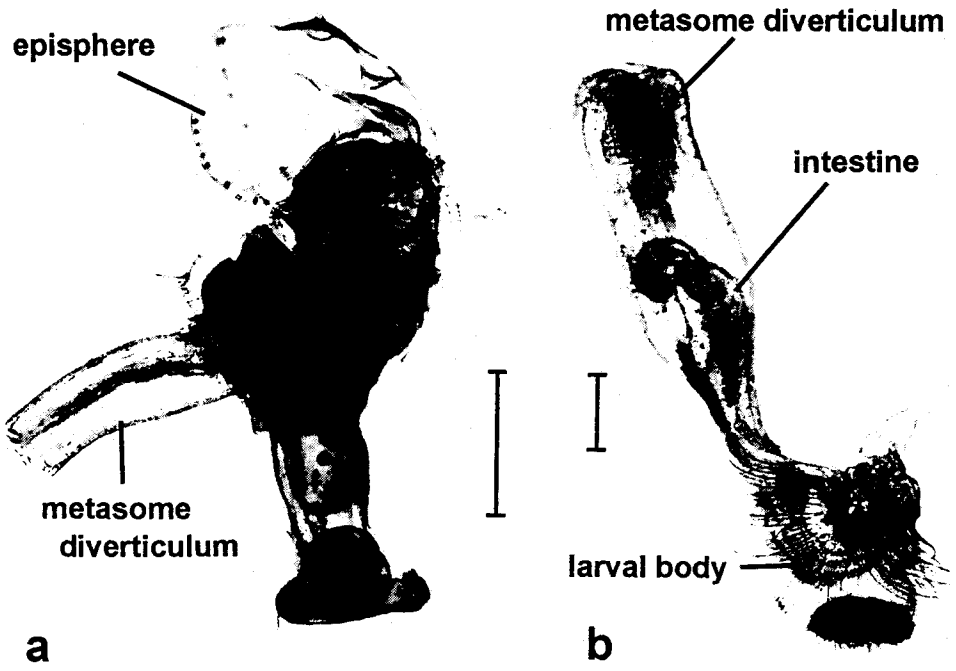

Fig. 7. Metamorphosis stages in larvae of *Phoronis mülleri*. 7a: h-aberrant metamorphosis stage as found in plankton containers. The metasome diverticulum is evaginated at right angles to the body axis. This metamorphosis does not reach completion. Scale bar 500 µm. 7b: Normal initial stage of metamorphosis. The metasome diverticulum is evaginated in the direction of the episphere. Scale bar 500 µm

Phoronis larva can sink the required 30-40 m to the sea floor. Metamorphosis in the water column is improbable and has been postulated on the basis of the presence of metamorphosis stages in plankton pails (Cori, 1939). Here, a high bacterial concentration that causes all *Phoronis* larvae, including even immature ones, to undergo metamorphosis, is rapidly reached due to decomposing plankton.

Generally, aberrant metamorphosis forms arise that have been depicted as "metamorphosis stages" in the literature of the past 100 years (Schneider, 1862; Ikeda, 1901; Siewing, 1969). Laboratory findings on seismic behaviour, carried out in a measuring cylinder 80 cm in height, can be substantiated by observations in the field. In quantitative studies of plankton tows in 0.5 and 5 m depth as well as in plankton samples in the vicinity of the "Kabeltonne", Helgoland, following larger wave movements, more mature larvae are not found in the sea, as is to be expected.

The mature larvae arrive at the sea floor after cessation of their ciliary beat, become "trapped" by the light mud and its adherent bacteria, and are forced to undergo metamorphosis. The secondary nerve complex serves as the triggering centre that ultimately gives the starting shot for the rapid transformation which lasts 15 min at the most.

In regions with pure sand substrates, the *Phoronis* larvae can rise to the surface

