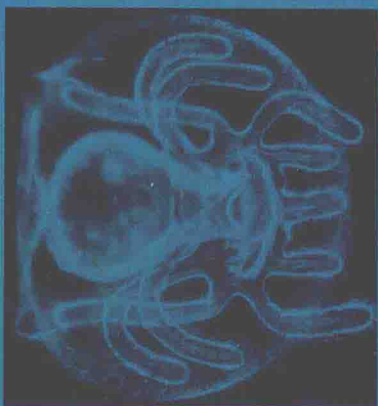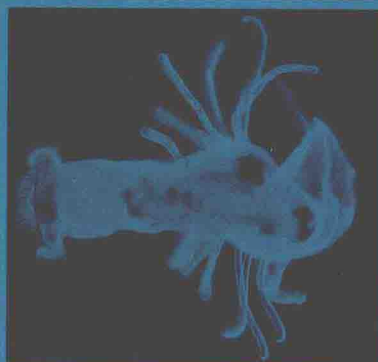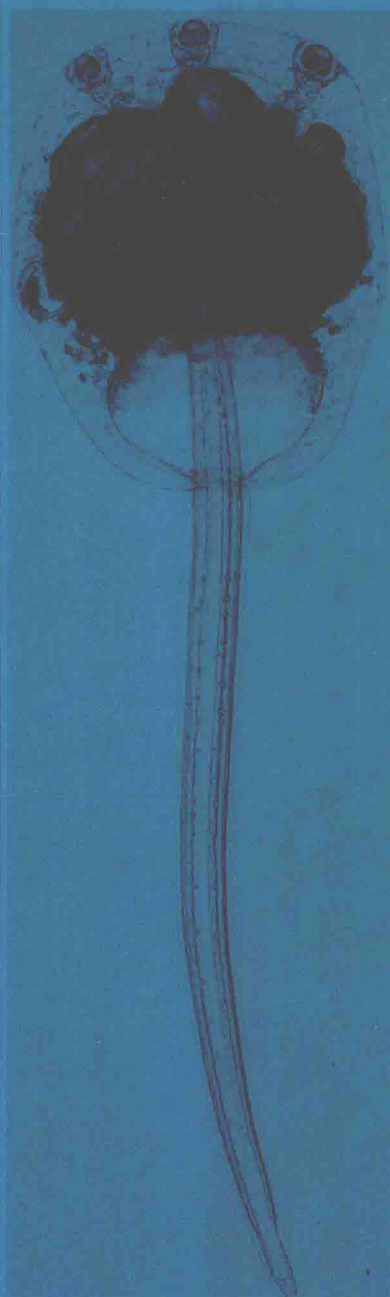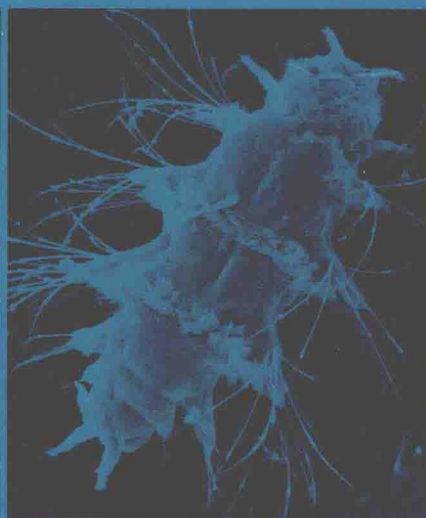# SETTLEMENT AND METAMORPHOSIS OF MARINE INVERTEBRATE LARVAE

Editors: **Fu-Shiang Chia** / **Mary E. Rice**

SETTLEMENT AND METAMORPHOSIS OF MARINE INVERTEBRATE LARVAE

Proceedings of the Symposium on Settlement and Metamorphosis of Marine Invertebrate Larvae, American Zoological Society Meeting, Toronto, Ontario, Canada, December 27-28, 1977

