FEEDING ACTIVITY AND DIET OF BENTHIC SUSPENSION FEEDERS RELATED TO METABOLIC REQUIREMENTS AND SESTON COMPOSITION

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Dieta i taxes d'alimentació de suspensívors bentònics marins en relació als seus requeriments metabòlics i a la composició del seston

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Al Pere i la Marta de Miralcamp

ELS MEUS PARES

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AGRAÏMENTS

Tot i que encara em falten alguns mesos per acabar aquesta tesi, no se perquè avui he decidit començar aquest apartat. Potser és que així em fa l'efecte que sòc més aprop del final...sigui com sigui començaré avui 17 d'Abril del 1998.

El meu primer pensament a l'hora de començar aquest apartat ha estat la meua familia. Vull agrair als meus pares la seua generositat i el seu recolzament incondicional. El seu suport, tot i que com a bons fills de la terra ferma no ho veien gens clar, ha estat imprescindible per mi durant tots aquest anys. A la meua germana Carme l'hi agraeixo la seua paciència i l'estar sempre apunt per ajudar-me. Al meu germà Anton i la meua cunyada Espe els he d'agrair sobretot el deixar-me compartir tant de prop els primers estadis de vida dels tres petardos de casa: La Pilar, l'Elena i la Marta. Elles sense saber-ho m'han ajudat a desconnectar en moltes ocasions i m'han ensenyat que fer un puzzle de la "Sirenita" potser tant o mes important que escriure un paràgraf més de tesi.

El Rafel ha estat al meu costat durant tots aquests anys. Amb ell vaig començar la meua aventura en el camp de la recerca estudiant misidacis de coves ja fa uns quants anys i des de llavors hem anat navegant junts per aquest mon. Ell ha estat en tots els aspectes, el millor col·laborador que he tingut. Amb ell he viscut moments inoblidables en llocs increïbles!!!, i d'ell he aprés moltissimes coses. El seu "infatigable" ajut en la feina d'aigua ha estat crucial per a mi en moments que ja em semblava haver esgotat totes les bateries. L'hi vull agrair sobretot recordant, aquest últims mesos, la PACIÈNCIA que ha tingut amb mi, el deixar-me disfrutar d'aquesta feina, haver aguantat inacabables converses a totes hores sobre els punts i comes d'aquesta tesi, ... ah !!! i fer que el meu anglès l'entengués algú més a part de nosaltres dos. També vull agrair a tota la familia de Vic, des dels més grans als més petits, les bones estones que he compartit amb ells i el seu interès per la nostra feina.

Els meus orígens en la recerca van començar al Departament D'Ecologia, gràcies a la Tecla que em va introduir al seu grup junt amb el Mikel Zabala, el Rafel i la Marta Carola. Els misidacis van ser el primer esglaó i van continuar les gorgonies, les garotes.... D'aquell temps en el departament d'Eco tinc molt bons records.... Recordo l'equip femení de Rem: la Mª José C, la Marta C, la Nuri M, la Montserrat R; les esquiades i sortides en bici: el Quim, el Miguel Àngel M, la Teresa A. el Javier R. l'Imma Llobet, la Marta P, l'Esperança G, a més de sopars i anades al cinema amb els marins i limnòlegs.

Vaig començar la tesi en un projecte que encapçalava el Josep-Maria Gili i vaig anar a l'Institut de Ciències del Mar. Tot i que llavors no n'era molt conscient ara veig la sort que he tingut d'anar a parar alli. Aquesta tesi tot i que pretent ser de bentos, hi ha molt de plancton (sobretot del més petit). Tot el que he aprés al respecte ho dec a la gent del

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La part de metodologia-instrumental d'aquesta tesi l'he d'agrair en primer lloc al Prof Armin Svoboda. Ell va venir a Barcelona i va ser el disenyador oficial de l'instrumental utilitzat. Despres, les aventures i desventures en la posta apunt dels circuits electrònics la vaig compartir amb l'Agustí Julià i el Jordi Parera. Jo no sabia ni soldar un cable i qualsevol cosa que fes xispes m'esgarrifava.... gràcies als coneixements de l'Agustí i del Jordi juntament amb el seu entusiasme, la cosa va anar canviant. Ells dos van fer que aquella caixa plena de cables i components electrònics ja no em fes "por". Els agraeixo l'haver trobat sempre un forat en les seves agendes per ajudar-me, l'haver estat partícips d'aquesta part de la tesi i el fer-me entendre el funcionament de la "bèstia".

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De les meves estades a l'Estartit el primer que em ve al cap es en Josep Mª Llenes (així sembla mes de Lleida, oi!!!). Tenir-lo a ell allí es com tenir algú de la familia. Ell m'ha solventat problemes logístics.... (recordo el rescat de les campanes amb la seva barca), m'ha proporcionat ampolles (millor dit ampolletes) de les que a mi m'agraden... i sobretot múltiples converses als griells, al port, a la botiga ... d'aquelles que et fan sentir que allí hi tens un amic. Faig extensiu també el meu agraïment a tota la familia Llenas-Elias per les seves atencions durant tot aquest temps.

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La gent del Servei Cientifico Tècnic, C:H:N: l'Isidre Casals i la Pilar Fernández i TOC: la Laia Balart, Coulter - Citómetre: Jaume Comas, sempre han mostrat un gran interès per la meua feina i m'han analitzat molt eficientment les piles de mostres que els he portat durant aquest anys.

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INTRODUCCIÓ GENERAL

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Les característiques físiques del medi marí permeten que una bona part de la matèria orgànica particulada (sigui viva o inert) es pugui mantenir en suspensió. Aquesta propietat ha permès el desenvolupament d'una estratègia capturadora de l'aliment característica del medi marí: l'estratègia suspensívora. Aquesta estratègia d'alimentació es compartida per un gran nombre de grups filogenèticament tan separats com esponges, celenteris, poliquets, briozous, tunicats.... Tots aquest grups són ampliament representats en els fons rocosos litorals arreu del mon, aportant en conjunt, la major part de la biomassa animal en aquestes zones. Els suspensívors bentònics marins, tant per la seva abundància com per la seva capacitat de processar gran volums d'aigua, actuen com a importadors de materia del plancton al bentos i com a tals semblen ser peces claus en el funcionament global dels sistemes rocosos litorals. Altrament, el desconeixement de la seva dieta natural enfront a l'ampli rang de partícules en suspensió limiten la comprensió dels factors que regulen l'energètica i la dinàmica demogràfica de les seves poblacions.

La major part d'aquest estudi s'ha dut a terme en la Mediterrània nord-occidental, típicament considerada una mar temperada càlida. La descripció en treballs previs i en el que aquí es presenta, dels paràmetres físics (temperatura, llum, hidrodinamisme, pluja) químics (nutrients) i biològics (producció primària i disponibilitat d'aliment), han fet palès una clara pauta estacional en aquesta zona (Margalef 1985, Zabala and Ballesteros 1989, Ballesteros 1989, García et al 1994, Font et al 1995, Garrabou 1997). En aquest sentit, la Mediterrània presenta una situació d'estratificació de la columna d'aigua durant els mesos d'estiu que correspon a un període de baixa producció biològica a les capes superficials. En contrast, l'hivern es caracteritza per ser un període de barreja on la producció es mes alta i es manté al llarg de tota la columna. Entre aquets dos períodes ben marcats, hi han dos períodes de transició caracteritzats per una forta inestabilitat (primavera i tardor)

Aquesta dinàmica general de canvi en la quantitat i composició de la matèria orgànica particulada suspesa en la massa d'aigua, de manera directa o indirecta es sospitava que havia d'estar afectant la dinàmica dels organismes bentònics i molt especialment els suspensívors Aquests organismes pel fet de ser sèssils, depenen de les preses que els corrents arrosseguen fins a les seves estructures capturadores. Això els obliga a ser directament depenents del que succeeix en la columna d'aigua. A més a més una sèrie d'observacions sobre la dinàmica d'alguns suspensívors bentònics mediterranis, efectuades per diferents autors en l'ultima dècada, vàren constatar que la producció secundaria (creixement somàtic i reproducció) era marcadament estacional solapant-se ambdós fenòmens en una mateixa època de l'any (Boero and Fresi 1986, Boero et al 1986, Llobet et al 1991, Becerro and Turon 1992, Garrabou 1997, Coma et al 1998). La majoria d'aquest treballs hipotetitzaren, que la dinàmica observada en les espècies podria estar directament

relacionada amb la disponibilitat d'aliment. Així, durant la (les) epoca(ques) amb restriccions d'aliment, no podria tenir lloc la producció secundaria. De fet les primeres evidències directes de restriccions tròfiques en la dinàmica d'alguns suspensívors bentònics varen ser aportades seguint una aproximació energètica, o sigui, avaluant les entrades (aliment) i les sortides (creixement, reproducció, respiració i excreció) (Coma et al 1998). En aquest estudi es va proposar l'estiu com una estació energèticament desfavorable en la Mediterrània per les dues espècies de cnidaris estudiades. Les restriccions durant l'estiu vindrien donades en primer lloc, per una disminució de la producció global a la columna d'aigua (caiguda en la matèria orgànica particulada), relacionada amb la menor producció primària (esgotament dels nutrients per sobre la termoclina). A més a més l'estratificació de la columna provocaria una ràpida sedimentació de la matèria orgànica suspesa, fent que el seston sigui poc accessible pels organismes sèssils. Juntament amb les possibles restriccions tròfiques, l'elevada temperatura que caracteritza l'època estival en la Mediterrània, incrementaria el metabolisme basal dels organismes poiquiloterms com el suspensívors bentònics, deixant menys marge per la producció secundaria. Tot i que aquesta primera aproximació energètica amb dues espècies de cnidaris bentònics recolzaven una restricció tròfica a l'estiu, algunes limitacions metodològiques en l'anàlisi de la dieta varen impedir afirmacions més rotundes sobre l'hipòtesi. De fet, la dieta en les dues espècies de suspensívors bentònics va estar avaluada mitjançant l'anàlisi dels continguts estomacals (Coma et al 1994, Coma et al 1995c). Això va deixar de banda el paper de grups de preses potencials les quals no deixaven cap remanent en els estómacs. Dins d'aquest grups de preses es podrien incloure els procariotes, protozous, alguns grups del fitoplancton, carboni dissolt.., altament dominants en les comunitats planctòniques tant en termes de biomassa (Stockner and Antia 1986) com de producció (Platt et al. 1983, Burkill et al. 1993).

Dins d'aquest marc general, aquesta memòria es va marcar com objectiu dos aspectes fonamentals en la biologia dels suspensívors bentónics marins: l'estudi qualitatiu i quantitatiu de les seves dietes naturals així com les variacions estacionals en la ingesta. A més a més, seguint una aproximació energètica, es pretenia veure si les espècies estudiades reflectien o no la suposada crisi tròfica estival. Val a dir que l'estudi de tots els paràmetres del balanç energètic tal i com es van tractar en el treball de Coma et al 1998 era altament laborios. Llavors es va optar per l'estudi del que es podria anomenar "excedent d'energia" (traducció de Energy Surplus, Sebens 1979, 1982) que seria l'energia obtinguda amb la ingestió d'aliment que queda un cop restada la despesa que suposa la respiració.

