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# CONTROL MEASURES FOR CHESAPEAKE BAY JELLYFISHES

1 April 1971 - 31 March 1972

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# CONTROL MEASURES FOR CHESAPEAKE BAY JELLYFISHES

1 April 1971 - 31 March 1972

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## ABSTRACT

Progress on the biological studies of scyphozoan jellyfishes is herein reported.

Morphological studies as an aid to identification of *Aurelia aurita*, *Cyanea capillata*, *Chrysaora quinquecirrha*, and *Rhopilema verrilli* are now completed. Further morphological evidence of "northern" and "southern" strains of *Aurelia aurita* based on life history studies is presented.

With respect to the strobilation process, nucleic acid and protein ratios are compared before, during, and after this form of asexual reproduction occurs. An ultrastructural analysis of polyps reveals that neurosecretory substances are associated with the very early stages (pre-strobilation) of strobilation.

Inhibitor studies carried out indicate that the free-swimming ephyrae are more sensitive to external agents than are the sessile polyps. This is not surprising because the polyps may survive a wide spectrum of environmental conditions in nature whereas the ephyrae are considerably more labile.

The seasonal distribution of *Chrysaora* polyps is reported for selected sites which are monitored each year. In spite of the low abundance of medusae in the summer of 1971, numerous polyps and cysts persist on natural shell substrate which may give rise to nuisance populations of medusae during 1972.

Uptake of glycine and serine, selected dissolved organic compounds of the natural waters, indicate that the dissolved organic fraction is not a prime nutrition source for *Chrysaora* ephyrae and polyps. Gastric cavity analysis of *Cyanea* medusae indicate that these organisms are omnivorous and primarily night feeders. The component fatty acids indicate significant differences between species, some differences associated with sex in the medusae, and significant differences between medusae and polyps of the same species.

Speculations on low *Chrysaora* and *Aurelia* populations during the summer of 1971 relate to possible lack of planktonic food sources of the ephyrae and young medusae and to the extreme hydrographic conditions during the spring of 1971.

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# CONTROL MEASURES FOR CHESAPEAKE BAY JELLYFISHES

## I. INTRODUCTION

With the enactment of Public Law 89-720, The Jellyfish Act, recognizing that certain coelenterates are a hazard to human activity and a deterrent to the recreational development of the Chesapeake Bay, several research teams were formed. These teams have as their objective the development of a degree of understanding of the biology of the jellyfish so that control of jellyfish populations may be accomplished without incurring unacceptable ecological costs. The research endeavors at VIMS are designed around specific objectives which will contribute to the ultimate attainment of the stated objective of jellyfish population control.

Since 1968, studies at VIMS have included both laboratory and field investigations under the coordination of Dr. Edwin B. Joseph, Assistant Director of Fisheries and Biological Oceanography. The coordination of the VIMS Jellyfish Project has continued since October 1971 under Dr. Paul L. Zubkoff, Head, Department of Environmental Physiology.

Field observations on abundance and location have permitted a general comparison of scyphozoan populations dominating the Lower Bay. Wide fluctuations of jellyfish populations (*Chrysaora quinquecirrha, Aurelia aurita,* and *Cyanea capillata*) have been observed during this period. The summer of 1969 was a year of great abundance for the sea nettle, *Chrysaora quinquecirrha,* whereas the summer of 1970 was a period of high populations which disappeared approximately 4-6 weeks earlier than the previous year. In contrast, the summer of

1971 was a year of very low abundance of both *Chrysaora quinquecirrha* and *Aurelia aurita*. In addition, during April of 1971, the greatest abundance of *Cyanea capillata* was observed since the program was initiated.

Although in 1971, polyps of *Chrysaora quinquecirrha* were observed undergoing strobilation at the same sites monitored in the previous years of large jellyfish populations, the Lower Bay waters were free of *Chrysaora quinquecirrha* for most of the summer. Thus, the summer of 1971 may be looked upon as a year in which these obnoxious organisms were under natural control. Unfortunately, that goal of jellyfish population control for which this project is striving was seen momentarily, but is still out of reach because we do not fully understand that observed phenomenon.

In light of the concerted efforts of developing an understanding of these organisms through biochemical, physiological, morphological, developmental and ecological studies over the past 4 years, a more thorough base of fundamental knowledge now exists for continued investigations.

The progress under the Cooperative Agreement for 1 April 1971 - 31 March 1972, Control Measures For Chesapeake Bay Jellyfishes, Project JF-3-9, Contract 14-17-0003-598, follows.

## II. PUBLICATIONS, ABSTRACTS, AND THESIS

These studies were conducted as part of the Cooperative Virginia-Maryland Jellyfish Research Program, unless otherwise noted by the asterisk. Funding of the Virginia subprogram is under Public Law 89-720, Jellyfish Act, Contract No. 14-17-0003-598 (Dr. Edwin B. Joseph, 1 April 1968 - 1 October 1972, or Dr. Paul L. Zubkoff, 1 October 1972 - 31 March 1972, Project Coordinator) from National Marine Fisheries Service of the National Oceanic and Atmospheric Administration, U. S. Department of Commerce and Virginia Institute of Marine Science, Commonwealth of Virginia.

Coordination meetings with the Maryland Jellyfish Research Team were held in September at VIMS, Gloucester Point, Virginia, and in February at Chesapeake Biological Laboratory, Solomons, Maryland. In addition, members of both groups held informal discussions at the Atlantic Estuarine Research Society Meeting in November (Plainview, New York). Minutes of these meetings have been forwarded to NMFS.

A report of the previously completed work does not appear in the 1971 Federal Aid Program Activities, National Marine Fisheries Service, USDC-NOAA. Studies completed since the start of the VIMS-NMFS Program are listed below:

### PUBLICATIONS

CALDER, D. R. 1971.

Nematocysts of polyps of *Aurelia*, *Chrysaora* and *Cyanea*, and their utility in identification.

Trans. Amer. Micros. Soc. 90: 269-274.

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Development of the sea nettle, *Chrysaora quinquecirrha* (Scyphozoa, Semaeostomeae).

Chesapeake Sci. 13: 40-44.

CALDER, D. R. 1972.

Nematocysts of the medusa stage of *Rhopilema verrilli* (Scyphozoa, Rhizostomeae).

Trans. Amer. Micros. Soc. 91: 213-216.

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Bibliography on the Scyphozoa, with selected references on Hydrozoa and Arthozoa.

Va. Inst. Mar. Sci. Spec. Sci. Rep. 59. 142 p.

CONES, H. N. JR. 1969*.

Strobilation of *Chrysaora quinquecirrha* polyps in the laboratory.

Va. J. Sci. 20: 16-18.

CONES, H. N. JR., and D. S. HAVEN. 1969*.

Distribution of *Chrysaora quinquecirrha* in the York River.

Chesapeake Sci. 10: 75-84.

JOSEPH, J. D., R. W. SCHMIDT, and P. L. ZUBKOFF. 1971.

Comparative biochemistry of jellyfish: Neutral lipids of *Aurelia*, *Chrysaora* and *Cyanea* polyps.

Amer. Chem. Soc., 6th Mid. Atlantic Reg. Meeting, Feb. 3-5, 1971, Baltimore, Md. Abstr. BIOL 10.

OAKES, M. J. and D. S. HAVEN. 1971.

Some predators of polyps of *Chrysaora quinquecirrha* (Scyphozoa, Semaeostomeae).

Va. J. Sci. 22: 45-46.

OLMON, J. and K. L. WEBB. 1970.

The effects of salinity and decreasing temperature on polyp stages of the jellyfish, *Aurelia aurita* and *Chrysaora quinquecirrha*.

Va. J. Sci. 21: 115. (Abstr.)

OLMON, J. and K. L. WEBB. 1971.

Salinity and temperature effects on setting planulae of Chesapeake Bay jellyfish.

Va. J. Sci. 22: 100. (Abstr.)

PERKINS, F. O., R. W. RAMSEY, and S. S. RAMSEY*. 1970.

The ultrastructure of muscle contraction in the jellyfish (*Chrysaora quinquecirrha*) fishing tentacle.

7 ieme Cong. Int. Micros. Electron. 777-778. (Abstr.)

PERKINS, F. O., R. W. RAMSEY, and S. S. RAMSEY. 1971*.

The ultrastructure of fishing tentacle muscle in the jellyfish *Chrysaora quinquecirrha*: A comparison of contracted and relaxed states.

J. Ultrastruct. Res. 35: 431-450.

## THESIS

DIETZ, M. A. 1971.

An ultrastructure study of strobilation in *Chrysaora quinquecirrha* with special reference to neurosecretion.

M.S. College of William and Mary, Williamsburg, Va.

## MANUSCRIPTS SUBMITTED FOR PUBLICATION

Black, R. E. Nucleic acid and protein levels in strobilating polyps of *Chrysaora quinquecirrha* and *Aurelia aurita*.


### Abstract

Levels of DNA, RNA, and protein were measured in scyphistomae of the scyphozoans, *Aurelia aurita* and *Chrysaora quinquecirrha* during strobilation. In synchronously developing populations of *Aurelia*, the amount of DNA per polyp increases about 3-fold during strobilation, whereas RNA and protein remain relatively constant. The RNA/DNA ratio drops by 50% during strobilation in *Chrysaora* and 28% to 50% in *Aurelia*. Specific RNA reserves are probably not accumulated in advance of strobilation, however, since strobilation is blocked immediately in both species by 1 to 3 μg/ml actinomycin D. Polyp size and DNA content are usually increased by prolonged incubation at temperatures of 12° to 15°C, which facilitates strobilation; however, the RNA/DNA and protein/DNA ratios of such polyps are not significantly increased by the cold conditioning.


Submitted to <u>Marine Biology</u>

Dietz, M. A. and K. L. Webb. An ultrastructural study of strobilation in *Chrysaora quinquecirrha* with special reference to neurosecretion.


## Abstract

Scyphistomae and strobilae of the scyphozoan *Chrysaora quinquecirrha* were fixed and sectioned for electron microscopy. The polyps were divided into four classes on the basis of their stage of development: non-strobilating (scyphistomae), neck-formation, segmentation, and metamorphosis. Ultrastructural preparations revealed neurosecretory cells containing numerous membrane-bounded granules in scyphistomae and necked polyps. Between neck-formation and segmentation the neurosecretory granules moved from the cell body to the axons of the neurites. By metamorphosis most of the neurosecretory product had disappeared from the axons. Other changes which appear to accompany strobilation included both a decrease in glycogen and contraction of muscles along the inner edge of the epidermis.


Submitted to J. Exper. Biol.

Olmon, J., K. L. Webb, and M. Bolus. Environmental factors influencing setting of planulae of *Aurelia, Chrysaora* and *Cyanea*. I. Planula density and chemical simulation of crowding.

## Abstract

*Aurelia aurita, Chrysaora quinquecirrha* and *Cyanea capillata* planulae were subjected to various conditions of crowding and held under controlled conditions until the planulae set and metamorphosed into polyps. Maximum numbers of polyps were formed at planula densities of 2-10 planulae per ml of sea water, of 5-12 planulae per $cm^2$ of substrate area. The effects of various concentrations of glycine, serine, glutamic acid, α-ketoglutaric acid, phosphate buffer and dimethylsulfoxide on larval cyst formation in *Cyanea* were tested. Dimethylsulfoxide partially inhibited larval cyst formation at a concentration of $10^{-2}$ M but the other chemicals tested had no significant effect.

Morales-Alamo, R. and D. S. Haven. Mouth shape of the scyphistoma of *Aurelia aurita* from Chesapeake Bay and its taxonomic significance.

Abstract

Scyphistomae (polyps) of *Aurelia aurita*, *Chrysaora quinquecirrha* and *Cyanea capillata* were reared in the laboratory from known parent medusae collected in the York River, Virginia. It was observed that the fully developed (16-tentacle stage) polyp of *Aurelia* possessed a circular mouth. This feature distinguishes *Aurelia* from *Chrysaora* and *Cyanea* in the same region and from polyps of *Aurelia* obtained from Woods Hole, Massachusetts, all of which have cruciform mouths. A circular mouth in Chesapeake Bay *Aurelia* also contrasts with previous descriptions of a cruciform mouth for *Aurelia* from Plymouth, England. This difference in mouth shape may not only be useful for identification of *Aurelia* in field collections in Chesapeake Bay but also raises the possible existence of subspecies of *Aurelia* from different geographic regions.

Submitted to Biol. Bull.

Webb, K. L., A. L. Schimpf, and J. Olmon. Free amino acid composition of scyphozoan polyps of *Aurelia aurita, Chrysaora quinquecirrha,* and *Cyanea capillata* at various salinities.


Summary

1. Free amino acid (FAA) composition of scyphozoan polyps of *Aurelia aurita* and *Chrysaora quinquecirrha* was found to be linearly related to salinity.
2. Glycine, the most concentrated amino acid in polyps of *Aurelia* and *Chrysaora,* was as much as 80% of the total free amino acid pool. The concentrations of FAA of *Cyanea* were more evenly distributed among a number of amino acids.
3. The polyps of *Aurelia aurita* from Chesapeake Bay seem to make up a population distinct from those of the Woods Hole, Massachusetts region. β-alanine constitutes a major fraction of the FAA of the Chesapeake Bay *Aurelia* polyps and is lacking in the Woods Hole population. The Chesapeake Bay *Aurelia* polyps also possess circular mouths rather than the typical cruciform mouth.
4. The FAA composition of *Chrysaora* polyps from the field was quite variable upon collection but became similar to laboratory raised polyps after 48 hours of starvation. It was concluded that quality and quantity of food and elapsed time since feeding greatly alter the FAA pools.


In Press Comp. Biochem. Physiol.

# COMPLETED MANUSCRIPTS

Calder, D. R. Laboratory observations on the life history of *Rhopilema verrilli* (Scyphozoa, Rhizostomeae).

## Summary

The life history of the rare scyphozoan *Rhopilema verrilli* is described from the planula to the young medusa stages. Planulae are retained within the gonadal tissue of the medusa until fully developed. On liberation, most planulae set and metamorphosed into tiny scyphistomae within 7-10 days. The scyphistoma differs from other described species in having an unusually large, clavate manubrium. The only means of asexual reproduction observed in the scyphistoma cultures involved the formation of podocysts. Strobilation was usually of the monodisc variety, although polydisc strobilation was not infrequent. The process of strobilation was completed within seven days at 20 C. Newly liberated ephyrae typically had eight pairs of lappets and eight rhopalia. Ephyral development resembled that of the closely related rhizostome *Rhizostoma pulmo*. The cnidome of the planula and scyphistoma consisted of atrichous isorhizas ("a" atrichs) and microbasic heterotrichous euryteles, while that of the strobila and ephyra consisted of "a" atrichs, euryteles, and holotrichous haplonemes.

## UNPUBLISHED INFORMAL PRESENTATIONS

DIETZ, M. A. and K. L. WEBB. 1971.

An ultrastructural study of strobilation in *Chrysaora quinquecirrha* with special reference to neurosecretion.

Estuarine Research Federation, Plainview, Long Island, New York.

MORALES-ALAMO, R., and D. S. HAVEN. 1971.

Circular mouth shape as a character peculiar to the scyphistoma of *Aurelia aurita* from Chesapeake Bay.

Estuarine Research Federation, Plainview, Long Island, New York.

ZUBKOFF, P. L. and J. D. JOSEPH. 1971.

Comparative biochemistry of jellyfish: Component fatty acids of the total lipids of *Aurelia*, *Chrysaora*, and *Cyanea*.

Estuarine Research Federation, Plainview, Long Island, New York.

## III. STATEMENT OF PROGRESS

### JOB. NO. 1 - PROBLEMS OF IDENTITY

OBJECTIVE: To further clarify the problems of identity of the several life stages of the abundant jellyfishes of the Chesapeake Bay.

D. R. Calder

The newly liberated ephyra is the least differentiated and, thus, the most difficult stage to identify in the development of a medusa. Detailed comparative studies on ephyrae of the four species of jellyfish (*Aurelia*, *Chrysaora*, *Cyanea*, and *Rhopilema*) have now been completed in order to identify morphological differences useful in identification. The use of nematocysts and life cycle studies have been employed.

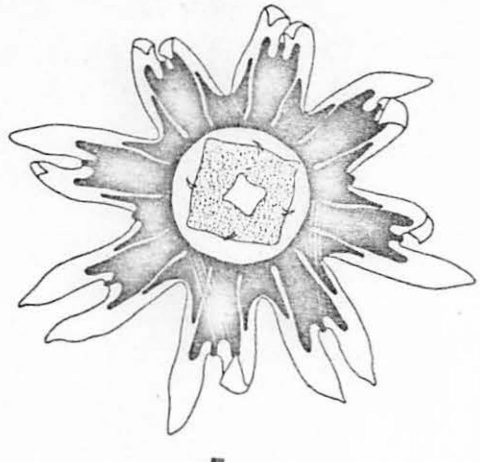
#### Studies on *Cyanea*

Polyps of *Cyanea* began to strobilate in the laboratory during autumn 1971, and observations were made on the ephyra and its development into the medusa (Figs. 1-6). Russell (1970) outlined differences useful in distinguishing *Cyanea* from *Aurelia*, *Chrysaora*, and *Rhizostoma*, but his studies were based on European specimens. Present observations, based on *C. capillata fulva* from Chesapeake Bay confirm that the differences characterizing *Cyanea* from Europe also apply to our local variety.

In previous reports, the nematocysts in ephyrae of *Aurelia* and *Chrysaora* were described, and the cnidome of the *Rhopilema* ephyra will be discussed elsewhere (manuscript in preparation). To complete the comparison, details on nematocysts in ephyrae of *Cyanea* are given in Table 1. The cnidome is similar to that described previously for

FIGS. 1-6. Stages in the development of <u>Cyanea capillata fulva</u>, from laboratory cultures.

1. Ephyra at liberation, 3 mm diameter.
2. Five-day-old ephyra, 5 mm.
3. Seven-day-old ephyra, 7 mm.
4. Eleven-day-old ephyra, 10 mm.
5. Fourteen-day-old ephyra, 13 mm.
6. Twenty-day-old medusa, 17 mm.



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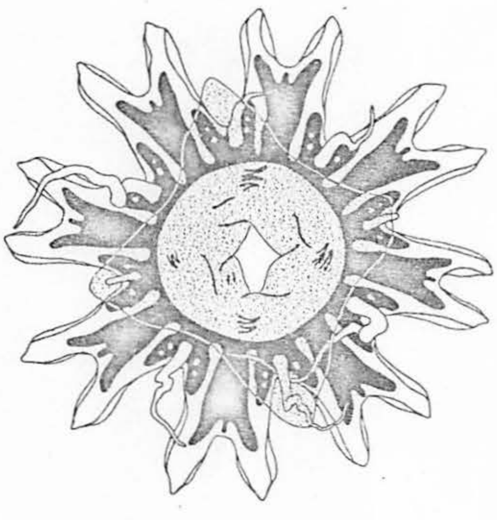

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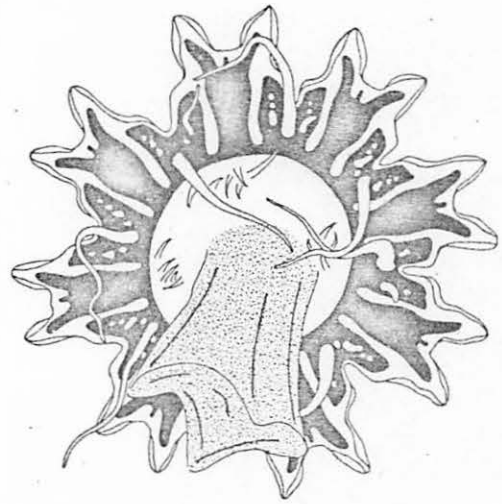

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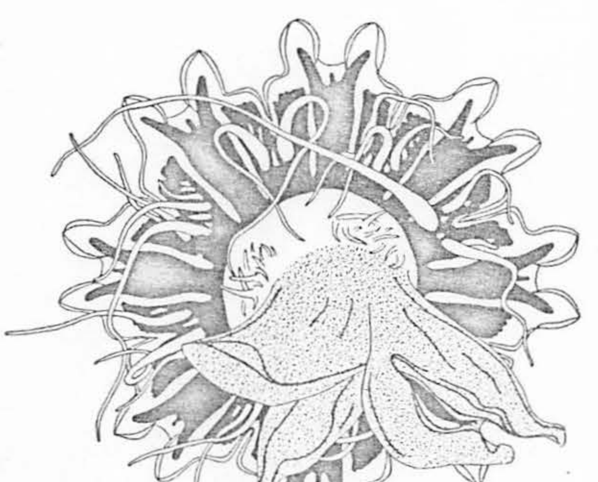

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TABLE 1. Nematocysts of the ephyra stage of <u>Cyanea capillata</u>.

| | "a" Atrichs | "A" Atrichs | "α" Atrichs | Holotrichs | Euryteles |
|---|---|---|---|---|---|
| No. examined | 25 | 1 | 25 | 25 | 25 |
| Length | 5.3-6.9 | 12.4 | 5.6-7.1 | 9.9-12.9 | 10.2-12.8 |
| Width | 3.8-4.9 | 7.9 | 2.1-3.4 | 8.7-11.0 | 7.1- 9.2 |
| Mean length | 6.0 | 12.4 | 6.7 | 11.6 | 11.2 |
| Mean width | 4.0 | 7.9 | 2.7 | 10.0 | 8.2 |
| Greatest L:W ratio | 1.66:1 | 1.57:1 | 3.18:1 | 122:1 | 1.58:1 |
| Smallest L:W ratio | 1.36:1 | 1.57:1 | 2.00:1 | 1.10:1 | 1.26:1 |
| Mean L:W ratio | 1.50:1 | 1.57:1 | 2.52:1 | 1.16:1 | 1.36:1 |

the medusa stage of *Cyanea*. However, "A" atrichs, which were relatively common in the medusa were usually undeveloped in the newly-liberated ephyra. Only two "A" atrichs were observed in the 15 ephyrae examined and only one of these nematocysts could be accurately measured. There were also marked differences in the relative abundance of "α" atrichs from one ephyra to another. These nematocysts were common to abundant in some of the newly-liberated ephyrae and rare or even absent in others. The "α" atrichs are concentrated in the oral region of the polyp (Calder, 1971) and do not become equally distributed among the ephyrae produced by a given strobila. Those formed distally on the strobila bear moderate or large numbers of these nematocysts, while those produced on the proximal end have relatively few or no "α" atrichs. Euryteles, "a" atrichs and holotrichs were abundant in all the ephyrae examined.

## Differences in Allopatric Populations of *Aurelia*

Differences in allopatric populations of *Aurelia aurita* have already been described. Morales-Alamo and Haven (1972) found differences in the mouth shape which distinguished the polyp of Chesapeake Bay *Aurelia* from Woods Hole, Massachusetts specimens. Webb, Schimpf and Olmon (1972) reported differences in the free amino acid composition of polyps from the same two locations. Earlier, Mayer (1914) had demonstrated physiological differences between medusae of *A. aurita* from Halifax, N. S., and Tortugas, Fla.