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Professor Robert L. Fernald, to whom this Symposium is dedicated.
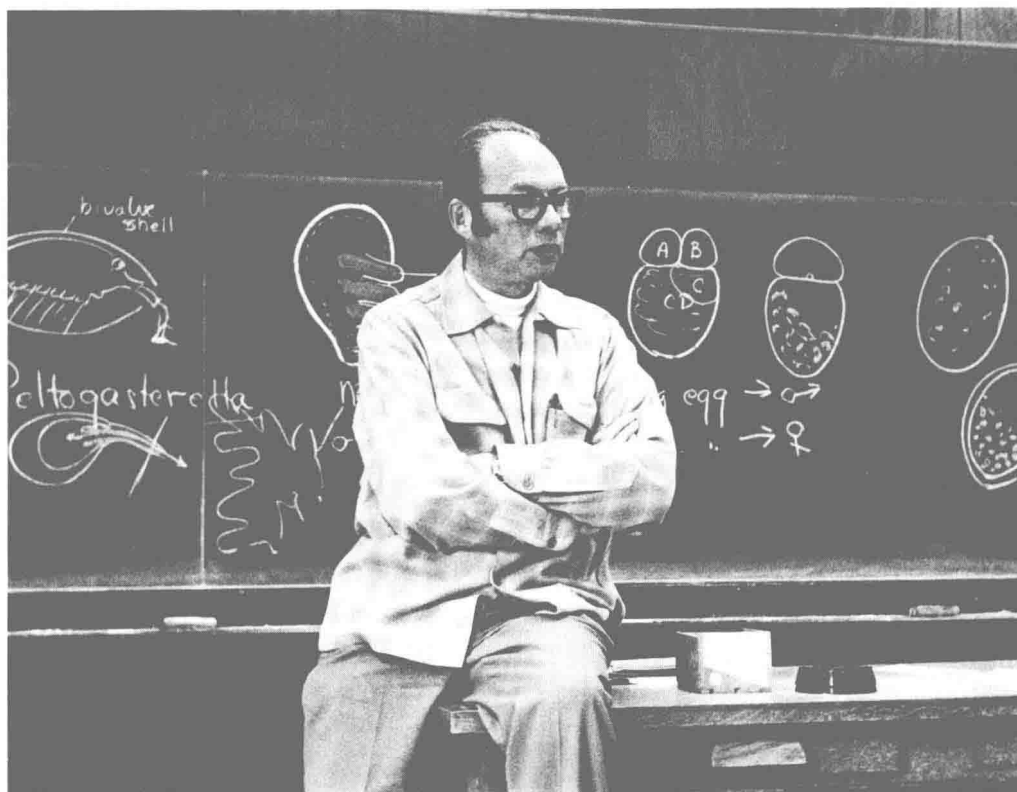
Robert L. Fernald received his Ph.D. in 1941 from the University of California, Berkeley. He taught in the Department of Zoology, University of Washington, Seattle, from 1946 to 1975 and was the Director of the Friday Harbor Laboratories for 14 years (1958–1972). He retired from the Zoology Department in 1975 and is now living in his house on Griffin Bay, San Juan Island. He continues to teach the course in Comparative Invertebrate Embryology at the Friday Harbor Laboratories.

PREFACE

During recent years considerable attention has been directed to studies of reproduction and development of marine invertebrates. This interest has been evidenced by the publication of numerous research papers, review articles (*e.g.*, Chia,[1] Mileikovsky,[2,3] Scheltema,[4] Stearns[5]), textbooks (Kume and Dan,[6] Reverberi[7]), reference books (Anderson,[8] Jägersten[9]), a multi-volume treatise (Giese and Pearse[10,11,12,13]), and various symposia. The American Society of Zoologists has sponsored the following series of symposia dealing with invertebrate development: Developmental Biology of Cnidaria in 1972,[14] Developmental Biology of Echinoderms in 1973,[15] Developmental Biology of Spiralia in 1974,[16] and Effects of Environmental Pollutants on Developing Invertebrates in 1976. Other recent sysmposia which have been devoted entirely or in part to invertebrate larvae are the Fourth European Marine Biology Symposium,[17] the Conference on Marine Invertebrate Larvae,[18] held in Rovinj, Yugoslavia, and the Symposium on Reproductive Ecology of Marine Invertebrates,[19] sponsored by the Belle W. Baruch Institute for Marine Biology and Coastal Research, University of South Carolina. None of these symposia has emphasized settlement and metamorphosis of marine larvae.

In view of the fact that there are at least 100,000 species of marine benthic invertebrates which have pelagic larval stages of two to several weeks'duration,[20] it is not surprising that problems of larval settlement and metamorphosis are diverse and complicated. In terms of larval settlement some very exciting discoveries, especially in relation to induction of settlement by specific substrata, have been made in recent years. This literature has been reviewed by Crisp[21,22] and Scheltema.[4] At present the literature on metamorphosis of marine larvae is rather scattered and remains largely at the level of descriptions of morphological changes. For most marine organisms our information can hardly compare with what is known about metamorphosis of insects and amphibians.[23]

It is our judgment that many more biologists in the near future will turn their attention to the mechanisms of induction of both settlement and metamorphosis, and it is expected that results of such investigations will contribute significantly to the general fields of developmental biology and marine ecology. It seemed, therefore, both timely and important to bring together persons working in the field of invertebrate development for an exchange of ideas and synthesis of existing information. In view of these considerations, the Symposium on Settlement and Metamorphosis of Marine Invertebrate Larvae, the proceedings of which are reported in this volume, was conceived and organized.

