



Chemistry, pharmacology and bioactivity of a novel apoptotic compound - a sex regulator in decapod crustaceans with promising environmental and medical applications





GIUSEPPE REVERBERI

Direttore dell'Istituto di Zoologia della Università di Palermo

Boll Zool. 4-6: 91-94 (1950)

La situazione sessuale di *Hippolyte viridis* e le condizioni che la reggono.

Continuando le ricerche sul problema della castrazione parasitaria, ho rivolto la mia attenzione a un piccolo decapode delle praterie di Posidonia, l'*Hippolyte viridis*, che può ospitare o la *Bo-pyrina virbii* o il *Phrixus virbii*: la prima che vive sotto il branchiostegite, il secondo sulla porzione ventrale dell'addome. Una notazione che ebbi modo di fare fin dalle prime raccolte e che confer-

.....

4° Per spiegare come, col cambiare delle condizioni somatiche (accrescimento) lo stato sessuale si inverte, e per restare nella teoria della determinazione genetica del sesso – sebbene in *Hippolyte* non ci siano cromosomi sessuali evidenti – può supporre che delle due componenti geniche l'una, quella maschile, si esprima solo a bassi livelli di energia di sviluppo l'altra, quella femminile, ad alti livelli.

Hippolyte inermis Leach (adult female)



Strategies of sexual inversion in *Hippolyte inermis* Leach (Crustacea, Decapoda) from a Mediterranean seagrass meadow

Valerio Zupo*

Stazione Zoologica "A. Dohrn", Laboratorio di Ecologia del Benthos, Punta S. Pietro, 80077 Ischia (Napoli)
Italy

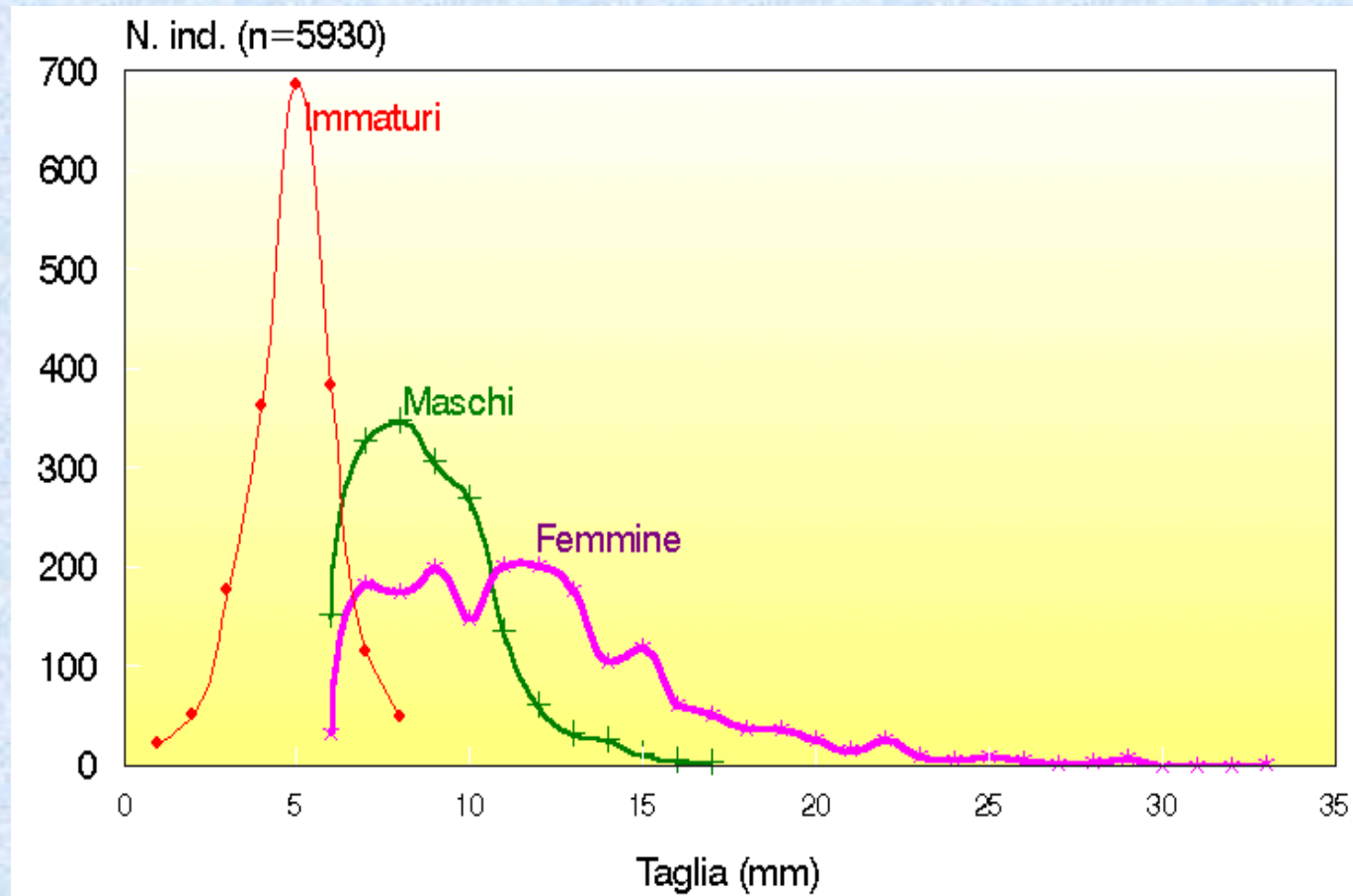
(Received 14 May 1993; revision received 1 December 1993; accepted 11 January 1994)