Present studies provide additional evidence that northern and southern populations of *A. aurita* along this coast differ from each other. Comparisons were made of the development of specimens from Texas, Virginia, Delaware and Massachusetts. Those from Texas, Virginia and Delaware showed one pattern of development (Fig. 7), those from Massachusetts another (Fig. 8). Polyp nematocyst studies corroborate these findings. *Aurelia* from Massachusetts differed from the Delaware, Virginia and Texas specimens in lacking the polyspira nematocyst type. Thus, caution should be exercised in extrapolation of information obtained on *Aurelia* from New England and other northern locations to populations elsewhere along this coast.

## "Red" and "White" Phases of *Chrysaora*

Preliminary observations lend support to the hypothesis that the color phases of *Chrysaora quinquecirrha* are genetic, at least in part. Polyps were reared from planulae produced by both red and white medusa phases. Medusae reared in the laboratory from "white" polyps did not develop any pigmentation (Calder, 1972). However, specimens reared from "red" polyps developed faint reddish pigmentation after attaining a diameter of 4-5 cm in the laboratory. As the medusae continued to grow, the pigment became more and more conspicuous. Unfortunately, the specimens died 51 days after liberation, having attained a diameter of 7 cm.

## Record of *Pelagia*

A fifth species of scyphozoan medusa has been found in Virginia waters for the first time. Specimens of *Pelagia noctiluca*, a species lacking a polyp stage, were found in Wachapreague Inlet on the eastern shore during October 1971. The medusa is a close relative of and resembles *Chrysaora quinquecirrha*, but possesses only eight tentacles and 16 lappets in the adult. The species is reported as

FIG. 7. Developmental pattern displayed by "southern" *Aurelia aurita* (from Texas, Virginia, Delaware).

1. Newly-liberated ephyra, 2.3 mm diameter.
2. Six-day-old ephyra, 4.5 mm.
3. Eight-day-old ephyra, 6.0 mm.
4. Twelve-day-old ephyra, 7.5 mm.
5. Twenty-day-old medusa, 12.0 mm.
6. Thirty-day-old medusa, 17.0 mm.

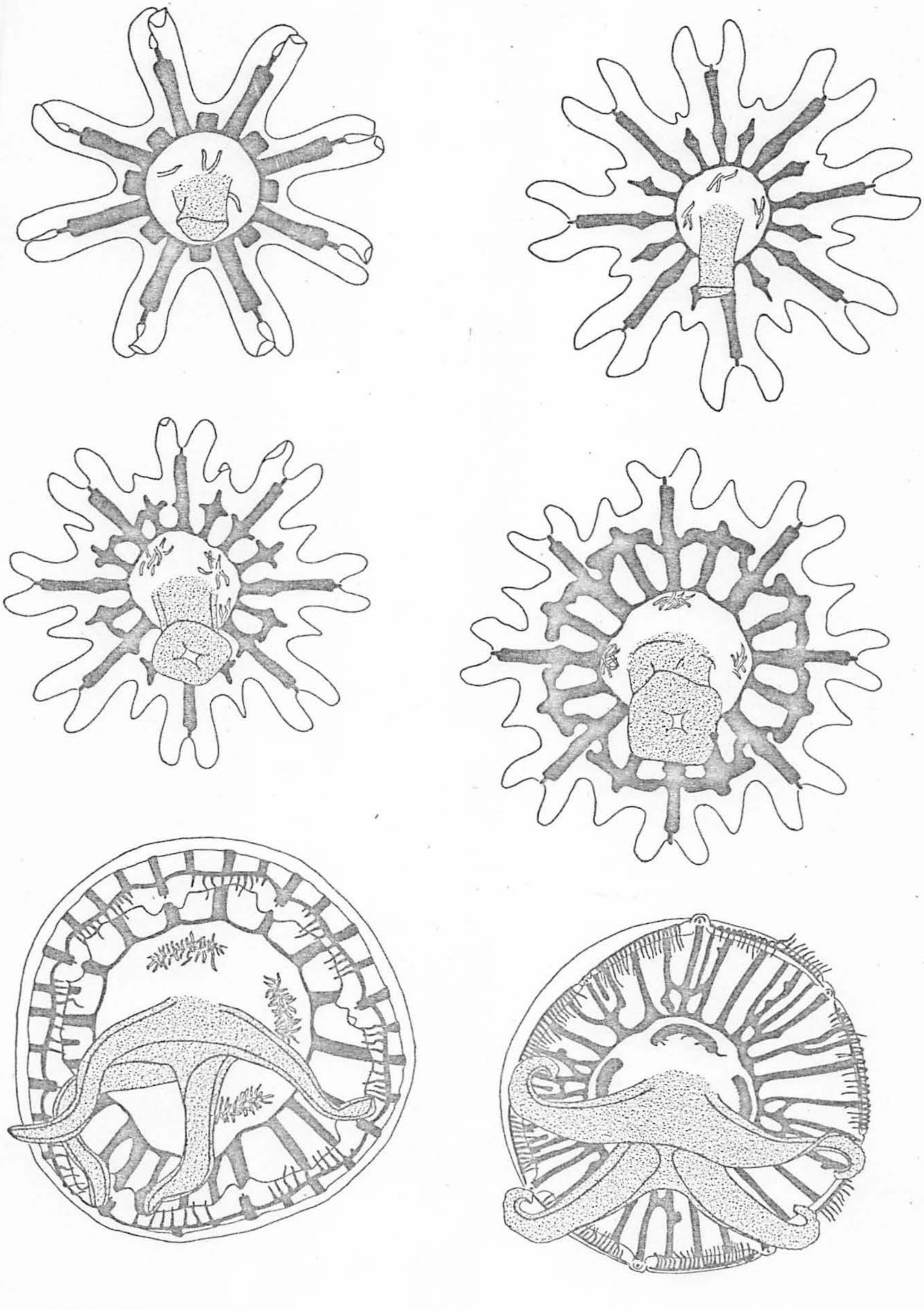
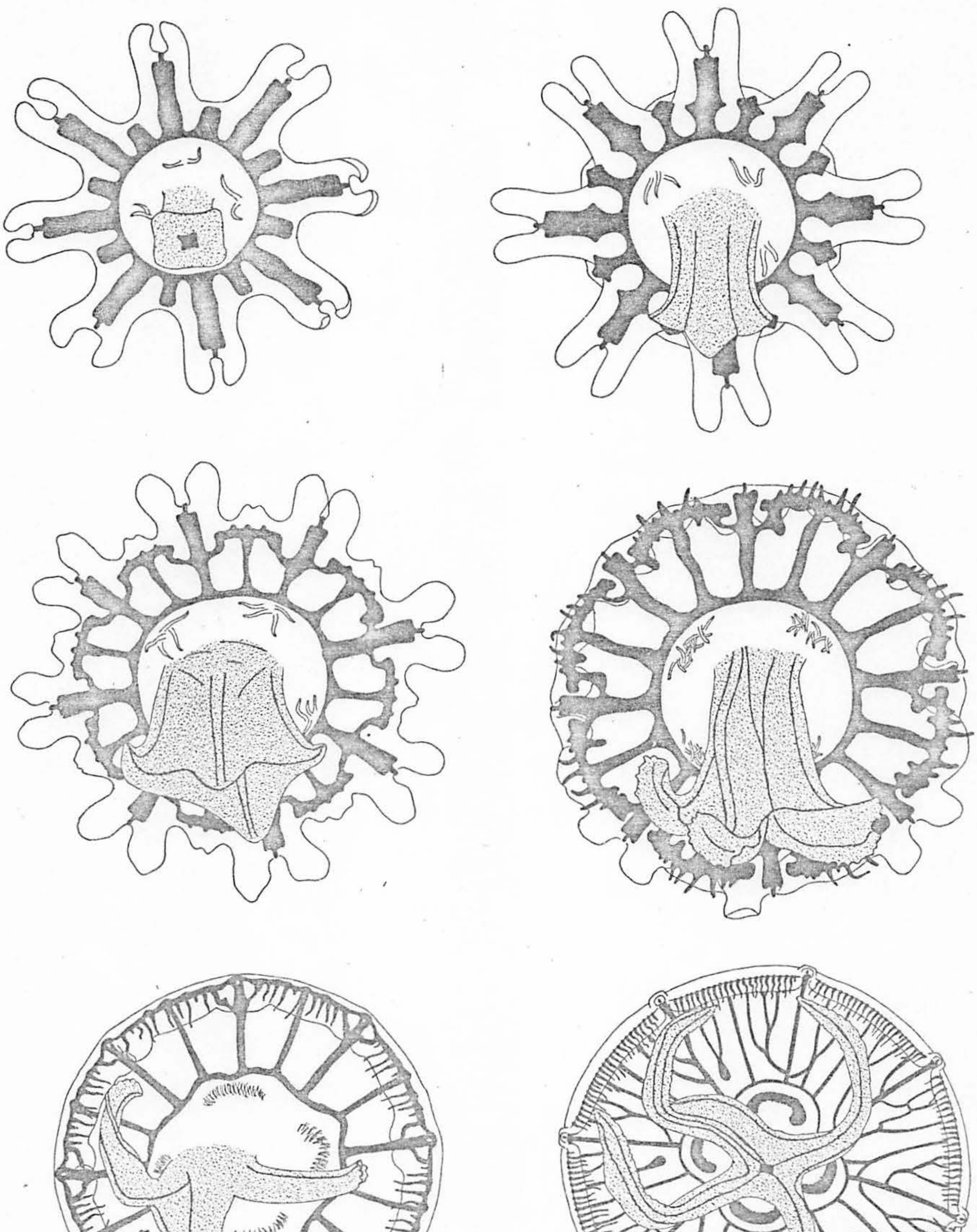


FIG. 8. Developmental pattern displayed by "northern" *Aurelia aurita* (from Woods Hole).

1. Newly-liberated ephyra, 3.0 mm diameter.
2. Three-day-old ephyra, 4.1 mm.
3. Seven-day-old ephyra, 5.9 mm.
4. Ten-day-old medusa, 7.5 mm.
5. Fourteen-day-old medusa, 10.0 mm.
6. Thirty-day-old medusa, 20.0 mm.



venomous by Halstead (1965). *P. noctiluca* is a pelagic scyphozoan, commonly found in the Gulf Stream, and its occurrence in local waters is undoubtedly attributable to offshore wind and current patterns.

## Additions to Polyp Collections

A list of the polyps in culture at VIMS was given in an earlier contract report. In addition to these we have obtained *Cassiopea* sp. from Bermuda, *Aurelia* sp. from Washington (state), and scyphistomae (probably *Aurelia aurita*) from New Brunswick and Prince Edward Island, Canada. The identity of these polyps will be established through life history studies as soon as the cultures strobilate.

## Nomarski Interference Microscopy

Polyps and early strobilae were examined using Nomarski differential interference microscopy to determine whether this optical technique would reveal color differences whereby early strobilae could be distinguished. Preliminary results from this study were negative; the only differences noted were morphological and just as easily distinguished under brightfield optics.

## Bibliography

A jellyfish bibliography (Calder, Cones and Joseph, 1971) was published since the last contract report. Work has continued on this project to keep the reference list up to date.

## Summary

This phase of the VIMS Jellyfish Program was added initially to resolve difficulties in the identification of scyphistomae in Chesapeake Bay. Reliable means have been described whereby the polyps of the four species of jellyfish occurring in the bay can be identified. Life histories of the four species have been followed from the ephyra to the medusa, thereby making identification of any stage in jellyfish development also possible. Differences in allopatric populations of *Aurelia aurita* have been explored. A bibliography of jellyfish literature has been assembled, published, and distributed. With this report the jellyfish taxonomy phase is completed and emphasis will be shifted to a Creek Plankton-Nutrition Integrated Study. Under this study taxonomic work will be directed at establishing zooplankton composition and dominance and the physical environmental conditions prior to and during strobilation and maturation of *Chrysaora* in Sarah's Creek, Virginia.

## LITERATURE CITED

CALDER, D. R. 1971.

Nematocysts of polyps of *Aurelia, Chrysaora* and *Cyanea,* and their utility in identification.

Trans. Amer. Micros. Soc. 90: 269-274.

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JOB NO. 2 - THE STROBILATION PROCESS

OBJECTIVE - To investigate further the process of strobilation as a possible weak link in the jellyfish life cycle.

1 - Nucleic Acid and Protein Levels in Strobilating Polyps of *Chrysaora quinquecirrha* and *Aurelia aurita*

R. E. Black

The scyphistoma (polyp) of the scyphozoan undergoes extensive cell division during strobilation, utilizing food reserves accumulated prior to strobilation, according to histological studies by Chuin (1930). Quantitative biochemical changes during this process have not been studied. As an initial study, it is desirable to obtain quantitative information about changes in DNA, RNA, and protein during strobilation, since these provide a basis for interpreting other biochemical events. Levels of these macromolecules in laboratory cultures of strobilating and nonstrobilating polyps of *Chrysaora quinquecirrha* and *Aurelia aurita* are reported.

*Aurelia* polyps were held at 12° to 15°C for one to several months in order to condition them for strobilation (Spangenberg, 1967). The polyps were then returned to 25°C and potassium iodide was added to a concentration of 1.25 μM. In cultures which had been cold conditioned for as long as 3 months, nearly synchronous strobilation was initiated in all polyps within 2 to 3 days after this treatment. Strobilation in *Chrysaora* was induced by returning the polyps to 25°C after a period of at least 2 months in the cold (Loeb, 1970). The initiation of strobilation usually required 3 to 21 days, and no synchrony was observed.

For nucleic acid and protein determination, 20-50 *Chrysaora* polyps, 50-100 *Aurelia* polyps, or 300-500 ephyrae of either species were homogenized and extracted thrice in cold 5% (W:V) trichloroacetic acid (TCA), twice in boiling ethanol-ether (3:1 V:V), and once in 5% TCA at 90°C for 20 minutes, according to the fractionation procedure of Schmidt and Thannhauser (1945). The Dische diphenylamine method was used for the estimation of DNA; the orcinol method of Mejbaum was used for RNA (cf. Schneider, 1957). Protein was determined by the method of Lowry et al. (1951) or by the biuret method as described by Layne (1957).

The RNA/DNA and protein/DNA ratios obtained for several batches of strobilating *Chrysaora* are shown in Table 1. The animals used in this study were collected from shells in March and held in the laboratory at 18°C for periods of 2 to 8 weeks prior to analysis. They were fed *Artemia* during this time. The RNA/DNA ratio decreases about 50% during strobilation. Although the protein/DNA ratio tends to decrease during strobilation, it is so variable that no conclusion can be drawn about its relation to development.

In order to interpret the changes in ratio noted above, it is necessary to compare the actual levels of macromolecules in polyps of comparable size at different stages of strobilation. Since each assay required 20-50 polyps, such a comparison required large numbers of polyps of uniform size, which could be induced to strobilate fairly synchronously. This requirement could not be met in *Chrysaora*, since synchrony is so poor, and it was necessary to use synchronously developing polyps of *Aurelia* for such measurements. The results of duplicate experiments on *Aurelia* polyps, preconditioned at 15°C for

3 months, are shown in Fig. 9. A 3-fold increase in DNA occurs in strobilating polyps but not in the nonstrobilating ones over the same period of time. The levels of RNA and protein vary considerably in different samples of polyps, but do not show major net changes over the 10-day experimental period in either strobilating or control polyps. Although the polyps were not fed during the experimental period, starvation did not result in a decrease in RNA or protein in the control polyps; it therefore seems reasonable to conclude that starvation cannot account for the drop in RNA/DNA ratio in strobilating polyps (Fig. 10). The increase in DNA during strobilation is not unexpected, since extensive cell division has been reported during this process (Chuin, 1930).

Budding occurred in both experimental and control *Aurelia* polyps during the above experiments. The buds were included with the polyps in the assays. Because of the short duration of the experimental period, no attempt was made to determine differences in budding rates. Approximately 30-50 buds were produced in each group of 50 polyps during the period.

Prolonged incubation at low temperature usually resulted in a considerable increase in the average size of polyps of both species. The large size and high RNA/DNA and protein/DNA ratios obtained in cold-treated, unstrobilated polyps of both species suggested that RNA and possibly protein are accumulated during the prolonged period of cold treatment prior to strobilation. This was investigated by comparing these ratios in conditioned and nonconditioned polyps of *Chrysaora* and *Aurelia*. The polyps used had been cultured from eggs or planulae. The cold conditioned *Chrysaora* had

FIG. 9. Nucleic acids and protein in cold-conditioned, strobilating (●——●) and nonstrobilating (o----o) polyps of *Aurelia*. KI (1.2 μM) was added to experimental polyps to induce strobilation, but was omitted from controls. ES: early strobila. LS: late strobila.

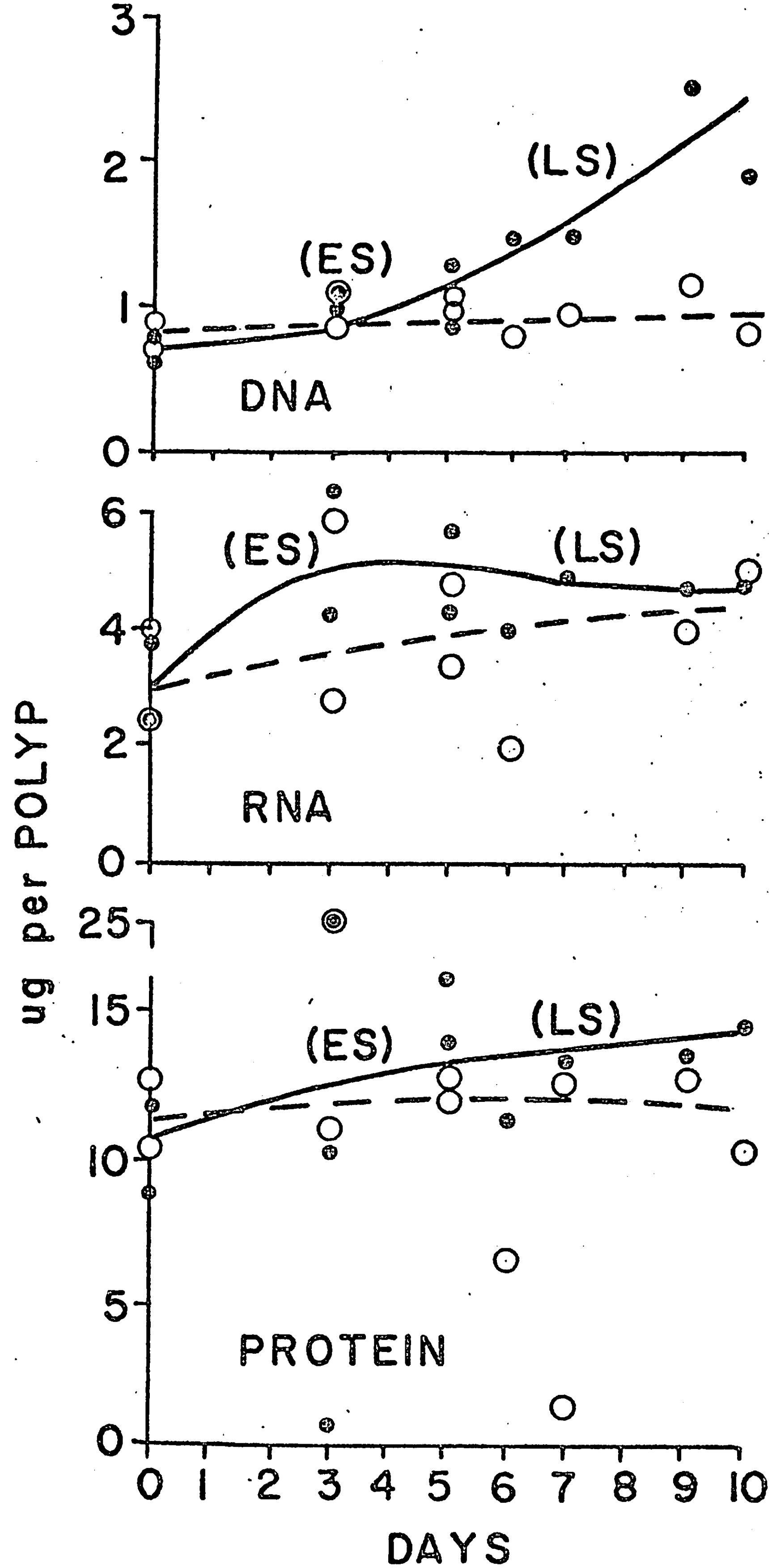
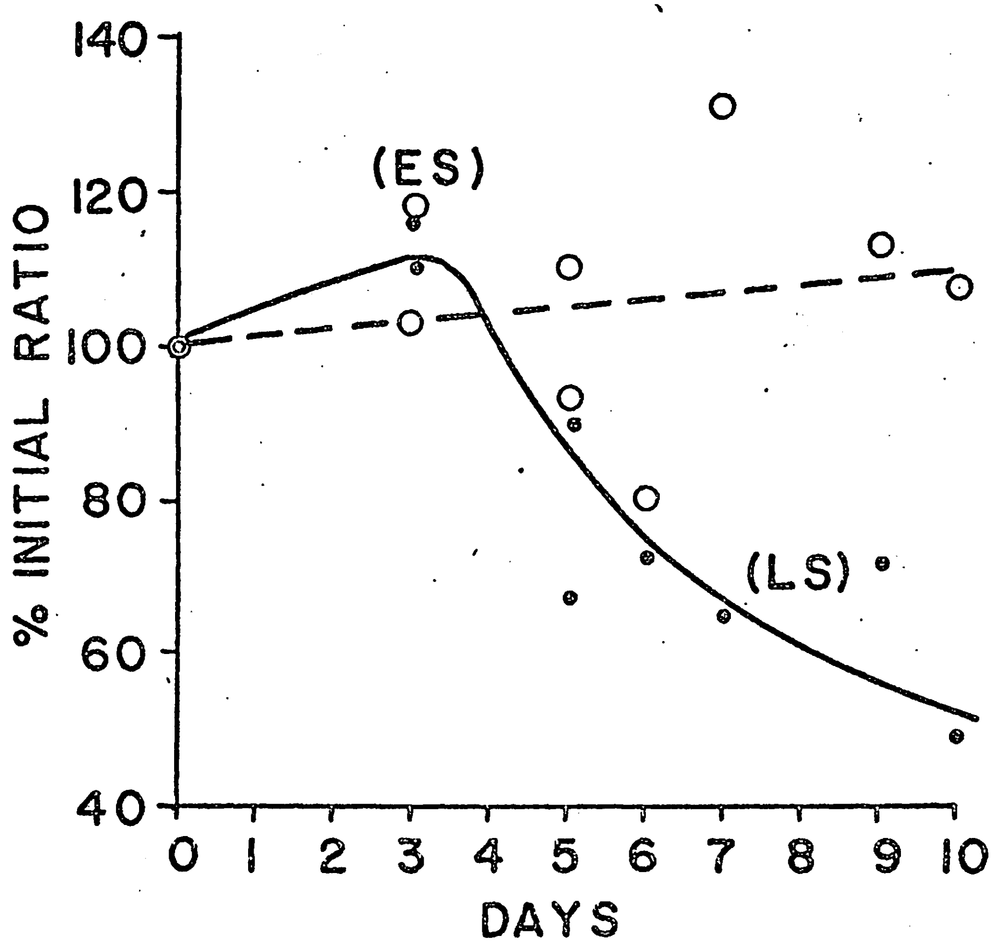


FIG. 10. Changes in RNA/DNA ratio in strobilating ( ——— ) and nonstrobilating ( ---- ) polyps of *Aurelia*. Polyps were starved during the experiment.



been maintained at 12° to 14°C for 6 months, while the *Aurelia* had been held at 15°C for 9 months. Controls were held at 25°C during the same period. The conditioned polyps selected for the assays were of considerably larger size than the nonconditioned controls. The ratios of RNA/DNA and protein/DNA were not greatly increased by the prolonged cold treatment (Table 3). The larger size of the conditioned polyps is reflected in their higher DNA content. These polyps probably possess larger cell numbers than do the controls. It will be noted that the ratios in these polyps are much higher than those presented in Table 2.