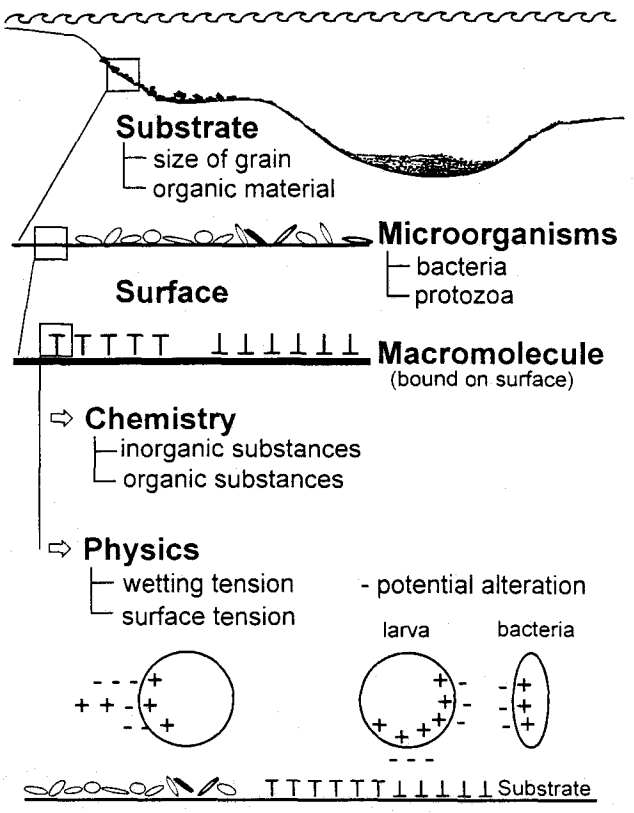

Fig. 8. Schematic representation of induction of metamorphosis in marine sessile invertebrates. The possible succession of adsorption to, and colonization of, solid surfaces in the sea as the preparatory step for the subsequent settlement by sessile or hemisessile invertebrates is depicted

again, when the weather calms, and drift farther on. As maturity and hypermaturity are approached, the threshold necessary for initiating metamorphosis decreases (Fig. 10), and the larvae react more strongly to wave movements and bacterial concentration. This leads to colonization of suboptimal areas (e.g. substrates with higher proportions of sand).

DISCUSSION

Substrates in the sea are subject to succession, just as are fields on the land, for example. Every small or larger stationary or solid surface is affected (Fig. 8). Initially, macromolecules (e.g. protein molecules) are deposited and these are used by bacteria as nutrition resources. Various protozoa sweep the bacteria into their gullets by ciliary action. If the surface is large enough and illuminated, then it is colonized by algae. When the light factor is not limiting, a large number of different invertebrates can colonize the surface and compete intraspecifically and interspecifically with each other for space. Finding the appropriate niche and eliminating the competition are the magic words for the survival of the species during colonization of substrates.

On account of their sessile or hemisessile form of life, the inhabitants in or on the substrate of the sea floor are relatively protected. Difficulties are encountered when further sexually produced generations are required. Progeny that become immobile in the immediate vicinity of their parents mean competition for nutrition and space and sometimes inbreeding.

The pelagic-benthic life cycle avoids both problems. The supply of food in the upper layers of the water is bountiful and the larvae drift and are able to colonize new substrates. When one compares this strategy with that of holometabolic insects, it is clear that competition for food in both groups is avoided by the larvae. The larval form in the holometabolic insects is merely a feeding stage, the adult is the reproductive and redistribution stage. The place where nutrition is available is sought by the adult as the eggs are laid. In marine larvae, in contrast, the larval stages represent the feeding and redistribution stages.

Thus, the choice of substrate is the most critical stage in the life cycle of sessile marine benthic invertebrates. The question is, how do the larvae with their modest arsenal of sense organs recognize the substrate that will be suitable for the survival of the adult animal? Experiments on the induction of metamorphosis in benthic invertebrates are almost invariably sucessful when the natural substrate is used (Jägersten, 1940; Wilson, 1952; Silén, 1954; Siewing, 1974). Frequently the substrate itself (grain size, surface, individual components) is ineffective, and it is probably the admixed organic components that are more responsible for the succession characteristic of the substrate (see Table 1). These components comprise the content of organic material, the arrangement of macromolecules and the surfaces covered with bacteria, microorganisms and algae (Fig. 10).

The interaction between the substrate and larvae of various animal groups has been studied very closely (Chia & Rice, 1978). In many cases, the various inducers disclosed here can be traced back to the bacterial population characteristic of the respective substrate in the sense of the ecology of colonization. In the sea, every surface – ranging from every grain of sand to scums, to slime, to organic material (proteins, arthropodin) and to different algae – every solid surface becomes colonized by bacteria (see above). Their numbers, species and the species composition depends upon the substrate. It is easily imaginable that Rhodophytes, such as *Laurencia pacifica*, have a bacterial composition on their surfaces quite different from that on the surfaces of Chlorophytes, such as *Ulva* spp. Both algae act as an ideal substrate for metamorphosis for different *Aplysia* species (Hadfield, 1978). For *Phoronis mülleri*, the inducing bacteria can only be ideally recruited on *Laminaria saccharina* (Herrmann, 1976).

Formerly, diatoms and sand were considered to be the triggering agents (Wilson, 1955), but it has been proven that induction can be initiated, free of the substrate, by using bacteria (Müller, 1969; Herrmann, 1975a, 1975b, 1976; Eiben et al., 1976). The amounts of inorganic and organic compounds such as Zn, Cu, Li, Cs, Rb and, for example, extracts of arthropods etc., used as inducers in the experiments, are not representative of the quantities found in the sea. These inducers and their physical effects can be considered as spare keys for inducing metamorphosis, albeit by using them, the induction of metamorphosis can be more precisely explained (Müller, 1973; Eiben, 1976; Berking, 1988).