The Symposium was sponsored by the American Society of Zoologists at its annual meeting in Toronto in December, 1977. Divided into three sessions, it extended over a period of one and one-half days (December 27, 28) and included 19 papers, consisting of reviews as well as reports of original research on settlement and metamorphosis in a total of 11 phyla. This publication of the proceedings of the Symposium, with reviews of the literature on settlement and metamorphosis, is intended to serve as a general reference for biologists and also as a reference for graduate students and investigators who are working on problems of invertebrate development. It is our hope that the contents of this volume will stimulate further research on these subjects and will point out new directions for future studies.

provided assistance from its Symposium Fund as well as through the Division of Invertebrate Zoology. Preparation of the publication was funded, in part, by the Fort Pierce Bureau of the Smithsonian Institution. Numerous individuals contributed their time, effort, and devotion to make the Symposium a success and this publication a reality. We wish to thank those persons who acted as chairmen of the three sessions of the Symposium: Richard M. Eakin, Eugene N. Kozloff and Arthur H. Whiteley. We express our gratitude to Mary A. Wiley, Business Manager of the American Society of Zoologists, for her cheerful efficiency and able assistance in every phase of our endeavors. We are grateful to the editors of Elsevier North Holland, J. G. Hillier and Margaret Quinlin, for their facilitation of our publication efforts. Special thanks go to Margaret Quinlin for her many helpful suggestions during the preparation of this volume and for her warm personal concern for our publication. And we are indeed indebted to Venka V. Macintyre who, with unusual dedication, diligence and expertise, provided invaluable editorial assistance and typed the entire volume for camera-ready copy.

This volume is dedicated to Robert L. Fernald, Professor of Zoology at the University of Washington, Seattle on the occasion of his retirement from the Department of Zoology and in recognition of his innumerable contributions over the past three decades to the field of comparative invertebrate embryology. Both as Director of the Friday Harbor Laboratories and as a teacher of embryology, Dr. Fernald has guided and inspired many students toward professions in teaching and research in invertebrate embryology and development. The fact that more invertebrate embryology classes are being taught today than ever before in so many places by his students and students' students is indeed a tribute to his achievements as an educator and a scholar. Each of the contributors to this Symposium has been influenced either directly or indirectly by his scholarship. We dedicate this book to him with appreciation and fond affection.

Fu-Shiang Chia
Mary E. Rice
May 1, 1978

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CONTENTS

MECHANISMS OF LARVAL ATTACHMENT AND THE INDUCTION
OF SETTLEMENT AND METAMORPHOSIS IN COELENTERATES: A REVIEW

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Literature is reviewed concerning behavior, mechanisms of attachment, induction of settlement and metamorphosis, and intercellular communication and sensory receptors in planulae. Behavioral responses of planulae, either taxic or kinesic, are shown to increase the opportunity for settlement on favorable substrates. Evidence is reviewed demonstrating that larval attachment is made either by glandular secretion or by firing of cnidocytes. In some species, organic surface films have been implicated as metamorphic triggers, either by providing an excitatory chemical cue, or by providing an attractive surface texture. In some symbiotic hydroids, components of the host species induce or enhance settlement and metamorphosis. Nervous and neuroid conduction, and diffusion of hormone-like chemicals are discussed as possible mechanisms for intercellular coordination during settlement and metamorphosis.

INTRODUCTION

The life cycle of most coelenterates includes a planula larval stage. Planulae are usually elliptical and are constructed of two body layers, ectoderm and endoderm, separated by a thin mesoglea. The endoderm of most planulae forms a solid core; only in a few species of actinarian planulae does the endoderm form a functional gastrovascular cavity. Relative to endoderm, the ectoderm displays a considerable degree of cellular differentiation. Characteristic ectodermal cell types include the sustentacular cells (usually ciliated), various gland cells, cnidocytes, and putative sensory cells.[1,2,3,4] In some actinarian planulae, ectodermal cells at the aboral end form a specialized structure known as the apical organ. This structure is suspected to perform a sensory role, either during feeding,[1,5] or during substratum selection.[6,7]

Planula motility, either swimming or crawling, is usually mediated by the activity of cilia. These organelles are characteristically distributed over the entire surface of the larva. In most coelenterates, metamorphosis transforms the motile planula into a sessile polyp. As a result, the planulae of many species, especially those which are symbiotic, possess the ability to select settlement substrates which enhance post-metamorphic survival.

In this review, we shall attempt to examine the literature concerning settlement and metamorphosis of planula larvae. By doing so, we hope to reveal those areas where information is inadequate or lacking, and where future research might be most beneficial.

PLANULA BEHAVIOR AND SITE SELECTION

Many species of planula possess a limited repertoire of behaviors which are directed by various types of environmental cues. These behaviors, defined as taxic or kinesic responses, influence the eventual site of settlement.

In the laboratory, free-swimming planulae often exhibit two major behavioral phases during larval life. The initial period is characterized by active swimming near the water surface. This is followed by a period in which the planulae descend to the bottom of the culture bowl where swimming becomes sluggish or is halted altogether. This behavior has been reported in the planulae of *Stomphia didemon*,[8] *Siderastrea radians*[9] and *Pocillopora damicornis*.[10] Some species such as *Coryne uchidai*[11] and *Favia fragum*,[12] commence crawling during the latter phase.

Harrigan[10] and Lewis[12] have shown that this behavioral transition in both *Pocillopora damicornis* and *Favia fragum*, results from a gradual reversal of the positive phototaxis exhibited by young planulae. In these cases the reversal is apparently dictated by endogenous programming. Whatever the causative mechanism of this behavior pattern, it would seem to facilitate an initial period of dispersal (none of the species mentioned possess a medusoid morph in the life cycle), followed by a period of contact with a settling substrate.