The high initial RNA/DNA and the absence of a major net increase in RNA suggested that sufficient RNA reserves might be available at the beginning of strobilation to permit this process to occur to some extent independently of concomitant RNA synthesis. If this were the case, blockage of the RNA transcription process by use of the inhibitor, actinomycin D, would not immediately block strobilation. Accordingly, the effects of acyinomycin D were tested on polyps in different stages of strobilation. Strobilation was blocked immediately at any stage by 1.0 μg/ml of this inhibitor in *Aurelia* or by 3.0 μg/ml in *Chrysaora*. Both initiation and completion of constrictions, as well as development of ephyral organs were prevented. It is concluded that continuous RNA synthesis is essential for all events of strobilation. Specific RNA reserves are probably not accumulated prior to strobilation.

The simplest explanation for the apparent decrease in the RNA/DNA ratio is that DNA synthesis and cell division occur more rapidly than RNA synthesis during this phase of development. If this

TABLE 2. Macromolecule ratios in strobilating polyps of *Chrysaora*. Values are μg per μg DNA ± standard deviation. Numbers of replicate batches of polyps (20 to 50 per batch) are in parenthesis.

| STAGE | RNA/DNA | PROTEIN/DNA |
|---|---|---|
| Unstrobilated Polyp | 10.1 ± 1.9 (8) | 30.5 ± 11.7 (8) |
| Early Strobila | 11.0 ± 1.1 (4) | 33.8 ± 11.6 (4) |
| Mid-Strobila | 7.3 ± 1.7 (5) | 18.1 ± 9.0 (5) |
| Late Strobila | 7.8 ± 1.1 (4) | 21.1 ± 8.2 (3) |
| Ephyra | 5.5 ± 1.5 (5) | 22.7 ± 10.0 (5) |
| Polyp After Strobilation | 5.4 ± 1.3 (2) | 18.0 ± 10.5 (2) |

TABLE 3. Nucleic acid and protein in conditioned and nonconditioned polyps. DNA values are in μg per polyp. Fifty polyps were assayed per batch, and n = number of relicate batches.

| | CONDITIONED | NONCONDITIONED |
|---|---|---|
| *Chrysaora* (n = 6) | | |
| DNA | 1.3 ± 0.2 | 0.6 ± 0.1 |
| RNA/DNA | 18.9 ± 3.7 | 15.3 ± 3.3 |
| Protein/DNA | 79 ± 18 | 104 ± 25 |
| *Aurelia* (n = 4) | | |
| DNA | 0.6 ± 0.2 | 0.4 ± 0.2 |
| RNA/DNA | 8.5 ± 5.8 | 6.7 ± 3.7 |
| Protein/DNA | 27 ± 20 | 25 ± 25 |

is correct, some of the new DNA produced during strobilation is not transcribed until later, during the growth and development of the released ephyrae. The major proportion of cellular RNA is ribosomal, and one may suspect that the synthesis of at least this RNA lags behind cell division during strobilation. This situation would be analogous to that found in cleaving eggs of sea urchins and some amphibians (Balinsky, 1970).

Summary

In *Aurelia*, the amount of DNA per polyp increases about 3-fold during strobilation, whereas RNA and protein remain relatively constant.

The RNA/DNA ratio drops by 50% during strobilation in *Chrysaora* and 28% to 50% in *Aurelia*.

Actinomycin D (1.0-3.0 μg/ml) blocks strobilation immediately in both species.

The size and DNA content of polyps are increased by prolonged treatment at 12° to 15°C prior to strobilation; however, the RNA/DNA and protein/DNA ratios are not significantly increased by cold conditioning.

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## 2 - An Ultrastructural Study of Strobilation in *Chrysaora quinquecirrha* With Special Reference to Neurosecretion

M. A. Dietz and K. L. Webb

The coelenterate nervous system controls a number of morphogenetic processes. Burnett, Diehl, and Diehl (1964) found growth and regeneration in *Hydra* to be controlled by neurosecretory cells. The initiation of sexuality in *Hydra* is also related to neurosecretory activity (Burnett and Diehl, 1964). Lesh and Burnett (1966) extracted an inducer from the hypostomal region of *Hydra*. This inducer appears to control the direction of cell differentiation of interstitial cells along the body. Destruction of nerve cells reduces inducer activity. This fact and the previous work with *Hydra* suggest that the inducer is a product of neurosecretory activity.

Strobilation is a unique metamorphic process by which jellyfish scyphistomae produce ephyrae that later develop into medusae. It is by this process that a change from the asexual to the sexual generation is accomplished. The changes in gross morphology which occur during strobilation have been described by a number of investigators, including Thiel (1938), Hyman (1940), Spangenberg (1968), and Loeb (1970).

Spangenberg, working with *Aurelia aurita,* uses the term 'strobilation' to refer to two separate processes: segmentation and metamorphosis. Loeb (1970), working with *Chrysaora quinquecirrha,* recognizes an additional process which she refers to as neck formation. Previous ultrastructural studies involving *Chrysaora quinquecirrha* have been concerned primarily with the nematocysts (Burnett and Sutton, 1969; Sutton and Burnett, 1969) and the tentacle muscles of the medusa

(Perkins, et al., 1971). The ultrastructure of scyphistomae and strobilae of *Chrysaora quinquecirrha* representing each of these stages of strobilation is examined in this study. On the basis of the findings in *Hydra* mentioned previously, attention is focused on the nerve elements, particularly the neurosecretory cells.

Strobilating polyps were randomly picked from the cultures and were divided into three classes on the basis of their stage of development:

1. Neck Formation: Polyps with a distinct constriction beneath the base of the tentacles (Fig. 3).
2. Segmentation: Polyps with a series of constrictions which divide the body into a number of segments. The number of segments is variable, and may range from one to 16. The tentacles are still present at this stage, and ephyrae are not yet being released (Fig. 4).
3. Metamorphosis: Polyps with mature ephyrae. The tentacles, septal muscles, and atrichous polyspiras nematocysts have been destroyed, and a number of new structures, including lappets, rhopalia, manubria, etc., have developed (Fig. 5).

Scyphistomae and strobilae were fixed in 3% glutaraldehyde and post-fixed in 1% osmium tetroxide according to the rapid method of Hayat and Giaquinta (1970). The osmolality of the fixatives were corrected to that of the culture medium with sodium chloride.

The polyps were cut through the hypostomal region, slightly below the bases of the tentacles (Fig. 1-b). All of the sectioning was done with a Porter-Blum Mt-2B ultramicrotome. The sections were stained with uranyl acetate and Reynold's lead citrate and were examined with a Zeiss EM 9S-2 electron microscope. A Zeiss Photoscope II was used for macro-photography. Sample sizes of 5-10 polyps were examined at each stage; results are based on the examination of 15-25 sections per polyp.

Examination of thin sections of *Chrysaora* scyphistomae reveals the typical coelenterate body plan with a loosely arranged epidermis and a dense, glandular gastrodermis separated by a collagenous mesoglea (Chapman, 1966). Large intracellular spaces are common in the epidermal layer. The epidermis is composed of epitheliomuscular cells, cnidoblasts, interstitial cells, and nerve cells. The nerve cells may be classified as neurosensory, ganglionic, or neurosecretory. Neurosensory cells are easily distinguished by the presence of an apical sensory hair. They are generally found between the epitheliomuscular cells with the apical flagellum projecting from the surface. Both ganglionic and neurosecretory cells occur at the bases of epitheliomuscular cells. They are similar in structure and are characterized by an irregular nucleus with several nucleoli, free ribosomes with little or no endoplasmic reticulum, glycogen granules in the perikaryon, microtubules, and complex Golgi. However, neurosecretory cells may be distinguished by the presence of electron-dense, membrane-bounded granules. These granules vary in size from 100-160 nm and may be seen in association with the Golgi or scattered throughout the perikaryon.

Neurites of neurosecretory and ganglionic cells in the scyphistoma often occur in groups and may be seen lying adjacent to the mesoglea (Fig. 9). Microtubules are frequently seen in the neurites, but neurosecretory granules are seldom present at this stage.

In the scyphistoma, glycogen granules are abundantly scattered through many of the cells of the epidermis (Fig. 6). Although some glycogen is always present, there appears to be a distinct decrease in the number of granules during strobilation (Fig. 7).

In addition to the decreases in glycogen, several other changes appear to accompany strobilation. The gross morphology of the polyp changes drastically. After the body becomes constricted into a series of segments, the tentacles are resorbed and feeding ceases until all of the segments have matured into ephyrae and are released. During this time there is frequently an increase in pigmentation, with the polyps ranging from light pink to red.

During neck-formation, segmentation, and metamorphosis, large bundles of muscle fibers appear along the inner edge of the epidermis (Figs. 10, 11). Although thin bands of muscle may be seen in this area in scyphistomae, they never occur in large bundles. It may be that the initial constrictions which occur during strobilation are the result of muscle contraction.

Several changes occur with respect to the neurosecretory material. In scyphistomae and during neck-formation, neurosecretory cells are easily recognized by the presence of neurosecretory granules (Fig. 8). Occasionally some neurosecretory granules may be seen in the neurites, but this is very infrequent. During segmentation and metamorphosis, very few cells are clearly identifiable as neurosecretory. Without the presence of the dense, membrane-bounded granules, ganglionic and neurosecretory cells are practically indistinguishable. In segmented polyps the disappearance of the dense, membrane-bounded granules from the neurosecretory cell body is accomplished by a dramatic increase in the occurrence of dense, membrane-bounded granules in the neurites (Figs. 10, 11). These granules range in size from 90-150 nm and are similar in appearance to the neurosecretory granules seen in earlier stages. During metamorphosis, most of the neurites are again devoid of granules or contain granules that appear less full. Neurosecretory cells are again difficult to distinguish (Fig. 12, 13).

The figures are reproduced in the Master of Science Thesis of Marsha Dietz, School of Marine Science, College of William and Mary.

JOB NO. 3 - NATURAL CONTROL AGENTS

OBJECTIVE: To explore the utility of natural toxins and diseases as control agents.

## Attempts to Culture Microbes From Jellyfish Tentacles

F. O. Perkins, D. S. Haven, and R. Morales

During August of 1971, *Chrysaora quinquecirrha* jellyfishes with degenerated tentacles were obtained from the Elizabeth River. The specimen, that had been frozen in seawater, was used for innoculating several plates of the various media tabulated. These microbial analyses were performed by the VIMS Department of Microbiology and Pathology, Dr. Frank O. Perkins, Chairman.

The only growth which was observed was that of *Aspergillus* and *Penicillium* molds. There were no growths identifiable as other fungi or protozoa.

In view of the lack of evidence of protozoans, microbes and other organisms, an identifiable parasitic organism for the jellyfish has not been found.

It should be emphasized at this time that these studies were necessarily of a superficial nature and have not been conducted in a concerted effort. It is quite possible that organisms could have been lost in transfer or preparation of the samples or that the media used were not appropriate. The availability of a parasitic organism still remains hypothetical at this time.

# MEDIA FOR MICROBE CULTURING

1. Fluid Thioglycollate Medium (Difco)
2. 10% Beef Serum (Difco)
3. Fullers Medium
4. Trypticase™ Soy Broth (BBL)
5. MV (gelatin, liver extract, yeast extract, peptone, glucose)
6. Cerophyl Seawater (Cerophyl Laboratories)
7. Nutrient Agar Medium (Difco)
8. Yeast - Peptone - Dextrose
9. Medium # 1 (modified MV containing cholesterol)
10. Goldstein's Medium

JOB NO. 4 - IDENTIFICATION OF INHIBITORS

OBJECTIVE - To identify substances that are capable of inhibiting strobilation, inducing it out of season, or causing direct mortality to any stage of the life cycle.

1 - Mortality Studies

D. S. Haven and R. Morales-Alamo

The last series of toxicity tests conducted on *Chrysaora* during this contract period was concluded on 1 July 1971. By that date the available supply of polyps had been depleted. Most of our efforts between July and September were spent collecting medusae and tending to the polyps being raised from them in the laboratory. The scarcity of medusae throughout the lower Chesapeake Bay in the summer of 1971, added to a lower than usual set of planulae in the laboratory, required that a greater length of time than usual be spent on field collections.

A total of 76 toxicity tests were conducted during the period 18 April to 1 July 1971, including 48 tests on polyps and 28 on ephyrae. Results of the test with Tetraphenylarsonium bromide had to be discarded because of mortalities in the controls. Polyps used in all but one test (that of 18 April with Malachite Green oxalate) were collected from natural oyster shell substrate collected at West Point on the Corrotoman River on 12 May. Ephyrae used were released in the laboratory by these polyps. With one exception (Malachite Green oxalate), chemicals tested on polyps had not been tested previously while those tested on ephyrae were selected from those most effective in previous tests on polyps of *Chrysaora* or ephyrae of *Aurelia*.

Results obtained in the April to June series of tests appear on Table 4. Table 5 combines the results obtained with *Chrysaora* ephyrae with those obtained in previous tests with other life stages of *Chrysaora* and *Aurelia*. Tabulated summaries of results obtained with the chemicals having the greatest effect to date on polyps and ephyrae of *Chrysaora* appear on Tables 6 and 7. An appendix to these tables gives complete names of chemicals.

Tubercidin and triphenyltin chloride are the two chemicals found to be most toxic among those tested so far. They have caused 100 percent mortality on all life stages tested at all concentrations. Tubercidin was completely effective on ephyrae of *Chrysaora* down to a concentration of 0.05. It has not been tested on polyps at concentrations lower than 1 ppm. Triphenyltin chloride had caused total mortality on polyps of *Chrysaora* and *Aurelia* and ephyrae of *Chrysaora* at 0.1 ppm and ephyrae of *Aurelia* at 1 ppm (the highest concentration tested).

The other two chemicals causing high mortalities were 2, 3-Dichloro-1,4-naphthoquinone and Bromsalans. 2, 3-Dichloro-1,4-naphthoquinone was much more effective on ephyrae of *Chrysaora* than on its polyps or the polyps of *Aurelia*. It caused 100 percent mortality on the ephyrae of *Chrysaora* at 0.1 ppm but none on the polyps of *Chrysaora* at 0.1 ppm, 1 ppm, or lower, nor on the polyps of *Aurelia* at 0.5 and 0.1 ppm. Bromsalans caused 100 percent mortality on ephyrae of *Chrysaora* at 0.1 ppm and on the polyps at the lowest concentration tested (1 ppm). It was not as effective on polyps of *Aurelia* as on ephyrae and polyps of *Chrysaora*, having caused a relatively low 20-25 percent mortality at 1 ppm and more at 0.5 and 0.1 ppm on the former.

TABLE 4

RESULTS OF TOXICITY TESTS ON EPHYRAE AND POLYPS OF CHRYSAORA, APRIL TO JUNE, 1971

JELLYFISH MCRTALITY STUDIES
PERCENT MORTALITY AFTER 24-HR EXPCSURE

CCDE FCR SPECIES C=CHRYSAORA A=AURELIA E=EPHYRAE P=PCLYPS M=MEDUSAE

| SPECIES | CCNCTCN | CHEMNBR | CHEMICAL | DATE | CHEMICAL CONCENTRATION (PPM) 10 | 5 | 1 | 0.5 | 0.1 | 0.05 | 0.01 | CARRIER | CARRIER CONTROL (EQUIVALENT PPM) 10 | 5 | 1 | WATER CONTROL WC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EPHYRAE OF CHRYSAORA | | | | | | | | | | | | | | | | |
| CE | N | 3 | 3'-CHLORO-3-NITROSALICYLANIL. | 052771 | ***** | ***** | 80.0 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 4 | CATECHCL | 052671 | ***** | ***** | 100.0 | 100.0 | 50.0 | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CE | N | 10 | 5-CHLORO-2-NITROPHENOL | 052871 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 13 | 4'-BRCMO-3-NITROSALICYLANIL. | 052771 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 17 | 3'-BRCMO-3-NITROSALICYLANIL. | 052771 | ***** | ***** | 70.0 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 18 | SYNERG. MIXT&4'-CL-4,3,NITRSAL | 052771 | ***** | ***** | 45.4 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 21 | 4'-BROMO-5-NITROSALCYLANIL. | 052771 | ***** | ***** | 100.0 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 25 | 4',5-DIBRCM-3-NITROSALICYLANIL | 052571 | ***** | ***** | 100.0 | 100.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 26 | P-ICCOANILINE | 060371 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 30 | ISCM. MIXT.4'-CL-4,3,NITRSAL. | 052871 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 31 | 4'-IODO-5-NITRCSALICYLANIL. | 052571 | ***** | ***** | 100.0 | 100.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 32 | 2'-CHLORO-3-NITROSALICYLANIL. | 052871 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 34 | 3'-ICDO-3-NITRCSALICYLANILIDE | 052871 | ***** | 0.0 | 0.0 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 39 | TETRAPHENYLARSCNIUM BROMIDE | 052671 | ***** | ***** | 29.9 | 33.3 | 0.0 | ***** | ***** | 2 | ***** | ***** | 20.0 | 37.5 |
| CE | N | 40 | SCDIUM TETRATHIONATE | 060371 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 46 | TRIPHENYLTIN CHLORIDE | 052671 | ***** | ***** | 100.0 | 100.0 | 100.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 47 | PENTACHLORCPHENOL | 052671 | ***** | ***** | 87.5 | 0.0 | 0.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 48 | 2,3-DICHLORO-1,4-NAPHTHOQUIN. | 052671 | ***** | ***** | 100.0 | 100.0 | 100.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 50 | MALACHITE GREEN OXALATE | 052571 | ***** | ***** | 100.0 | 100.0 | 90.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 53 | BRCMSALANS | 052571 | ***** | ***** | 100.0 | 100.0 | 100.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 55 | C-9491 | 052771 | ***** | ***** | 100.0 | 100.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 57 | C-9491 OXYGEN ANALOG | 052671 | ***** | ***** | 14.2 | 0.0 | 0.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 77 | 5-IODOURIDINE | 060371 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 80 | 5-CHLORCURIDINE | 060371 | ***** | ***** | 100.0 | 0.0 | 0.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 82 | PSEUDOURIDINE | 060371 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 86 | TUBERCIDIN | 052571 | ***** | ***** | 100.0 | 100.0 | 100.0 | 100.0 | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 92 | P-AMINOPHENOL | 052571 | ***** | ***** | 100.0 | 100.0 | 0.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| POLYPS OF CHRYSAORA | | | | | | | | | | | | | | | | |
| CP | N | 99 | DEOXYCYTIDINE | 061471 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 100 | 5-BROMODEOXYURIDINE | 061471 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 101 | DEOXYGUANOSINE | 061471 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 102 | ADENOSINE MONOPHOSPHORIC AC. | 061571 | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | 0.0 | 0.0 | ***** | 0.0 |
| CP | N | 103 | 5-BROMODEOXYCYTIDINE | 061471 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 104 | 5-AZACYTIDINE | 061571 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 105 | GUANOSINE 2H2O | 061571 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 106 | 6-MERCAPTOPURINE RIBOSIDE | 061771 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 107 | ADENINE | 061771 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 108 | 2,8-DIHYDROXYADENINE | 061771 | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | 0.0 | 0.0 | ***** | 0.0 |

TABLE 4 (continued)

JELLYFISH MORTALITY STUDIES
PERCENT MORTALITY AFTER 24-HR EXPOSURE

CODE FOR SPECIES C=CHRYSAORA A=AURELIA E=EPHYRAE P=POLYPS M=MEDUSAE

| SPECIES | CONC N | CHEM NUMBER | CHEMICAL | DATE | CHEMICAL CONCENTRATION (PPM) 10 | 5 | 1 | 0.5 | 0.1 | 0.05 | 0.01 | CARRIER | CARRIER CONTROL (EQUIVALENT PPM) 10 | 5 | 1 | WATER CONTROL WC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CP | N | 109 | HYPOXANTHINE | 062271 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 3 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 110 | 6-METHOXYPURINE | 061771 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 111 | XANTHINE | 062271 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 3 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 112 | 6-THIOXANTHINE | 062271 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 3 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 113 | 6-CHLOROPURINE | 062271 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 3 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 114 | 2-HYDROXY-6-METHYL PURINE | 062271 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 3 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 115 | PURINE RIBOSIDE | 062271 | ***** | ***** | 0.0 | ***** | ***** | ***** | ***** | 3 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 115 | PURINE RIBOSIDE | 063071 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 116 | 2'-DEOXYINOSINE | 062371 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 117 | XANTHOSINE | 062371 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 118 | PURINE | 062371 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 119 | ADENOSINE-N1-OXIDE | 062371 | ***** | 100.0 | 36.3 | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 119 | ADENOSINE-N1-OXIDE | 062871 | ***** | 100.0 | 100.0 | 0.0 | 0.0 | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 120 | 6-ISOPROPOXYPURINE | 062371 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 121 | 6-IODOPURINE | 062371 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 122 | 6-BENZYL M AMINOPURINE | 062471 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 123 | 2'-DEOXYADENOSINE | 062471 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 128 | KINETIN(6-FURFURYLAMIN PURINE | 062871 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 129 | 6-HYDRAZINOPURINE | 062871 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 130 | ADENINE-N1-OXIDE | 062871 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 131 | CORDYCEPIN | 062871 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 132 | 7-METHYL XANTHOSINE | 062871 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 133 | 6-HYDROXYLAMINPURIN RIBOSIDE | 062871 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 134 | 1-METHYL ADENOSINE | 062971 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 135 | 6-CHLOROPURINE RIBOSIDE | 062971 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 136 | KINETINRIBOSIDE | 062971 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 137 | PUROMYCIN AMINONUCLEOSIDE | 062971 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 138 | 6-SELENO-PURINE | 062971 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 139 | 6-AMINO-2,8-DICHLOROPURINE | 062971 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 140 | BLUE TETRAZOLIUM | 063071 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 2 | ***** | 0.0 | 0.0 | 0.0 |
| CP | N | 141 | P-TERT.-BUTYLPHENOL | 063071 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 2 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 142 | 4-CHLORO-2-NITROANILINE | 063071 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 143 | CHLORAL HYDRATE | 063071 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 1 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 144 | BISMARCK BROWN | 063071 | ***** | 100.0 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 145 | CETYL PYRIDINIUM BROMIDE | 070171 | ***** | 28.5 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 146 | BENZOTRIAZOLE | 070171 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 2 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 149 | CAFFEINE | 070171 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 150 | CETYL PYRIDINIUM CHLORIDE | 070171 | ***** | 100.0 | 100.0 | 83.3 | ***** | ***** | ***** | 0 | ***** | 0.0 | ***** | 0.0 |