Abstract

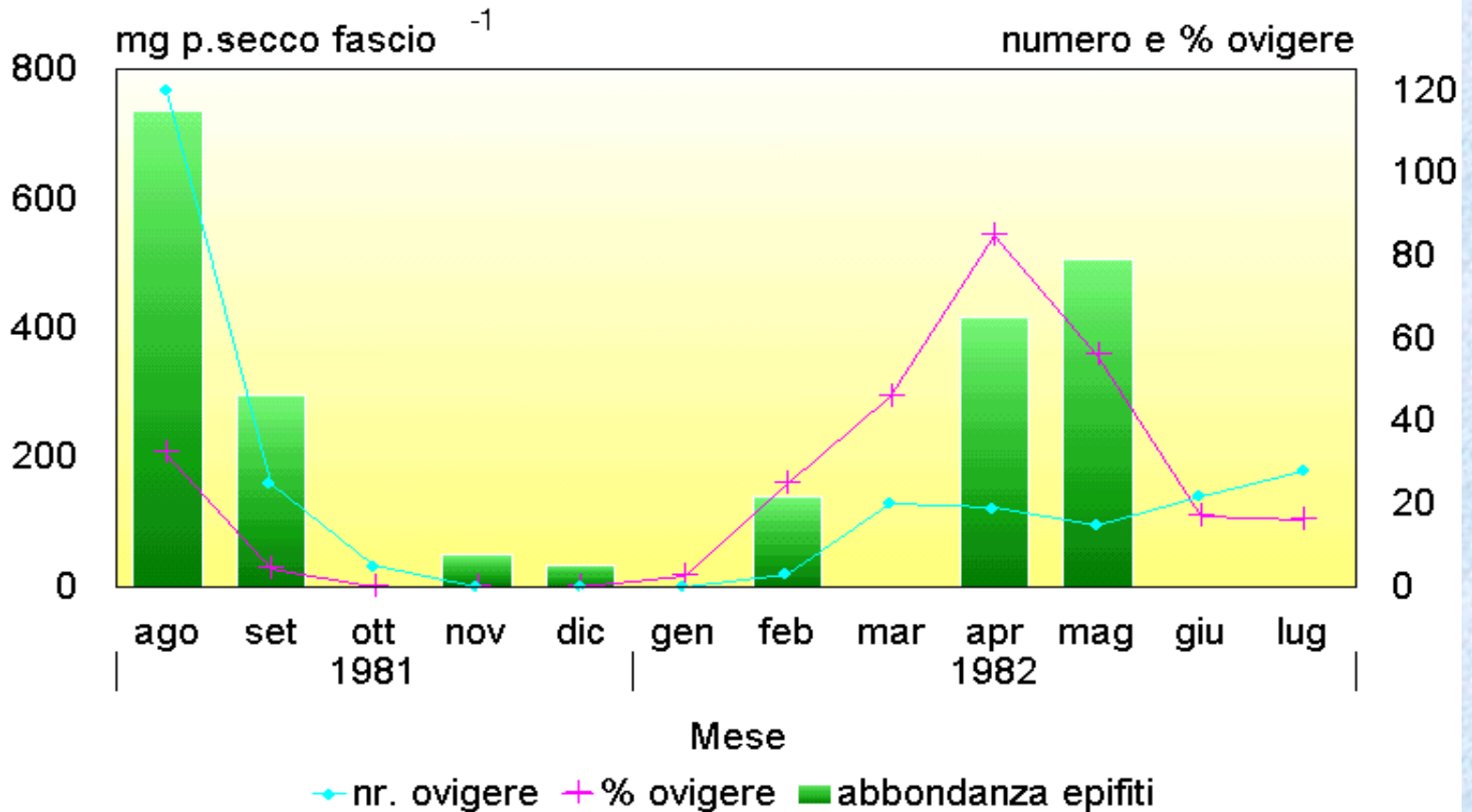
The population of the shrimp *Hippolyte inermis* Leach was investigated for one year along a transect through a bed of *Posidonia oceanica* (L.) Delile. Two reproductive periods per year were observed and two types of females were identified: one type is small, does not pass through a male stage and spawns in September to produce the next year's male generation. The other is large, passes through a male stage, and spawns in April to produce sufficient males and females for the reproductive period in September. This unusual strategy of sexual inversion could be an adaptation to overcome problems related to predation pressure and seasonal availability of food in *P. oceanica* seagrass meadows.