Es varen escollir 3 espècies de suspensívors bentònics de filums diferents: una gorgonia, una esponja i un ascidi. L'elecció de *Paramuricea clavata* com espècie de gorgonia, va venir donada per l'abundant informació que és te sobre la dinàmica d'aquesta espècie, així com el coneixement de la seva dieta estudiada com s'ha dit abans amb l'anàlisi dels continguts estomacals (Coma et al 1994, Coma et al 1998). De la mateixa manera, *Halocynthia papillosa* es una espècie d'ascidi solitari del que hi han uns coneixements

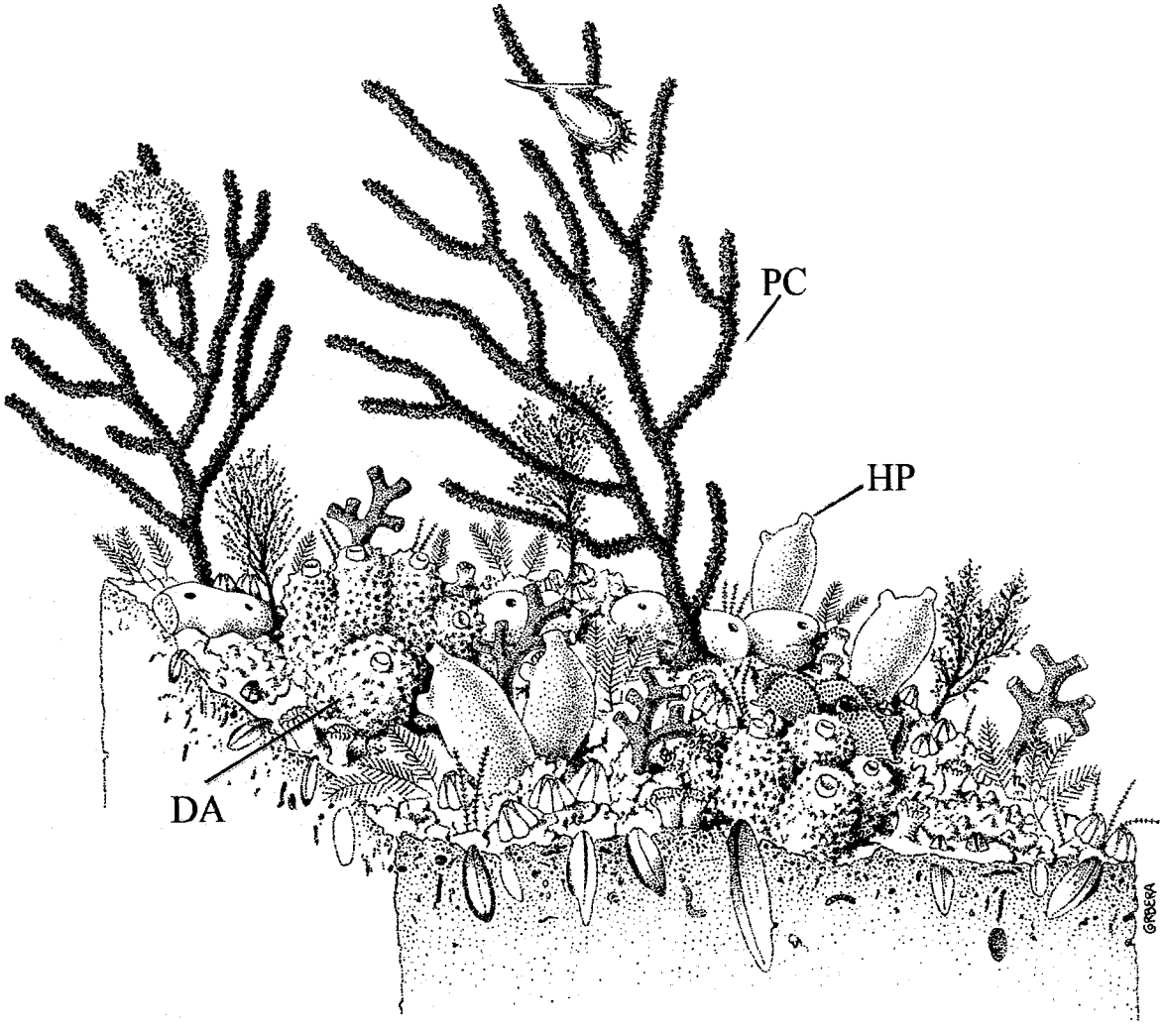

Fig.1. Vista general de la comunitat amb les espècies estudiades, DA: *Dysidea avara*, HP: *Halocynthia papillosa*, PC: *Paramurcicea clavata*.

previs de la seva dinàmica (Becerro and Turon 1992) a més a més de ser fàcil de manipular. Tot i que no hi han dades de la dinàmica de l'esponja *Dysidea avara*, la seva abundància i facilitat de manipulació, va fer que fos escollida com a objecte d'estudi (Fig. 1).

Les incubacions in situ amb campanes de circuit tancat va ser la metodologia escollida per dur a terme aquest estudi, tant la part d'alimentació com de respiració, degut a que podia ser aplicada a totes les espècies. Per l'estudi de la captura de preses (sobretot les de talla petita), en suspensívors actius com esponges i ascidis s'han descrit bàsicament dos sistemes de mesura. Els mètodes directes es basen en la comparació entre les

concentracions de preses trobades a l'aigua ambient (que serà filtrada pel suspensívor) i la concentració de preses de la corrent exhalant (aigua que surt de l'organisme un cop passada per les xarxes capturadores) (Pile et al 1996, Pile et al 1997). Els mètodes indirectes es basen en la mesura de la concentració de preses al principi i al final d'una incubació d'un o varis organismes en un recipient tancat (Patterson et al. 1991). Els dos mètodes, directe i indirecte tenen els seus inconvenients. El mètode directe exigeix una isolació de la corrent exhalant de l'aigua ambient (per això cal que la recol·lecció de l'aigua no es faci a una velocitat superior a la velocitat de la corrent d'exhalació) que pot ser dificultosa en alguns organismes i impossible en altres com per exemple els suspensívors passius com les gorgonies. Per utilitzar els mètodes indirectes, cal assegurar que s'eviten una sèrie de fenòmens inherents a les incubacions: la sedimentació de partícules quan no hi ha un flux adient a l'interior del recipient incubador, la caiguda d'oxigen al llarg del temps d'incubació que pot fer canviar l'activitat filtradora dels organismes, la desaparició de preses al llarg de l'incubació, la producció d'amoni produïts per la remineralització de la matèria orgànica i que pot arribar a ser tòxica a partir d'uns determinats valors.

En l'estudi de l'alimentació, l'optimització del volum d'aigua necessari per a l'anàlisi dels components del plàncton que es volien quantificar com a possibles preses dels suspensívors estudiats, va ser un punt clau a l'hora del disseny de les campanes incubadores. En el capítol metodològic d'aquesta tesi es presenta el protocol seguit per l'anomenada quantificació, que creiem que pot ser útil per un estudi similar aplicat a la gran majoria de suspensívors bentònics. A més a més, el dispositiu utilitzat havia d'assegurar en els experiments d'alimentació, un flux constant que no permetés la sedimentació de les partícules tancades dins la campana així com simular al màxim les condicions ambientals (gairebé sempre sotmeses a un cert moviment de la massa d'aigua). En l'estudi de la respiració s'havia d'assegurar que no es formessin gradients en la concentració d'oxigen provocats pel fenomen de buidat de les capes més properes als organismes. A més, es volia obtenir de manera fiable una sèrie de mesures de consum d'oxigen, a les diferents hores del dia. Tots aquests requisits es varen aconseguir amb el disseny del dispositiu experimental que es descriu en l'Anex II. Previ als experiments d'alimentació, mitjançant una sèrie de proves amb els organismes que es volien estudiar, utilitzant diferents biomasses i temps d'incubació, es va decidir els temps d'incubació mínims per detectar una caiguda significativa de tots els tipus de preses que es volien estudiar.

Un cop dissenyats i posats a punt els dispositius experimentals (aparells) i el protocol d'obtenció i tractament de les mostres, es va procedir al disseny experimental. Com s'ha comentat anteriorment, aquest estudi pretenia abordar la dinàmica en les taxes d'alimentació i respiració de les espècies escollides en funció de les condicions del medi (sobretot disponibilitat d'aliment i temperatura). Per això, la part experimental va ser duta a terme in situ i de manera estacional, de cara a cobrir de manera més fidel possible les condicions naturals a les que estan sotmesos els suspensívors bentònics en la zona

estudiada. Degut a la variació estacional en el cicle de producció biològica en la Mediterrània, l'estudi mensual en termes qualitatius i quantitatius de l'abundància i composició de les preses potencials en la capa d'aigua més propera al fons, ens va permetre una descripció de l'aliment amb el que podien comptar aquest organismes. A més una sèrie d'experiments d'alimentació duts a terme estacionalment i experiments de respiració duts a terme cada 2-3 mesos, varen ser plantejats per assolir els objectius proposats.

La presentació d'aquest treball s'ha fet en una sèrie de capítols ordenats de la següent manera. Un primer capítol on s'explica la metodologia emprada en l'obtenció de mostres al camp i el seu posterior tractament al laboratori. Aquesta metodologia es aplicada a totes les espècies estudiades tot i que alguns detalls propis de cadascuna venen especificats en els conseqüents capítols. Un segon capítol descriu un cicle anual de la composició del pico- nano- i microplancton així com carboni particulat detritic i dissolt en la capa d'aigua propera a les comunitats bentòniques estudiades. Els capítols 3, 4 i 5 descriuen els resultats de la dieta i taxes d'alimentació al llarg de l'any de les espècies Mediterrànies estudiades, l'esponja *Dysidea avara*, l'ascidi *Halocynthia papillosa* i la gorgonia *Paramuricea clavata*. El capítol 6 presenta les dades de respiració al llarg de l'any de les tres espècies estudiades així com una valoració de "l'excedent d'energia" calculada utilitzant les dades d'ingesta dels capítols anteriors. En aquest capítol també es discuteix l'hipòtesi bàsica de partida sobre una possible crisi tròfica estival en la Mediterrània. Seguidament es presenten els dos capítols següents com annexes. L'annex I presenta les dades d'un estudi puntual de l'alimentació i respiració d'una espècie de gorgònia tropical, *Plexaura flexuosa*. La peculiaritat i interès de l'estudi d'aquesta espècie ve donat per la gran quantitat d'algues simbionts que presenta en contrast a l'espècie de gorgonia Mediterrània sense simbions, exclusivament heterotròfica. Finalment i com annex II es presenta la descripció del dispositiu instrumental dissenyat i posat a punt per assolir els objectius d'aquesta tesi, amb un èmfasi especial en la part corresponent a respiració.

Tot i que de manera global aquesta memòria va ésser plantejada per aconseguir uns objectius comuns, cadascun dels capítols, excepte el de metodologia, han estat plantejats amb l'estructura d'articles de revistes científiques. Per la presentació de la memòria s'ha unificat el format i s'han fet comunes les parts de metodologia i bibliografia. De totes maneres seguint l'última normativa de la Universitat de Barcelona, en el *Curriculum Vitae* presentat juntament amb aquesta memòria, es fa pales el nombre d'autors així com el seu ordre dels articles publicats o pendents de publicació.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

The physical properties of seawater allow living creatures and particulate matter to remain in suspension, thereby creating a niche for a trophic strategy that does not occur on land: suspension feeding. The importance of this feeding strategy is particularly apparent in benthic marine communities, in which most animal groups have morphological structures capable of exploiting suspended particles as a potential food source. Because of their abundance, certain benthic suspension feeders have been shown to have a major impact within the ecosystems in which they dwell (Gili & Coma 1998). However, the natural diets of most suspension feeders are poorly known, and our lack of knowledge about their feeding habits has become a limiting step in understanding the factors that constrain populations.