RESULTS OF TOXICITY TESTS ON DIFFERENT LIFE STAGES OF CHRYSAORA AND AURELIA.
INCLUDES ONLY CHEMICALS TESTED ON CHRYSAORA EPHYRAE IN APRIL-JUNE 1971

JELLYFISH MORTALITY STUDIES
PERCENT MORTALITY AFTER 24-HR EXPOSURE

CODE FOR SPECIES C=CHRYSAORA E=EPHYRAE
A=AURELIA P=POLYPS
M=MEDUSAE

| SPECIES | CCNDTCN | CHEMNBR | CHEMICAL | DATE | CHEMICAL CONCENTRATION (PPM) 10 | 5 | 1 | 0.5 | 0.1 | 0.05 | 0.01 | CARRIER | CARRIER CONTROL (EQUIVALENT PPM) 10 | 5 | 1 | WATER CONTROL WC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CE | N | 3 | 3'-CHLORO-3-NITROSALICYLANIL. | 052771 | ***** | ***** | 80.0 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 3 | 3'-CHLORO-3-NITROSALICYLANIL. | 082770 | 76.4 | 29.6 | ***** | 0.0 | ***** | ***** | ***** | 1 | 0.0 | 0.0 | ***** | 0.0 |
| CP | N | 3 | 3'-CHLORO-3-NITROSALICYLANIL. | 111870 | 100.0 | 12.5 | 0.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| AE | N | 3 | 3'-CHLORO-3-NITROSALICYLANIL. | 010571 | 100.0 | 100.0 | 81.2 | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CE | N | 4 | CATECHOL | 052671 | ***** | ***** | 100.0 | 100.0 | 50.0 | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CM | N | 4 | CATECHOL | 090370 | 100.0 | 100.0 | 100.0 | 100.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 4 | CATECHOL | 100170 | 59.9 | 44.4 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | N | 4 | CATECHOL | 090470 | 50.0 | 50.0 | 0.0 | 0.0 | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| AE | N | 4 | CATECHOL | 010571 | 87.5 | 85.7 | 0.0 | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CE | N | 10 | 5-CHLORO-2-NITROPHENOL | 052871 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 10 | 5-CHLORO-2-NITROPHENOL | 101370 | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| AE | N | 10 | 5-CHLORO-2-NITROPHENOL | 010571 | 37.5 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CE | N | 13 | 4'-BROMO-3-NITROSALICYLANIL. | 052771 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 13 | 4'-BROMO-3-NITROSALICYLANIL. | 111970 | 87.5 | 100.0 | 0.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| AE | N | 13 | 4'-BROMO-3-NITROSALICYLANIL. | 010671 | 100.0 | 80.0 | 77.7 | ***** | ***** | ***** | ***** | 1 | 0.0 | 0.0 | 0.0 | 0.0 |
| AP | N | 13 | 4'-BROMO-3-NITROSALICYLANIL. | 111970 | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| CE | N | 17 | 3'-BROMO-3-NITROSALICYLANIL. | 052771 | ***** | ***** | 70.0 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 17 | 3'-BROMO-3-NITROSALICYLANIL. | 112470 | 72.7 | 83.3 | 0.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| CP | N | 17 | 3'-BROMO-3-NITROSALICYLANIL. | 010871 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| AP | N | 17 | 3'-BROMO-3-NITROSALICYLANIL. | 112470 | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| CE | N | 18 | SYNERG. MIXT&4'-CL-4,3,NITRSAL | 052771 | ***** | ***** | 45.4 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 18 | SYNERG. MIXT&4'-CL-4,3,NITRSAL | 112570 | 66.6 | 100.0 | 0.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| CE | N | 21 | 4'-BROMO-5-NITROSALCYLANIL. | 052771 | ***** | ***** | 100.0 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 21 | 4'-BROMO-5-NITROSALCYLANIL. | 112770 | 70.0 | 71.4 | 0.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| AE | N | 21 | 4'-BROMO-5-NITROSALCYLANIL. | 010671 | 100.0 | 100.0 | 100.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | 0.0 | 0.0 | 0.0 |
| AP | N | 21 | 4'-BROMO-5-NITROSALCYLANIL. | 112770 | 100.0 | ***** | ***** | ***** | ***** | ***** | ***** | 0 | 0.0 | ***** | ***** | 0.0 |
| CE | N | 25 | 4',5-DIBROM-3-NITROSALICYLANIL | 052571 | ***** | ***** | 100.0 | 100.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 25 | 4',5-DIBROM-3-NITROSALICYLANIL | 120170 | 100.0 | 100.0 | 100.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| CP | N | 25 | 4',5-DIBROM-3-NITROSALICYLANIL | 010871 | ***** | ***** | 58.3 | 50.0 | 0.0 | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| AE | N | 25 | 4',5-DIBROM-3-NITROSALICYLANIL | 010571 | 100.0 | 100.0 | 100.0 | 100.0 | 37.5 | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| AP | N | 25 | 4',5-DIBROM-3-NITROSALICYLANIL | 120170 | 100.0 | 83.3 | ***** | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| AP | N | 25 | 4',5-DIBROM-3-NITROSALICYLANIL | 010871 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| CE | N | 26 | P-IODOANILINE | 060371 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 26 | P-IODOANILINE | 120270 | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| AP | N | 26 | P-IODOANILINE | 120270 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |

TABLE 5 (continued)

JELLYFISH MORTALITY STUDIES
PERCENT MORTALITY AFTER 24-HR EXPOSURE

CODE FOR SPECIES C=CHRYSAORA E=EPHYRAE
A=AURELIA P=POLYPS
M=MEDUSAE

| SPECIES | CONC | CHEM NBR | CHEMICAL | DATE | CHEMICAL CONCENTRATION (PPM) 10 | 5 | 1 | 0.5 | 0.1 | 0.05 | 0.01 | CARRIER | CARRIER CONTROL (EQUIVALENT PPM) 10 | 5 | 1 | WATER CONTROL WC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CE | N | 30 | ISOM. MIXT.4'-CL-4,3,NITRSAL. | 052871 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 30 | ISOM. MIXT.4'-CL-4,3,NITRSAL. | 120870 | 55.5 | 66.6 | 0.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| AP | N | 30 | ISOM. MIXT.4'-CL-4,3,NITRSAL. | 120870 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CE | N | 31 | 4'-IODO-5-NITROSALICYLANIL. | 052571 | ***** | ***** | 100.0 | 100.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 31 | 4'-IODO-5-NITROSALICYLANIL. | 120870 | 100.0 | 100.0 | 100.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| CP | N | 31 | 4'-IODO-5-NITROSALICYLANIL. | 010871 | ***** | 100.0 | 100.0 | 42.8 | 0.0 | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| AP | N | 31 | 4'-IODO-5-NITROSALICYLANIL. | 120870 | 80.0 | 0.0 | ***** | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CE | N | 32 | 2'-CHLORO-3-NITROSALICYLANIL. | 052871 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 32 | 2'-CHLORO-3-NITROSALICYLANIL. | 120970 | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| AE | N | 32 | 2'-CHLORO-3-NITROSALICYLANIL. | 010671 | 87.5 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | 0.0 | 0.0 | 0.0 |
| AP | N | 32 | 2'-CHLORO-3-NITROSALICYLANIL. | 120970 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CE | N | 34 | 3'-IODO-3-NITROSALICYLANILIDE | 052871 | ***** | 0.0 | 0.0 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 34 | 3'-IODO-3-NITROSALICYLANILIDE | 120970 | 33.3 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| AP | N | 34 | 3'-IODO-3-NITROSALICYLANILIDE | 120970 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CE | N | 39 | TETRAPHENYLARSONIUM BROMIDE | 052671 | ***** | ***** | 29.9 | 33.3 | 0.0 | ***** | ***** | 2 | ***** | ***** | 20.0 | 37.5 |
| CP | N | 39 | TETRAPHENYLARSONIUM BROMIDE | 121670 | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | 0.0 | 0.0 | 0.0 | 0.0 |
| AP | N | 39 | TETRAPHENYLARSONIUM BROMIDE | 121670 | 100.0 | 57.1 | ***** | ***** | ***** | ***** | ***** | 2 | 0.0 | 0.0 | 0.0 | 0.0 |
| CE | N | 40 | SODIUM TETRATHIONATE | 060371 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 40 | SODIUM TETRATHIONATE | 121670 | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | 0.0 | 0.0 | 0.0 | 0.0 |
| AP | N | 40 | SODIUM TETRATHIONATE | 121670 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | ***** | 2 | 0.0 | 0.0 | 0.0 | 0.0 |
| CE | N | 46 | TRIPHENYLTIN CHLORIDE | 052671 | ***** | ***** | 100.0 | 100.0 | 100.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 46 | TRIPHENYLTIN CHLORIDE | 011471 | 100.0 | 100.0 | 100.0 | ***** | ***** | ***** | ***** | 2 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 46 | TRIPHENYLTIN CHLORIDE | 012771 | ***** | ***** | 100.0 | 100.0 | 100.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| AE | N | 46 | TRIPHENYLTIN CHLORIDE | 011471 | ***** | 100.0 | 100.0 | 100.0 | ***** | ***** | ***** | 2 | ***** | 100.0 | ***** | 100.0 |
| AP | N | 46 | TRIPHENYLTIN CHLORIDE | 011471 | 100.0 | 100.0 | 100.0 | ***** | ***** | ***** | ***** | 2 | ***** | 0.0 | ***** | 0.0 |
| AP | N | 46 | TRIPHENYLTIN CHLORIDE | 012771 | ***** | ***** | 100.0 | 100.0 | 100.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 47 | PENTACHLOROPHENOL | 052671 | ***** | ***** | 87.5 | 0.0 | 0.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 47 | PENTACHLOROPHENOL | 011471 | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | 2 | ***** | 0.0 | ***** | 0.0 |
| CP | N | 47 | PENTACHLOROPHENOL | 012871 | 63.6 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | 0.0 | ***** | ***** | 0.0 |
| AE | N | 47 | PENTACHLOROPHENOL | 011471 | ***** | 100.0 | 100.0 | 100.0 | ***** | ***** | ***** | 2 | ***** | 100.0 | ***** | 100.0 |
| AP | N | 47 | PENTACHLOROPHENOL | 012871 | 100.0 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | 0.0 | ***** | ***** | 0.0 |
| CE | N | 48 | 2,3-DICHLORO-1,4-NAPHTHOQUIN. | 052671 | ***** | ***** | 100.0 | 100.0 | 100.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 48 | 2,3-DICHLORO-1,4-NAPHTHOQUIN. | 012071 | 100.0 | 100.0 | 0.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| CP | N | 48 | 2,3-DICHLORO-1,4-NAPHTHOQUIN. | 012771 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| AP | N | 48 | 2,3-DICHLORO-1,4-NAPHTHOQUIN. | 012071 | 100.0 | 100.0 | 100.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |

TABLE 5 (continued)

JELLYFISH MORTALITY STUDIES
PERCENT MORTALITY AFTER 24-HR EXPOSURE

CODE FOR SPECIES C=CHRYSAORA A=AURELIA E=EPHYRAE P=POLYPS M=MEDUSAE

| SPECIES | CONDITION | CHEM NUMBER | CHEMICAL | DATE | CHEMICAL CONCENTRATION (PPM) 10 | 5 | 1 | 0.5 | 0.1 | 0.05 | 0.01 | CARRIER | CARRIER CONTROL (EQUIVALENT PPM) 10 | 5 | 1 | WATER CONTROL WC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AP | N | 48 | 2,3-DICHLORO-1,4-NAPHTHOQUIN. | 012771 | ***** | ***** | 80.0 | 40.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 59.9 | 0.0 |
| CE | N | 50 | MALACHITE GREEN OXALATE | 052571 | ***** | ***** | 100.0 | 100.0 | 90.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 50 | MALACHITE GREEN OXALATE | 012071 | 100.0 | 100.0 | 100.0 | ***** | ***** | ***** | ***** | 2 | 0.0 | ***** | ***** | 0.0 |
| CP | N | 50 | MALACHITE GREEN OXALATE | 012771 | ***** | ***** | 100.0 | 100.0 | 83.3 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 50 | MALACHITE GREEN OXALATE | 041871 | ***** | ***** | 100.0 | 33.3 | 0.0 | 0.0 | 0.0 | 2 | 0.0 | ***** | ***** | 0.0 |
| AP | N | 50 | MALACHITE GREEN OXALATE | 012071 | 100.0 | 100.0 | 100.0 | ***** | ***** | ***** | ***** | 2 | 0.0 | ***** | ***** | 0.0 |
| AP | N | 50 | MALACHITE GREEN OXALATE | 012771 | ***** | ***** | 100.0 | 100.0 | 20.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CE | N | 53 | BROMSALANS | 052571 | ***** | ***** | 100.0 | 100.0 | 100.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 53 | BROMSALANS | 012171 | 100.0 | 100.0 | 100.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| AP | N | 53 | BROMSALANS | 012171 | 100.0 | 66.6 | 25.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| AP | N | 53 | BROMSALANS | 012771 | ***** | ***** | 20.0 | 0.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 59.9 | 0.0 |
| CE | N | 55 | C-9491 | 052771 | ***** | ***** | 100.0 | 100.0 | 0.0 | ***** | ***** | 1 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 55 | C-9491 | 012171 | 100.0 | 100.0 | 0.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| AP | N | 55 | C-9491 | 012171 | 100.0 | 100.0 | 0.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| | | 0 | | | ***** | ***** | ***** | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | ***** |
| CE | N | 57 | C-9491 OXYGEN ANALOG | 052671 | ***** | ***** | 14.2 | 0.0 | 0.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 57 | C-9491 OXYGEN ANALOG | 012871 | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| AP | N | 57 | C-9491 OXYGEN ANALOG | 012871 | 100.0 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 1 | 0.0 | ***** | ***** | 0.0 |
| CE | N | 77 | 5-IODOURIDINE | 060371 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 77 | 5-IODOURIDINE | 030971 | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | 0.0 | ***** | ***** | 0.0 |
| CP | U | 77 | 5-IODOURIDINE | 030971 | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | 0.0 | ***** | ***** | 0.0 |
| CE | N | 80 | 5-CHLOROURIDINE | 060371 | ***** | ***** | 100.0 | 0.0 | 0.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 80 | 5-CHLOROURIDINE | 031071 | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 0 | ***** | ***** | ***** | 0.0 |
| CP | U | 80 | 5-CHLOROURIDINE | 031071 | 0.0 | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | 0.0 | ***** | ***** | 0.0 |
| CE | N | 82 | PSEUDOURIDINE | 060371 | ***** | ***** | 0.0 | 0.0 | 0.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CP | U | 82 | PSEUDOURIDINE | 031071 | 100.0 | 100.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | 100.0 | ***** | ***** | 0.0 |
| CP | N | 82 | PSEUDOURIDINE | 061471 | ***** | 0.0 | 0.0 | ***** | ***** | ***** | ***** | 2 | ***** | 0.0 | ***** | 0.0 |
| CE | N | 86 | TUBERCIDIN | 052571 | ***** | ***** | 100.0 | 100.0 | 100.0 | 100.0 | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 86 | TUBERCIDIN | 031771 | 100.0 | 100.0 | 100.0 | ***** | ***** | ***** | ***** | 2 | 0.0 | ***** | ***** | 0.0 |
| CP | U | 86 | TUBERCIDIN | 031771 | 100.0 | 100.0 | 100.0 | ***** | ***** | ***** | ***** | 2 | 0.0 | ***** | ***** | 0.0 |
| AP | N | 86 | TUBERCIDIN | 031771 | 100.0 | 100.0 | 100.0 | ***** | ***** | ***** | ***** | 2 | 0.0 | ***** | ***** | 0.0 |
| CE | N | 92 | P-AMINOPHENOL | 052571 | ***** | ***** | 100.0 | 100.0 | 0.0 | ***** | ***** | 2 | ***** | ***** | 0.0 | 0.0 |
| CP | N | 92 | P-AMINOPHENOL | 032571 | 93.7 | 59.9 | 0.0 | ***** | ***** | ***** | ***** | 2 | 0.0 | ***** | ***** | 0.0 |
| CP | U | 92 | P-AMINOPHENOL | 032571 | 100.0 | 68.1 | 0.0 | ***** | ***** | ***** | ***** | 2 | 0.0 | ***** | ***** | 0.0 |
| AP | N | 92 | P-AMINOPHENOL | 032571 | 100.0 | 100.0 | 25.0 | ***** | ***** | ***** | ***** | 2 | 0.0 | ***** | ***** | 0.0 |

TABLE 6. Percent mortality of *C. quinquecirrha* polyps after 24-hour immersion in different concentrations of the chemicals found to be the most effective to date.

| Chemical | Concentration (PPM) 1.0 | 0.5 | 0.1 |
|---|---|---|---|
| 4',5-Dibromo-3-nitrosalicylanilide | 58.3 | 50.0 | 0.0 |
| 4'-Iodo-5-nitrosalicylanilide | 100.0 | 42.8 | 0.0 |
| Triphenyltin chloride | 100.0 | 100.0 | 100.0 |
| Malachite Green Oxalate | 100.0 | 33.0 | 0.0 |
| Bromsalans | 100.0 | - | - |
| Tubercidin | 100.0 | - | - |
| Cetyl pyridinium chloride | 100.0 | 83.3 | - |
| Adenosine-$N^1$-oxide | 100.0 | 0.0 | 0.0 |

TABLE 7. Percent mortality of *C. quinquecirrha* ephyrae after 24-hour immersion in different concentrations of the chemicals found to be the most effective to date.

| | CONCENTRATION (PPM) 1 | 0.5 | 0.1 | 0.05 |
|---|---|---|---|---|
| 3'-Chloro-3-nitrosalicylanilide | 80.0 | 0.0 | 0.0 | -- |
| Catechol | 100.0 | 100.0 | 50.0 | -- |
| 3'-Bromo-3-nitrosalicylanilide | 70.0 | 0.0 | 0.0 | -- |
| 4'-Bromo-5-nitrosalicylanilide | 100.0 | 0.0 | 0.0 | -- |
| 4',5-Dibromo-3-nitrosalicylanilide | 100.0 | 100.0 | 0.0 | -- |
| 4'-Iodo-5-nitrosalicylanilide | 100.0 | 100.0 | 0.0 | -- |
| Triphenyltin chloride | 100.0 | 100.0 | 100.0 | -- |
| Pentachlorophenol | 87.5 | 0.0 | 0.0 | -- |
| Malachite Green oxalate | 100.0 | 100.0 | 90.0 | -- |
| Bromsalans | 100.0 | 100.0 | 100.0 | -- |
| C-9491 | 100.0 | 100.0 | 0.0 | -- |
| Tubercidin | 100.0 | 100.0 | 100.0 | 100.0 |
| p-Aminophenol | 100.0 | 100.0 | 0.0 | -- |

## APPENDIX A

CODE NUMBERS AND COMPLETE NAMES OF CHEMICALS USED IN JELLYFISH TOXICITY STUDIES

| | | |
|---|---|---|
| 1 | 3-TRIFLUOROMETHYL-4-NITROPHENOL | |
| 2 | 3-FLUORO-4-NITROPHENOL | |
| 3 | 3'-CHLORO-3-NITROSALICYLANILIDE | |
| 4 | CATECHOL | |
| 5 | 3-TRIFLUOROMETHYL-2-NITROPHENOL | |
| 6 | 2,5-DICHLORO-4-NITROPHENOL | |
| 7 | 3,4,6-TRICHLORO-2-NITROPHENOL | |
| 8 | 2-CHLORO-4-NITROPHENOL | |
| 9 | 2-BROMO-4-NITROPHENOL | |
| 10 | 5-CHLORO-2-NITROPHENOL | |
| 11 | 3-CHLORO-4-NITROPHENOL | |
| 12 | 3-TRIFLUOROMETHYL-4-NITROPHENOL (NA SALT) | |
| 13 | 4'-BROMO-3-NITROSALICYLANILIDE | |
| 14 | 3'-FLUORO-3-NITROSALICYLANILIDE | |
| 15 | 3-NITROSALICYLIC ACID | |
| 16 | 3-NITROSALICYL CL | |
| 17 | 3'-BROMO-3-NITROSALICYLANILIDE | |
| 18 | SYNERGISTIC MIXTURE | 3'-CHLORO-5-NITROSALICYLANILIDE |
| | | 4'-CHLORO-5-NITROSALICYLANILIDE |
| | | 4'-CHLORO-3-NITROSALICYLANILIDE |
| | | 3'-CHLORO-3-NITROSALICYLANILIDE |
| 19 | 2,4,6-TRICHLOROANILINE | |
| 20 | PROPIOANILINE | |
| 21 | 4'-BROMO-5-NITROSALICYLANILIDE | |
| 22 | 5-ETHYL-6-PHENYL-META-THIAZANE-2,4-DIONE | |
| 23 | 4'-FLUORO-3-NITROSALICYLANILIDE | |
| 24 | 4'-IODO-3-NITROSALICYLANILIDE | |
| 25 | 4',5-DIBROMO-3-NITROSALICYLANILIDE | |
| 26 | P-IODOANILINE | |
| 27 | 5-ETHYL-6-NITRO-PHENYL-META-THIAZANE-2,4-DIONE | |
| 28 | 2'-CHLORO-5-NITROSALICYLANILIDE | |
| 29 | P-BROMOANILINE | |
| 30 | ISOMERIC MIXTURE | 4'-CHLORO-4-NITROSALICYLANILIDE |
| | | 4'-CHLORO-3-NITROSALICYLANILIDE |
| 31 | 4'-IODO-5-NITROSALICYLANILIDE | |
| 32 | 2'-CHLORO-3-NITROSALICYLANILIDE | |
| 33 | 3,5-DINITROSALICYLIC ACID | |
| 34 | 3'-IODO-3-NITROSALICYLANILIDE | |
| 35 | 3-BROMO-4-NITROPHENOL | |
| 36 | TETRAPHENYLARSONIUM CHLORIDE | |
| 37 | TETRAETHYAMMONIUM CHLORIDE | |
| 38 | PYROCATECHOL | |
| 39 | TETRAPHENYLARSONIUM BROMIDE | |
| 40 | SODIUM TETRATHIONATE | |
| 41 | 1-ACETYL-2-PHENYLHYDRAZINE | |
| 42 | 2,4,5-TRICHLOROPHENOXYPROPIONIC ACID | |
| 43 | 2-CHLOROQUINOLINE | |
| 44 | P-AMINOACETOPHENONE | |
| 45 | 2,5-PIPERAZINEDIONE | |
| 46 | TRIPHENYLTIN CHLORIDE | |
| 47 | PENTACHLOROPHENOL | |
| 48 | 2,3-DICHLORO-1,4-NAPHTHOQUINONE | |
| 49 | 1,2,3,4,5,6-HEXACHLOROCYCLOHEXANE | |
| 50 | MALACHITE GREEN OXALATE | |
| 51 | 2-(2',4',5'-TRICHLOROPHENOXY) PROPIONIC ACID | |
| 52 | ABATE | |
| 53 | BROMSALANS | |
| 54 | AMIDITHION | |
| 55 | C-9491 | |
| 56 | AMETRYNE | |
| 57 | C-9491 OXYGEN ANALOG | |
| 58 | ATRATONE | |
| 59 | TERT-BUTYL CHLORIDE | |
| 60 | TETRAMETHYLUREA | |
| 61 | PROPYL CHLORIDE | |
| 62 | PYRIDAZINE | |
| 63 | ISOMERIC MIXTURE | 3'-CHLORO-5-NITROSALICYLANILIDE |
| | | 3'-CHLORO-3-NITROSALICYLANILIDE |
| 64 | 4'-CHLORO-5-NITROSALICYLANILIDE | |
| 65 | 3'-CHLORO-5-NITROSALICYLANILIDE | |
| 66 | 1,2,5,6-TETRAHYDROPYRIDINE | |
| 67 | N-BUTYL PROPIONATE | |
| 68 | 1,2,3,4-TETRAHYDROQUINOLINE | |
| 69 | PYRIDINE | |
| 70 | BROMOPHOS-ETHYL | |
| 71 | CARBOPHENOTHION | |
| 72 | DDVP | |
| 73 | QUINOLINE | |
| 74 | BUTONATE | |
| 75 | 3-AMINOQUINOLINE | |