Key words: Food; *Hippolyte inermis*; life cycle; *Posidonia oceanica*; Sex reversal

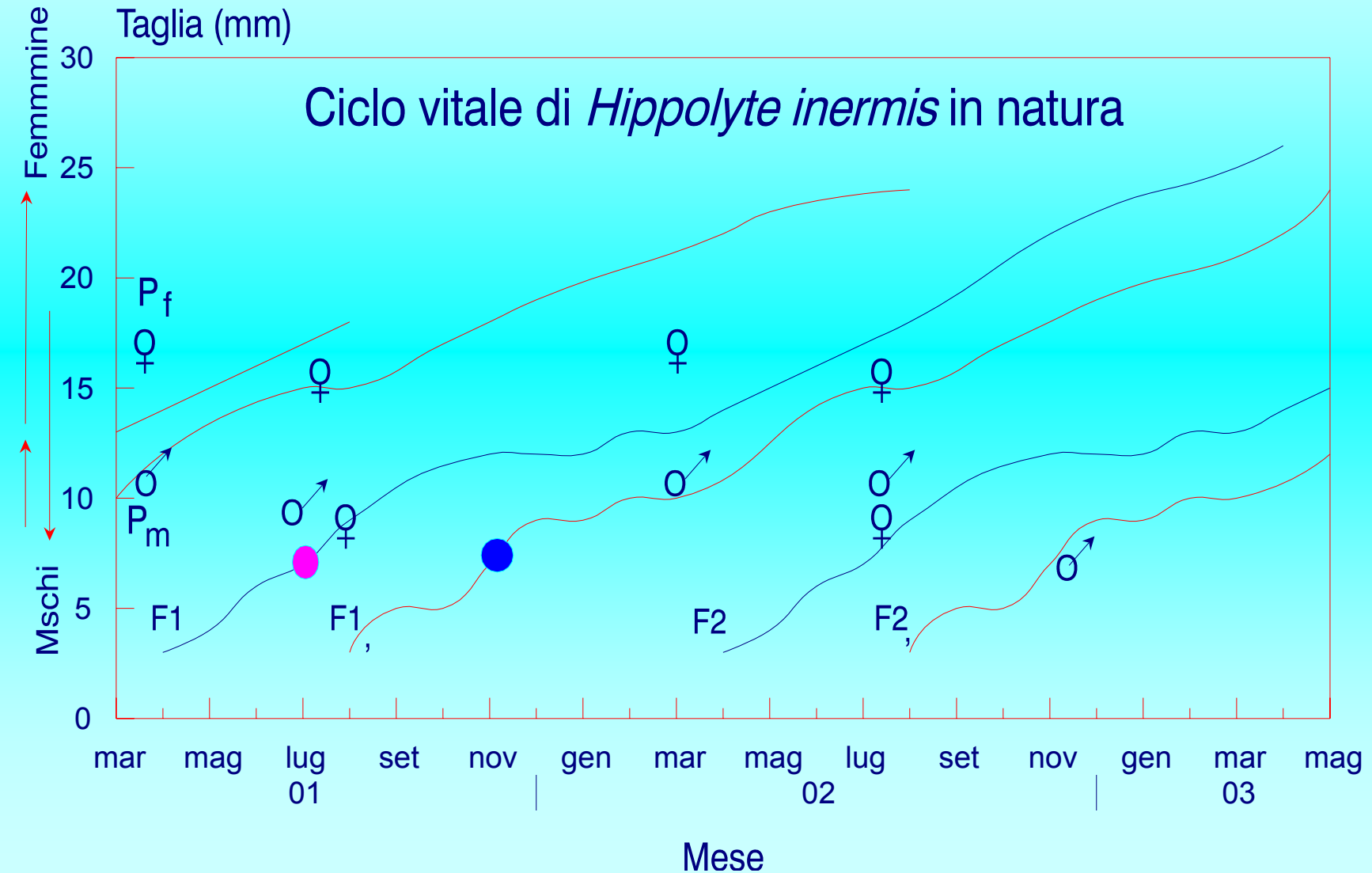
Sex ratio in the field



Relationships with epiphytes



Results of the first field investigation





Influence of diet on sex differentiation of *Hippolyte inermis* Leach (Decapoda: Natantia) in the field