There are far more kinds of bacteria present in the sea than there are animals and

Table 1. List of diverse inducers in metamorphosis of marine larvae (see also Fig. 8)

Factor	Species/phylum	Authors
Factor substrate		
Size of grain		
Hard rock with some mud	*Aporrhais* sp. (Gastropoda/Prosobranchia)	Yonge (1937)
Sand	*Placopecten magellanicus* (Lamellibranchiata)	Culliney (1975)
Sand	*Mercenaria mercenaria* (Lamellibranchiata)	Keck et al. (1971)
Sand 0.1–0.9 mm	*Nassarius obsoletus* (Gastropoda/Prosobranchia)	Scheltema (1961)
Fine sand 0.2–0.45 mm	*Ophelia bicornis* (Polychaeta)	Wilson (1948)
Fine sand 0.05–0.1 mm	*Owenia fusiformis* (Polychaeta)	Wilson (1932)
Fine sand/silt	*Golfingia misakiana* (Sipunculida)	Rice (1978)
Muddy sand	*Notomastus* sp. (Polychaeta)	Wilson (1937)
Sandy mud 0.047 mm	*Scolecolepis fulginosa* (Polychaeta)	Day & Wilson (1934)
Content of organic material		
3 %	*Owenia fusiformis* (Polychaeta)	Wilson (1932)
6.9 %	*Scolecolepis fulginosa* (Polychaeta)	Day & Wilson (1934)
Rich in detritus	*Melinna cristata* (Polychaeta)	Nyholm (1950)
Mud	*Armandia brevis* (Polychaeta)	Hermans (1978)
Mud from adult habitat	*Nassarius obsoletus* (Gastropoda/Prosobranchia)	Scheltema (1961)
Factor surface		
Structure of surface		
Fibrous, spiny surface	*Tubularia larynx* (Gnidaria/Hydrozoa)	Barnes & Powell (1950)
Silk material	*Mytilus edulis* (Lamellibranchiata)	Bayne (1965)
Covered with microorganisms		
Living organic film	*Ophelia bicornis* (Polychaeta)	Wilson (1955)
Film of bacteria	*Ostrea edulis* (Lamellibranchiata)	Cole & Knight-Jones (1949)
Film of bacteria	*Spirorbis borealis* (Polychaeta)	Knight-Jones (1951)
Film of bacteria (*Pseudomonas, Flavobact.*)	*Protodrilus symbioticus* (Archiannelida)	Gray (1966)
Surface with bacteria	*Cassiopea xamachana* (Cnidaria/Scyphozoa)	Wieker (1975)
Film of microorganisms	*Bugula flabellata* (Tentaculata/Bryozoa)	Crisp & Ryland (1960)
Film of microorganisms (bacteria, diatoms, flagellata)	*Spirorbis borealis* (Polychaeta)	Meadows & Williams (1963)
Film of bacteria and diatoms	*Pocillopora damicornis* (Cnidaria/Anthozoa)	Harrigan (1972)
Film of microorganisms	*Haminoea solitaria* (Gastropoda/Opisthobranchia)	Harrigan & Alkon (1979, cited in Chia & Rice, 1978, p. 168)
Film of microorganisms	*Elysia chlorotica* (Gastropoda/Opisthobranchia)	Harrigan & Alkon (1979, cited in Chia & Rice, 1978, p. 168)
Aerobic bacteria	*Hydractinia echinata* (Cnidaria/Hydrozoa)	Müller (1969)
Facultatively aerobic bacteria	*Phoronis mülleri* (Tentaculata/Phoronida)	Herrmann (1975a)

Table 1 (continued)