Some planulae change their reaction to light only after reception of a specific chemical cue. Williams[13] documented this phenomenon in the crawling planula of *Clava squamata*, a hydroid epiphytic on fronds of the algae, *Ascophyllum nodosum*. Planulae of this species are normally positively phototactic. However, if older planulae are exposed to directional light in the presence of *Ascophyllum*, they become negatively phototactic. The effect of this algal-induced photonegativity, and the tendency of the planulae to settle in pits and grooves on the surface of the plant thallus, promote settlement in sites protected from desiccation and extreme water currents, respectively.[13]

A second example of this type of behavior is exhibited by the planulae of *Hydractinia echinata*, a colonial hydroid characteristically found on the shells of hermit crabs. Schijfsma[14] observed both positive phototaxis and photokinetic responses by the crawling planulae of this hydroid. However, the planulae became indifferent to light when a hermit crab was present in the bowl. *Hydractinia* planulae are not directly attracted to hermit crabs;[14] their eventual transfer to the shell of the crab depends upon the chance of the crab dragging its shell past the planulae.[15,16] This evidence suggests that the light reactions of *Hydractinia* planulae facilitate dispersal, but a mechanism exists to inhibit these dispersive activities in the proximity of a hermit crab.

In its natural habitat, the scyphistoma of *Cyanea capillata* is typically found hanging upside down from the lower face of overhanging objects. During an investigation of this scyphozoan, Brewer[17] noticed that the planulae increase their activity and swim upward in sea water with a high concentration of dissolved carbon dioxide. Such conditions may develop in calm water at the surface of sediments. Brewer[17,18] has suggested that upward swimming by planulae which encounter such conditions will enhance the possibility of contact with the underside of objects.

Prior to settlement, some species of planulae are capable of responding to the texture and contour of the substrate. This is particularly true of crawling planulae. Crawling rate by planulae of *Clava squamata* increases on glossy surfaces and decreases on rough surfaces.[13] In addition, the planulae of this species, as well as the crawling planulae of *Sertularella muirensis* and *Favia fragum*, will settle preferentially in pits and grooves on the surface of the substratum.[13,19,12]

Williams[20] has described congregating behavior by the crawling planulae of the hydroids, *Nemertesia antenina* and *N. ramosa*. He attributed the apparent gregariousness of these species to positive thigmotaxis.

MECHANISMS OF PLANULA ATTACHMENT

Two types of substrate attachments by planulae are recognized. The first, which is weak and transient, may be called temporary attachment. For example, the swimming planulae of both *Favia fragum* and *Tealia crassicornis* occasionally attach to objects but subsequently resume swimming.[12,6] The second type, which is much stronger and sometimes permanent, is the settlement attachment. Settlement attachments are always accompanied by metamorphosis.

Two types of ectoderm cells, gland cells and cnidocytes, may potentially establish both temporary and settlement attachments.

Gland Cells

When the planulae of the sea pen, *Ptilosarcus gurneyi*, are offered suitable substrate, they become covered with a mucous secretion.[21] In a comparative fine structural study of the pre- and post-metamorphic stages of *P. gurneyi*, Chia and Crawford[4] identified two types of gland cells in the planula which were not present in the polyp. Assuming these are not merely developing stages of polyp secretory cells, their products must be functional only during larval life—presumably to facilitate settlement attachment.

Vandermeulen[3,22] compared the epidermal ultrastructure of the planula and polyp of the coral *Pocillopora damicornis*. The aboral epidermis of the planula contained four morphologically distinct secretory cells. However, in the six-hour settled polyp, only one secretory cell, the calicoblast cell, was found in the aboral epidermis. Vandermeulen[3] has suggested that the secretions of at least some of the planula gland cells facilitate settlement attachment.

The planulae of both *Phialidium gregarium* and *Hydractinia echinata* secrete a mass of viscous, mucoid material at the onset of metamorphosis.[23,24] Bonner[23] demonstrated that the settlement process in the planula of *Phialidium gregarium* is coincident with the disappearance of PAS-positive gland cells from the ectoderm. Prior to settlement, these intensely staining cells are more abundant at the aboral end of the planula, the end which fixes to the substrate. Similarly, Nyholm[25] found mucous cells at the aboral end of the planula of *Protanthea simplex*. On this evidence, he also suggested that mucus was responsible for substrate attachment during the settlement process.