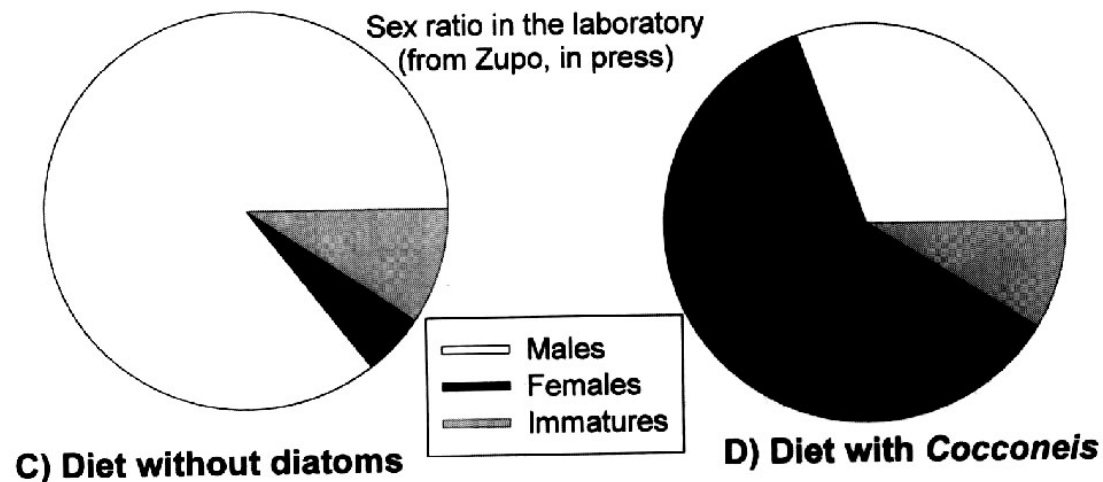
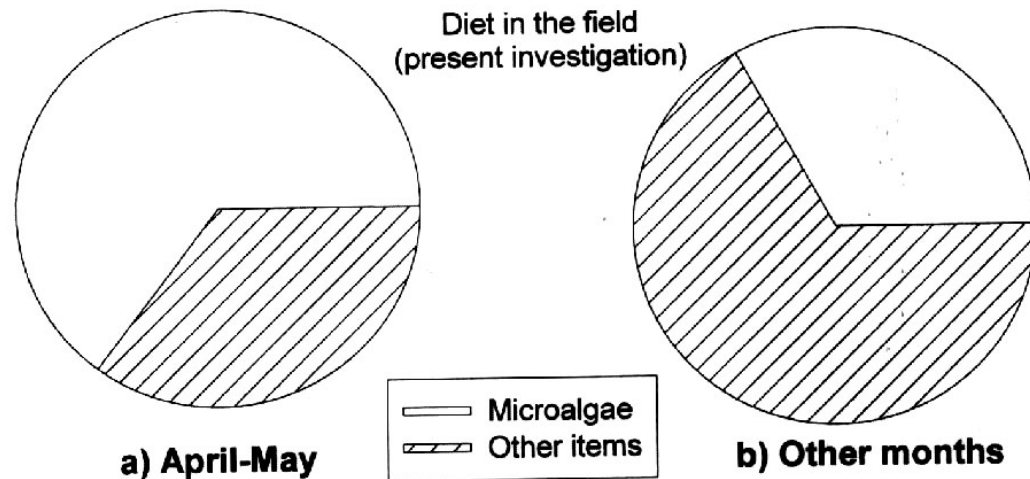
Valerio Zupo

Stazione Zoologica 'A. Dohrn' di Napoli, Laboratorio di Ecologia del Benthos, Punta S. Pietro, I80077 Ischia, Italy. E-mail: vzupo@alpha.szn.it

Key words: *Hippolyte inermis*, shrimp, food, sex reversal, development, *Posidonia oceanica*

Abstract

The gut contents of the shrimp *Hippolyte inermis* were investigated for 1 year along a depth transect through a seagrass bed. Besides size, sex and weight of all individuals were recorded. The diets of immature and adult individuals were compared to detect any influence of food on sex development, since previous investigations indicated a correlation of the life cycle of this protandric species with the abundance of algal food in the environment, and laboratory experiments demonstrated the effect of diatoms of the genus *Cocconeis* on the direct development of females. Results indicated that the shrimp is an opportunistic herbivore, able to feed on both plant and animal items, with a preference for macroalgae and diatoms present on the leaves of *Posidonia oceanica*. Small females, deriving from direct differentiation, had a diet significantly different from that of males. The difference was due to a larger abundance of microalgae in the guts of young females. The influence of microalgal food on the sex reversal mechanism of this species, previously detected through laboratory experiments, was demonstrated to control the life cycle of *H. inermis* in the field.