Factor	Species/phylum	Authors
Covered with microorganisms		
Bacteria (0.8 µm)	*Nassarius obsoletus* (Gastropoda/ Pulmonata)	Scheltema (1961)
Bacteria (vibrio)	*Cassiopea andromeda* (Cnidaria/ Syphozoa)	Neumann (1979)
With bacteria and algae	Larvae of *Mytilus* sp. (Lamellibranchiata)	Scheer (1945)
Film of bacteria (*Fucus serratus*, Phaeophyta)	*Alcyonidium polyoum* (Tentaculata/ Bryozoa)	Crisp & Ryland (1960)
Organisms		
Ophlitaspongia pennata (Porifera)	*Rostanga pulchra* (Gastropoda/ Opisthobranchia)	Chia & Rice (1978)
Cliona celata (Porifera)	*Membranobalanus orcutti* (Crustacea/ Cirripedia)	Newman & Ross (1976)
Porites lobata (Cnidaria)	*Philippia radiata* (Gastropoda/ Prosobranchia)	Hadfield (1976)
Tubularia indivisa (Cnidaria)	*Trinchesia aurantia* (Gastropoda/ Opisthobranchia)	Swennen (1961)
Alcyonium digitatum (Cnidaria)	*Tritonia hombergi* (Gastropoda/ Opisthobranchia)	Thompson (1962)
Kirchenpauaria pinnata (Cnidaria)	*Eubranchus exiguus* (Gastropoda/ Opisthobranchia)	Tardy (1962, cited in Chia & Rice, 1978, p. 178)
Porites compressa (Cnidaria)	*Phestilla sibogae* (Gastropoda/ Opisthobranchia)	Hadfield & Karlson (1969)
Living coral epithelium	*Boscia anglica* (Crustacea/Cirripedia)	Moyse (1971)
Electra pilosa (Bryozoa)	*Adalaria proxima* (Gastropoda/ Opisthobranchia)	Hadfield (1976)
Electra crustulenta (Bryozoa)	*Doridella obscura* (Gastropoda/ Opisthobranchia)	Perron & Turner (1977)
Filamentous algae	*Mytilus edulis* (Lamellibranchiata)	Bayne (1965)
Unidentified blue-green algae (Cyanophyta)	*Dolabella auricularia* (Gastropoda/ Opisthobranchia)	Switzer-Dunlap & Hadfield (1977)
Lyngbya majuscula (Cyanophyta)	*Stylocheilus longicauda* (Gastropoda/ Opisthobranchia)	Switzer-Dunlap & Hadfield (1977)
Ulva fasciata (Chlorophyta)	*Aplysia juliana* (Gastropoda/ Opisthobranchia)	Switzer-Dunlap & Hadfield (1977)
Ascophyllum nodosum (Phaeophycea)	*Clava squamata* (Cnidaria/Hydrozoa)	Williams (1965, cited in Chia & Rice, 1978, p. 3)
Laurencia pacifica (Rhodophyta)	*Aplysia californica* (Gastropoda/ Opisthobranchia)	Kriegstein et al. (1974)
Callithamnion halliae (Rhodophyta)	*Aplysia brasiliana* (Gastropoda/ Opisthobranchia)	Strength & Blankenship (1979, cited in Chia & Rice, p. 169)
Laurencia sp. (Rhodophyta)	*Aplysia dactylomela* (Gastropoda/ Opisthobranchia)	Switzer-Dunlap & Hadfield (1977)
Chondrococcus hornemanni (Rhodophyta)	*Aplysia parvula* (Gastropoda/ Opisthobranchia)	Switzer-Dunlap (1978)
Lithophyllum + *-thamnion* sp. (Rhodophyta)	*Tonicella lineata* (Mollusca/ Polyplacophora)	Barnes & Gonor (1973)

Table 1 (continued)

Factor	Species/phylum	Authors
Arrangement of macro-molecules		
Calcareous shells (1–2 days in SW)	*Protodrilus rubropharyngeus* (Archiannelida)	Jägersten (1940)
Protein network (Quinone tanned protein)	*Balanus crenatus* (Crustacea/Cirripedia)	Knight-Jones (1953)
Dense film of extracts (monomolecule)	*Balanus balanoides* (Crustacea/ Cirripedia)	Crisp & Meadows (1962)
Arthropodin bounded on surfaces	*Balanus* sp. (Crustacea/Cirripedia)	Crisp & Meadows (1963)
Mucus of host polychaete	*Proboscidactyla flavicirrata* (Cnidaria/ Hydrozoa)	Nishihira (1967)
Chemical factor		
Inorganic		
Zn	*Echinus larvae (Echinodermata)*	*Runnström & Runnström (1919)*
K-bichromate	*Botryllus schlosseri* (Tunicata)	Zinkin (1938)
Na, Cu	*Bugula neritina* (Tentaculata/Bryozoa)	Lynch (1961)
Cu, Fe, Al	*Ascidia* larvae (Tunicata)	Grave & Nicoll (1939)
Cu	*Tubularia larynx* (Cnidaria/Hydrozoa)	Prefinch & Downing (1949)
Li, Cs, Rb	*Hydractinia echinata* (Cnidaria/Hydrozoa)	Müller (1973)
K, Cs	*Bowerbankia gracilis* (Tentaculata/ Bryozoa)	Eiben (1976)
Cs, Rb	*Phoronis psammophila* (Tentaculata/ Phoronida)	Herrmann (1979)
Cs	*Psammechinus miliaris* (Echinodermata)	Herrmann (1983)
Cs	*Polygordius appendiculatus* (Annelida)	Herrmann (1986)
Cs	*Asterias rubens* (Echinodermata)	Herrmann (in prep.)
Cs	*Paracentrotus lividus* (Echinodermata)	Herrmann (in prep.)
Rb, Cs, Hg	*Phoronis mülleri* (Tentaculata/Phoronida)	Herrmann (1994, present paper)
Hyperacidity	*Phallusia, Acidella, Ciona* (Tunicata)	Berrill (1947)
Organic		
Vinegar acid, apple acid	*Teredo norvegica* (Lamellibranchiata)	Harington (1921)
Amino-acid	*Ascidia* larvae (Tunicata)	Grave & Nicoll (1939)
Strychnin	*Botryllus schlosseri* (Tunicata)	Zinkin (1938)
Quinone tanned protein	*Balanus crenatus* (Crustacea/Cirripedia)	Knight-Jones (1953)
Protein-carbohydrate compl. (ovalbumin)	*Semibalanus balanoides* (Crustacea/ Cirripedia)	Larman & Gabbott (1975)
Shellfish glycogen	*Crassostrea virginica* (Lamellibranchiata)	Keck et al. (1971)
Pankrea. caseinhydrolysate	*Cassiopea andromeda* (Cnidaria/ Syphozoa)	Hofmann & Brand (1987)
Epoxide of d-tocotrienol $C_{27}H_{40}O_3$	*Coryne urchidai* (Cnidaria/Hydrozoa)	Kato et al. (1975)
DMSO	Larvae of *Ascidia* (Tunicata)	Cloney (1978)
Pine wood	*Teredo navalis* (Lamellibranchiata)	Culliney (1975)
Wood	*Bankia goudi* (Lamellibranchiata)	Culliney (1975)
Coal from sugar	*Ophelia bicornis* (Polychaeta)	Wilson (1952, 1955)
Arthropodin	*Balanus* sp. (Crustacea/Cirripedia)	Crisp (1953)