Finally, it is known that crawling planulae employ mucus for adherence to the surfaces over which they creep.[13]

Cnidocytes

The temporary attachments exhibited by some planulae result from the discharge of mechanosensitive nematocysts. If an object, such as a coverslip or mollusc shell, is dragged past the planula of *Hydractinia echinata,* the planula will attach and transfer to this object by discharging atrichous isorhiza nematocysts.[15,16] Müller *et al.*[16] have stated that "zeta potentials produced by the movement of the object, in addition to the impulse of collision, constitutes the condition that stimulates the larva to fire its nematocysts. . . ." However, this nematocyst-mediated attachment is not followed by metamorphosis unless further, specific triggers are provided (see section on induction of settlement). It is interesting to recall that tentacle atrichous isorhizas are used by hydra for adherence to the substrate during somersaulting locomotion.[26]

A second example is provided by the actinula larva of *Tubularia larynx* (the planula stage of this hydrozoan is passed within the parent body). This larva possesses a number of stiff tentacles which radiate outward from the body proper. The tentacle tips, which are swollen with large numbers of cnidocytes, temporarily attach to surfaces which they contact.[27] Attachments were observed even on clean glass surfaces. Haws[28] has shown that discharge of tentacle nematocysts is responsible for this adhesion, and the readiness of the nematocysts to respond to mechanical contact increases with the age of the actinula.

Cnidocytes which are sensitive to specific chemical or contact-chemical stimuli may also facilitate attachments between the planula and a substrate. Donaldson[29] has made a detailed study of such a phenomenon in the planula of a symbiotic hydroid, *Proboscidactyla flavicirrata*. Members of this genus live only on the tube rims of certain sabellid polychaetes.[30] Campbell[31] showed that the free-swimming planula of *Proboscidactyla flavicirrata* readily attaches to the tentacular pinnules of the host sabellid worm by discharging nematocysts. The

experiments of Donaldson[29] indicate that contact-chemoreception of some factor from sabellid body surfaces provides the exclusive stimulus for the discharge of microbasic eurytele nematocysts. The nematocysts do not respond to tissues from other organisms or to simple mechanical stimulation applied in the presence of host mucus or tissue extracts. Following temporary attachment to the pinnules, the planulae soon elongate and the cnidocytes lose their sensitivity to sabellid tentacles. Instead, the effective stimulus for the cnidocytes becomes the tube of the worm. If the planulae contact the worm tube, nematocysts will discharge and the planulae will transfer to the tube and metamorphose. Because the orientation of the planulae is unimportant for attachment to the tentacles and the tube, Donaldson[29] has suggested that either two functional populations of cnidocytes are intermingled or only one type exists whose specificity can be altered.

Microbasic euryteles are often found in other hydrozoan, as well as scyphozoan planulae.[1] In addition, all anthozoan planulae are provided with spirocysts, whose ultrastructure strongly suggests an adhesive function.[32] In an examination of the planulae of *Anthopleura elegantissima*, we have found that the aboral half of the planula contains five times more spirocysts than the oral half.

INDUCTION OF LARVAL SETTLEMENT AND METAMORPHOSIS

Species of coelenterate planulae vary in their degree of dependence on environmental factors for the induction of settlement and metamorphosis. As in many other groups of invertebrates, the planulae of generalist species exhibit little or no dependence on external inducing factors, while specialist species (in habitat or diet) are likely to be obligatory to environmental induction.

The planulae of *Phialidium gregarium* will settle on a variety of substrates, including glass, wood, plastic and algae.[33] Similarly, many other hydrozoan planulae, including *Aequorea aequorea*, and *Sarsia tubulosa* will settle in glass bowls without requiring apparent physical or chemical inducing factors (personal observations). However, these observations should not be considered conclusive proof against the existence of subtle discriminatory abilities by the planula. An instructive example is provided by *Hydractinia echinata*. When Schijfsma[14] cultured the crawling planula of this hydroid in bowls containing sand and provided it with continuous circulation of fresh sea water, the planulae began to settle and metamorphose two days after fertilization. He concluded that these planulae metamorphose according to an endogenous time table. However, Müller and his associates[34,35,24,36,37,38] reinvestigated settlement by *H. echinata* planulae and found that if planulae were cultured under sterile conditions, they would not metamorphose. These investigators found that certain marine, gram-negative bacteria emit a product at the end of their exponential growth phase which induces metamorphosis in planulae of *H. echinata*. The effective bacteria are regularly found in natural sea water, on marine substrates, and on hermit crab shells, and they can be cultured under aerobic conditions in solutions of meat extract. Two observations indicate that the effective bacteria do not exert their inductive capacity by conferring an attractive surface texture on substrates: (a) if the bacterial cells are removed by filtration, the supernatant will effectively induce metamorphosis; (b) when planulae are suspended in solutions of the bacterial inducer, they may metamorphose without attachment to a substrate.

If the bacterial culture is subjected to osmotic shock prior to filtration (a procedure which is known to enhance release of surface-bound enzymes and secretion of leakage products[24]), the supernatant, but not the bacterial cells, has inductive capacity.