Most of this study was carried out in the north-west Mediterranean, typically considered to be a warm temperate sea. The descriptions in previous works and in that which is presented here, of the physical (temperature, light, hydrodynamism, rainfall), chemical (nutrients) and biological (primary production and food availability) parameters, have shown a clear seasonal pattern in this zone (Margalef 1985, Zabala & Ballesteros 1989, Ballesteros 1989, Garcia et al 1994, Font et al 1995, Garrabou 1997). In this sense, the Mediterranean presents a situation of stratification of the column of water during the summer months which corresponds to a period of low biological production in the superficial layers. In contrast, winter is characterised as being a mixed period with a high production which is maintained throughout the whole column. Between these two well defined periods there are two transition periods characterised by strong instability (spring and autumn).

It was suspected that this general dynamic, either directly or indirectly, could be affecting that of the benthic organisms, and specifically that of benthic suspension feeders. These organisms, being sessiles, depend on prey (in the form of particulate organic matter) which the currents displace to their capturing structures; something which obliges them to be totally dependent on whatever happens in the water column. Furthermore, a series of observations regarding the seasonal dynamic of some Mediterranean benthic suspension feeders (including ascidians) made by different authors over the last decade showed that the secondary production (somatic and reproductive growth) of some of these Mediterranean species was markedly seasonal, and in some occasions both phenomena overlapped in the same time of year (Boero & Fresi 1986, Boero et al 1986, Llobet et al 1991, Becerro & Turon 1992, Garrabou 1997, Coma et al 1998). Some studies hypothesised that the dynamic observed in the species could be directly related to food availability. Thus, during the time(s) of year when food supply is restricted, secondary production would rarely take place. In fact the first direct evidence of trophic restrictions in the dynamic of

Mediterranean benthic suspension feeders was reached following an energetic approach, that's to say, evaluating the entrances (food) and exits (growth, reproduction, respiration and excretion) (Coma et al 1998). In this study, summer was proposed as a season that is trophically unfavourable in the Mediterranean for the two species of cnidarians studied. The restrictions throughout the summer owed in the first place, to a reduction in the overall production of the water column (reduction in the quantity of particulate organic matter), related to less primary production (exhaustion of nutrients above the thermocline). Moreover the stratification of the column would provoke a fast sedimentation of the particles, making the seston less accessible to the sessile organisms. Together with the possible trophic restrictions, the high temperature which characterises the summer period in the Mediterranean, would increase the basal metabolism of poikilotherm organisms such as benthic suspension feeders, leaving less of a margin for secondary production. Although this first energetic approach with two species of benthic cnidarians supported the existence of this summer trophic restriction, some methodological shortcomings rendered the evidence inconclusive: the diet of these two species was estimated through stomach contents analysis (Coma et al 1994, Coma et al 1995c), and prey that did not leave any traces was overlooked. Within this category, organisms such as prokaryotes, protozoa, some phytoplankton groups, and even dissolved carbon are included. They are also dominant in plankton communities, both in terms of biomass (Stockner & Antia 1986) and production (Platt et al 1983, Burkill et al 1993).

Within this general framework, the main goals of the this work were two aspects of the biology of benthic marine suspension feeders: the qualitative and quantitative study of their natural diet, as well as the seasonal variation of the ingestion rate. Furthermore, from an energetic approach, we wanted to assess whether or not the energetics of the species can show evidences of the trophic hypothesis. The study of all the parameters of the energetic balance as they were treated in the work of Coma et al (1998) was tremendously laborious. Therefore the study of "Energy Surplus" (Sebens 1979, 1982) was opted for; the excess energy of the ingestion of food once the basal metabolic expenditure estimated through respiration was deducted.

Three benthic suspension feeder species from three different phylums were chosen: a gorgonian, an ascidian and a sponge. The choice of *Paramuricea clavata* as a species of gorgonian was related to the abundant information which there is about the dynamic of the species as the knowledge of its diet studied through the analysis of stomach contents (Coma et al. 1994, Coma et al.1998). In the same way, *Halocynthia papillosa* was a species of solitary ascidia of which there was some previous knowledge of its dynamic (Becerro & Turon 1992) as well as being easy to manage. In spite of the non-availability of data regarding the dynamic of the sponge *Dysidea avara*, its abundance and easy of handling made that it was chosen as object of the study (Fig. 1).

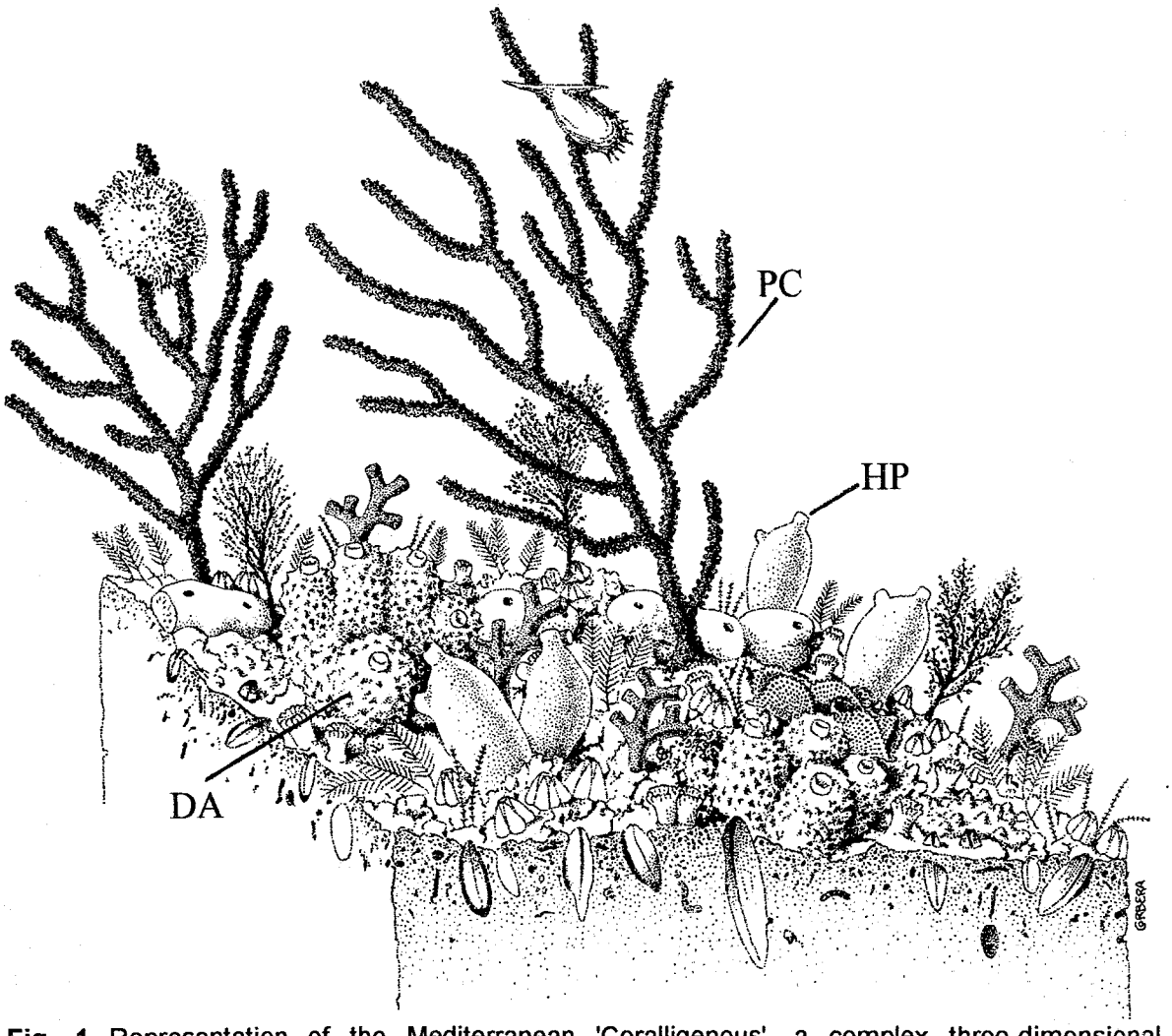

Fig. 1 Representation of the Mediterranean 'Coralligenous', a complex three-dimensional sublittoral community dominated by suspension feeders. DA: *Dysidea avara*, HP: *Halocynthia papillosa*, PC: *Paramuricea clavata*.

The *in situ* incubation using closed circuit chambers was the methodology chosen to bring about this study, as much for food as for respiration, because it was a methodology applicable to all of the species. For the study of the capture of small prey in active suspension feeders such as sponges and ascidians, two basic systems of measuring have been described. The direct methods are based on the comparison of the concentrations of prey found in the surrounding water (filtered by the suspension feeders) and the concentration of prey from the exhaled current (water which leaves the organism once it has passed through the capturing structures (Pile et al. 1996, Pile et al. 1997). The direct methods are based on the measuring of the concentration of prey at the beginning and at the

end of the incubation of one or various organisms in a closed recipient (Patterson et al. 1991). The two methods, directly and indirectly, have their inconveniences. The direct method demands a separation between the exhaling current and the surrounding water, in order to achieve this it is essential that water collection is not done at a higher speed to that of the exhaling current. This could be extremely difficult or even impossible in passive suspension feeders such as gorgonians. In order to use the indirect methods, one has to be sure that a series of phenomena inherent to incubation are avoided: sedimentation of particles when there is not enough of a flow inside the incubating recipient, the fall of oxygen over the incubation period which could affect the filtering activity of the organisms, the disappearance of prey over the incubation period, the production of ammonia due to the remineralisation of the organic material which could prove toxic above certain levels.

In the design of the incubation chambers, as far as the quantification of the food intake of planktonic components was concerned, a key factor was the assessment of the optimal volume for its analysis. In the methodological part of this work the protocol followed is presented. This will perhaps be useful in similar studies on any other suspension benthic feeder. Moreover, the ideal device used had to guarantee a constant flux in order to avoid any sedimentation of the particles inside the chamber. Additionally, the conditions inside the chamber should resemble the real ones as much as possible (i.e., almost always subject to a certain movement of the water mass).

As for the respiration studies gradients in the oxygen concentrations had to be avoided. These gradients are naturally built up as the organisms deplete the oxygen content of the closest layers of water. On top of that, reliable, frequent and comparable measurements of oxygen concentration were needed throughout a daily cycle. All these requirements were met in the design of the experimental device described in Annexe II. Previous to the food intake experiments an assessment was made to determine the minimum incubation times needed in order to obtain a detectable drop in the concentration of all the prey studied.

Once the experimental devices were designed and set up, along with the protocol of acquisition and treatment of samples, the experiments were designed. As previously stated, the approach was aimed at determining food intake and dynamics of the three chosen species; how they change in response to fluctuating environmental situations (with special emphasis on food availability and temperature). This was the reason for the experiments being performed *in situ* and seasonally distributed, with the aim of obtaining the most natural conditions to those which these species are subjected to. Owing to the seasonal variation in the Mediterranean biological production cycle, the monthly study in both quantitative and qualitative terms of the abundance and composition of potential prey in the layer of water closest to the seabed, allowed a description of available food for the suspension feeders in the zone. There were also a series of food intake experiments which

were undertaken seasonally and experiments on respiration which took place every two-three months, and were decided on in order to bring about the proposed objectives.