Table 1 (continued)

Factor	Species/phylum	Authors
Organic		
Organic material of bacteria	*Lytechinus pictus* (Echinodermata)	Cameron & Hinegardner (1974)
Inner shell of chicken eggs	*Bowerbankia gracilis* (Tentaculata/Bryozoa)	Hasper (1913)
Tube cement	*Sabellaria alveolata* (Polychaeta)	Wilson (1968)
Tube cement	*Sabellaria spinulosa* (Polychaeta)	Wilson (1970)
Humic substances	*Teredo navalis* (Lamellibranchiata)	Culliney (1975)
Skeleton of gorgonians	*Conopea galatea* (Crustacea/Cirripedia)	Patton (1963, cited in Chia & Rice, 1978, p. 211)
Muscle extract of adult	*Ostrea edulis* (Lamellibranchiata)	Bayne (1969)
Extract of arthropods	*Semibalanus balanoides* (Crustacea/Cirripedia)	Crisp & Meadows (1963)
Extract of gorgonian axial skeleton	*Alcyonium* sp. (Cnidaria/Anthozoa)	Bourdillon (1954)
Sargassum extract	*Coryne urchidai* (Cnidaria/Hydrozoa)	Nishihira (1968)
Physical factor		
Charact. of surface of sandgrain	*Ophelia bicornis* (Polychaeta)	Wilson (1955)
Impulse 150 V, 1 msec, 1 sec	*Arbacia punctulata* (Echinodermata)	Cameron & Hinegardner (1974)
Electro-kinetic potential	*Phoronis mülleri* (Tentaculata/Phoronida)	Herrmann (1976)
Wetting-tension	*Bowerbankia gracilis* (Tentaculata/Bryozoa)	Eiben (1976)
Alteration of surface tension	*Phoronis mülleri* (Tentaculata/Phoronida)	Herrmann (1994, present paper)
Colour of the surface		
Colour	*Spirorbis* sp. (Polychaeta)	Neu (1933)
Green light (530–545 µm)	*Balanus improvisus* (Crustacea/Cirripedia)	Neu (1933)
Green light is avoided	*Balanus amphitrite* (Crustacea/Cirripedia)	Neu (1933)

plants together. Normally, bacteria function destructively in ecosystems. In the sea they have another purpose, viz. to lead the marine larvae to their species-specific substrate. In fact, they can be regarded as "ecological ushers".

This function can be more exactly shown for *Phoronis mülleri*, but is thoroughly applicable to other larvae that are also induced by bacteria to undergo metamorphosis.

The collapse of plankton blooms (e.g. *Noctiluca scintillans*) raises the nutritive content and the bacterial concentration, thereby causing *Phoronis* larvae to become sensitized and slightly activated. Both reactions amplify the seismic behaviour of the larvae, which during August storms sink to the sea floor where light mud with its adherent bacteria further activate the larvae until, enveloped with bacteria at the appropriate concentration and composition, they are induced to undergo metamorphosis. The metasome diverticulum evaginates irrevocably (Fig. 7b). The logarithmic growth phase of bacteria in the species-specific substrate is maintained by the flow of nutrients from the collapsing blooms of plankton.

Induction of metamorphosis by bacteria occurs in other marine larvae e.g. *Hydractinia echinata* (Müller, 1969, 1973), *Cassiopea andromeda* (Hofman & Brand, 1987; Neumann, 1979), *Phoronis psammophila* (Herrmann, 1981) and *Psammechinus miliaris* (Cameron & Hinegardner, 1974; Herrmann, 1981). The methods employed in older studies also indicate that various larvae are stimulated to undergo metamorphosis by bacteria.