(Zupo, 2001)

Effect of microalgal food on the sex reversal of *Hippolyte inermis* (Crustacea: Decapoda)

Valerio Zupo*

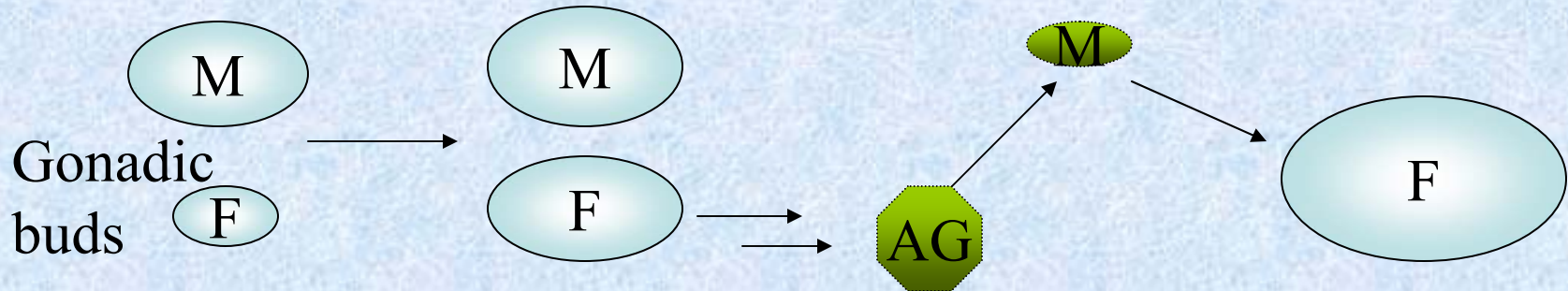
Stazione Zoologica 'A. Dohrn' di Napoli, Laboratorio di Ecologia del Benthos, Punta San Pietro, 80077 Ischia (Naples), Italy

ABSTRACT: The effect of diatoms of the genus *Cocconeis* on the sex reversal of the shrimp *Hippolyte inermis* Leach was examined in the laboratory. Randomised experiments were carried out to investigate the functional response of shrimps to various diets. The benthic diatom *Cocconeis neothumensis* was offered, alternatively, during the larval phase and during the postlarval phase, and the results obtained with shrimps produced by individual females were compared. Results demonstrated that diets based on green alga *Enteromorpha* sp. or dry commercial food did not influence the normal protandric development, as most shrimps at sexual maturation were males. Conversely, diets containing *C. neothumensis* did influence the protandric development, as most shrimps at sexual maturation were females and the sex ratio was significantly different from that obtained with diets not containing *C. neothumensis*. These results provide an explanation of the patterns observed in the field, and are in accordance with the seasonal abundances of diatoms in the leaf stratum of *Posidonia oceanica*.