Müller and associates have also attempted to determine the physiological mechanism by which the stimulus activates metamorphosis. If various concentrations of the inductive bacteria, or the isolated product, were plotted against the percent metamorphosis effected by each dose, the resultant dosage-response curve exhibits Michaelis-Menten saturation kinetics[38,35,24] (Fig. 1).
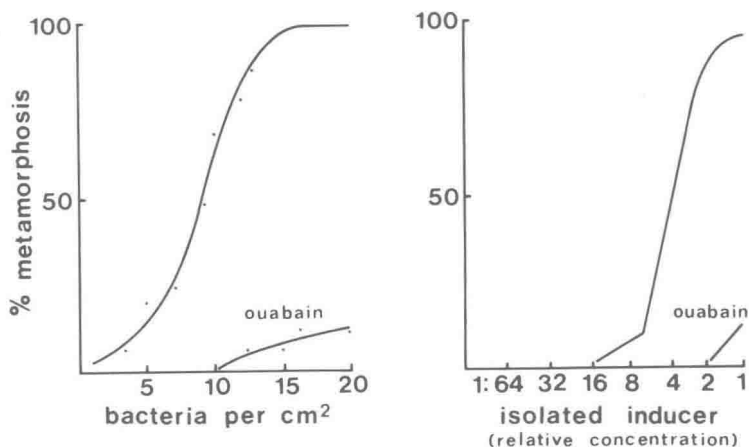
Fig. 1. Induction of metamorphosis in *Hydractinia echinata* by bacteria and the isolated inducer. The effect of ouabain is shown in each case (from Müller, 1973).

This evidence suggested that an enzyme or carrier system was involved in the larval response.

It was further discovered that bacterial induced metamorphosis was inhibited by ouabain, but that ouabain inhibition could be abolished at high concentrations of the isolated bacterial product. Therefore, Müller[35,24] suggested that the inducer may operate by stimulating the Na^+/K-ATPase of larval cell membranes.

Supporting evidence for this hypothesis was subsequently derived from experiments using various monovalent cations. It was found that the effect of the bacterial inductor could be imitated by Cs^+, Li^+, Rb^+, and K^+.[38,36,24,37] These experiments indicate that the planula receptor which receives the inductive stimulus possesses membrane-bound binding sites for cations. Experiments using various combined concentrations of K^+ and Ca^{++} revealed that the percent metamorphosis was optimal when the relative ratio of these ions was K^+/Ca^{++}=40. This is the Gibbs-Donnan ratio. Ouabain was found to antagonize the inductive capacity of all the monovalent cations except potassium (Fig. 2). From this and other results, it was concluded that potassium induction was based on a passive event controlled by the Gibbs-Donan principle, but Cs^+ induction and induction by the bacterial product was an active event—apparently stimulating the activity of the carrier system. These observations are similar to experimental results obtained with the Na^+/K^+-ATPase of other systems.[24,36]

Müller et al.[16] have recently shown that the distinctive shell vibrations produced by a hermit crab provide a second type of stimulus for metamorphosis in *H. echinata* planulae. Metamorphosis takes place on inhabited shells even when these have been previously sterilized. The mechanism of this induction has not been determined.

The planula of the athecate hydroid, *Coryne uchidai*, provides another example of a settlement response elicited by a chemical stimulus. *Coryne uchidai* is an epiphytic hydroid associated with certain Sargasso-algae.[39] In laboratory experiments, planulae which were offered a choice of a number of algal species displayed greater settlement on Sargasso-algae.[40] In a subsequent experiment, the behavior of planulae was compared both in the presence and in the absence of solutions of *Sargassum* extract.[11] Planulae in the control group initially displayed swimming activity, but gradually they began crawling on the bottom and side of the culture bowls. None of the planulae in this group metamorphosed before three days after their release. However, in the presence of *Sargassum* extract, the swimming and crawling activity of planulae was markedly attenuated. In this group, metamorphosed polyps began to appear within one day
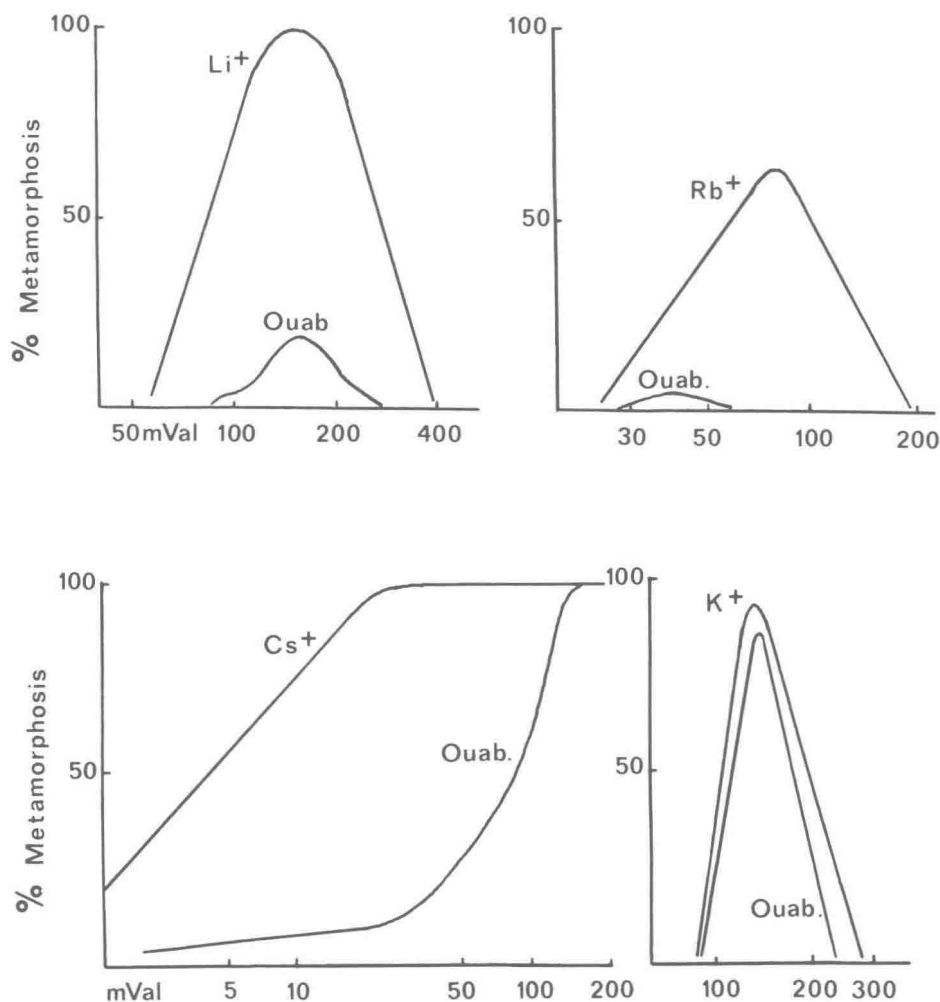
Fig. 2. Induction of metamorphosis in *Hydractinia echinata* by monovalent cations. The effect of ouabain is shown in each case (from Müller, 1973).