For *Phoronis mülleri* and *Hydractinia echinata* it has been shown that the bacterial inducers are not identical; attempts to induce metamorphosis by exchanging the inducer bacteria failed. Bacteria that induced metamorphosis in *Ph. mülleri* were also effective in eliciting metamorphosis in *Ph. psammophila*. A tenfold higher concentration of bacteria was necessary, however, even though induction with the same concentration of anorganic compounds was effective for both species (Herrmann, 1979).

The ion concentrations used in the experiments did not in any way represent the concentrations found in natural seawater. Rubidium occurs in the highest concentration (0.17 mg $\times$ l^{-1}), followed by cesium (0.0005 mg $\times$ l^{-1}) and mercury (0.00003 mg $\times$ ml^{-1}). These ions represent minor trace elements in seawater (Goldberg, 1965). The concentration used in the experiments exceeded the natural quantity by 3500-fold (RbCl) to a millionfold. Thus, induction of metamorphosis using inorganic compounds is an artificial induction. These cations can be compared with a spare key, in a lock-and-key system. The lengthier time taken for induction to occur and the slower process of the ensuing activities than those observed when induction is induced by bacteria shows that this key does not quite fit.

Metamorphosis could be triggered with CsCl in other larvae besides *Phoronis mülleri*, e.g. in *Hydractinia echinata* (Spindler & Müller, 1972), *Phoronis psammophila* (Herrmann, 1979), *Psammechinus miliaris* (Herrmann, 1983), *Polygordius appendiculatus* (Herrmann, 1986) and *Laeospira (Spirorbis) borealis* (Herrmann, in prep.).

Differences between *Hydractinia echinata* and *Phoronis mülleri* in the effective concentrations of cations are shown here because many studies have been carried out (Müller & Buchal, 1973; Schwoerer-Böhning et al., 1990).

Induction in *Phoronis* with CsCl shows a larger effective range than with RbCl. Compared to *Hydractinia*, however, the range is quite narrow. The concentration needed to trigger metamorphosis with RbCl is exclusive for both species, whereas for CsCl it

Table 2. Comparison of the induction of metamorphosis in *Phoronis mülleri* and *Hydractinia echinata* using cations (concentrations are given as final concentrations in seawater)

Compound parameter	*Phoronis mülleri*	*Hydractinia echinata*
CsCl/SW (mol $\times$ l^{-1})		
Effective range	0.015–0.075	0.007–0.4
Optimal concentration	0.05 –0.07	0.06 –0.3
RbCl/SW (mol $\times$ l^{-1})		
Effective range	0.01 –0.028	0.03 –0.2
Optimal concentration	0.012–0.023	0.08
Induction time	minimum: 9 min (stored in the solution!)	120–180 min and then replacement in SW

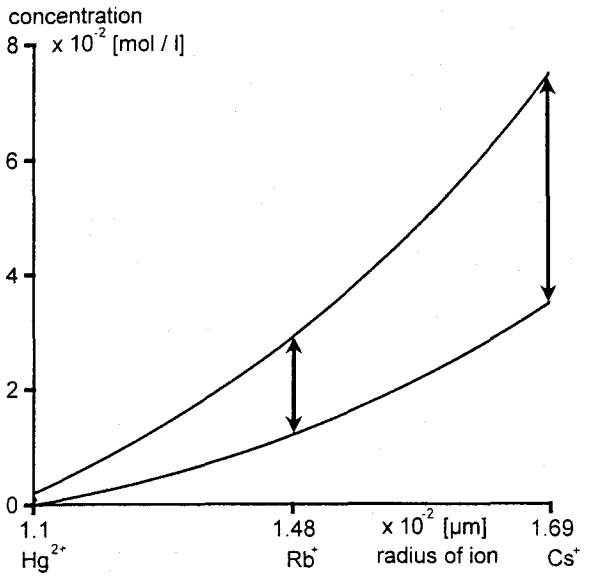

Fig. 9. Comparison of the ranges of concentrations for inducing metamorphosis in *Phoronis mülleri* and their relationship to the ion radiuses of $HgCl_2$, RbCl and CsCl

overlaps. The concentration range for induction in *Hydractinia* with CsCl includes that for RbCl. In *Phoronis*, the optimal concentration ranges are separate.

The variation in the optimal concentration ranges for Rb, Cs and Hg are probably attributable to the ion radiuses of these cations (Fig. 9). Cs^+ has an ion radius of 1.69×10^{-3} µm, that of Rb^+ is 1.48×10^{-3} µm, and thus is significantly smaller. A mixture of both ions can take on a higher configuration than either alone. It is possible that the much-reduced triggering concentration when both are applied together, is related to this fact (Herrmann, in prep.). This is an indication that the configuration of the cations in the immediate vicinity of the larval epithelium plays an important role in inducing metamorphosis.