This work is presented in a series of chapters ordered as follows. A first chapter explains the methodology used to obtain samples in the field and their posterior treatment in the laboratory. This methodology is applicable to all of the species studied but some particular details related specifically to each one are detailed in the following chapters. A second chapter describes an annual cycle of the composition of pico-, nano-, and micro-plankton together with detrital particulate carbon and dissolved in the layer of water closest to the benthic communities studied. Chapters 3, 4 and 5 describe the results of the diet and feeding rates over an annual cycle of the three Mediterranean species, the sponge *Dysidea avara*, la ascidia *Halocynthia papillosa* y la gorgonian *Paramuricea clavata*. Chapter 6 presents the data of respiration over the year of the three species together with an evaluation of the "Energy Surplus" calculated using the data of ingestion of previous chapters. In this chapter the parting hypothesis regarding a possible summer trophic crisis in the Mediterranean is discussed. There are then two Annexe chapters. Annexe I presents the data of a specific study about feeding and respiration of the tropical species, *Plexaura flexuosa*. The peculiarity and interest of the study of this species lies in the great amount of symbiotic algae which presents a contrast to the non-symbiotic Mediterranean gorgonian which is exclusively heterotrophic. Finally and as Annexe II, the description of the instrumental device designed and employed in order to obtain the objectives of this thesis with a special emphasis on the part corresponding to respiration.

Although on a global scale this work was established in order to obtain common objectives, each chapter, except the methodology, has been set up with the structure of an article for scientific magazines. For the presentation of the work, the format has been unified and the methodological and bibliographical parts put together. In any case, following the latest norm of the University of Barcelona, in the *Curriculum Vitae* presented together with this work, the number of authors is reflected as well as their order as much in the published articles as in those awaiting publication.

1

MATERIALS AND METHODS FOR FEEDING EXPERIMENTS

I. STUDY SITE

Sampling was conducted at a coastal station (15 m depth) near the Medes Islands Marine Reserve (NW Mediterranean Sea, 42° 3' N, 3° 13' E; Fig. 1.1) between October 1995 and November 1996. The Islands are inhabited and located at approximately one mile from the mainland and about four miles from the Ter river.

II. FIELD SAMPLING

Incubations for feeding experiments were conduced in hemispherical U.V.-transparent Plexiglas chambers approximately 3L in volume. The chambers (one experimental- with organisms-, and one control) were placed at 15 m. The chambers have and inlet and an outlet apertures connected to a common piece of PVC tubing, so the system becomes closed. An electric pump was placed at the outlet aperture which during normal operation forces water through the system at a speed of 1.2 cm s^{-1} (this flow becomes turbulent inside the chambers). For more details about the chambers see annexe II. Whole specimens, were removed from their substrate and cleaned from macroepibionts. Removed specimens were attached to PVC posts. These organisms were kept in their natural environment and with conspecifics until used in incubation experiments performed *in situ*. At the beginning of each experiment, an specimen on a PVC post was placed on the base of the experimental chamber. During this period, the inlet and outlet apertures were not connected, so that the system worked as an open-flow one. 3 replicate water samples of 500 ml were collected from the outlets of both chambers (initial water samples) and preserved for further analysis (see below). At this point, inlet and outlet apertures were connected and the system was running as a closed-flow one. After 1 to 3 hours depending of the specie, the chambers were taken to the surface and 3 replicate water samples were collected again from both chambers (final water samples). Grazing was calculated from decreases in prey concentration in the experimental chamber relative to the control chamber. The potential prey items included:

* Procayotic picoplankton
  - -Heterotrophic bacteria
  - -*Synechococcus* sp (cyanobacteria)
  - -*Prochlorococcus* sp.

* Eucaryotic picoplankton (picoeucaryotes)	-Naked flagellates < 2 μm
* Eucaryotic nanoplankton (nanoeucaryotes)	-Naked flagellates > 2 μm (nanoflagellates) -Small dinoflagellates (unable to be distingued with inverted microscope) -Coccolithophores (unable to be counted with acid Lugol's).
* Microplankton	-Ciliates, -Phytoplankton (diatoms and dinoflagellates)

Moreover, dissolved organic carbon (DOC) and detrital particulate organic carbon smaller than 100 μm (POC), were also considered as a potential food. Water volume used for the analysis of DOC, POC, pico and nanoplankton was previously screened by a 100 μm net to avoid larger plankters. Five experiments were carried out each season, and therefore a total of twenty experiments were performed throughout the year cycle. Nevertheless, phytoplankton and ciliates only were analysed in 3 experiments of each season.

III. Samples Preservation and Analysis

Picoplankton and Nanoplankton

For analysis of heterotrophic bacteria, *Prochlorococcus sp.*, *Synechococcus sp.*, autotrophic pico and nanoeucaryotes, 2 ml subsamples were withdrawn from each of 6 replicates and preserved with paraformaldehyde (1.0 % final concentration), kept cold and dark for less than 30 min, and frozen in liquid nitrogen (Campbell et al. 1994). Afterwards they were stored at -80 °C or in dry ice. Samples were analyzed using a Coulter EPICS 753 flow cytometer (Coulter Electronics Corporation, Hialeah, Florida) equipped with two 5 W argon lasers and a Micro-Sampler-Delivery-System. The flow cytometer was set up for UV (220 mW) and 488 nm (1W) colinear analysis. Hoechst 33342 (DNA-specific fluorochrome) was used to stain DNA according to Monger and Landry (1993). Five parameters were collected in list mode and analyzed with custom-designed software (CYTOPC by Daniel Vaulot): red fluorescence (from chlorophyll *a*), orange fluorescence (from phycoerythrin), blue fluorescence (from DNA stained with Hoechst 33342), forward- and right- angle light scatter signals (FALS and RALS). For statistical purposes sample size for analysis was chosen to provide more than 10000 events per sample, therefore, 1 ml of samples were analysed for picoeucaryotes and nanoeucaryotes and 100 ml for heterotrophic bacteria, *Prochlorococcus sp.*, *Synechococcus sp.*

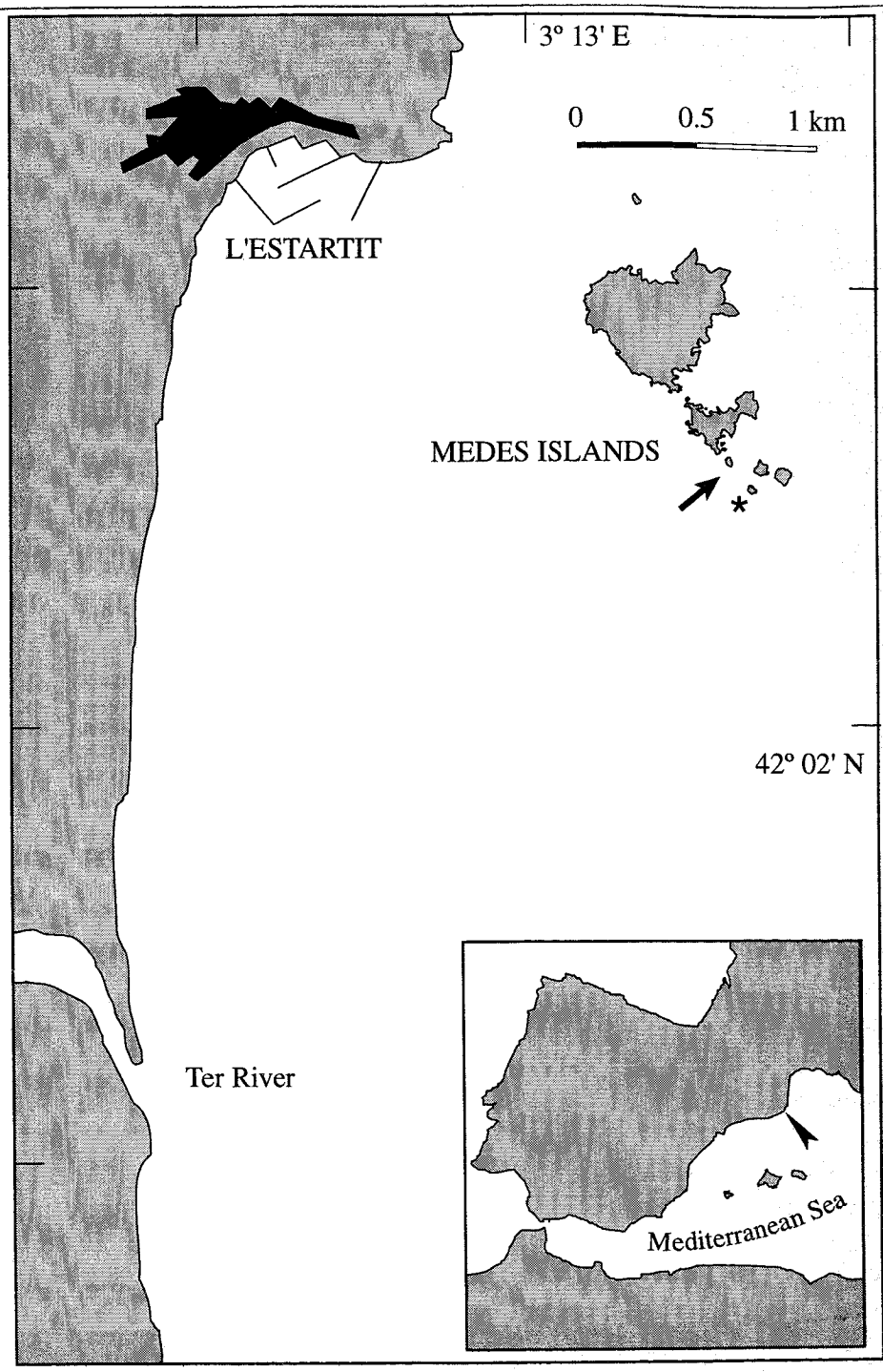

Fig. 1.1. Map of the study area (Medes Islands, NW Mediterranean Sea). Arrow and asterisk showing the sampling site and the temperature monitoring site, respectively.

For the quantification of heterotrophic nanoflagellates, 50 ml of water from 4 replicate were preserved with formaldehyde (0.5 % final solution). Subsamples of 20 ml were stained with DAPI, filtered onto 0.2 μm filters, and heterotrophic nanoflagellates were counted by epifluorescence microscopy (Porter and Feig 1980). The same subsamples stained with DAPI were used in order to measure cell size (length and width) of heterotrophic bacteria, *Synechoccoccus,* pico- and nanoeucaryotes and heterotrophic nanoflagellates. For heterotrophic bacterial size, over a hundred cells were measured using framegrabber and software for image analysis as described in Massana *et al.* (1997). For *Synechococcus sp,* pico- and nanoeucaryotes and heterotrophic nanoflagellates we used the micrometric rule of the microscope to measure cells length and width. Cells smaller than 2 μm were attributed to picoeucaryotes and the cells larger than 2 μm to nanoeucaryotes. Autotrophic nanoeucaryotes and heterotrophic nanoflagellates were measured together, and a common mean biovolume per month were calculated. It was not possible to measure *Prochlorococcus* due to the difficulty of observing the cells with epifluorescence microscopy; we used a mean size of 0.7 μm calculated for the Mediterranean by Vaulot *et al.* (1990).