after release of the planulae. Nishihira[11] has therefore suggested that some chemical factor from Sargasso-algae enhances settlement and metamorphosis by causing ciliary arrest in the planulae.

Kato *et al.*[41] have succeeded in extracting and purifying the chemical component of *Sargassum tortile* which stimulates settlement and metamorphosis of *Coryne uchidai*. The most potent molecule was found to be the epoxide of δ-tocotrienol ($C_{27}H_{40}O_3$).

Nishihira[19,42] also examined substrate selection by a second epiphytic hydroid of Sargasso-algae, *Sertularella muirensis*. Field experiments showed that these planulae preferentially settle on certain species of *Sargassum*, even when ecological factors other than the algae were controlled. However, the mechanism of selection was not investigated.

The chemical induction of settlement and metamorphosis of *Proboscidactyla flavicirrata* by the mucus of the host polychaete has already been reviewed in the previous section on planula attachment.

Settlement and metamorhposis of some species of planula are enhanced by the presence of a thin organic surface film. Such films may provide an excitatory chemical cue, as in *H. echinata*,

or they may influence settlement by conferring an attractive surface texture on the substrate. A possible example of the latter is found in the planula *Pocillopora damicornis*. The optimal settlement substrate for this species is a thin organic film comprised of green filamentous algae, diatoms, and bacteria.[10] However, they will also settle favorably on such chemically diverse substrates as coralline algae, brown algae, and films of bacteria and diatoms alone. Substrates avoided by the planulae include clean sand, clean inorganic surfaces, red algae, and encrusting animals such as ascidians.

Nishihira[43] examined the distribution of newly settled polyps of several epiphytic hydroids of marine grasses. *Clytia edwardsia* and *Tubularia radiata* are characteristically found on eel grass (*Zostera marina*) while *Plumularia undulata* grows on surf grass (*Phyllospadix iwatensis*). In every case, settlement densities were a function of the age of the blade, the older portions of the plant receiving the heaviest settlement. Nishihira[43] suggested that this phenomenon is related to the diatom and microbial film present on older parts of the host plants. If so, the attractiveness of the film may be either its chemical nature or its surface texture.

The planula of *Ptilosarcus gurneyi* is competent to metamorphose at six days post-fertilization. However, metamorphosis will be delayed considerably unless specific substrate qualifications are fulfilled.[21] It was found that planulae preferentially metamorphose on sand samples from sea pen beds and further experimentation proved that the organic component of the sand actively induced settlement and metamorphosis.

In experiments performed by Williams,[20] crawling planulae of *Clava squamata* and *Kirchenpaueria pinnata* were observed to preferentially settle around attached polyps of their respective species. In addition, more planulae of *K. pinnata* settled on surfaces previously coated with planula mucus, than on clean surfaces or on surfaces covered with a bacterial mucous film. Williams[20] concluded that in these species planula settlement was enhanced by an intraspecific factor present in the mucous secretions of both the pre- and post-metamorphic stages.

Siebert[8] has reported that adults of the anemone *Stomphia didemon* can be found on many types of hard substrates (rocks, bottles, old shells). The planulae of this species will begin to settle on coarse sand and fine gravel at eight days, but settlement on broken scallop shells and on plain glass bowls requires almost twice as long. No explanation was given for the differential attractiveness of these substrates.