The motility of bacteria used as inducers of metamorphosis is an essential factor, an indication that intimate contact with the larval epithelium is essential. It may be postulated that there, a receptor system exists that is common to all the larvae mentioned above. The epidermis may be implicated here, for all planktonic larvae live under essentially similar physiological conditions of the external medium. This speculation agrees with the opinion of Müller & Buchal (1973), who maintained that the receptive system is localized in the cell membrane and possesses binding sites for cations.

Contact of the larva with the real solid surface can trigger nervous stimuli which act synergistically with bacterial induction, thus lowering the strength of the inductive stimulus needed. This can be seen in *Phoronis mülleri* in cases where contact of the secondary nervous system with a sand grain or a piece of lint makes induction effective immediately. For certain mollusc larvae, Hadfield (1978) postulated that the stimulus must be perceived strictly through surface mechanoreceptors.

The results using bacteria, cations and tensides as inducers point to changes in the

larval epithelium which lead to increased pressure in the coelomic spaces due to active processes in the cell membrane. Müller & Buchal (1973) implicated Na-K-ATPase that plays a role in the transport of monovalent cations in the cell membrane, a process that can be blocked by ouabain.

The same induction time (9 min) for all cations in *Phoronis* suggests that the mechanism of action of the cations is via the epithelium. The brief induction time using bacteria as inducers excludes structural changes in the larva and, for the same reason, no hormonal processes (neurosecretion) can take place. The secondary nerve complex gives the starting signal for muscle contraction in the whole body. Muscle contraction and the amply filled coelomic spaces in the *Phoronis* larva press the metasome diverticulum, the muscle sheath of *Phoronis*, outward. In the majority of the aberrant metamorphoses, the induction of metamorphosis was incomplete or the inducer was present in less than threshold amounts.

The natural surface tension of seawater is lowest in the winter months (56 dyne $\times$ cm^{-1}), increases slightly during the spring and rises rapidly between the end of July and the beginning of August by 10 dynes $\times$ cm^{-1} to reach 67 dynes $\times$ cm^{-1} (Gunkel, 1968). This is exactly the order of magnitude necessary to trigger metamorphosis in *Phoronis mülleri* experimentally. It can be assumed that induction in the natural substrate is caused by a double sensitization due to bacteria and to an increase in surface tension.

The change in surface tension as the ultimate mechanism of induction in early experiments cannot be excluded. The factors reported by earlier authors, such as substrate grain size (Wilson, 1932, 1937), various concentrations of organic compounds (Day & Wilson, 1934), surfaces colonized by microorganisms (Knight-Jones, 1951, 1953; Crisp & Meadows, 1963; Gray, 1966) and inorganic compounds (Grave & Nicoll, 1939; Lynch, 1961; Crisp, 1956, 1974), indicate that a change in surface tension may well have played a role as the inducer (Fig. 8).

The wetting action of *Bowerbankia gracilis* (Eiben, 1976) and changes in electrokinetic potential (Herrmann, 1976) have been given as general physical causes for the induction of metamorphosis.

It is assumed that tensides affect the membrane, causing a redisposition of membrane lipids as has been postulated by Rockstroh (1967) for ciliates. The induction of metamorphosis by bacteria and anorganic compounds may also take place this way. It is apparent that all inducers of metamorphosis in *Phoronis mülleri* that have been tested up to now (decomposing planktonic organisms, bacteria and anorganic compounds) attain a similar value in surface tension (63-64 dynes $\times$ cm^{-1}) as for induction by tensides. This may mean that change in surface tension is an essential characteristic of the induction. Tensides, like those used in the experiments, are components of soaps that reach rivers (concentration in surface water 0.01 mg $\times$ l^{-1}) and river mouths (0.3 mg $\times$ l^{-1}) (Bock & Mann, 1971). In seawater the straight chained molecules like Marlon^R A (10 mg $\times$ l^{-1}) decompose within 14 days. Other tensides take about 20 days (Bock & Schöberl, 1977), so that the tenside content of the seawater around Helgoland is much less than the concentration used in the experiments.