76 N-BUTYL CHLORIDE
77 5-IODOURIDINE
78 DIHYDROURACIL
79 5-BROMOURIDINE
80 5-CHLOROURIDINE
81 6-AZAURIDINE
82 PSEUDOURIDINE
83 URIDINE
84 ADENOSINE
85 DEOXYADENOSINE
86 TUBERCIDIN
87 ACETONITRILE
88 ACETANILIDE
89 ACETALDOXIME
90 2-AMINOANTHRAQUINONE
91 P-AMINOAZOBENZENE
92 P-AMINOPHENOL
93 ACETOPHENONE
94 ALIZARINE YELLOW R
95 M-AMINOACETOPHENONE
96 ABIETIC ACID
97 ACETAL
98 ACETAMIDE
99 DEOXYCYTIDINE
100 5-BROMODEOXYURIDINE
101 DEOXYGUANOSINE
102 ADENOSINE MONOPHOSPHORIC ACID
103 5-BROMODEOXYCYTIDINE
104 5-AZACYTIDINE
105 GUANOSINE 2H2O
106 6-MERCAPTOPURINE RIBOSIDE
107 ADENINE
108 2,8-DIHYDROXYADENINE
109 HYPOXANTHINE
110 6-METHOXYPURINE
111 XANTHINE
112 6-THIOXANTHINE
113 6-CHLOROPURINE
114 2-HYDROXY-6-METHYL PURINE
115 PURINE RIBOSIDE
116 2'-DEOXYINOSINE
117 XANTHOSINE
118 PURINE
119 ADENOSINE-N1-OXIDE
120 6-ISOPROPOXYPURINE
121 6-IODOPURINE
122 6-BENZYL M AMINOPURINE
123 2'-DEOXYADENOSINE
124 6-BENZYLMERCAPTO M PURINE
125 6-AMINO-3-DIMETHYLALLYLPURINE
126 XANTHINE-3-N-OXIDE
127 6-HISTAMINO PURINE
128 KINETIN (6-FURFURYLAMINO PURINE)
129 6-HYDRAZINOPURINE
130 ADENINE-N1-OXIDE
131 CORDYCEPIN
132 7-METHYL XANTHOSINE
133 6-HYDROXYLAMINOPURINE RIBOSIDE
134 1-METHYL ADENOSINE
135 6-CHLOROPURINE RIBOSIDE
136 KINETINRIBOSIDE
137 PUROMYCIN AMINONUCLEOSIDE
138 6-SELENO-PURINE
139 6-AMINO-2,8-DICHLOROPURINE
140 BLUE TETRAZOLIUM
141 P-TERT-BUTYLPHENOL
142 4-CHLORO-2-NITROANILINE
143 CHLORAL HYDRATE
144 BISMARCK BROWN
145 CETYL PYRIDINIUM BROMIDE
146 BENZOTRIAZOLE
147 ANTHRAQUINONE
148 CALCIUM GLUTAMATE
149 CAFFEINE
150 CETYL PYRIDINIUM CHLORIDE

The only other two chemicals tested which produced significant mortalities at 0.1 ppm on the ephyrae of *Chrysaora* were Malachite Green oxalate and Catechol. At 0.1 ppm Malachite Green oxalate caused a mortality of 90 percent on the ephyrae. In previous tests it caused only 20 percent on the polyps of *Aurelia* and none o the polyps of *Chrysaora*. Catechol caused 50 percent mortality on ephyrae of *Chrysaora* at 0.1 ppm and 100 percent at 0.5 and 1 ppm. In previous tests it also caused 100 percent mortality on medusae of *Chrysaora* at the lowest concentration tested (0.5 ppm). However, it had been previously shown to be much less effective on polyps of *Chrysaora* and ephyrae of *Aurelia*; it caused no mortality on these at 1 ppm and less than 100 percent at 5 and 10 ppm.

Four other chemicals caused 100 percent mortality on ephyrae of *Chrysaora* at 0.5 ppm but none at 0.1 ppm. These were 4,5-Dibromo-3 nitrosalicylanilide, 4'-Iodo-5-nitrosalicylanilide, C-9491 and p-Aminophenol. All were also much more effective on ephyrae than on polyps.

Fifteen of the chemicals tested at 1 ppm in this series caused mortalities greater than 70 percent on the ephyrae of *Chrysaora* and another one caused a mortality of 45 percent. One other chemical, C-9491 oxygen analog, caused a lower mortality of 14 percent at 1 ppm.

Results of the tests conducted on polyps of *Chrysaora* during this period showed that only two chemicals caused mortality in this series of tests (Bismarck Brown and Cetyl pyridinium bromide), which caused 100 and 28 percent mortality, respectively, at 5 ppm.

Most of the chemicals tested on polyps in this series were analogues of the purine and pyrimidine bases of the nucleic acids. Only one of these, Adenosine-$N^1$-oxide, had any effect on the polyps.

The results obtained so far reinforce the suggestion that ephyrae are more sensitive than polyps to the effect of chemicals.

2 - Metabolic Inhibitors of *Chrysaora* Strobilation

P. L. Zubkoff

Inhibitors are used to provide insight into metabolic processes of jellyfish. The selection of inhibitors is made with the objectives that analogues of coenzymes, naturally occurring substrates for macromolecular synthesis, and other "small" molecules will interfere with known enzymatic pathways.

The inhibitors which have been selected can be roughly grouped into (1) a spectrum of components which may interfere with metabolism in general and (2) more specific components whose most likely action will interfere with either RNA, protein, lipid or carbohydrate synthesis. In some cases, molecules which will most likely interfere with electron transfer mechanisms are also employed.

The initial selection of the nucleic acid precursor analogues includes components which contain the halogen substitutions and other modifications of the purine and pyrimidine rings.

Two components show a remarkable degree of inhibition in the 24-hour polyp assay: tubercidin and adenosine-$N^1$-oxide. Both of these components are adenine analogues. Tubercidin is distinguished by having a carbon atom in the seven position of the purine ring; adenosine-$N^1$-oxide has an oxidized nitrogen atom at the $N^1$ position. It is assumed that these components compete directly with adenine containing coenzymes which are present in very small quantities.

Since adenine or adenosine is a component of the ubiquitous energy compounds of the cell, ATP, it is most difficult at this time to pinpoint a direct site for which these two compounds may have their effects. Some potential sites of interference include: (1) ATP formation, (2) NADH formation and metabolism, (3) coenzyme A biosynthesis and functioning, and (4) cyclic AMP mediated hormonal actions.

Two Initial Hypotheses

In view of the sensitivity of the polyps to both tubercidin and adenosine-$N^1$-oxide, we are proposing as an initial hypothesis that the ATP pool of the polyp is small. A likely site of action of these two inhibitors is by non-competitive inhibition of enzymes intimately involved with either the ATP formation or regeneration from AMP and ADP.

A second hypothesis is that enzymes associated with formation of NADH are non-competitively inhibited.

Experiments will be conducted during another contract period to define the presumed metabolic processes.

JOB NO. 5 - ECOLOGICAL ROLE OF JELLYFISHES

OBJECTIVE: To clarify further the ecological role of jellyfishes with special reference to their position in the food chain.

## 1 - Seasonal Distribution and Abundance of Medusae

R. Morales-Alamo and D. S. Haven

We continued to monitor the abundance of medusae at Gloucester Point by making daily counts along the length of the main pier at VIMS. Counts for the period were started on 29 March.

The average daily number of medusae counted is summarized for a 5-day period in Table 1. Medusae of *Cyanea* continued to be as abundant through the first week of May as they had been since mid-February. An especially large concentration of medusae was observed on April 8 when the number in the area usually counted along the pier was estimated at 11,000. Thousands more were present in a wide band along the VIMS waterfront. Numbers decreased gradually from May on and no medusae were seen after mid-June. Throughout winter and spring the diameter of the medusae of *Cyanea* was around 60 mm.

No medusae of *Aurelia* were seen from the pier in the summer of 1971 during the regular monitoring schedule. Only one *Aurelia* was reported by VIMS personnel from the immediate Gloucester Point area; a male medusa was collected by J. Olmon on 9 August. Several small medusae of *Aurelia*, 75 to 125 mm in diameter, were seen in the Ware and Rappahannock rivers early in the summer. However, no other fully grown *Aurelia* were seen at any of the locations monitored by us during the summer in lower Chesapeake Bay.

Medusae of *Chrysaora* were very scarce in the Gloucester Point area during the summer of 1971. Only 10 medusae were seen from the VIMS pier between 14 June and 23 July at the times counts were made. The first medusa was seen on July 6 and the last one on July 21. Medusae were seen at other times and locations in the Gloucester Point area but they were few and were present for only a short period of time. Two technicians collecting medusae from our piers were not able to catch any more than 25 medusae in any 4-hour period, and this number was reached on only a few occasions.

Our observations, and reports obtained from residents in localities throughout the Virginia tributaries of Chesapeake Bay, confirmed an extreme scarcity of medusae of *Chrysaora* in the summer of 1971. The greatest concentration seen by us this summer was observed at Dixie on the Piankatank River early in July. The number of medusae was estimated at 75 per square meter as far as could be seen from the pier where observation was made. Most of the medusae were around four inches in diameter and immature but looked healthy. When we returned to this same location six weeks later the medusae were seen only in small numbers, less than one per square meter and were small (50-75 mm). At this time, many appeared to be dying; the tentacles were degenerating, pulsations were weak, and the tissue had an opaque appearance.

Mature medusae of *Chrysaora* were collected at Gloucester Point, Sarah's Creek, Piankatank River and Hampton River from the first week in August to the first week in September. Although medusae were small (50-75 mm) and looked sick, we were able to rear in the laboratory thousands of polyps from these medusae. No medusae were found at these locations after the first week in September (they were gone from Sarah's Creek by mid-August). The last medusae seen by us in the summer of 1971 were found in the Elizabeth River on September 12.

TABLE 8. Average daily number of medusae counted on weekly 5-day periods from VIMS pier, Gloucester Point, Virginia, 29 March - 3 September 1971.

| Period | *Cyanea* | | | *Chrysaora* | | |
|---|---|---|---|---|---|---|
| | Average No. of Medusae | Size Range (in.) | Range of Daily Size Modes (in.) | Average No. of Medusae | Size Range (in.) | Range of Daily Size Modes (in.) |
| 29 Mar. - 2 Apr. | 63 | 1-5 | 2-3 | | | |
| 5 Apr. - 9 Apr. | 3680 | 1-5 | 2-3 | | | |
| 12 Apr. - 16 Apr. | 1844 | 1-5 | 2 | | | |
| 19 Apr. - 23 Apr. | 539 | 1-5 | 2 | | | |
| 26 Apr. - 30 Apr. | 134 | 1-3 | 2 | | | |
| 3 May - 7 May | 1249 | 1-5 | 2-3 | | | |
| 10 May - 14 May | 159 | 1-4 | 2 | | | |
| 17 May - 21 May | 71 | 1-4 | 2 | | | |
| 24 May - 28 May | 18 | 1-4 | 2 | | | |
| 31 May - 4 June | 12 | 1-3 | 2-3 | | | |
| 7 June - 11 June | 7 | 2-3 | 2-3 | | | |
| 14 June - 18 June | 0 | | | 0 | | |
| 21 June - 25 June | 0 | | | 0 | | |
| 28 June - 2 July | 0 | | | 0 | | |
| 5 July - 9 July | 0 | | | (3)* | 2-5 | |
| 12 July - 16 July | | | | (1)* | 2 | |
| 19 July - 23 July | | | | (6)* | 2-4 | |
| 26 July - 30 July | | | | 0 | | |
| 2 Aug. - 6 Aug. | | | | 0 | | |
| 9 Aug. - 13 Aug. | | | | 0 | | |
| 16 Aug. - 20 Aug. | | | | 0 | | |
| 23 Aug. - 27 Aug. | | | | 0 | | |
| 30 Aug. - 3 Sept. | | | | 0 | | |

* = Actual number of medusae counted during period.

2 - Polyps and Podocysts on Bottom Substrate

R. Morales-Alamo and D. S. Haven

Samples of bottom substrate (oyster shells) were collected in the fall of 1971 at several stations in the major western shore tributaries of the Chesapeake Bay to investigate the abundance and condition of polyps following the unusual scarcity of medusae during the summer. Twenty stations were sampled in September and October in the Great Wicomico, Rappahannock, Piankatank, York and James rivers. Eight stations were sampled in Sarah's Creek (York River) and 4 in Back Creek in December.

Polyps and/or podocysts of *Chrysaora* were found at all stations but one (Table 9). The latter was Tue Marsh light at the mouth of the York River where, instead, we found 1020 larval cysts of *Cyanea*. The numbers of polyps and podocysts found at all other stations were similar to those found in previous sampling periods and were large enough to suggest that the sessile stage populations of *Chrysaora* were not affected adversely by whatever factor inhibited the development of medusae populations. They also indicate that a sufficient stock is in existence at present for production of medusae populations in 1972 in the large numbers usually found in the lower Chesapeake Bay tributaries.

Sub-samples of the polyps collected on 8 and 10 December at Sarah's Creek and Back Creek were examined for nematocyst types. Out of 142 examined 15 were *Cyanea*. The rest were *Chrysaora*.

A single sample of oyster shells was collected at West Point on the Corrotoman River on 12 May 1971 to obtain polyps for toxicity tests. Although not recorded, the number of polyps on the shells was

TABLE 9. Number of polyps and podocysts on natural oyster shell substrate collected at different stations, Fall 1971.

| Station | Date | No. Shells Examined | No. Shells With Polyps and/or Podocysts | No. Polyps | No. Cysts | Mean No. Polyps and Cysts Per Shell | Mean No. Polyps and Cysts Per Shell Having Either |
|---|---|---|---|---|---|---|---|
| <u>Great Wicomico River</u> | | | | | | | |
| Haynie Point | 24 Sept. | 10 | 10 | 33 | 87 | 12.0 | 12.0 |
| Off Crane Creek | 24 Sept. | 10 | 8 | 24 | 43 | 6.7 | 8.4 |
| Whaley Flats | 24 Sept. | 10 | 10 | 37 | 88 | 12.5 | 12.5 |
| Fleet Point | 24 Sept. | 15 | 13 | 74 | 124 | 13.2 | 15.2 |
| <u>Rappahannock River</u> | | | | | | | |
| Corrotoman River | | | | | | | |
| Corrotoman Point | 15 Oct. | 10 | 3 | 22 | 24 | 4.6 | 15.3 |
| West Point | 15 Oct. | 15 | 11 | 375 | 313 | 45.9 | 62.5 |
| <u>Piankatank River</u> | | | | | | | |
| Burton Point | 27 Sept. | 20 | 10 | 41 | 40 | 4.1 | 8.1 |
| Palace Bar (old) | 27 Sept. | 20 | 12 | 104 | 127 | 11.5 | 19.2 |
| Palace Bar (new) | 27 Sept. | 10 | 10 | 58 | 102 | 16.0 | 16.0 |
| 3 Branches | 27 Sept. | 20 | 9 | 54 | 10 | 3.2 | 7.1 |
| Island Bar | 27 Sept. | 20 | 5 | 9 | 0 | 0.4 | 1.8 |
| Ginney Point | 27 Sept. | 19 | 11 | 30 | 153 | 9.6 | 16.6 |
| Cape | 27 Sept. | 20 | 4 | 18 | 0 | 0.9 | 4.5 |
| <u>York River</u> | | | | | | | |
| Aberdeen Creek | 23 Sept. | 10 | 6 | 115 | 112 | 12.7 | 21.2 |
| Aberdeen Rock | 23 Sept. | 20 | 11 | 40 | 22 | 3.1 | 5.6 |
| Pages Rock | 23 Sept. | 20 | 4 | 33 | 16 | 2.4 | 12.2 |
| Timberneck Creek | 23 Sept. | 20 | 4 | 29 | 10 | 1.9 | 9.7 |
| Tue Marsh Light | 23 Sept. | 12 | 6* | 0 | 0 | - | - |

TABLE 9. (Continued)

| Station | Date | No. Shells Examined | No. Shells With Polyps and/or Podocysts | No. Polyps | No. Cysts | Mean No. Polyps and Cysts Per Shell | Mean No. Polyps and Cysts Per Shell Having Either |
|---|---|---|---|---|---|---|---|
| Sarah's Creek | | | | | | | |
| 1 | 8 Dec. | 20 | 8 | 119(2/20)** | 165 | 14.2 | 35.5 |
| 2 | 8 Dec. | 40 | 3 | 20(0/ 7) | 83 | 2.6 | 34.3 |
| 3 | 8 Dec. | 20 | 15 | 202(3/20) | 470 | 33.6 | 44.8 |
| 4 | 8 Dec. | 20 | 9 | 49(2/20) | 143 | 9.6 | 21.3 |
| 5 | 8 Dec. | 40 | 6 | 24(0/ 9) | 23 | 1.2 | 7.8 |
| 6 | 23 Sept. | 20 | 11 | 96 | 126 | - | - |
| 6 | 8 Dec. | 20 | 9 | 126(3/20) | 466 | 29.6 | 65.8 |
| 7 | 8 Dec. | 20 | 9 | 168(2/20) | 322 | 24.5 | 54.4 |
| 8 | 8 Dec. | 40 | 8 | 30(3/20) | 122 | 3.8 | 19.0 |
| Back Creek | | | | | | | |
| 1 | 10 Dec. | 10 | 3 | 1(0/ 1) | 51 | 5.2 | 17.3 |
| 2 | 10 Dec. | 20 | 2 | 0 | 8 | 0.4 | 4.0 |
| 3 | 10 Dec. | 20 | 3 | 0 | 41 | 2.0 | 13.7 |
| 4 | 10 Dec. | 20 | 3 | 5(0/ 5) | 42 | 2.3 | 15.6 |
| James River | | | | | | | |
| Wreck Shoal | 6 Oct. | 10 | 6 | 36 | 98 | 13.4 | 22.3 |

* = 1020 *Cyanea* larval cysts also found on these shells.

** = $\frac{\text{Number of polyps identified as } Cyanea}{\text{Number of polyps examined for nematocyst types}}$

TABLE 10. Numbers of polyps and cysts on oyster shells. Sarah's Creek, 8 December 1971.

| Station | No. Shells Examined | No. Shells With Polyps and/or Cysts | No. Polyps | No. Cysts | No. Polyps Per Shell | No. Cysts Per Shell | No. Polyps and Cysts Per Shell | No. Polyps and Cysts Per Shell Having Either |
|---|---|---|---|---|---|---|---|---|
| 1 | 20 | 8 | 119 | 165 | 5.9 | 8.2 | 14.2 | 35.5 |
| 2 | 40 | 3 | 20 | 83 | 0.5 | 2.1 | 2.6 | 34.3 |
| 3 | 20 | 15 | 202 | 470 | 10.1 | 23.5 | 33.6 | 44.8 |
| 4 | 20 | 9 | 49 | 143 | 2.4 | 7.1 | 9.6 | 21.3 |
| 5 | 40 | 6 | 24 | 23 | 0.6 | 1.2 | 1.2 | 7.8 |
| 6 | 20 | 9 | 126 | 466 | 6.3 | 23.3 | 29.6 | 65.8 |
| 7 | 20 | 9 | 168 | 322 | 8.4 | 16.1 | 24.5 | 54.4 |
| 8 | 40 | 8 | 30 | 122 | 0.7 | 3.0 | 3.8 | 19.0 |

as abundant as has usually been the case for that station. Examination in the laboratory showed that most of the polyps were strobilating. Within a period of one week these polyps produced hundreds of ephyrae. This was as expected for that time of the year and suggested that medusa populations would be as abundant as usual in the summer. Failure of the expected medusa populations to materialize must have been the result of adverse environmental conditions affecting further development and growth of the ephyrae and not due to reduced polyp populations or to failure of the latter to strobilate.