Although larva of *Tealia crassicornis* will eventually settle on wax and plain glass dishes, metamorphosis occurs 15 days earlier if tubes of the polychaete *Phyllochaetopterus* are present.[6] Siebert and Spaulding[44] have reported that *Phyllochaetopterus* tubes also enhance metamorphosis of *Cribrinopsis fernaldi* planulae. Paradoxically, however, the habitat of these anemones is quite different from that of *Phyllochaetopterus*. The polychaete is an inhabitant of sand and mud flats, while *Tealia* and *Cribrinopsis* are found on rocky substrates.

Finally, Theodor[45] has reported that the planulae of *Eunicella stricta* preferentially settle on coral debris or coral skeletons with attached pieces of coralline algae, bryozoan zooecia, and worm tubes; few planulae settle on clam shells or broken pieces of glass or plastic.

INTERCELLULAR COMMUNICATION AND SENSORY RECEPTORS

Depending on the species of planula, the metamorphic trigger may be either simple contact with a surface, or a specific type of physical or chemical cue. Regardless of the nature of the trigger, settlement and metamorphosis involve many types of cellular processes: ciliary arrest, nematocyst discharge, glandular secretion, cellular differentiation, and bodily shape changes. Coordination between the many cells seems necessary and some form of intercellular communication and integration must be involved. Although the actual mechanism of communication remains speculative at the present time, there are three possibilities: nervous conduction, neuroid conduction, or diffusion of hormone-like chemicals.

Histological studies have reported the presence of a "fibrillar layer" located immediately below the ectodermal epithelium.[1,2,3,4] Widersten,[1] Lyons,[2] and Chia and Crawford[4] have suggested that this layer is composed of nerve cell processes, while Vandermeulen[3] believes the layer is formed of elongate basal processes of the overlying ectodermal cells. Whatever their identity, the fibers exhibit ultrastructural characteristics of axons , including microtubules and possible neurosecretory vesicles.

Although neuroid conduction has been shown in many types of coelenterate epithelia,[46] no report is available demonstrating such activity in the ectodermal epithelium of planulae. Fine structural studies have failed to show gap junctions between planula ectoderm cells.[2,3,4]

Circumstantial evidence for nervous or neuroid conduction comes from the studies of Müller's group[38,24,36,37] on metamorphosis of *Hydractinia echinata* planulae. It will be recalled that the bacterial inducer may act via stimulation of the Na^+/K^+-ATPase. This system is responsible for maintaining the electrochemical potential across cell membranes. Conceivably, increased activity of the Na^+/K^+-ATPase may transduce the chemical stimulus of the bacterial metabolite into an ionic generator potential. Unfortunately, electrophysiological experiments on metamorphosis in *H. echinata* planulae have not been performed.

At present, evidence for intercellular communication via hormone-like chemicals is only conjectural. When planulae of *Proboscidactyla flavicirrata* attach to tentacle pinnules of the host sabellid, the cnidocytes require 30 seconds to become desensitized to sabellid tissue and subsequently require several minutes to acquire sensitivity to the worm tube (respecification).[29] It has also been necessary to maintain both desensitization and respecification. Donaldson[29] has pointed out the discrepancy between the actual time required for conduction of the information, and the time which might be expected if nervous or neuroid conduction was responsible. Based on this evidence he proposed that a metabolite, which diffuses from the planula-pinnule attachment site, is responsible for altering the sensitivity of the planula's cnidocytes. Müller *et al.*[16] have reported that conduction of "pacemaker" impulses in metamorphosing *H. echinata* planulae is a slow process. In this case, however, the actual rate of conduction was not specified.

There is considerable controversy concerning the identity of the cells which might receive the settlement trigger. In some cases the receptive field seems to be distributed over the entire surface of the planula. For example, if planulae of *H. echinata* are cut transversely into several pieces and each piece exposed to a sufficiently large concentration of the bacterial inducer, each piece, except the tail, will metamorphose into the corresponding part of the polyp. Donaldson[29] similarly found that all portions of the planula were receptive to both the sabellid tentacle stimulus and the worm tube stimulus. In this case, the extent of the sensitivity is correlated with the uniform distribution of the cnidocytes, which appear to be the receptor cells initiating attachment and possibly metamorphosis.

Due to the fact that planulae swim and crawl with the aboral end foremost, and settlement attachment takes place with the aboral end, investigators have often searched for sensory cells in this region. The most obvious aboral specialization is the apical organ, present only in some actinarian planulae. We have made a preliminary fine structural study of this organ in the planula of *Anthopleura elegantissima*. It is composed of a number of very tall columnar cells, each giving rise to a long cilium (Fig. 3). In life, the cilia tend to adhere together in the form of a tuft which is capable of some bending movement.[47,1,17] However, the cilia of the tuft are not fused, as has been stated by Siebert.[7] The ciliary rootlets of these cells are exceedingly long; they extend to the base of the columnar cells. Widersten[1] described uptake of both methylene blue and silver stain by the apical organ cells in the planula of *Metridium senile.* In *A. elegantissima* we have found nerve-like processes concentrated just below the columnar cells of the apical organ (Fig. 3). These processes contain both clear and dense-cored vesicles (Fig. 4). The settlement qualifications of *A. elegantissima* are still unknown. Siebert[7] was unsuccessful in his