Cell biovolume was calculated from length and width by approximation to the nearest geometric figures. Carbon content was then estimated from literature conversion factors as follows: Heterotrophic bacteria 0.22 pg C μm^{-3} (Fry 1988); *Prochlorococcus* 0.133 pg C μm^{-3} (Simon and Azam 1989); *Synechococcus* 0.357 pg C μm^{-3} (mean value of: Björnsen 1986, Kana and Glibert 1987, Verity *et al.* 1992); Pico- nano-eucaryotes pg C = $0.433 \times (\mu m^3)^{0.863}$ (Verity *et al.* 1992).

Flow cytometer counts were compared to epifluorescence microscopy counts. In order to establish this comparison, 32 samples stained with DAPI were used to count heterotrophic bacteria, *Synechococcus sp.* and autotrophic nanoflagellates by both epifluorescence microscopy (Porter and Feig 1980) and flow cytometry as described above.

Phytoplankton and ciliates

For analysis of phytoplankton and ciliates, 2 replicate 350-ml water samples were preserved with acid Lugol's (1 % final concentration). For each sample, 100-ml subsamples were allowed to settle and observed with an inverted microscope using the Utermöhl technique. Dominant groups of diatoms and dinoflagellates were quantified in this study. The microscope was provided with a color CCD video camera connected to a video recorder. Images of the organisms for measurement were recorded and digitized, and sizing was made using image analysis software (NIH image). For each sampling day, twenty individuals of the most common groups were measured; the volumes were estimated from the length and width measurements assuming ellipsoidal, cylindrical or conical shapes (Edler 1979). Carbon content was then estimated from literature conversion factors;

phytoplankton pg C $cell^{-1}$= 0.109 x $(\mu m^3)^{0.991}$ (Montagnes *et al.* 1994); ciliates 0.19 pg C μm^{-3} (Putt and Stoecker 1989).

Total particulate organic carbon

Particulate organic carbon was measured by filtering 60-ml (4 replicates) on pre-combusted GF/F glass fibre filters. Filters were then frozen in liquid nitrogen and kept at -80°C until analysis. Previous to analysis, filters were dried at 60 °C for 24 h and exposed to hydrochloric acid vapors for 48 h in order to destroy inorganic material. Then filters were dried again and analyzed with a C:H:N autoanalyser Perkin-Elmer 240.

Dissolved organic carbon

For dissolved organic carbon, 20-ml water samples (4 replicates) were filtered through pre-combusted GF/F glass fibre filters. The filtered water was stored in glass tubes at -20 °C until analysis. Analysis was conducted by high-temperature catalytic oxidation with an autoanalyser Shimadzu TOC-5000.

IV. DATA ANALYSIS

Depletion rates were calculated by assuming exponential growth and clearance of prey (Frost 1972; Saiz 1993). Thus, the prey growth rate is computed k (h^{-1})

$$k = \frac{\ln(C_1 / C_0)}{t_1 - t_0} \qquad (1)$$

where C_0 and C_1 are the prey concentrations in the chamber at the initial time t_0 and at the final time t_1. The clearance rate CR (volume swept clear $biomass^{-1}$ $time^{-1}$) is computed:

$$CR = V\frac{g}{b} \qquad (2)$$

where V is the volume of the chamber, b is the organisms biomass and g is the grazing coefficient (h^{-1}), computed as:

$$g = k_c - k_o \qquad (3)$$

where k_c is the prey growth rate in the control chamber, and k_o is the apparent growth in the chamber with organisms. Finally, the ingestion rate I (prey ingested $biomass^{-1}$ $time^{-1}$) is:

$$I = CR\ C \qquad (4)$$

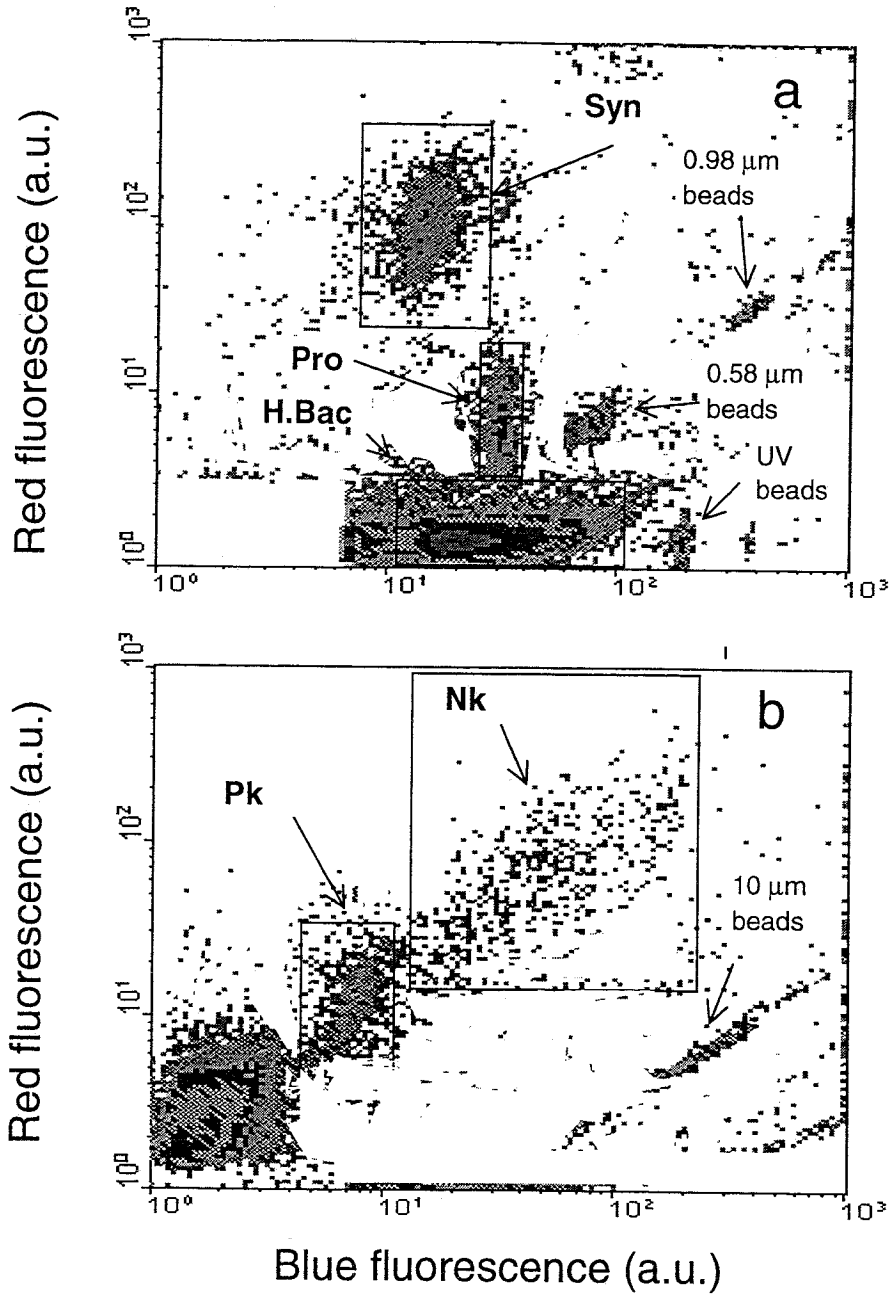

Fig. 1.2. Pico- and nanoplankton populations identified by Flow Cytometry. Red fluorescence results from chlorophyll *a* excitation and blue fluorescence from DNA stained with Hoechst 33342. (a) Picoplankton: H. Bac: heterotrophic bacteria, Pro: *Prochlorococcus*, Syn: *Synechococcus*-type cyanobacteria. (b) Pico- and nanoeucaryotes: Pk: autotrophic picoeucaryotes, and Nk: autotrophic nanoeucaryotes. a.u.: arbitrary units.

where C is the monthly average prey concentration.

The significance of predation on each kind of prey was tested by comparing growth rates of prey in control and experimental chambers with a two-tailed Wilcoxon test (Sokal and Rohlf 1981).

IV. METHODOLOGICAL CONSIDERATIONS

Different microbial populations were distinguished by flow cytometry (Fig. 1.2). Comparison between flow cytometer counts (FCT) and epifluorescence microscope counts (EMC) showed a good correlation in all type of cells (Table 1.1). For heterotrophic bacteria and autotrophic nanoflagellates the slope of the regression line fit to the data was not significantly different than 1.0 (Table 1.1). However, the high value of the nanoflagellate intercept indicate that for low abundance, flow cytometry was counting more cells than microscopy. For *Synechococcus* the slope was significantly different from 1.0 (Table 1.1), showing that flow cytometer was counting about 6 % more cells than epifluorescence microscopy. Regarding picoeucariotes, we were not able to quantify them by epifluorescence microscopy in order to check the flow cytometer counts, but other authors (Buck *et al.* 1996) have reported a good correlation between both methods.

Table 1.1. Parameters of the regression equation between flow cytometer counts (FCT) and epifluorescence microscope counts (EMC), and t-test analysis testing if the slope of the regression line fit to the data was not significantly different from 1.

					t-test		
FCT-EMC	Intercept	Slope	r^2	p	t	df	p
Heterotrophic bacteria	-1.60×10^4	1.11	0.87	< 0.0001	1.25	30	0.221
Synechococcus	162	1.16	0.91	< 0.0001	2.39	30	0.023
Autotrophic nanoeucaryotes	179	0.89	0.43	< 0.005	0.58	30	0.567

2

SEASONAL VARIATION OF POC, DOC AND THE CONTRIBUTION OF MICROBIAL COMMUNITIES TO THE LIVE POC IN A SHALLOW NEAR-BOTTOM ECOSYSTEM AT MEDES ISLANDS (NORTH-WESTERN MEDITERRANEAN)

I. ABSTRACT

Microbial planktonic communities (i.e. bacteria and protozoa), phytoplankton, DOC and POC were seasonally examined at Medes Islands (North-Western Mediterranean) to assess their variation in abundance and composition through the year in a near-bottom littoral ecosystem. From October 95 to November 96 samples were collected between 2 and 6 times per month at 0.5 m above the bottom. Mean DOC and POC values through the year were 2560 ± 180 (SE) μg C l^{-1} and 387 ± 35 μg C l^{-1}, respectively. All year long detrital organic carbon (detrital = total POC - live carbon) represented the main POC fraction, and mean live carbon was 24 ± 9 μg C l^{-1}. Winter and spring were the seasons with maximum values of POC and spring and summer had the maximum values of DOC. Heterotrophic bacteria, with a mean abundance of 5.16 ± 0.08 10^5 cells ml^{-1}, was the main contributor to live carbon (26 ± 7 %). During winter, heterotrophic bacteria biomass decreased 40 % due to a decrease in mean biovolume per cell. *Synechococcus sp* and *Prochlorococcus sp* abundance were 2.24 ± 0.09 10^4 cells ml^{-1} and 1.05 ± 0.07 10^4 cells ml^{-1}, respectively. However, while *Synechococcus sp* were present all year long, *Prochlorococcus sp* were not observed from April to July. Mean phytoplankton (i.e. diatoms and dinoflagellates) abundance was 2.06 ± 0.40 10^4 cell l^{-1} with biomass reaching a maximum during the winter months, the period with the lowest temperature and the highest nutrient concentration. The size composition of live carbon showed two clearly distinguishable periods during the year. From December to March, live carbon was dominated in biomass by microplankton, while from April to November, pico and nanoplankton cells were dominant. Overall, the dynamic of the near-bottom planktonic communities was characterised by a low biomass of heterotrophic bacteria, *Synechococcus, Prochlorococcus,* phytoplankton and ciliates, and by a high temporal heterogeneity of the different planktonic communities in contrast to previous water column studies. This pattern is discussed in relation to the physical and chemical characteristics of the environment as well as to the potential role that benthic communities may be exerting in the control of the dynamic of the near- bottom planktonic communities.