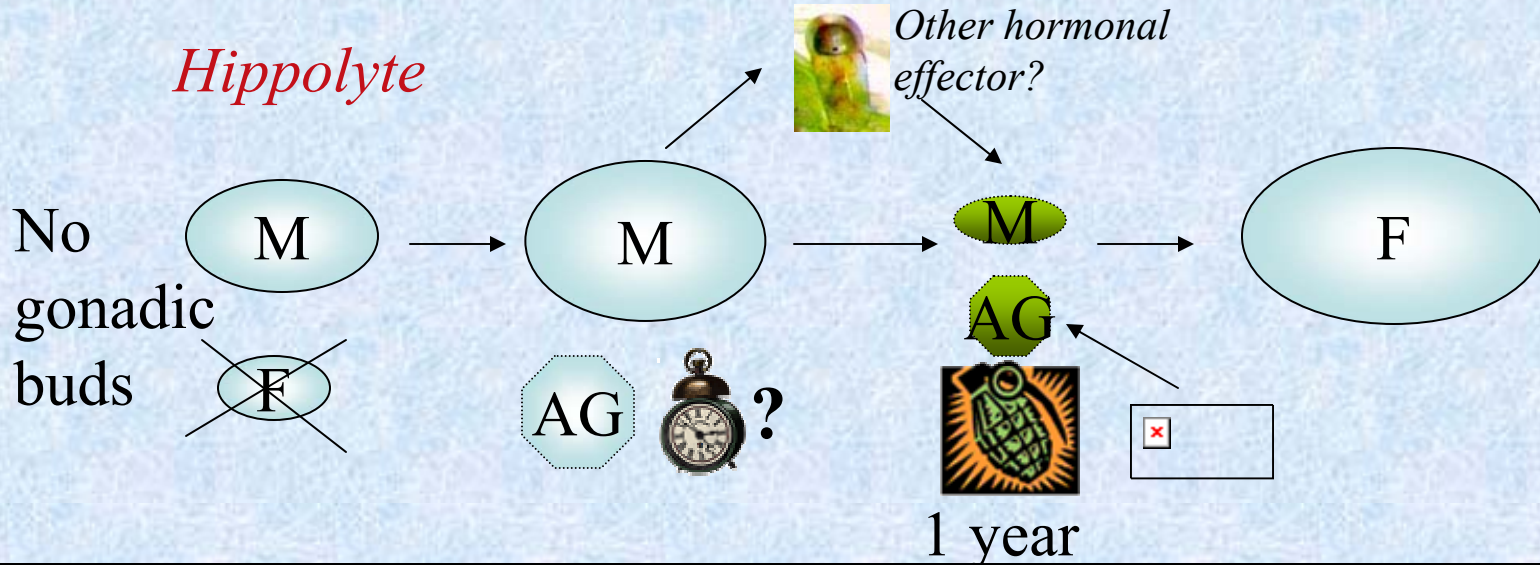
KEY WORDS: Diatom · Shrimp · Sex change · Adaptation · Feeding

How food may influence sex?

Other decapods



Hippolyte



A high-contrast, black and white photograph showing a close-up of a person's face. The lighting is dramatic, with deep shadows and bright highlights. The person's mouth is open, and their hand is visible near their face, suggesting a state of distress, pain, or intense emotion. The image has a grainy, artistic quality.

Petit Format Guignozzi

The diagram illustrates the cell cycle and its potential outcomes. On the left, four floppy disks are stacked vertically, each representing a different state: green (top), red, blue, and black (bottom). To the right of these disks are four labels: 'PROLIFERAZIONE' (aligned with the green disk), 'ARRESTO E QUIESCENZA' (aligned with the red disk), 'DIFFERENZIAMENTO' (aligned with the blue disk), and 'MORTE' (aligned with the black disk). In the center, a circular arrow represents the cell cycle, with four segments labeled: 'PROLIFERAZIONE' (green), 'DIFFERENZIAMENTO' (blue), 'ARRESTO E QUIESCENZA' (red), and 'MORTE' (black). To the right of the circular arrow, four large, dark, arrow-shaped blocks point towards the right, labeled: 'DANNO' (top), 'ONTOGENESI' (top-right), 'OMEOSTASI NUMERICA' (bottom-right), and 'INFEZIONI' (bottom). A small black box with the letter 'M' is visible on the far right edge.