TABLE 11. Number of polyps and cysts on each shell having either, Sarah's Creek, 8 December 1971.

| STATIONS 1 | | 2 | | 3 | | 4 | | 5 | | 6 | | 7 | | 8 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| P | C | P | C | P | C | P | C | P | C | P | C | P | C | P | C |
| 22 | 15 | 13 | 28 | 6 | 7 | 1 | 0 | 1 | 0 | 19 | 31 | 38 | 73 | 1 | 24 |
| 3 | 10 | 5 | 50 | 25 | 25 | 14 | 20 | 4 | 4 | 8 | 10 | 2 | 0 | 7 | 14 |
| 2 | 16 | 2 | 5 | 2 | 80 | 7 | 4 | 4 | 5 | 57 | 187 | 0 | 3 | 0 | 3 |
| 36 | 32 | | | 17 | 30 | 1 | 13 | 1 | 0 | 5 | 7 | 27 | 34 | 0 | 6 |
| 6 | 0 | | | 38 | 37 | 2 | 3 | 12 | 10 | 0 | 100 | 69 | 110 | 6 | 54 |
| 39 | 67 | | | 8 | 18 | 2 | 67 | 2 | 4 | 0 | 6 | 16 | 20 | 2 | 5 |
| 0 | 5 | | | 18 | 33 | 14 | 25 | | | 7 | 5 | 0 | 10 | 10 | 5 |
| 11 | 20 | | | 28 | 34 | 7 | 2 | | | 30 | 100 | 2 | 31 | 4 | 11 |
| | | | | 3 | 17 | 1 | 9 | | | 0 | 20 | 14 | 41 | | |
| | | | | 9 | 22 | | | | | | | | | | |
| | | | | 2 | 4 | | | | | | | | | | |
| | | | | 6 | 11 | | | | | | | | | | |
| | | | | 15 | 58 | | | | | | | | | | |
| | | | | 21 | 75 | | | | | | | | | | |
| | | | | 4 | 19 | | | | | | | | | | |
| 119 | 165 | 20 | 83 | 202 | 470 | 49 | 143 | 24 | 23 | 126 | 466 | 168 | 322 | 30 | 122 |

P = Polyps

C = Podocysts

TABLE 12. Number of polyps and cysts on oyster shells, Back Creek, 10 December 1971.

| Station | No. Shells Examined | No. Shells With Polyps and/or Cysts | No. Polyps | No. Cysts | No. Polyps Per Shell | No. Cysts Per Shell | No. Polyps and Cysts Per Shell | No. Polyps and Cysts Per Shell Having Either |
|---|---|---|---|---|---|---|---|---|
| 1 | 10 | 3 | 1 | 51 | 0.1 | 5.1 | 5.2 | 17.3 |
| 2 | 20 | 2 | 0 | 8 | 0 | 0.4 | 0.4 | 4.0 |
| 3 | 20 | 3 | 0 | 41 | 0 | 2.0 | 2.0 | 13.7 |
| 4 | 20 | 3 | 5 | 42 | 0.2 | 2.1 | 2.3 | 15.6 |

TABLE 13. Number of polyps and cysts on individual shells, Back Creek, 10 December 1971.

| STATION | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1 | | 2 | | 3 | | 4 | |
| P | C | P | C | P | C | P | C |
| 0 | 8 | 0 | 3 | 0 | 11 | 4 | 5 |
| 1 | 0 | 0 | 5 | 0 | 6 | 0 | 17 |
| 0 | 43 | | | 0 | 24 | 1 | 20 |
| 1 | 51 | 0 | 8 | 0 | 41 | 5 | 42 |

P = polyps

C = Podocysts

## 3 - Amino Acid Uptake By Jellyfish

K. L. Webb

The possible biological significance of net uptake of small organic compounds by marine invertebrates may involve several factors. The obvious factor of contribution by such compounds to the energy supply of the organism has been discussed at great length by Stephens (e.g. 1968). An additional possibility is the influence of these compounds on morphogenetic processes, for example, sexual differentiation in *Hydra* (Loomis, 1964). Dissolved free amino acids (DFAA) may also contribute to the free amino acid (FAA) pools of marine invertebrates. It has been demonstrated that marine invertebrates have more concentrated FAA pools than fresh water invertebrates (Simpson et al. 1959) and that the total concentration of these pools is linearly related to salinity (Lynch and Wood 1966). This latter observation has recently been extended to jellyfish polyps (Webb et al. 1972). These pools must be maintained from some source either external or internal. A consideration of the above observations indicated that it might be informative to determine if there is a net uptake of FAA from the environment by jellyfish polyps or ephyrae of a magnitude to materially affect these pools.

Transfer of dissolved materials from the environment to the organism of necessity involves transfer via membrane transport mechanisms. A linear relationship between uptake rate and substrate concentration is sometimes considered indicative of diffusion uptake while a hyperbolic relationship is considered indicative of an active or metabolically dependent uptake. It must be noted, however, that at low substrate concentrations the hyperbola appears linear. Biologists usually consider uptake of dissolved materials by organisms an active process, especially when it occurs against a concentration gradient; in which case the diffusive movement would be outward. Biological membranes have long been known to be permeable in both directions (e.g. Christensen 1962) and we will thus consider it a "net" uptake only if more material is being transferred from the outside in (uptake) than from the inside out (loss).

A. Uptake of Glycine-$C^{14}$ By Ephyrae of *Chrysaora quinquecirrha*

To get a preliminary idea of uptake of glycine from the environment by ephyrae of *Chrysaora quinquecirrha*, ephyrae were incubated for about 1.5 hours in solutions of artificial seawater of 20 o/oo (10 ml) containing various concentrations of added C-14 glycine (see Table 14 and Fig. 12). Four concentrations were used in duplicate and 55 ephyrae were used for incubation. Uptake from media was determined by measuring loss from media as well as activity in an ethanol extract of the ephyrae by liquid scintillation counting. The data were in good agreement.

Uptake rates from concentrations approximating environmental concentration (90 nM/l) to an order of magnitude more concentrated were linear and thus kinetic parameters are not calculated; however, uptake but not loss at environmental levels of glycine is directly measured. Since this is not a measure of net uptake no calculations can be made of the energetic significance of these numbers even if we had guesses of ephyra respiration rates. It is possible, however, to make a first cut estimate of the turnover time of the glycine pool of ephyrae utilizing dissolved FAA of the environment. This estimate is 8.3 days based on the following calculations and data: One ephyra is estimated to contain 1.5 nM of glycine (and 0.72 nM taurine, 0.23

TABLE 14. Glycine-$C^{14}$ removed from 20 o/oo ASW

by ephyrae of *Chrysaora quinquecirrha*.

| nM/l Glycine | Pico moles Glycine Removed per hr per ephyrae |
|---|---|
| 89 | 6.2 |
| 89 | 7.1 |
| 178 | 15.1 |
| 178 | 12.7 |
| 356 | 25.2 |
| 356 | 23.1 |
| 890 | 54.5 |
| 890 | 60.7 |

Estimated environmental concentration 87.6 nM/l glycine (± 25.2 std. deviation, Webb & Wood 1967).

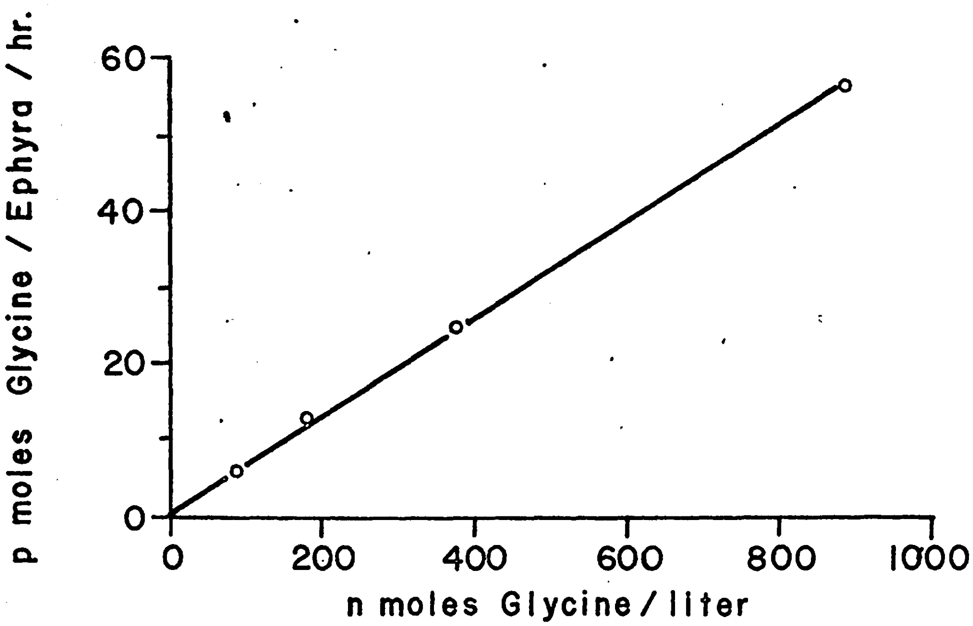


FIGURE 12. Glycine uptake by ephyrae of *Chrysaora*.

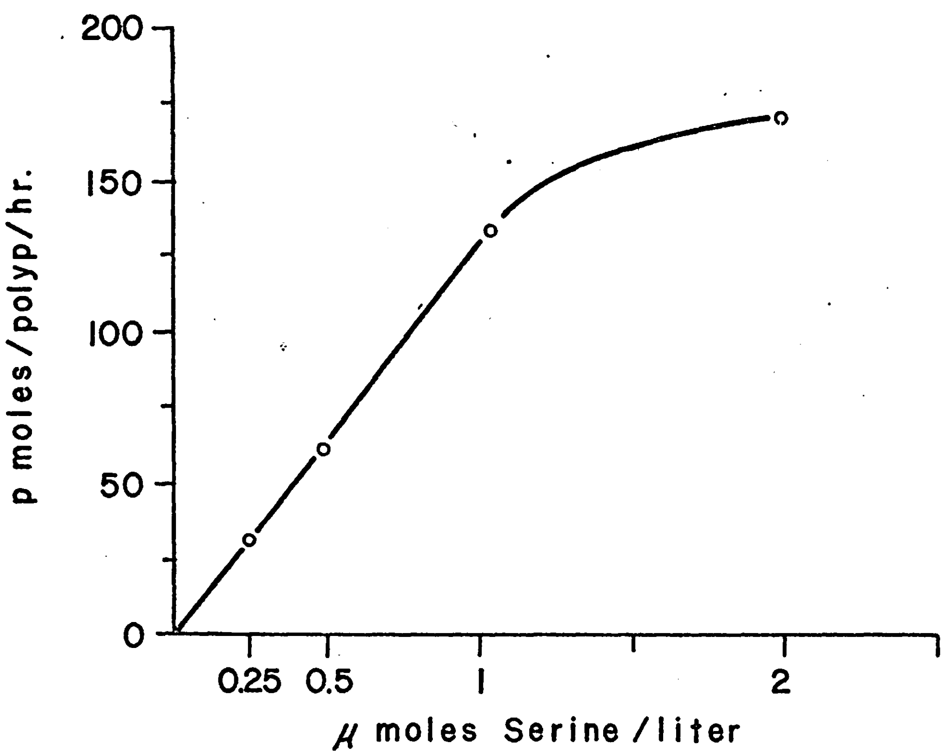


FIGURE 13. Serine uptake by polyps of *Chrysaora*.

glutamic acid, 0.07 nM serine and lesser amounts of other FAA based on an analysis of *Aurelia* ephyrae (std. deviation estimated to be 30% of mean value) and the input is calculated to be pico moles per ephyrae per hour from a solution of 90 nM/l glycine.

It is consequently concluded that uptake of glycine by ephyrae is probably not a process of significance and further experimentation was not pursued.

B. Uptake of Serine-$C^{14}$ By Polyps of *Chrysaora quinquecirrha*

*Chrysaora* polyps were incubated in C-14 serine containing 20 o/oo artificial seawater in lots of 32 for about 1 hour (see Table 15 and Fig. 13). Loss of activity from media was measured and chromatograms of FAA were run on ethanol extracts utilizing the flow cell/liquid scintillation counter/amino acid analyzer system.

Polyps analyzed contained a total of 51.7 nM FAA per polyp. Of this 39 nM was glycine and 2.8 nM was serine. From the linear portion of the uptake curve it is calculated that at a concentration of 30 nM/l serine the polyps would remove serine at a rate of 3.1 pico moles per hour per polyp. Thus, turnover as a result of this input would take 903 hours or 38 days and is probably of little significance at serine concentrations expected in the York River.

Predicted Vmax is 500 pico moles serine $hr^{-1}$ $polyp^{-1}$ or about 160 times greater than the estimated rate in the environment.

Of the C-14 activity recovered, 73.8% was in serine and 20.7% in glycine. The remaining activity was roughly evenly distributed among glutamic acid, alanine and several unidentified compounds presumed to be organic acids. The metabolic pathway which

TABLE 15. Serine-$C^{14}$ removed from 20 o/oo ASW by polyps of *Chrysaora quinquecirrha*.

| Serine nM/l | nM Removed $hr^{-1}$ $polyp^{-1}$ |
|---|---|
| 250 | .0303 |
| 500 | .0593 |
| 1000 | .134 |
| 2000 | .178 |

Estimated environmental concentration serine 32.9 nM/l (12.4 std. deviation, Webb & Wood 1967).

converts serine to glycine thus presumably has been demonstrated in *Chrysaora* polyps. The source of the large glycine pool in this organism is still questionable but it is reasonably certain that it is not primarily the result of direct accumulation from the dissolved glycine of the environment.

C. Uptake of Glycine-$C^{14}$ By Polyps of *Chrysaora quinquecirrha*

An experimental design was followed similar to that in item 1 using 20 minute incubations at 4 concentrations in replicate. When the results were obtained indicating that we had not approached saturation substrate concentrations, an additional four incubations were carried out at higher substrate concentrations. These data are included in Table 16 and Fig. 14. Artificial seawater at 20 o/oo at 25°C was used. Media and polyps were analyzed for amino acids and for C-14.

Environmental glycine concentration is considered to be 90 nano moles per liter (±25 std. deviation, Webb and Wood 1967) and at this concentration uptake by these polyps would be 23 pico moles of glycine per polyp per hour. Assuming one polyp contains 39 nM glycine (Webb et al. 1972), it would take 1700 hours or 70 days to replace the contained glycine at this uptake rate. These polyps contained between 42 and 45 nM glycine per polyp.

Of the C-14 recovered from the polyps more than 99% was still in glycine, a small amount was recovered in serine and organic acids.

D. Loss of Glycine By Polyps of *Chrysaora quinquecirrha*

A first attempt to measure the outward movement of glycine

TABLE 16. Glycine-$C^{14}$ uptake by polyps of *Chrysaora quinquecirrha*

| μM/l | Uptake nM/polyp/hr. |
|---|---|
| 1.79 | .435 |
| 1.79 | .456 |
| 2.68 | .756 |
| 2.68 | .750 |
| 4.46 | 1.17 |
| 4.46 | 1.15 |
| 8.02 | 2.17 |
| 8.02 | 2.04 |
| 14.6 | 2.86 |
| 29.8 | 3.66 |
| 58.4 | 5.44 |
| 116.8 | 5.18 |

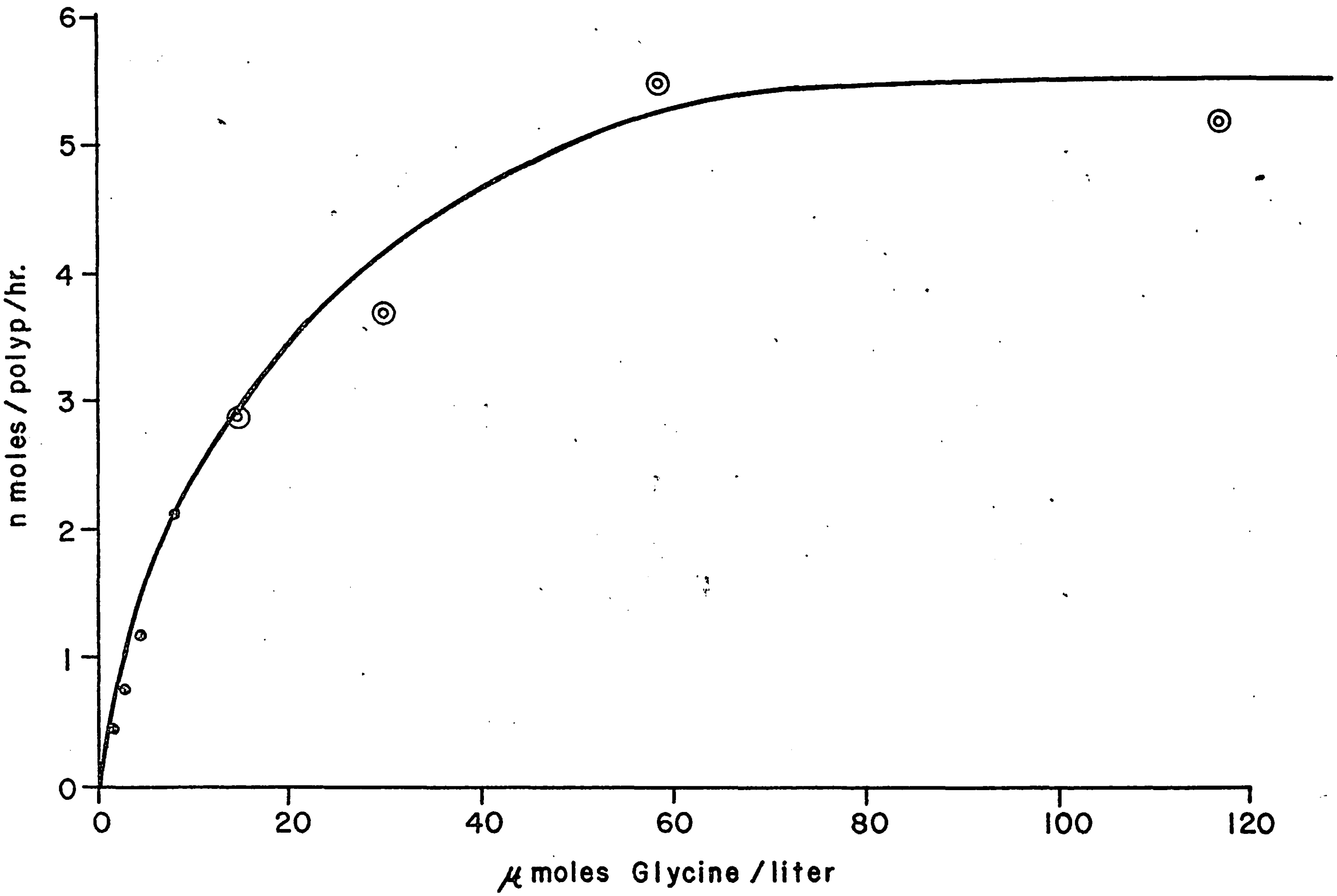


FIGURE 14. Glycine uptake by polyps of *Chrysaora*.

from *Chrysaora* polyps was made using the C-14 technique as our approach to the question of determining the direction of net movement. We labelled the internal glycine FAA pool with C-14 glycine by two successive 1.5 hour incubations of 18 polyps in a 60 μmolar solution of glycine-$C^{14}$ in 20 o/oo ASW. Polyps were subsequently rinsed twice in ASW (0.1 μM glycine), incubated again for 20 minutes in unlabelled ASW to remove the freely adhering glycine-$C^{14}$ and then divided into three groups for separate incubations of 20, 60 and 60 minutes. C-14 appearing in the media was assayed and assumed to be in glycine although this assumption was not checked. Incubated polyps were extracted and the FAA and their C-14 content determined in order to determine the specific activity of the glycine. This information is necessary to correct the activity appearing in the media to a quantity of glycine. Loss rates measured were 0.2 nM/polyp/hr for the 20 minute incubation and 0.8 for the hour incubation. These numbers are at least an order of magnitude greater than the rates of uptake measured at environmental concentrations of glycine and it would thus appear that the net movement of glycine is outward from jellyfish polyps in the environment. It appears that in laboratory or specialized environmental situations where the glycine concentrations exceed 3 μM (the concentration to provide an uptake of about 0.8 nM/polyp/hr, Table 3), a net uptake might occur. These considerations assume that the internal and not the external concentrations control the rate of exit. An extensive discussion is presented by Stephens (1968) who consistently overestimates environmental concentrations of materials.

## Discussion and Conclusions

Uptake rate constants for glycine and serine for *Chrysaora* polyps are compared to those from the literature for other marine invertebrates and York River bacteria (Table ). The transport constant ($K_t$) = the concentration of substrate at which the uptake is half the maximum value. A small number indicates high substrate affinity. Vmax = the maximum rate of uptake when uptake sites are saturated. A comparison of $K_t$ values indicates that *Chrysaora* polyps may be effective at competing for glycine with the other marine invertebrates listed (Table 17) but not natural bacterial populations. This interpretation supports our previous analysis based on turnover time of FAA pools as a result of uptake, i.e. that direct uptake of DFAA from the environment cannot materially affect the concentration of internal FAA pools in *Chrysaora* polyps if we consider what rates at environmental concentrations of DFAA actually are. Loss from the organism would further reduce the significance.

Although the uptake process may not be of energetic significance to the jellyfish polyp in the environment, the labelling technique will still prove useful in elucidating metabolic pathways within the organism.

TABLE 17. Kinetic parameters of uptake for amino acids by various marine invertebrates.

| Organism | Substrate | Vmax | $K_t$ |
|---|---|---|---|
| *Chrysaora* polyp | Serine | 0.5* | 3.6 µM |
| *Chrysaora* polyp | Glycine | 6.25* | 19.0 µM |
| Nereis limnicola[1] | Glycine | 0.13** | 37.0 µM |
| Golfingia[1] | Glycine | 0.10** | 100.0 µM |
| Nereis succinei[1] | Glycine | 1.6 ** | 110.0 µM |
| Clymenella[1] | Glycine | 10.0** | 200.0 µM |
| York River bacteria[2] | Glycine | | 0.13µM |
| York River bacteria[2] | Serine | | 0.77µM |

1. Data from Stephens 1967.
2. Data from Hobbie, Crawford and Webb 1968.

* nM $polyp^{-1}hr^{-1}$

** nM $g^{-1}hr^{-1}$

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## 4 - Gastric Cavity Contents of *Cyanea capillata*

J. D. Joseph and E. B. Joseph

Beginning on March 16, 1971, and continuing through May 19th medusae of *Cyanea capillata* were collected twice daily from the VIMS pier, when weather and water conditions permitted. Collections were made at approximately 8:30 a.m. and 2:00 p.m. In most cases the medusae ranged from 20 to 50 mm in diameter.

Medusae were placed on the dorsal surface of the exumbrella and a cruciform incision made through the ventral surface to expose the contents of the gastrovascular cavity. All extraneous material was removed and preserved in 90% methanol for later identification.

Visible animal remains were observed in *Cyanea capillata* during most of this period. A listing of the results is tabulated. As can easily be seen, it appears that in late March the amphipods were ingested to a large extent by the *Cyanea*. Further analyses which are not completed indicates that the *Cyanea* are quite omnivorous. We believe that *Cyanea* may be ingesting planktonic forms as well as the larger forms.

It is most unusual that such organisms as eel elvers, which are particularly hardy species have been found on several occasions in gastrovascular cavity of the *Cyanea*. This preliminary analysis of food ingested by the *Cyanea* medusae reflect organisms which are generally available to the medusae during this period of the year.