II. INTRODUCTION

Planktonic communities show a high spatio-temporal heterogeneity along the water column both at mesoscale (Denman and Powell 1984) and small scale (Owen 1989). Physical processes influence plankton distribution enhancing community development in high-energy layers where the different species adapt to special conditions of mixing and turbulence. (e.g. Mackas et al. 1993). This phenomena is well known in the plankton communities along the water column, specially throughout or in hydrographic structures such as the haloclines, but few work have been carried out in near-bottom layers. This spatial structure consists of multiple sublayers which allow habitat partitioning by planktonic organisms. The small-scale distribution enables species to concentrate and exploit a turbulent, high energy habitat such as the thermocline (Longhurst 1985). But, a similar phenomena may also occur in near-bottom layers where topography strongly affects currents and the dynamic of local water masses (Holloway 1992).

In littoral areas, the vertical structure of plankton communities could be more homogeneous due to continuous water mixing. However, near the bottom, the environmental and biological conditions for the development of plankton communities are strongly modified by substratum-current interactions (Riedl 1971) and by the supply of nutrients by benthic organisms (Graf and Rosenberg 1997). In extensive shallow marine ecosystems such as the coral reefs, planktonic communities are strongly affected by structure (space and volume) and activity (predation and food supply) of benthic communities (Sorokin 1994). Indeed, planktonic communities are actively exploited by benthic suspension-feeders, which partially return the organic matter captured from the water column, through detritic and dissolved forms or through meroplanktonic larvae (Graff 1992). However, although quantitative data on suspension feeder diet and capture rates under natural conditions are still scarce, suspension feeders are seen to be able to capture important amounts of planktonic prey items and therefore, the grazing pressure on the water column planktonic communities by macroinvertebrates appears to be much greater than previously thought (Reiswig 1990, Coma et al. 1995, Pile et al. 1996).

During the last decade, it has been shown that picoplankton (cells < 2 μm) and nanoplankton (cells 2 - 20 μm) dominate pelagic planktonic community in terms of biomass (Stockner and Antia 1986) and production (Platt et al. 1983, Burkill et al. 1993). Pico- and nanoplankton communities include procaryotic (heterotrophic bacteria, cyanobacteria and proclorophytes) and eucaryotic organisms (autotrophic and heterotrophic flagellates). As a consequence of this relevance, an important amount of research has been conducted to study the dynamics of these planktonic communities in the water column and their trophic interactions with other groups of plankters (Sherr and Sherr 1991, Van Wambeke et al. 1996).

In shallow littoral ecosystems despite the research on the dynamics of impact of the benthos on the associated planktonic microbial and algal communities (Stuart and Klumpp 1984, Fielding and Davis 1989, Velimirov and Walenta-Simon 1993), little is known about the seasonal changes of the whole particulate organic matter pool, including microbes, phytoplankton and dissolved and detrital organic carbon. Moreover, little is known about their variation in relation to the seasonal changes of the physical and chemical characteristics of the water.

The coastal waters of the western Mediterranean, as in other temperate seas, are characterised by two peaks of plankton production and biomass during the year, related to mesoscale hydrographic structures. An autumn peak develops after the disappearance of the summer thermocline, and a late-winter early-spring peak develops with the onset of water column stability. In both situations nutrient supplies enhance phytoplankton development (Estrada et al. 1985). The production dynamics of the near-bottom planktonic communities should not be the same as those from the water column. First, the sublittoral zone receives a major input of detrital and dissolved organic matter (DOM) from runoff and from benthic organisms such as algae and invertebrates; these coastal and benthic inputs represent more substrata for bacteria activity (Mann 1982, Delille et al. 1997). Second, near-bottom planktonic communities are subject to higher and more variable turbulence due to the bottom effect; this turbulence may directly or indirectly affect the dynamics of microbes and phytoplankton through the resuspension process (Wainright 1990). Third, in coastal zones during episodes of high primary production (i.e. phytoplankton blooms), a small percentage of this production is consumed by zooplankton with the main fraction transferred directly to the benthos (Kiørboe 1993). And finally, near-bottom planktonic communities are actively predated by benthic suspension feeder groups such as bivalves, sponges, ascidians, and cnidarians. These benthic macroinvertebrates can prey on micro- pico- and/or nanoplankton including procaryotic and eucaryotic organisms (Reiswig 1990, Stuart and Klumpp 1984, Pile et al. 1996), so they could directly control the abundance of these planktonic populations.

In this study we analysed the composition, abundance and seasonal variation of dissolved and particulate organic matter in a North-Western Mediterranean near-bottom ecosystem as an example of temperate sea. This work was focused to examine if the particular environmental and biological conditions of this ecosystem may make the dynamic of their planktonic communities to differ from that observed in previous planktonic communities studies in the water column. To assess this goal, seasonal changes in abundance and biomass of the near-bottom phytoplankton and microbial communities as well as the concentration of dissolved organic carbon (DOC) and particulate organic carbon (POC) were estimated in relation to physical and chemical characteristics of the water. The role of benthic organisms as a potential source of influence in the composition and dynamic of the near-bottom planktonic communities was also considered.

III. METHODS

Sampling was conducted at a coastal station near the Medes Islands Marine Reserve (NW Mediterranean Sea; see figure 1.1) between October 1995 and November 1996. Sampling took place between 2 (every 2 weeks) and 6 (every 5 days) times per month, amounting to a total of 48 sampling days distributed through the year. Samples were collected by scuba-divers at 0.5 m above the bottom using six 500 ml replicate plastic bags. See Chapter 1 for the groups of the seston which has been studied as well as the samples preservation and analysis. Water used for the analysis of DOC, POC, pico and nanoplankton was screened by a 100 μm net to avoid larger plankters. Temperature was measured *in situ* using an electrode (EOT 196 WTW) at the same time the samples were collected. Samples were processed in the laboratory 20 min after sampling. Vertical profiles of the sea water temperature from a permanent station located about 100 m from the study site, and the Ter river runoff (Fig. 1.1) were provided by the Medes Islands meteorological station (Pascual 1996). Vertical profiles of the water temperature were used to estimate the "Stratification Coefficient", defined as the variance around the mean temperature in a thermocline spread over 15 m. High values of the stratification coefficient indicate a high temperature difference from the top to the bottom of the thermocline, and therefore stratification of the water column. Low values of the stratification coefficient indicate a small temperature difference over the 15 m depth interval, and therefore mixing of the water column.

A multiple regression analysis was used to establish the relationship between DOC and the different POC groups with environmental and biological variables such as water temperature, stratification coefficient, irradiance, nutrients, runoff and macroalgae production. Macroalgae production include the production of the most abundant benthic algae (i.e., *Rissoella, Cystoseira* and *Halimeda* species) though the year (data from Ballesteros 1991). A backward stepwise procedure was used to exclude non relevant variables (Sokal and Rohlf 1981). Variables were square root transformed when normality (Kolmogorov-Smirnov test) and/or the heterosdacity (Levene's test) requirements were not fulfilled.

IV. RESULTS

Physical and chemical variables

Water temperature showed minimum values (12-14 °C) from December to March and maximum values during the summer months (20-23 °C) (Fig. 2.1a). Fall and winter were the seasons with lowest stratification coefficients (i.e. mixing of the water column). During spring the water column gradually became stratified until summer when the

maximum stratification coefficient values were observed (Fig. 2.1a). The highest nutrients concentration values were present in fall and winter. Nutrient concentration decreased during spring and the minimum values were recorded in summer (Fig. 2.1b; from Garrabou 1997). The river runoff was highest during the winter months, while minimum values were recorded during the summer period (Fig. 2.1c). The nutrients concentration variation along the year positively correlated with the river runoff (r^2=0.65, $p<0.0001$) but it did not exhibit a significant correlation with the stratification coefficient.

The highest values of irradiance through the year were during the summer months. Irradiance values decrease during autumn and winter. During spring, irradiance values gradually increased towards the summer months (Fig. 2.1c; from Garrabou 1997).

Heterotrophic bacteria

Heterotrophic bacterial abundance through the year ranged between 3 and 9 10^5 bacteria ml^{-1}. Abundance were similar through the year, except for May, August and November 1996 when lower values were observed (ANOVA, $F_{(13,316)} = 4.68$, $p<0.0001$; Turkey' post hoc test; Fig. 2.2a). The size range found for heterotrophic bacteria was from 0.2 to 2.3 μm length (0.5 ± 0.3 SD). Heterotrophic bacteria were smaller from January to April than during the rest of the sampled period (ANOVA, $F_{(9,4078)} = 28.91$, $p<0.0001$; Turkey' post hoc test), so biomass was 40 % lower during these three months (Fig. 2.2b,c). Mean values for cell abundance, biovolume and carbon per litre are shown in table 2.1. The variation of the heterotrophic bacteria biomass along the year correlated positively with water temperature and negatively with irradiance. Heterotrophic bacteria biomass did not show a significant relationship with either dissolved or detrital organic carbon (Table 2.2).

Prochlorococcus sp, Synechococcus sp

A *Prochlorococcus sp.* population was detectable in the water from August to March (Fig. 2.2a). The highest abundance and biomass values were found from September to November and minimum values were observed from December to March (Fig. 2.2b,c). From April to July it was not possible to distinguish a clear population of *Prochlorococcus sp* using flow cytometry because of the few cells present in the samples (< 300 cells ml^{-1}; Fig. 2.2a). *Synechococcus sp* cells (cyanobacteria) size range found at the studied place was from 0.4 to 2.4 μm length (1.3 ± 0.5 SD). *Synechococcus* cells were always present in the water through the year. They were most abundant from August to November (Fig. 2.2a), and low cyanobacteria abundance was observed during winter and spring. This temporal pattern was clearer in biovolume and carbon terms (Fig. 2.2b,c). Mean annual values of abundance, biovolume and biomass for *Prochlorococcus sp* and *Synechococcus sp* are shown on table 2.1. *Prochlorococcus sp* and *Synechococcus sp* abundance showed a good correlation during the period in which there was a clear *Prochlorococcus sp*

population (August to March; Pro ml^{-1} = -1719 + 0.7 (Syn ml^{-1}); Pro = *Prochlorococcus*, Syn = *Synechococcus*, n = 97, r^2=0.74; p<0.0001). *Synechococcus* biomass correlated