In experiments, anionic tensides are toxic for fish and decapods, whereas nonionic tensions are more poisonous for mussels (Swedmark et al., 1971). The survival rate of sea animals varies; for example, balanids can live longer than 14 days in 10 ppm (Bock & Mann, 1971), whereas *Ph. mülleri* larvae survive 2 h, at the most, at 1 ppm. The induction

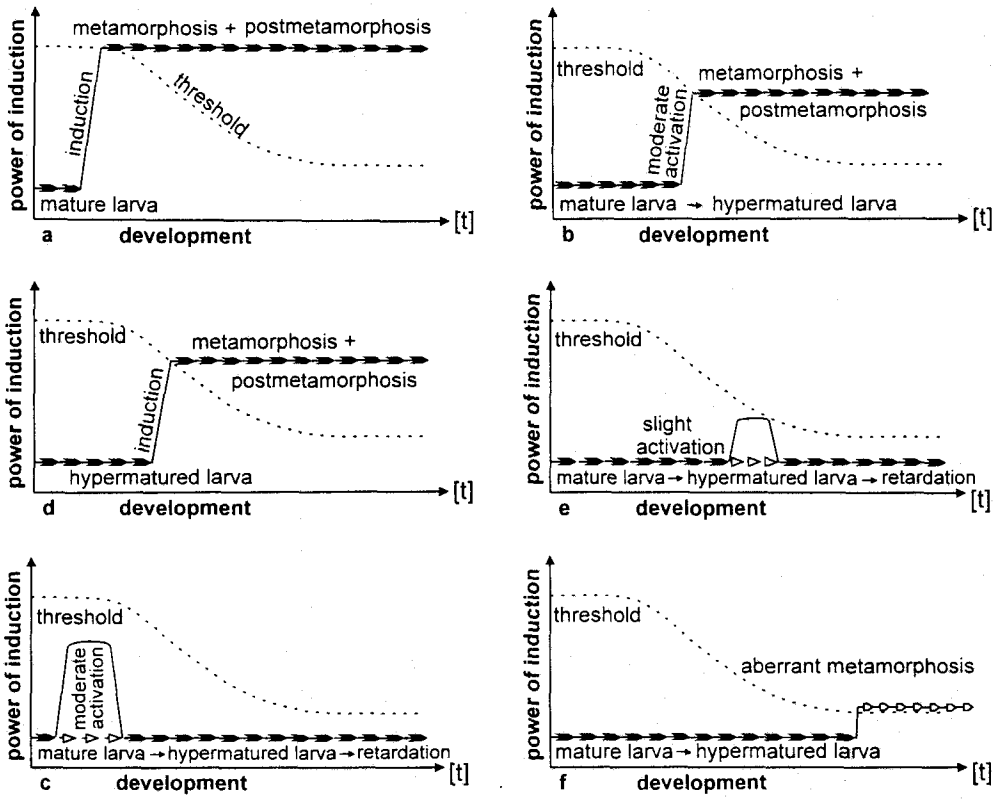

Fig. 10. Model concept of the induction of metamorphosis in response to bacteria or cations as determined for *Phoronis mülleri*. 10a: The threshold value sinks with increasing maturity of the larva. 10b: Minimal stimulation suffices to induce an overly mature larva to undergo metamorphosis. Larvae that have been maintained for prolonged periods of time in the laboratory and are thus retarded. They respond to a minimal stimulus, but aberrant forms usually arise (10d). Subthreshold stimulation cannot induce metamorphosis in a larva mature for metamorphosis (10c, e), but can initiate metamorphosis in overly mature larvae (10f). Weak induction suffices in retarded larvae

of metamorphosis in *Ph. mülleri* by tensides took place at concentrations at which histolysis of the larva or of its individual parts was imminent. It is likely that tensides do not play an essential role in the triggering of metamorphosis in the sea, even though mud adsorbs surface-active substances strongly.

The interaction between the induction of metamorphosis and the maturity of the larva can be shown schematically (Fig. 10). Metamorphosis denotes the transformation into another life form as initiated by activation (induction). The strength of induction necessary depends upon the threshold value which varies with the age of the animal. A larva just ready for metamorphosis requires a stronger stimulus (Fig. 10a) than an overly mature larva (Fig. 10b). A moderate activation is not enough to induce metamorphosis in a ripe larva (Fig. 10c), but is sufficient to activate an overly mature one (Fig. 10d). Only a slight induction suffices to start the process in an overly mature larva (Fig. 10e) that is in retardation, but in this case the metamorphosis is generally aberrant (Fig. 10f).

The hypothetical schemes of the induction of metamorphosis apply not only to *Phoronis mülleri* and *Phoronis psammophila*, but are also valid for other marine larvae. Thus, Echinoplutei of *Psammechinus miliaris* kept at 12 °C until they were overly mature, could be activated by raising the temperature a few degrees, or induction occurred due to the slight coating on the culture vessel.

Metamorphosis in marine animals also means the transition to another habitat. This process has a lot in common with the change of host in parasites, e.g. in cercaria of *Opisthorchis viverrini* (Haas et al., 1990). Both larvae have to be guided by external influences to recognize the substrate appropriate for their survival and to perform the transition by bodily transformation. In parasites it is the macromolecules of the skin, in ocean larvae it is bacteria that show the way and function as "ecological ushers"!

An exact sequence of different ethnological and ecological parameters, functioning independently of one another according to the lock-and-key principle, is required so that the transition from the planktonic to the benthic phase can proceed in a regular fashion.

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