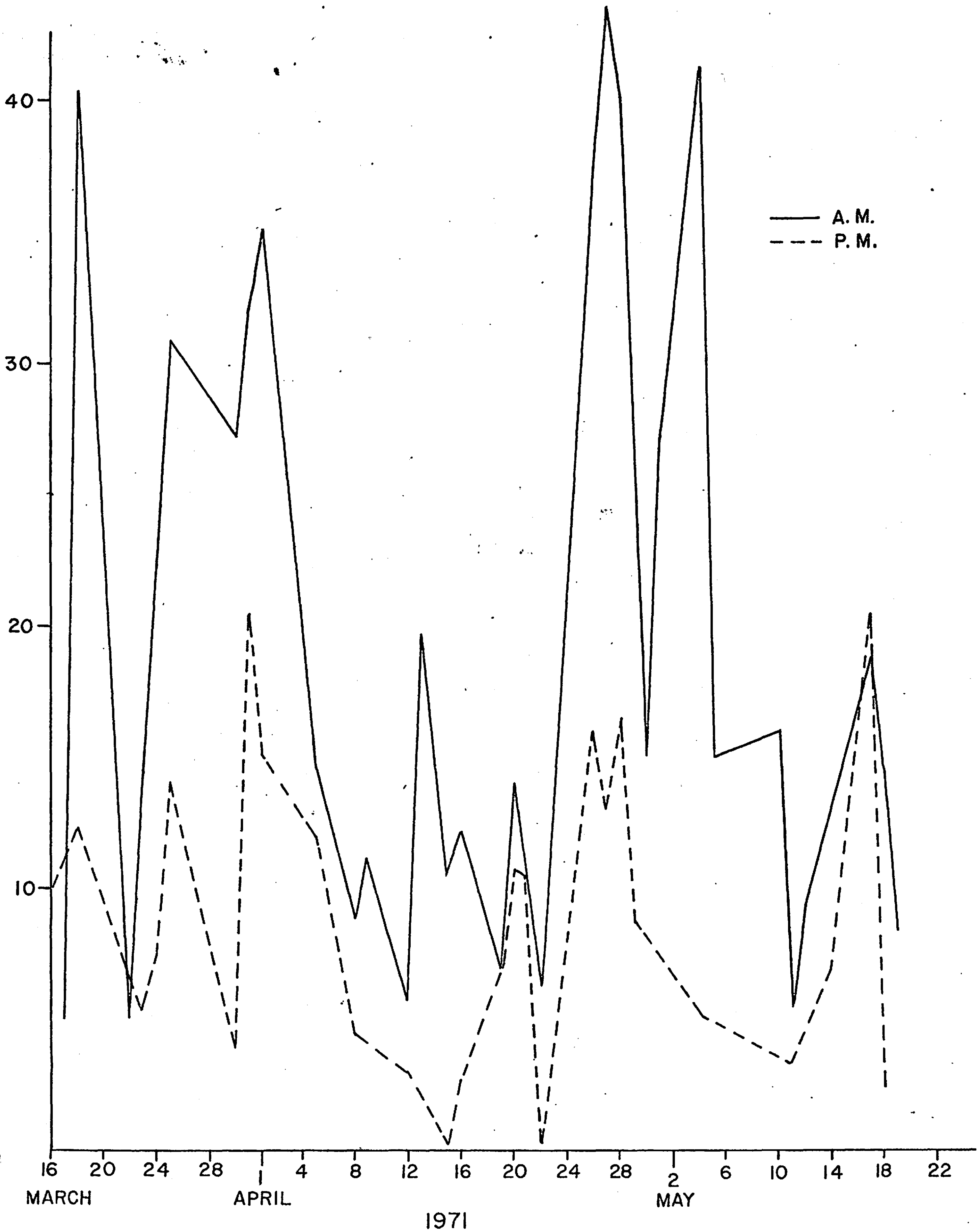
*Cyanea* showed a diurnal response in relation to the amount of food in its gastrovascular cavity; there was a higher percentage of identifible components in the morning samples. In general, the percentage of organisms having identifible components was markedly reduced in the organisms captured in the afternoon. We interpret these findings that the *Cyanea capillata* are primarily night-time feeders. However, the feeding analysis has not been interpreted with reference to either the tidal cycle or to available organisms in the habitat.

Later in the year, similar analyses on *Chrysaora quinquecirrha* were carried out. Unfortunately, *Chrysaora* medusae were not amenable to this analysis. Undoubtedly, the *Chrysaora* are ingesting planktonic forms and not the large forms that *Cyanea* locates. The types of organisms which serve as nutrition sources for *Chrysaora* are still largely unknown.

TABLE 18. Gastric cavity contents of *Cyanea capillata* medusae.

| | | Food Type | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Date | Time | Eel elvers | Gobys | Polychaetes | Mysid shrimp | Palaemonid sh. | Amphipods | Comaceans | Isopods | Flying Insects | Unident. Worm-like | Oligochaete | Caprellid amphipod |
| 3-16-71 | AM | 1 | 1 | 1 | | | | | | 2 | | | |
| | PM | | | | | | | | | | | | |
| 3-17 | AM | 1 | | | | | 1 | | | | | | |
| 3-18 | AM | 1 | | 2 | 3 | | 6 | 3 | | | | | |
| | PM | 1 | | | 1 | 2 | 3 | | | | | | |
| 3-22 | AM | | | | | | 2 | | | | | | |
| | PM | 1 | | 1 | 1 | | | | | | | | |
| 3-23 | AM | | | | 2 | | 5 | | | | | | |
| | PM | 1 | | 3 | | | | | | | | | |
| 3-24 | PM | | | 1 | | | | | | | | | |
| 3-25 | AM | | | 4 | | | 26 | | | | | | |
| | PM | | 1 | 3 | | | 2 | | 1 | | | | |
| 3-30 | AM | 2 | 1 | 1 | | | 15 | | | | | | |
| | PM | 1 | | | | | 1 | | | | 1 | | |
| 3-31 | AM | 1 | | | 1 | | 50 | | | | | | |
| | PM | 3 | 1 | 2 | | | 20 | | | | | | |
| 4- 1 | AM | | | | 1 | | 13 | | | | | 1 | |
| | PM | | | | 1 | | 15 | | | | 1 | | |
| 4- 5 | AM | | | 1 | 1 | | 1 | | | | | | |
| | PM | | | 1 | | | 2 | | | | | | 3 |
| 4- 7 | AM | | | | | | 5 | | | | | | |
| | PM | | | | | | 2 | | | | | | |
| 4- 8 | AM | | | 1 | | | 3 | | | | | | |
| 4-12 | AM | | | | 1 | | 1 | | | | | | |
| | PM | | | 1 | | | | | | | | | |
| 4-13 | AM | | | 5 | | | 1 | | | | | | |
| | PM | | | | | | | | | | | | |

FIG. 15. Relative percentages of *Cyanea capillata* having partially digested organisms in the gastric cavity.



## 5 - Neurophysiological Studies on *Cyanea capillata*

Drs. M. A. Patton and L. M. Passano came to VIMS in January 1972 for approximately 12 weeks in order to conduct neurophysical studies on *Cyanea capillata*.

This study was sponsored primarily by the National Science Foundation and the University of Wisconsin. The VIMS-NMFS Jellyfish Investigation provided space for their studies and occasionally personnel for aiding with collections.

The investigation will contribute to the general understanding of the nervous system of coelenterates and may possibly aid in developing an approach to control of jellyfishes, if specific inhibitors of neurotransmitters may be discovered.

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The coelenterates are the simplest animals to have a nervous system. It seems probable, therefore, that their nervous systems should be the simplest and most easily studied in the animal kingdom. The work of A. J. Romanes (1874-85) and G. A. Horridge (1953-57) seems to bear this out, at least for the system controlling swimming in the scyphozoa. The swimming muscles are located on the subumbrellar surface of the animal. All muscles contract during a swim pulse, acting against the elastic bell mesoglea; there are no antagonistic muscles, the elastic recoil of the mesoglea produces the recovery stroke. The power stroke seems to be controlled by only two nerve nets, the "giant fiber nerve net" and the "diffuse nerve net" (Horridge's terms).

Romanes demonstrated that the pulses of the giant fiber nerve net emanated from one of the eight marginal sense clubs, or rhopalia, and passed through the rest of the net, exciting the muscles as it went. Because these pulses could follow tortuous artificial pathways cut from the subumbrellar surface, he concluded that they were conducted through an unpolarized two-dimensional network of nerve fibers, demonstrated anatomically by his colleague, Schaffer.

Romanes also demonstrated the existence of the diffuse nerve net which modifies both the generation of giant fiber pulses by the rhopalia and the muscles' response to these giant fiber pulses. He further showed that the two nets were independent, since their signals could cross without interference. Horridge confirmed Romanes' ideas, morphologically and behaviorally, demonstrating the existence of two nerve nets in the ephyra of *Aurelia*.

Since it has been impossible to record systematically from the single cells of these medusae, further study has been of a limited nature. Both Horridge and Passano (1965) have been able to record electrical correlates of activity in the two nerve nets, but these appear to be multicellular recordings and cannot be used to determine how the individual cell functions in the nerve net. It is the hope of this study to impale single cells with microelectrodes. If we can do this, we may learn how the individual cell contributes to the function of the nerve net. Specifically, we may learn whether every cell in the nerve net responds when the pulses are conducted and whether the cell is bidirectional. Furthermore, we may learn how to measure the resting and action potentials and, by changing the ionic compositions of the bathing solution, the ionic basis of these potentials. Also, synaptic potentials and integration might be studied, especially if it is possible to record from two synaptically-connected nerve cells.

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## 6 - Jellyfish Biochemistry: Component Fatty Acids of Medusae Lipids

P. L. Zubkoff and J. D. Joseph

The near-shore waters of the Lower Chesapeake Bay are a particularly suitable habitat for supporting extensive populations of jellyfishes. During the summer months the "sea nettle", *Chrysaora quinquecirrha* DESOR predominates while the "moon-jelly", *Aurelia aurita* occurs to a somewhat lesser extent (HAVEN and MORALES-ALAMO). During the winter months the "lions mane", *Cyanea capillata* is particularly abundant; a pronounced peak in population numbers occurred during April of 1971. A rare species, *Rhopilema verrilli* has been observed in these waters in late autumn and early winter (CALDER 1972).

Two varieties of *Chrysaora* medusae are commonly observed in the Lower Chesapeake Bay: a red pigmented form and a white, translucent form. These forms possess similar nematocyst complements (CALDER, 1970) and similar free amino acid compositions (WEBB, 1970). Both forms produce a sting when contact with the human is made.

Since the fatty acids are constituents of both structural and energy reserve lipids, we believe that a detailed investigation of these fatty acids will provide essential data on which hypotheses on the mechanisms of morphogenesis and growth may be tested. Fatty acid esters have been identified with the phospholipid, mono-, di-, and triglycerides, sterol and wax fractions of the jellyfish lipids. In addition, component "X" of the *Chrysaora* polyp, postulated as an alkenyl diglyceride (JOSEPH, SCHMIDT and ZUBKOFF, 1971) has a fatty acid complement which is identical to that of the triglycerides.

There are two general classes of lipids: the phospholipids and the neutral lipids. The phospholipids are usually associated with structural components of cellular material, whereas the neutral lipids are usually associated with metabolically active components and energy reserves. The neutral lipids may be classified into approximately 7 groups, as can be seen with thin-layer chromatography using silica gel and hexane: ether: acetic acid: 1) hydrocarbons, 2) waxes and simple esters, 3) alkyl diglycerides, 4) triglycerides, 5) sterols, 6) fatty acids, 7) mono- and diglycerides.

One can extract the lipids from the cellular material with non-polar solvents such as chloroform and ether to obtain the neutral lipids, or one can use a mixture of polar and non-polar solvents, such as chloroform-methanol (BLIGH and DYER, 1959) for the extraction of the total lipids which includes the neutral and phospholipids. We employed this latter process for this study in order to extract all the lipids from the source material. After extraction, all fatty acids, both free and esterified, were converted to their respective methyl esters by the esterification procedure employing $BF_3$/methanol (METCALFE and SCHMITZ, 1961). Benzene (4.3 ml) and methanol (5.0 ml) were added to the $BF_3$ reagent (5.0 ml) just prior to use. To ensure the esterification of refractory lipids, methylation was carried out after saponification in alcoholic KOH.

The esters were analyzed on a 6 ft. 2 mm I.D. glass column packed with 5% DEGS on Chromosorb WAW-DMCS, 80-100 mesh, in a Hewlett-Packard 7620 gas chromatograph, using helium as a carrier gas with a flow of 30 ml/min, oven temperature at 180°C. The flame ionization detector was operated at 230°C, with a hydrogen flow of 25 ml/min and an air flow of 500 ml/min. The injection port was maintained at 230°C.

Under these conditions, chromatography of the esters was completed, through the 22:6ω3 ester, in less than 50 minutes. The samples were also analyzed on a 3% SE-30 column (Chromosorb WAW-DMCS, 80-100 mesh) under the same conditions or with 3% EGSS-X at 150°C.

The retention times of the chromatographic peaks were measured from the front of the solvent peak to the peak tops of the unknowns and the retention times (RRT) relative to that of stearic acid (18:0) were calculated. The unknown peaks were identified by comparison of the RRT with those of known methyl esters (Applied Science Labs), with those of a cod liver oil secondary standard (courtesy of Dr. R. G. Ackman, Halifax, N. S.), and through the use of the log retention time plot and separation factor techniques. The chromatograms were quantitated by the method of Carroll.

Fatty Acid Desaturation

Most organisms, with the exception of bacteria, may introduce double bonds into the carbon chain of unsaturated fatty acids. In animals, this introduction of double bonds, separated by methylene groups, occurs between the first double bonds and the carboxyl group; in plants, it occurs between the first double bond and the methyl terminal. Distinct families of polyunsaturated fatty acids develop, of which the most important are ω9 (oleic), ω6 (linoleic) and ω3 (α-linolenic).

Because linoleic (18:2ω6) and other ω6 fatty acids derived from it are necessary for higher animals to remain healthy, the 18:1ω6 and 18:2ω6 and other derived fatty acids are known as *essential* fatty acids. Animals may convert dietary 18:2ω6 into other members of the ω6 family by a series of alternate desaturations and elongations. Interconversions, however, between member fatty acids of different families do not occur in animals (GURR and JAMES, 1971).

The predominant polyunsaturated fatty acid in mammals is 20:4ω6 while fish have large amounts of 22:5ω3 and 22:6ω3. It is of considerable interest that a relatively large quantity of 20:4ω6 accumulates in *Chrysaora* polyps maintained on either brine shrimp nauplii (*Artemia salina*) or copepods (*Nitocra spinipes*).

Of the 24-30 component fatty acids identified and quantitated, the major differences between groups are noted as follows:

1. *Cyanea*

The fatty acids of *Cyanea* medusae from Virginia and Canadian waters are remarkably similar with the exception of the 18:1ω9, 20:4ω6 and 22:6ω3. Considering the wide geographic distribution of these two species (one from the North Atlantic and the other from the Chesapeake) the similarities are rather remarkable. In addition, the North Atlantic *Cyanea* is quite toxic to the human whereas the Chesapeake *Cyanea* is not. We wish also to point out that the *Cyanea* in Virginia is a wintering form, often yellow in appearance, whereas *Cyanea* exists in the Canadian coastal waters during the summer and has a subtle violet pigment.

2. *Aurelia*

Upon examination of *Aurelia* medusae of both male and female it is noted that the male has half the amount of 20:4ω6 and 2½ times as much 22:6ω3 as the female. With the exception of these two fatty acids, the fatty acid content of both male and female *Aurelia* are quite similar.

3. *Chrysaora*

In the Chesapeake the white and red forms of *Chrysaora* are quite common. In North Carolina a brown form of *Chrysaora* also exists. In June 1971, male and female medusae of both the red and the white form of *Chrysaora* were collected from a tributary creek of the Chesapeake within 10 minutes. These animals were undoubtedly exposed to the same environmental conditions. As can be seen from Table 19, the component fatty acids of the red and white forms are almost identical. Differences associated with sex are prominent with respect to 20:4ω6/22:1ω9 and 22:6ω3.

*Chrysaora* polyps cultured in the laboratory show high 18:1ω9 and low 20:4ω6, 20:5ω3 and 22:6ω3 when compared to medusae. Although these are laboratory cultured polyps and the medusae are isolated from nature, we do interpret this difference as indicating a shift in lipid metabolism associated with morphogenesis from polyp to medusae. The polyp, with its adult life ahead, probably has greater energy reserves than the medusae which possess large amounts of fatty acids associated with structural components ($C_{20}$ and greater fatty acids). However, further work is necessary to substantiate this interpretation.

The component fatty acids of the total lipids vary among the 3 species. Within the species, variations are associated with sex in *Aurelia*. Similarity of fatty acid distribution is noted in *Cyanea* from two geographical regions, in the red and white forms of Chesapeake *Chrysaora*, and in male and female *Chrysaora* (Table 20).

Fatty acids of jellyfish tissues (Table 22) and polyps (Table 23) have been completed and are being evaluated.

TABLE 19. Fatty acids of red and white *Chrysaora* medusae.

| | ♀ R % | ♀ W % | ♂ R % | ♂ W % |
|---|---|---|---|---|
| 12:0 | .086 | .156 | .082 | .119 |
| 13:0 | .038 | .041 | .065 | .009 |
| 14:0 | 1.13 | 1.20 | 1.36 | 1.01 |
| 14:1 / iso 15 | .192 | .218 | .214 | .143 |
| 15:0 | 1.67 | .892 | 1.55 | .715 |
| 15:1 / iso 16 | - | .873 | - | .692 |
| 16:0 | 12.90 | 13.94 | 10.74 | 13.19 |
| 16:1 | 3.61 | 3.59 | 3.01 | 2.49 |
| 16:4ω3 | .864 | - | + | - |
| 17:0 | 5.27 | - | 3.41 | 1.73 |
| iso 18 | - | 2.60 | - | 2.59 |
| 18:0 | 10.49 | 9.78 | 8.02 | 8.92 |
| 18:1ω9 | 6.04 | 6.67 | 5.23 | 5.70 |
| 18:2ω6 | .749 | .879 | .635 | .774 |
| 18:3ω6 / 20:0 | 1.55 | 1.53 | 2.12 | 1.85 |
| 18:3ω3 / 20:1ω9 | 1.48 | 1.39 | 1.35 | 1.34 |
| 18:4ω3 / 20:2ω9 | .825 | .773 | .466 | .512 |
| 20:2ω6 | .576 | + | .305 | .542 |
| 20:3ω6 | .159 | - | .134 | - |
| 22:0 | .348 | .567 | .222 | .433 |
| 20:4ω6 / 20:3ω3 / 22:1ω9 | 14.22 | 13.35 | 19.53 | 18.15 |
| 20:4ω3 | 1.37 | 1.50 | 1.18 | 1.14 |
| 20:5ω3 / 22:2ω6 | 3.13 | 7.74 | 9.97 | 8.11 |
| 22:3ω6 | - | - | - | - |
| 22:4ω6 / 24:1ω9 | 11.12 | 7.28 | 4.44 | 4.01 |
| 22:4ω3 | 8.32 | 6.42 | 4.80 | 5.17 |
| 22:5ω3 | 4.59 | 5.69 | 5.37 | 5.70 |
| 22:6ω3 | 9.27 | 12.55 | 15.80 | 14.95 |

TABLE 20. Component fatty acids of total lipid of jellyfish.

| | *Cyanea* | | *Aurelia* | | *Chrysaora* | | |
|---|---|---|---|---|---|---|---|
| | VIMS | PEI | ♂ | ♀ | Polyp | ♂ | ♀ |
| 16:0 | 7.53 | 8.50 | 12.49 | 12.53 | 11.42 | 9.56 | 11.95 |
| 18:0 | 1.72 | 4.67 | 12.25 | 13.15 | *4.03* | *9.98* | *9.83* |
| 18:1ω9 | *6.78* | *1.04* | 1.68 | 0.50 | *32.71* | *3.23* | *3.22* |
| 20:4ω6 | *4.90* | *7.19* | *12.34* | *25.50* | *5.51* | *33.14* | *37.25* |
| 20:5ω3 | 22.43 | 24.06 | 16.84 | 15.41 | *1.24* | *5.27* | *2.90* |
| 22:6ω3 | *25.47* | *20.65* | *22.21* | *9.85* | *1.73* | *8.72* | *6.42* |

Values are expressed as percent of total fatty acids. Major differences within species are italicized.

TABLE 21. Fatty acids of jellyfish medusae.

| | *Chrysaora* ♂ | *Chrysaora* ♀ | *Aurelia* ♂ | *Aurelia* ♀ | *Rhopilema* | *Cyanea* (Y.R.) | *Cyanea* Gulf of St. L. |
|---|---|---|---|---|---|---|---|
| 14:0 | 1.39 | 1.05 | .68 | 1.23 | 1.46 | 1.89 | 2.12 |
| 14:1 / iso 15 | .15 | .11 | + | + | .27 | – | – |
| 15:0 | 1.95 | 2.34 | 1.14 | .71 | .78 | .59 | .88 |
| 15:1 / iso 16 | – | – | – | 1.11 | – | – | – |
| 16:0 | 9.56 | 11.95 | 12.49 | 12.53 | 7.36 | 7.53 | 8.50 |
| 16:1 | 1.75 | 2.09 | 1.30 | 3.39 | 1.59 | 4.58 | 3.31 |
| 16:2 | – | – | – | 1.97 | – | – | – |
| 16:4ω3 | .51 | – | – | – | – | – | – |
| 17:0 | 2.37 | 4.06 | 1.91 | 1.46 | 1.41 | 2.34 | 2.05 |
| 17:1 | – | – | – | – | – | – | – |
| 18:0 | 9.98 | 9.83 | 12.25 | 13.15 | 13.87 | 1.72 | 4.67 |
| 18:1ω9 | 3.23 | 3.22 | 4.07 | 3.52 | 3.41 | 4.52 | 6.01 |
| 18:2ω6 | .52 | .60 | 1.70 | .53 | .21 | 1.24 | .64 |
| 18:3ω6 / 20:0 | .89 | .90 | .14 | .38 | – | .70 | 1.06 |
| 18:3ω3 / 20:1ω9 | .70 | .81 | 2.11 | .57 | .78 | 5.37 | 5.85 |
| 18:4ω3 / 20:2ω9 | .84 | .98 | 1.68 | .51 | .58 | 6.78 | 1.04 |
| 20:2ω6 | .38 | – | + | + | – | 2.25 | .57 |
| 20:3ω6 / 22:0 | .31 | .30 | .26 | + | – | .30 | 2.23 |
| 20:4ω6 / 20:3ω3 / 22:1ω9 | 33.14 | 37.25 | 12.34 | 25.50 | 10.05 | 4.60 | 7.19 |
| 20:4ω3 | 1.36 | – | .95 | – | 1.21 | 1.45 | .72 |
| 20:5ω3 / 22:2ω6 | 5.27 | 2.90 | 16.84 | 15.41 | 36.95 | 22.43 | 24.06 |
| 22:3ω6 | – | – | – | – | – | – | .68 |
| 22:4ω6 / 24:1ω9 | 8.86 | 8.96 | 2.23 | 3.63 | 1.81 | 1.29 | 1.08 |
| 22:4ω3 | 4.07 | 4.06 | 1.81 | 2.09 | .34 | 1.08 | 1.57 |
| 22:5ω3 | 3.97 | 2.14 | 3.86 | 2.43 | 6.81 | 3.80 | 5.07 |
| 22:6ω3 | 8.72 | 6.42 | 22.21 | 9.85 | 11.10 | 25.47 | 20.65 |

TABLE 22. Fatty acids of white *Chrysaora* tissues.