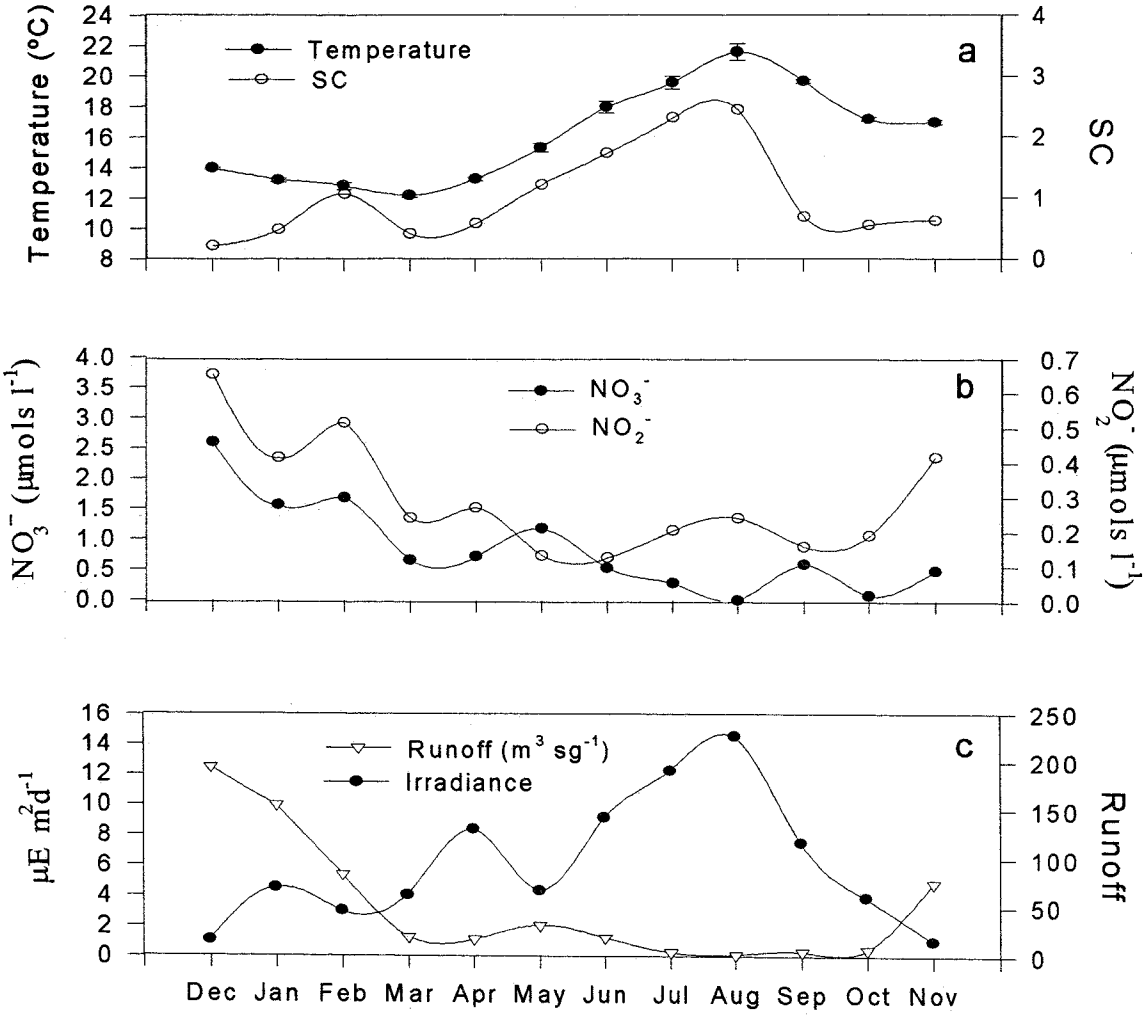

Fig. 2.1. (a) Average temperature monthly values ± SE at the sampling site (15 m) and Stratification Coefficient (SC) for the area. (b) Monthly values of nutrients, (c) Irradiance (data from Garrabou 1997), and Ter River runoff.

positively with water temperature (Table 2.2). *Prochlorococcus* biomass showed a positive correlation with nutrients concentration and negative correlation with irradiance (Table 2.2).

Pico- nano-eucaryotes, Phytoplankton and Ciliates

Autotrophic pico- and nanoeucaryotes and heterotrophic flagellates were always present in the samples (Fig. 2.3a). Except in spring, picoeucaryotes were more abundant than nanoeucaryotes and heterotrophic nanoflagellates all year around. Mean annual values of abundance, biovolume and biomass per each group are shown in table 2.1. The size range found for each group was for picoeucaryotes from 0.80 to 1.60 μm length (1.2 ± 0.3 SD) and for nanoeucaryotes and heterotrophic flagellates from 2.40 to 9.60 μm length

Table 2.1. Annual mean (± SE) abundance for the different groups of cells expressed as cell number (Cells ml^{-1}), biovolume ($\mu m^3 l^{-1}$), and biomass in Carbon units ($\mu g C l^{-1}$).

	Cell number Cell ml^{-1}	Biovolume $\mu m^3 l^{-1}$	Carbon $\mu g C l^{-1}$
Heterotrophic Bacteria	$5.16 \pm 0.08 \times 10^5$	$2.14 \pm 0.04 \times 10^7$	4.72 ± 0.09
Prochlorococcus sp	$1.05 \pm 0.07 \times 10^4$	$1.87 \pm 0.14 \times 10^6$	0.55 ± 0.04
Synechococcus sp	$2.24 \pm 0.09 \times 10^4$	$9.96 \pm 0.39 \times 10^6$	3.56 ± 0.14
Picoeucaryotes	1640 ± 72	$2.75 \pm 0.14 \times 10^6$	1.10 ± 0.05
Autotrophic Nanoeucaryotes	657 ± 24	$1.46 \pm 0.05 \times 10^7$	4.14 ± 0.14
Heterotrophic Nanoeucaryotes	633 ± 36	$1.39 \pm 0.07 \times 10^7$	3.07 ± 0.17

(3.79 ± 1.39 SD). In biomass units, autotrophic nanoeucaryotes and heterotrophic nanoflagellates were always dominant over picoeucaryotes. Nanoeucaryotes and heterotrophic nanoflagellates were specially abundant in spring (Fig. 2.3b; Fig. 2.4a). Picoeucaryotes biomass showed a positive correlation with nutrient concentration and a negative correlation with macroalgae production (Table 2.1). Nanoeucaryotes biomass correlated positively macroalgae production (Table 2.1).

Phytoplankton abundance through the year varied from 1.1×10^3 to 170×10^3 cells l^{-1} [$20.6 \pm 4.1\ 10^3$ (SE)]. Ciliates were much less abundant, with mean values of 358 ± 30 cells l^{-1} (between 80 - 990 cells l^{-1}; Fig. 2.3c,e). Phytoplankton size ranges varied for each group: pennate diatoms from 6.1 to 348 μm length (70 ± 22 SD); for centric diatoms from 8.6 to 288 μm length (65 ± 27 SD); for dinoflagellates from 8.4 to 49 μm length (28 ± 4 SD) and for ciliates from 10 to 72 μm length (38 ± 12 SD). The general trend of phytoplankton cell abundance and biomass showed two clearly distinguishable periods: one from December to June, clearly dominated by diatoms, and a second one from July to November, dominated by dinoflagellates (Fig. 2.3c,e). During the period dominated by diatoms, it was possible to distinguish two peaks of high phytoplankton abundance, one during March (late winter bloom) and another one from late May to early June (spring bloom; Fig. 2.3c). The winter bloom was dominated by pennate diatoms of the genus

Nitzchia and the spring bloom was dominated by centric diatoms of the genus *Chaetoceros*. Phytoplankton biomass was about 3 times higher during the winter period (from December to February; Fig. 2.3d, Fig. 2.4b), mainly due to the centric diatom of genus *Ditylum*. The late winter and spring blooms were dominated in biomass, as in number of cells, by pennate diatoms of the genus *Nitzchia* and centric diatoms of the genus *Chaetoceros* respectively. In September, it was possible to distinguish a short term peak dominated in cell number by diatoms, mainly of the genus *Thalassionema*, and in terms of biomass by the centric diatoms genus *Rhizosolenia*. Dinoflagellates were always present through the year, but it was from July to November when their relative contribution in number of cells was more important (Fig. 2.3e). However, even during this period, the biomass of diatoms and ciliates was as important as the dinoflagellate biomass (Fig. 2.3f, Fig. 2.4c).

The biomass of both diatoms and dinoflagellates correlated positively with nutrient concentration, and negatively with water temperature and macroalgae production (Table 2.2). Ciliate biomass showed a positive correlation with water temperature and nutrient concentration and a negative correlation with the stratification coefficient (Table 2.2).

Detrital and dissolved organic carbon

Total particulate organic carbon calculated from C:H:N analysis, that includes live carbon and detritus, showed a annual mean value of 387 ± 35 SE μg C l^{-1} (Fig. 2.5a). Detrital organic carbon (hereafter detrital POC) through the year was on average 17 ± 3 SE times larger than live carbon. Winter and spring were the periods with the largest amount of detrital organic carbon, which was on average 34 ± 4 SE times larger than live carbon, with a C/N relationship of 17 ± 4 SE, Summer and autumn were the seasons with the lowest detrital organic carbon values, which were about 6 ± 2 times larger than live carbon and with a mean C/N relationship of 4 ± 2 SE. Detrital POC correlated positively with the Ter runoff and macroalgae production. Detrital POC did not show any relationship with any phytoplankton group biomass (Table 2.2).

Dissolved organic carbon (DOC) showed a mean ± SE annual value of 2560 ± 180 μg C l^{-1}. As general pattern, two periods of high values of DOC can be distinguished, one in spring (May: mean ± SE 4240 ± 658 μg C l^{-1}) and one in summer (from late July to September: mean ± SE 3800 ± 309 μg C l^{-1}; Fig. 2.5c). DOC correlated positively with Ter runoff and macroalgae production (mainly due to *Cystoseira* and *Halimeda* species). No significant relationship was observed between DOC, detrital POC concentration, or with any phytoplankton group biomass (Table 2.2).