PROLIFERAZIONE

ARRESTO E QUIESCENZA

DIFFERENZIAMENTO

MORTE

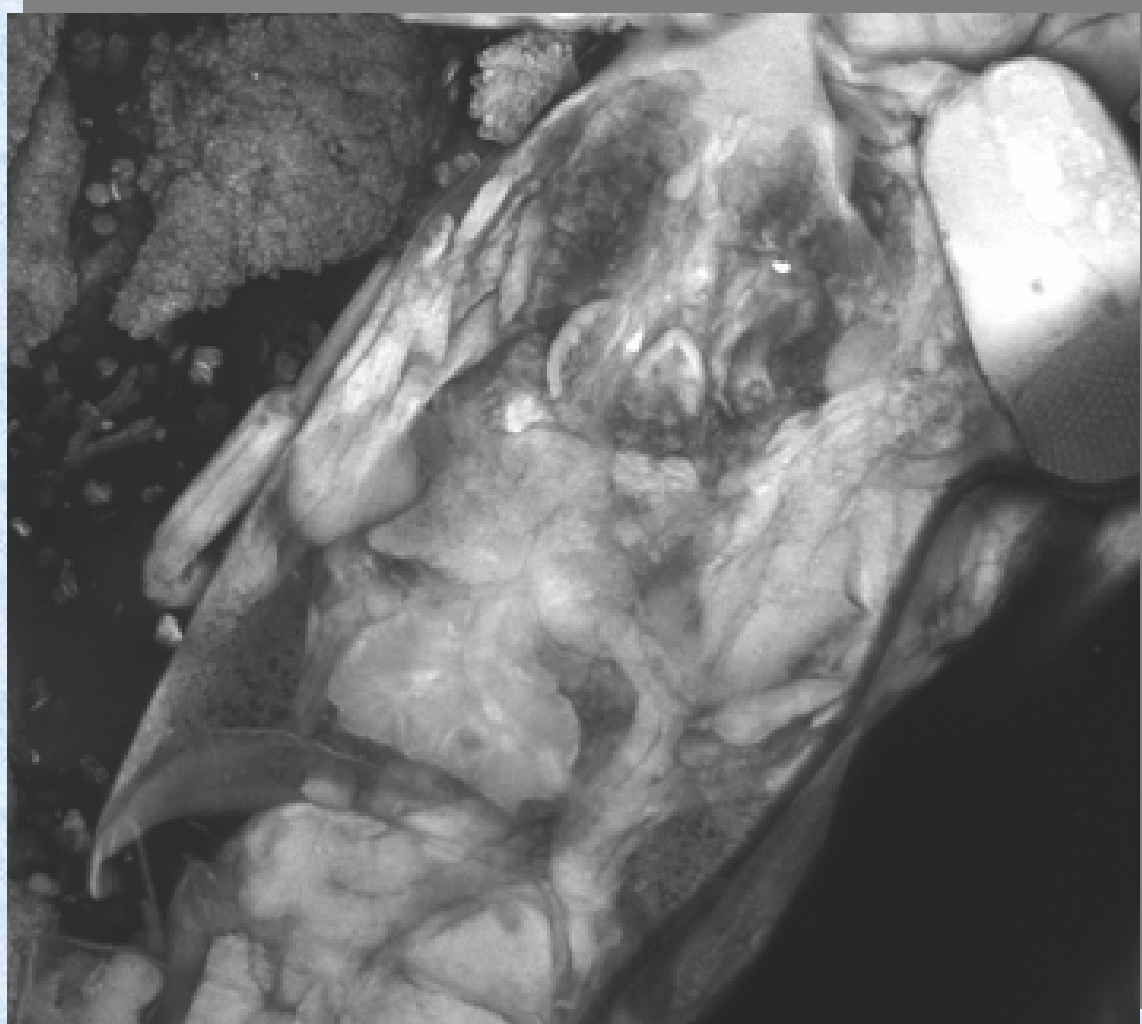
TO

Diagramma circolare che mostra i processi biologici:

- PROLIFERAZIONE
- DIFFERENZIAMENTO
- ARRESTO E QUIESCENZA
- OMEOSTASI NUMERICA
- INFEZIONI
- ONTOGENESI
- DANNO

Un grande freccia nera indica la progressione.

ma di morte cellulare che concorre al modellamento del divduo in via di sviluppo. Come risulta dallo schema so, nell'adulto questo programma presiede poi all'osi numerica delle popolazioni cellulari e rappresenta, sorprendentemente, una risposta attiva della cella agli stimoli lesivi indotti da agenti fisici, chimici e bi-



TUNEL (rapid detection of apoptosis)

E.U. Pharmapox

Chemistry, pharmacology and bioactivity of a novel apoptotic compound - a sex regulator in decapod crustaceans with promising environmental and medical applications



We aim at isolating, characterizing and investigating the bioactivity, the mechanism of action and the biotechnological applications of a new, peculiar factor present in marine diatoms.

Objectives (3 workpackages)

The main objectives to be reached in 33 months are:

WP 1: *to extract, isolate and purify the apoptotic compound(s) from diatoms;*

WP 2: *to characterize and study the bioactivity of this compound(s) as a sex regulator in decapod crustaceans;*