| | Medusa ♂ | Gonad ♂ | Oral Arms & Tent. ♂ | Medusa ♀ | Gonad ♀ | Oral Arms & Tent. ♀ |
|---|---|---|---|---|---|---|
| 14:0 | 1.01 | 1.04 | .726 | 1.20 | 1.40 | .532 |
| 14:1 | .143 | - | - | .218 | - | - |
| iso 15 | | | | | | |
| 15:0 | .715 | 1.19 | 1.78 | .892 | 1.64 | 1.60 |
| 15:1 | .692 | - | - | .873 | - | - |
| iso 16 | | | | | | |
| 16:0 | 13.19 | 13.86 | 8.10 | 13.94 | 12.42 | 9.09 |
| 16:1 | 2.49 | 1.50 | 1.49 | 3.95 | 1.61 | 1.39 |
| 16:2 | - | - | - | - | - | - |
| 16:4 | - | - | - | - | - | - |
| 17:0 | 1.73 | 2.81 | 4.52 | - | 1.90 | 4.82 |
| 17:1 | 2.59 | - | - | 2.60 | - | - |
| iso 18 | | | | | | |
| 18:0 | 8.92 | 8.50 | 9.19 | 9.78 | 9.43 | 10.08 |
| 18:1ω9 | 5.70 | 5.39 | 3.60 | 6.67 | 7.53 | 3.57 |
| 18:2ω6 | .774 | .792 | .524 | .879 | 1.02 | .304 |
| 18:3ω6 | 1.85 | 1.49 | 2.18 | 1.53 | 1.07 | .709 |
| 20:0 | | | | | | |
| 18:3ω3 | 1.34 | 1.09 | .712 | 1.39 | 1.64 | .205 |
| 20:1ω9 | | | | | | |
| 18:4ω3 | .512 | .116 | .093 | .773 | .324 | .243 |
| 20:2ω9 | - | - | - | - | - | - |
| 20:2ω6 | .542 | .443 | .511 | + | .355 | .526 |
| 20:3ω6 | .433 | .150 | .119 | .567 | .417 | .313 |
| 22:0 | | | | | | |
| 20:4ω6 | | | | | | |
| 20:3ω3 | 18.15 | 9.50 | 26.26 | 13.35 | 18.34 | 29.31 |
| 22:1ω9 | | | | | | |
| 20:4ω3 | 1.14 | 1.01 | .635 | 1.50 | .825 | 1.61 |
| 20:5ω3 | 8.11 | 16.14 | 9.48 | 7.74 | 9.93 | 8.63 |
| 22:2ω6 | | | | | | |
| 22:3ω6 | - | - | - | - | - | - |
| 22:4ω6 | 4.01 | 2.15 | 4.67 | 7.28 | 3.85 | 5.64 |
| 24:1ω9 | | | | | | |
| 22:4ω3 | 5.17 | 3.19 | 6.53 | 6.42 | 5.90 | 6.58 |
| 22:5ω3 | 5.70 | 7.60 | 5.57 | 5.69 | 4.56 | 5.07 |
| 22:6ω3 | 14.95 | 21.99 | 13.28 | 12.55 | 15.84 | 9.78 |

TABLE 23. Fatty acids of jellyfish polyps.

| | *Cyanea* | *Aurelia* | *Cassiopea* | *Chrysaora* |
|---|---|---|---|---|
| 12:0 | - | .26 | .62 | .39 |
| 13:0 | 1.21 | .63 | .49 | .40 |
| 14:0 | 1.15 | .65 | .62 | .75 |
| 14:1 / iso 15 | .38 | .21 | .41 | .59 |
| 15:0 | .82 | .24 | .35 | .33 |
| 15:1 / iso 16 | - | - | - | .81 |
| 16:0 | 14.97 | 10.22 | 15.23 | 11.42 |
| 16:1 | 8.12 | 7.27 | 9.79 | 9.40 |
| 16:2 | - | - | .75 | 1.07 |
| 17:0 | - | 1.31 | 2.88 | - |
| 17:1 | - | - | - | - |
| 18:0 | 3.15 | 7.36 | 5.68 | 4.03 |
| 18:1ω9 | 26.66 | 28.46 | 45.67 | 32.71 |
| 18:2ω6 | 3.63 | 5.39 | 9.34 | 6.05 |
| 18:3ω6 | - | 1.73 | - | - |
| 20:0 | .43 | .72 | 1.02 | .23 |
| 18:3ω3 / 20:1ω9 | 7.85 | 12.92 | 17.96 | 14.70 |
| 18:4ω3 | - | 1.80 | 3.47 | 2.92 |
| 20:2ω6 | 5.94 | 3.09 | 2.72 | 1.31 |
| 22:0 | .73 | .90 | 1.55 | 1.74 |
| 20:4ω6 / 22:1ω9 | 2.17 | 4.76 | 6.64 | 5.51 |
| 20:4ω3 | 2.11 | .90 | .61 | .39 |
| 20:5ω3 | 9.05 | .20 | .69 | 1.24 |
| 22:3ω6 / 24:0 | 8.41 | .66 | 1.55 | 2.29 |
| 22:4ω6 / 22:3ω3 | - | 1.23 | .89 | - |
| 22:4ω3 | - | 1.30 | - | - |
| 22:5ω3 | - | - | - | - |
| 22:6ω3 | - | 5.02 | 1.47 | 1.73 |

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## IV. SPECULATIONS ON 1971 JELLYFISH POPULATIONS

### 1 - Copepod Abundance

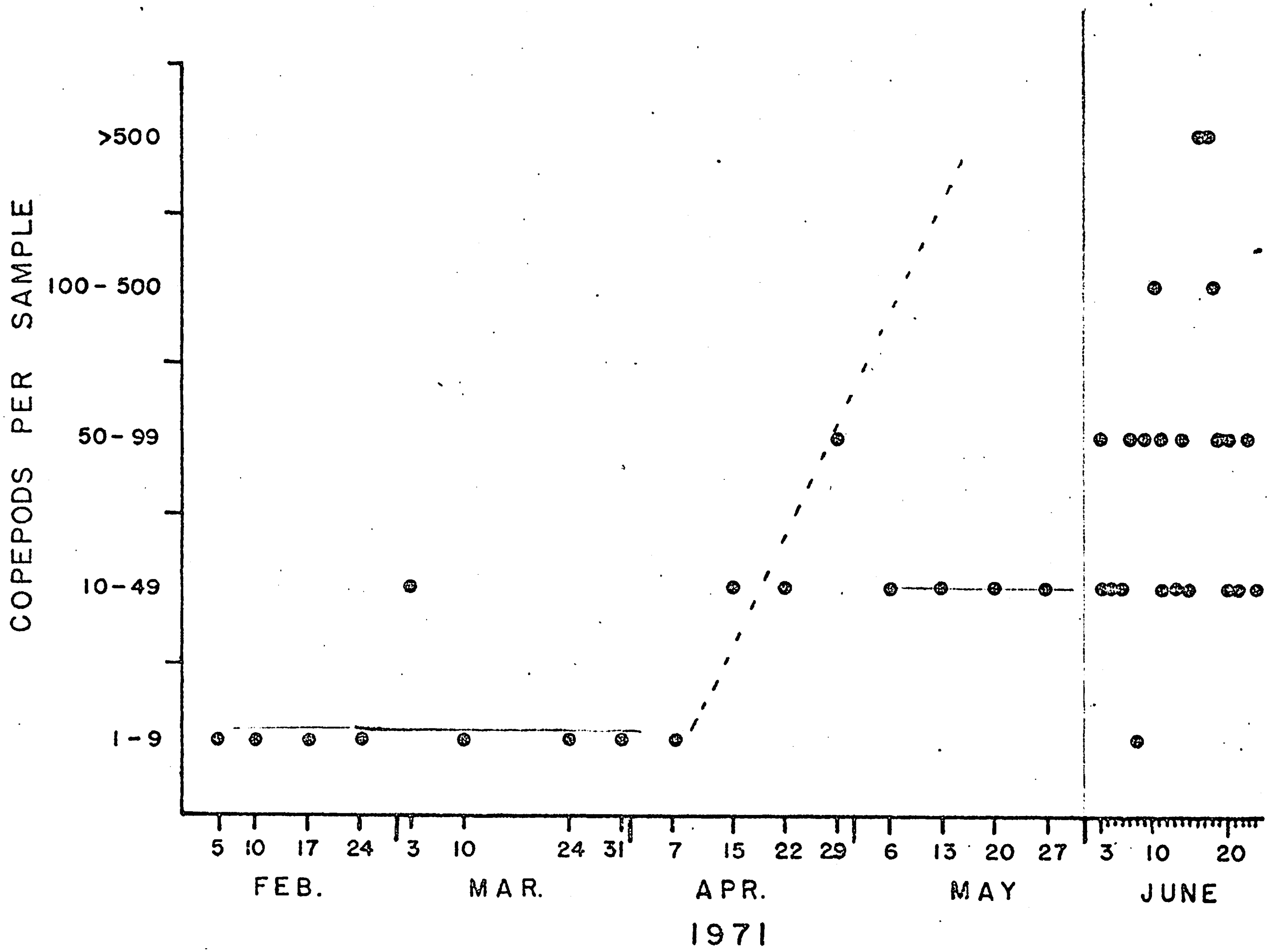
P. L. Zubkoff

A speculation on the lack of jellyfish centers on the possible availability of copepods as a planktonic food source for the developing medusae. It is speculated that *Acartia tonsa*, the predominant copepod of the York River, might be a suitable source of food.

Copepods were sampled at weekly intervals between February and May of 1971 and daily in June of 1971 by J. Weaver, a graduate student at VIMS. A plankton net was towed along the length of the *Pathfinder* pier at VIMS. A semi-quantitative approximation of the copepod abundance is presented in Figure 16. As can be seen, the relatively low abundance existed between February and mid-April. More organisms were available in mid-April and May. It is hypothesized that by the end of April greater copepod abundance would be expected (dotted line). However, environmental effects could have drastically reduced the population or prevented the normal seasonal abundance. The higher numbers in June are assumed to be a delayed abundance of the copepod populations. It is believed that such high numbers should be encountered in the beginning of May, the time that ephyrae would be liberated.

However, in retrospect, it turns out that sampling between February and the end of May was carried out during the day, whereas that during June was in the evening. Copepod distribution is known to have a diurnal response. The appearance of copepods during the night can be considerably greater than that during the day. However, disregarding the samples obtained in June, we still look on the semi-qualitative numbers as a potentially important aspect for the ephyra and medusa development. This interpretation has let us to an investigation of the stream flow data.

FIG. 16. Day/night.



2 - Stream Flow Data

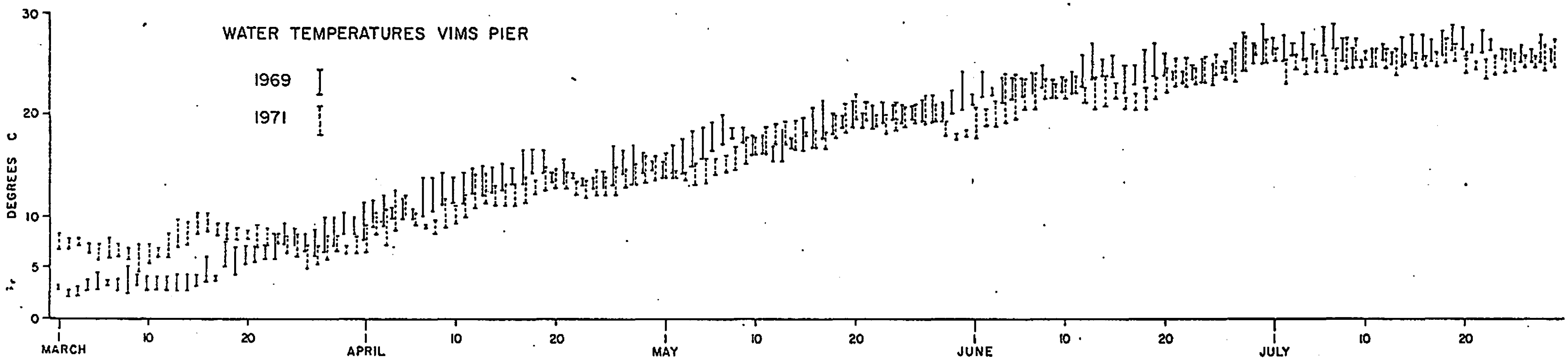
P. L. Zubkoff and J. E. Warinner, III

In view of the unusually small jellyfish populations that occurred in the summer of 1971, we analyzed the stream flow data of the York River which may be representative of the integrated fresh water input of the lower Chesapeake Bay. The daily range of water temperatures was taken at VIMS pier. Since the *Chrysaora* strobilation process usually occurs in mid-spring in the lower Chesapeake, only the mid-winter to early summer data were studied. The data for 1969, a year of particularly large jellyfish populations, and 1971 are compared.

The temperature ranges for the years 1969 and 1971 have been plotted in Figure 17. As can be seen immediately for 1969 and 1971 the greatest differences in temperature for 1969 and 1971 occurred during the month of March. For the first 20 days of March, 1969, the range of temperature was approximately 3 to 5°C while that for 1971 was 7 to 9°C. For the rest of the time between mid-March to mid-July the temperatures were approximately the same.

Since *Chrysaora* polyps and cysts exist during March, it is most unlikely that the temperature differences there would have any major adverse effects on the jellyfish population of 1971. It can be seen that for the active period of strobilation, probably late April to mid-May, that the temperatures for 1969 and 1971 were virtually identical. Any anomolous temperature effect in the environment during April to June of 1971 is clearly ruled out as being a major cause of the small 1971 jellyfish populations.

FIGURE 17.


WATER TEMPERATURES VIMS PIER
1969
1971
DEGREES C
30
20
10
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MARCH
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APRIL
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MAY
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JUNE
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JULY
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20

The York River fresh water discharge data, collected by the Geological Survey, U. S. Department of the Interior at the most seaward gauging stations of the Mattaponi River and the Pamunkey River*, which form the York River, are presented in Figure 18. Again, the year of 1969 (upper curves) is contrasted with the year of 1971 (lower curves). In 1969 the discharge ranged between 1000 and 4000 cubic feet per second (cfs) during February and April; during May the discharge was of the order of 1000 to 2000 cubic feet per second. The average discharge was approximately 1500 cubic feet per second from February to July. The salinity during this period of time ranged between 19 and 22.5 o/oo, eventually getting as high as 22.5 o/oo for June and July. The low salinity at 19 o/oo occurred for a brief period at the beginning of April. From February to June, there were 5 periods of major fresh water input.

In contrast to 1969, the year of 1971 was a year of particularly heavy fresh water input during February, mid-April, mid-May and early June. The river discharge ranged from 1000 to 7000 cubic feet per second for extensive periods with a maximum of 12,000 cfs at the beginning of June. The extent of fresh water input appears to be so heavy that the salinity dropped from the beginning of February to 20 o/oo down to a minimum of 15 o/oo in mid-March. Average values in salinity have been approximately 17 o/oo during March and April. A minimum of 14.5 o/oo was reached at the first of June, the period of maximum rainfall.

These discharge data indicate that the year 1969 with salinities of the order of 19-22 o/oo and average river discharges of

* Mattaponi River near Beulaville and Pamunkey River near Hanover.

approximately 1500 cubic feet per second are favorable environmental parameters conducive to supporting large jellyfish populations. In contrast, the year of 1971 has extensive periods of approximately 17 o/oo salinity, considerably lower than that of 1969 and discharge at least twice that of 1969.

We interpret these 1971 discharge data as being a major consideration for the environmental parameters which adversely affect the jellyfish populations. If one assumes that the period of strobilation is going to be from mid-April to mid-May, the salinity at 18-19 o/oo is certainly within the tolerance range for survival of the newly liberated ephyrae. Indeed, consistent with this interpretation, strobilating polyps were collected in the Corrotoman River on the 12th of May 1971. Unfortunately, no physical data were collected at that time. An observation in the Upper Bay (Cargo, personal communication) also indicated strobilating polyps occurring in early spring at their normal period of strobilation.

If one assumes that ephyrae were liberated in their normal populations between mid-April and mid-May, then there would be a large ephyrae population in the waters. These ephyrae (free floating embryonic forms of medusae) would rapidly undergo development and maturation into medusae between mid-May and the beginning of July or approximately 6-8 weeks--an increase in size from 2-3 mm in diameter to 30-50 mm in diameter, or a minimum of 10-fold increase in diameter. This increase in diameter corresponds to a 200-400 fold increase in area, and by conservative assumptions to 1000-fold increase in volume. If one assumes that this increase in mass occurs over an 8-week period, this represents an average doubling in volume or mass every 7 days.

Without considering specific cellular components or rates of synthesis, in order to increase in mass, ephyrae would have to ingest an enormous quantity of nutritive material. We speculate at this time that the heavy rainfalls exhibited in the early part of May and the early part of June disrupted the normal processes of jellyfish development by interrupting the planktonic food chains which would normally serve as nutrition sources for the developing ephyrae. We further speculate that since the period of mid-May to mid-June would be the most active period of ephyrae development, the very rapid drop in salinity from 19 o/oo to 15 o/oo from 10 May to 1 June subjected the polyps and ephyrae to a perturbed condition. In addition, the polyps were placed under severe stress and the newly liberated ephyrae could not undergo their normal pattern of development because of a concerted lack of nutritive plankton. In effect, the heavy rain waters perturbed the planktonic jellyfish forms and dispersed populations of planktonic organisms to such extent that existing ephyrae were incapable of survival. Under the severly stressed condition we speculate that the ephyrae simply disintegrated at the early but sensitive stage of their development, thus leading to a reduced population of medusae later in the summer.

Other data which are consistent with the change in normal development of the medusae have been depicted in the lipid analysis. In general, fatty acids of carbon chain less than 18 are associated with energy reserves while those of carbon chain greater than 20 are associated with structural lipids. Consistent with this observation, polyps have total fatty acids of carbon lengths predominantly $C_{18}$ and less while medusae are shown to have carbon lengths of $C_{20}$ and greater.

If one examines medusae collected in July of 1971 with those of frozen specimens obtained from the 1969 samples, one observes that the quantities of fatty acids of the 1971 medusae are intermediate between those of the polyps and those of the medusae of 1969. We interpret this finding to mean that those medusae which survived and developed during the late spring of 1971 were subjected to severe metabolic or nutritive stresses and that development occurred somewhat impaired.

These analyses of (1) copepod distribution, (2) fatty acid analysis, and (3) York River discharge data led to the filing of the amendment and the design of the nutrition-plankton study.

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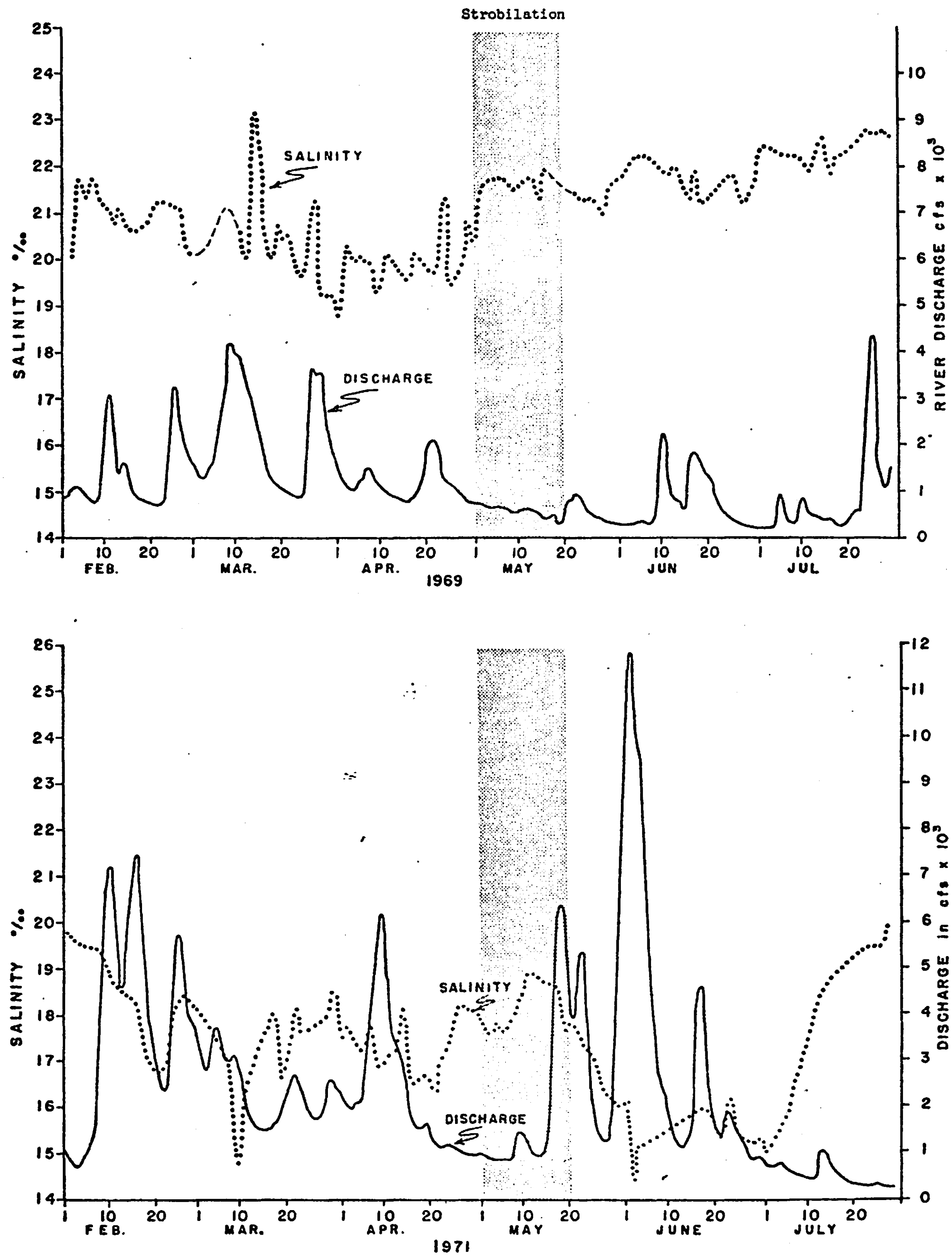
Water Resources Data for Virginia, Part 1. Surface Water Records

FIG. 18. York River fresh water discharge and salinity.



## V. DESIGN OF PLANKTON NUTRITION STUDY

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Two creeks were examined for suitability as study areas for a plankton-nutrition program: Back Creek, located 1.5 miles south of the York River, and Sarah's Creek, located on the north shore of the York River six miles from the mouth. Back Creek is relatively broad and has depths of seven feet for two miles. Sarah's Creek has two branches (northwest and northeast), with depths of seven feet for about 0.8 miles up both branches (U. S. Coast Pilot, 1971). Samples indicated a greater concentration of polyps in Sarah's Creek, particularly in the northeast branch, where large accumulations of shell were found. A regular sampling program was therefore undertaken in Sarah's Creek beginning 22 February 1972.

Stations were occupied at four locations: Station 1, just outside the creek (37° 15' 08" N, 76° 29' 00"W); Station 2, just inside the creek (37° 15' 24" N, 76° 28' 42" W); Station 3, adjacent to several oyster houses in the northeast branch (37° 15' 50" N, 76° 28' 00" W); Station 4, in the northwest branch (37° 15' 43" N, 76° 29' 02" W). On station, physical data, including air and water temperature, salinity, turbidity, dissolved oxygen, wave height, tide stage and current velocity were collected. Using a Clarke-Bumpus sampler, two five minute plankton tows were taken, the first preserved for zooplankton identification and counts, the second for biochemical analysis. A five minute tow was also made using a quarter meter net for *Chrysaora* ephyrae and young medusae.

Records were taken Monday through Friday on air temperature, water temperature, salinity, turbidity and wave height at a pier near Station 3 (37° 15' 37" N, 76° 28' 02" W). Records of minimum and maximum air temperature, wind velocity and direction, solar radiation and precipitation were kept at Gloucester Point (37° 14' 52" N, 76° 30' 03" W).

Oyster shells were collected using hand tongs at Station 3, and polyps on these shells were examined for morphological and cytological indications of strobilation. Because holotrichous haploneme nematocysts develop before morphological signs of strobilation, squashes were made to determine presence or absence of this nematocyst category. Data from this study should facilitate precise determination of the onset of strobilation and delineation of the environmental conditions extant at that time. Such information is necessary if an effective control program on polyps is to be undertaken.