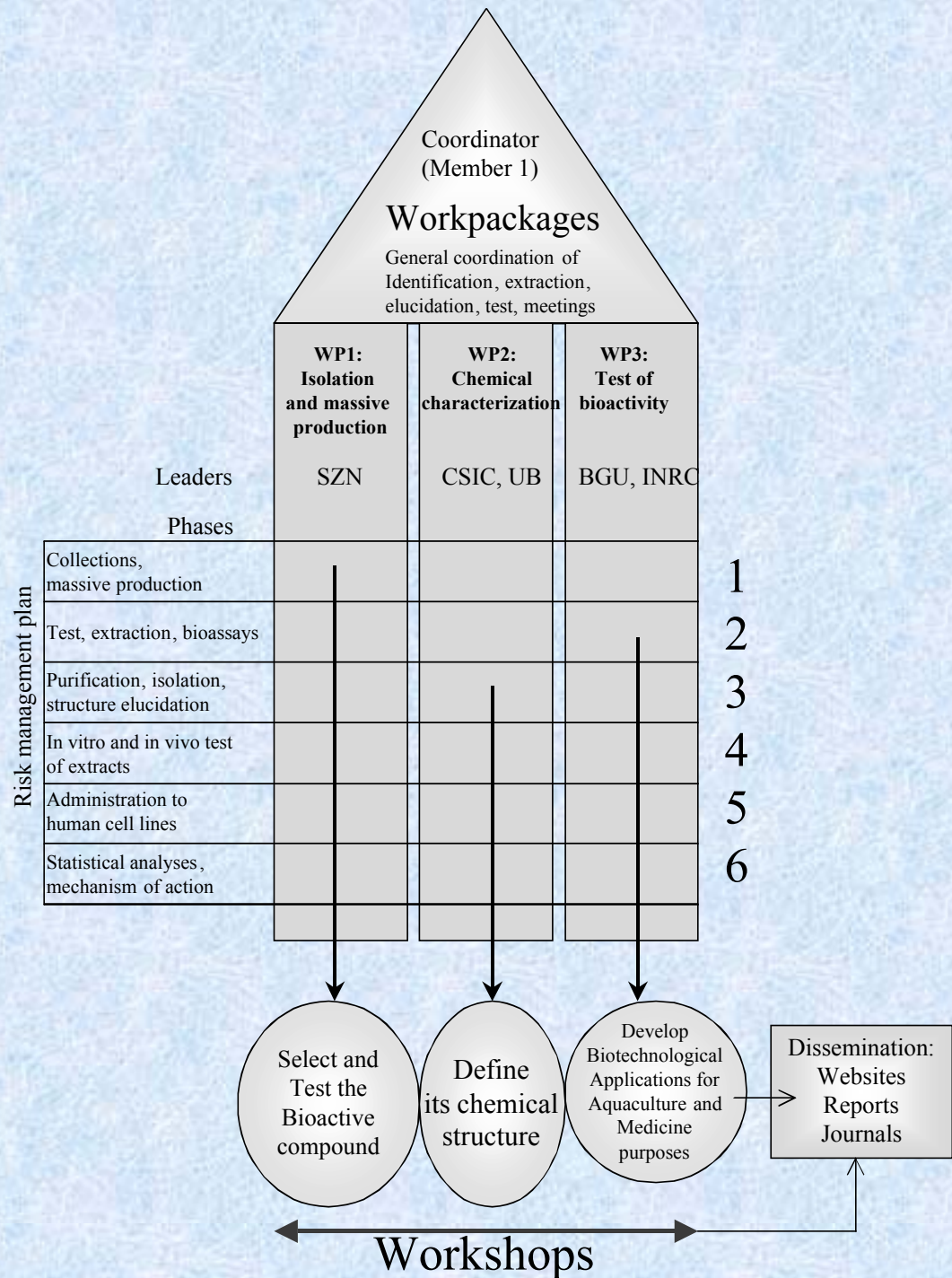
WP 3: *to investigate its possible biotechnological applications by testing its antitumoral activity and aquaculture efficacy.*

Multidisciplinarity

List of Participants

| Partic. Role* | Partic. no. | Participant name | Participant short name | Country | Date enter project** | Date exit project** |
|------------------|----------------|---|---------------------------|---------|-------------------------|------------------------|
| CO | 1 | Stazione Zoologica “A. Dohrn” | SZN | Italy | 1 | 33 |
| CR | 2 | Ben Gurion University of the Negev | BGU | Israel | 1 | 33 |
| CR | 3 | Consejo Superior de Investigaciones Cientificas | CSIC | Spain | 1 | 33 |
| CR | 4 | Universitat de Barcelona | UB | Spain | 1 | 33 |
| CR | 5 | Istituto Nazionale per la Ricerca sul cancro | INRC | Italy | 1 | 33 |

Structure of the project



Work to be done...

WP1

- Diatom collection
- Diatom isolation
- Diatom culture
- Massive cultures

WP2

- extraction of fractions

WP3

- Food preparation
- Sampling of shrimps
- Production of postlarvae
- Dried diatom bioassay
- Fraction bioassay
- Analysis of apoptosis

- Fraction Further purification
- Structure elucidation
- Synthesis

Crustacean cell lines
In vivo tests
In vitro tests
Tests on human cells

- Compound bioassay

Data analysis- Spread of biotechnologies