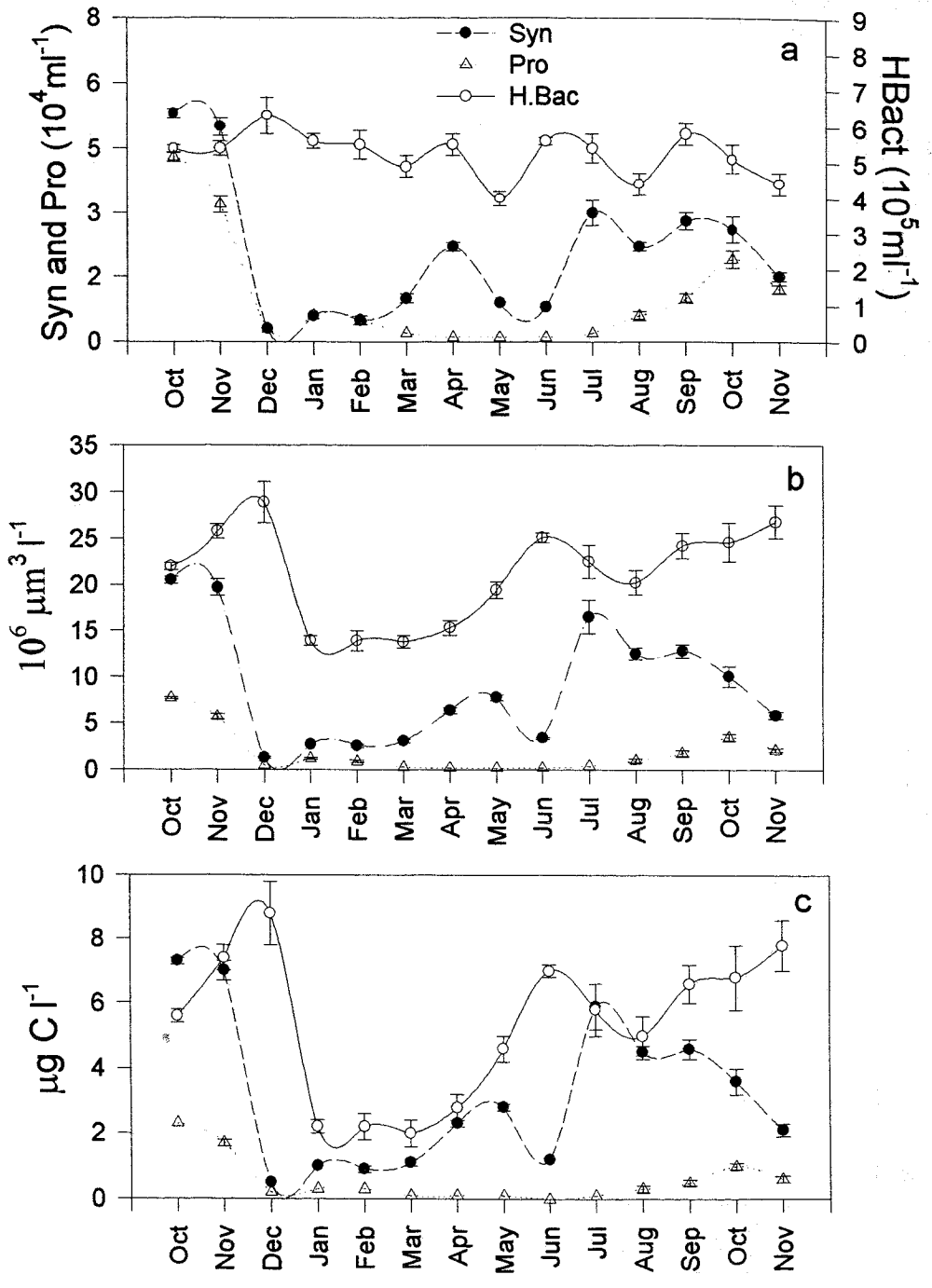

Fig. 2.2. Monthly averages (± SE) for H.Bac: heterotrophic bacteria, Syn:*Synechococcus* and Pro: *Prochlorococcus* abundance expressed as (a) cells ml^{-1}, (b) biovolume in µm^3 l^{-1}, and (c) carbon biomass in µg C l^{-1}.

Live carbon composition

The overall contribution of the pico- nano- and microplankton groups to the live carbon content of the water through the year showed that heterotrophic bacteria and the photosynthetic nanoeucaryotes were groups with the highest and more constant contributions (mean ± SE: 26 ± 7 % and 21 ± 7%) to the total carbon (Fig. 2.7b).

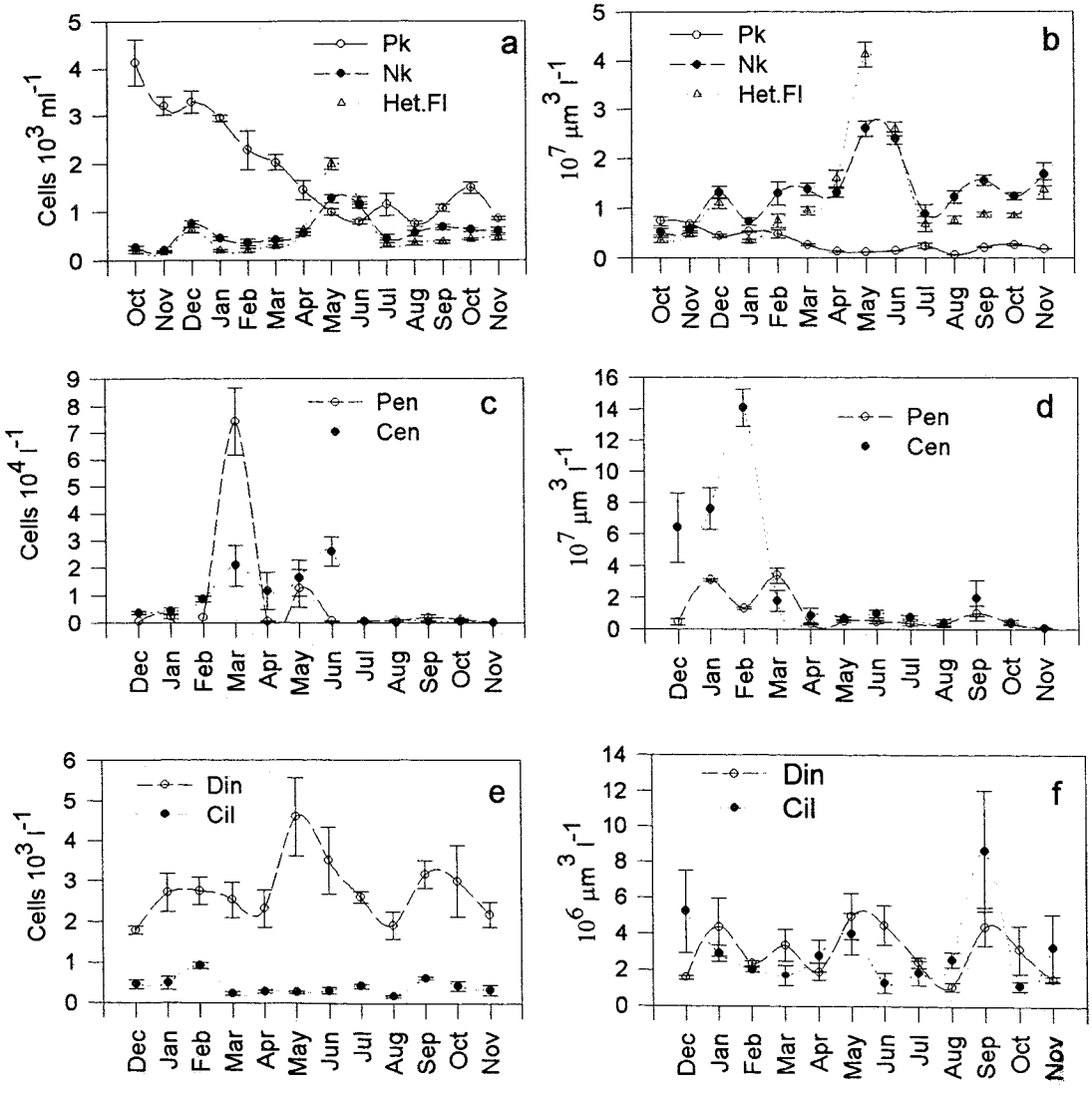

Fig. 2.3. Monthly averages (± SE) of pico- and nanoplankton abundance expressed as (a) cells ml^{-1}, (c,e) cells l^{-1}, and (b,d,f) biovolume expressed as μm^3 l^{-1}. Pk: picoeucaryotes, Nk: autotrophic nanoeucaryotes, Het.Fl: heterotrophic flagellates, Pen: pennate diatoms, Cen: centric diatoms, Din: dinoflagellates, and Cil: ciliates.

Table 2.2. Partial correlation coefficients from the multiple regression analysis. Dependent variables in row and independent variables in column. HBac: heterotrophic bacteria, Syn: *Synechococcus*, Pro: *Prochlorococcus*, Pk: picoeukaryotes, Nk: Nanoeukaryotes, Diat: diatoms, Din: dinoflagellates, Cil: ciliates, POC: detrital particulate organic carbon and DOC: disolved organic carbon). All variables in biomass units (µg C l-1). P: production, (--): analysis not performed, ns: not significant, ***: p<0.0001, **: p<0.001, *: p<0.1..

Variables	HBac	Syn	Pro	Pk	Nk	Diat	Din	Cil	POC	DOC
Temperature (°C)	0,83	0.93 ***	ns	ns	ns	-0.72 *	-0.65 *	0.82 *	--	--
SC	ns	ns	ns	ns	ns	ns	ns	-0.74 *	ns	ns
Irradiance (mE m^2 d^{-1})	-0.78*	ns	-0.61 *	ns	ns	ns	ns	ns	--	--
Nutrients (mmols l^{-1})	ns	ns	0.38 *	0.81 *	ns	0.75 *	0.76 *	0.75 *	--	--
Ter River runoff (m^3 sg^{-1})	ns	ns	ns	ns	ns	ns	ns	ns	0.85 **	0.67 *
Macroalgae P (g C $m^{-1}d^{-1}$)	ns	ns	ns	-0.59 *	0.66 *	-0.76 *	-0.75 *	ns	0.69 **	0.86 **
POC	ns	ns	ns	--	--	--	--	--	--	ns
DOC	ns	ns	ns	--	--	--	--	--	ns	--
Nk	--	--	--	--	--	--	--	--	ns	ns
Pk	--	--	--	--	--	--	--	--	ns	ns
Diat	--	--	--	--	--	--	--	--	ns	ns
Din	--	--	--	--	--	--	--	--	ns	ns

The contribution of the other groups was important in some periods. Diatoms contributed to 37 ± 13 SE % of the total carbon from December to March, and *Synechococcus* sp contributed to 29 ± 6 SE % of the total Carbon during summer (from July to September). The groups with minor annual contribution in terms of carbon were *Prochlorococcus* sp, picoeucaryotes, dinoflagellates and ciliates; overall these groups contributed to mean ± SE 2.4 ± 2.6 % of the total carbon (Fig. 2.5b). Total live carbon estimated from cell counts showed a annual mean value of 24 ± 9 SE μg C l^{-1}. February was the month with the highest live carbon values and August was the period with the lowest values (Fig. 2.5b).

V. DISCUSSION

The seasonal variation of water temperature and nutrient concentration were the environmental variables which better explained the changes observed in the contribution of the different planktonic groups to the pool of live carbon. During winter, period of low temperature and high nutrient concentration (Fig. 2.1), large phytoplankton cells were the dominant groups. In fact, during the winter months, diatoms contributed to about 40 % of the live carbon. In summer, period of high temperature and low nutrients (Fig. 2.1), small cells such as *Synechococcus* and autotrophic nanoeucaryotes were the dominant groups. This fact appears to be related to the surface to volume ratio of the cells because the uptake of nutrients increases with the increase of this ratio (Kiørboe 1993). Then, under situations of low nutrients such as the summer conditions in the Mediterranean, small cells may have an advantage in taking up nutrients.

Heterotrophic bacteria abundance at Medes Islands throughout the year was similar to those documented for surface waters in other Mediterranean coastal waters (Villefranche sur mer: 2-7 10^5 cells ml^{-1}; Ferrier-Pagés and Rassoulzadegan 1994), and agree with values of abundance in offshore oligotrophic waters (Azam et al. 1983). Surface waters of Blanes bay in the coastal Mediterranean showed a similar annual mean value but with a higher range of variation in bacteria abundance (annual mean: 5.02 10^5 cells ml^{-1}; range: 0.7-14 10^5 cells ml^{-1}; Vaqué et al. 1997). However, despite the agreement in bacterial abundance, the heterotrophic bacteria biomass that we observed was lower (mean annual value = 5 μg C l^{-1}) than that documented in Blanes bay (10 μg C l^{-1} in 1995, Vaqué 1996; 8.56 μg C l^{-1} in 1996, Vazquez-Dominguez personal communication). The differences were mainly because mean heterotrophic bacteria biovolume in the present study was lower than that of Blanes bay.

Despite the high DOC values observed during spring and summer, similar to highly productive systems such as kelp beds (Fielding and Davies 1989), the bacteria abundance reported during these periods was always one or two orders of magnitude lower than that

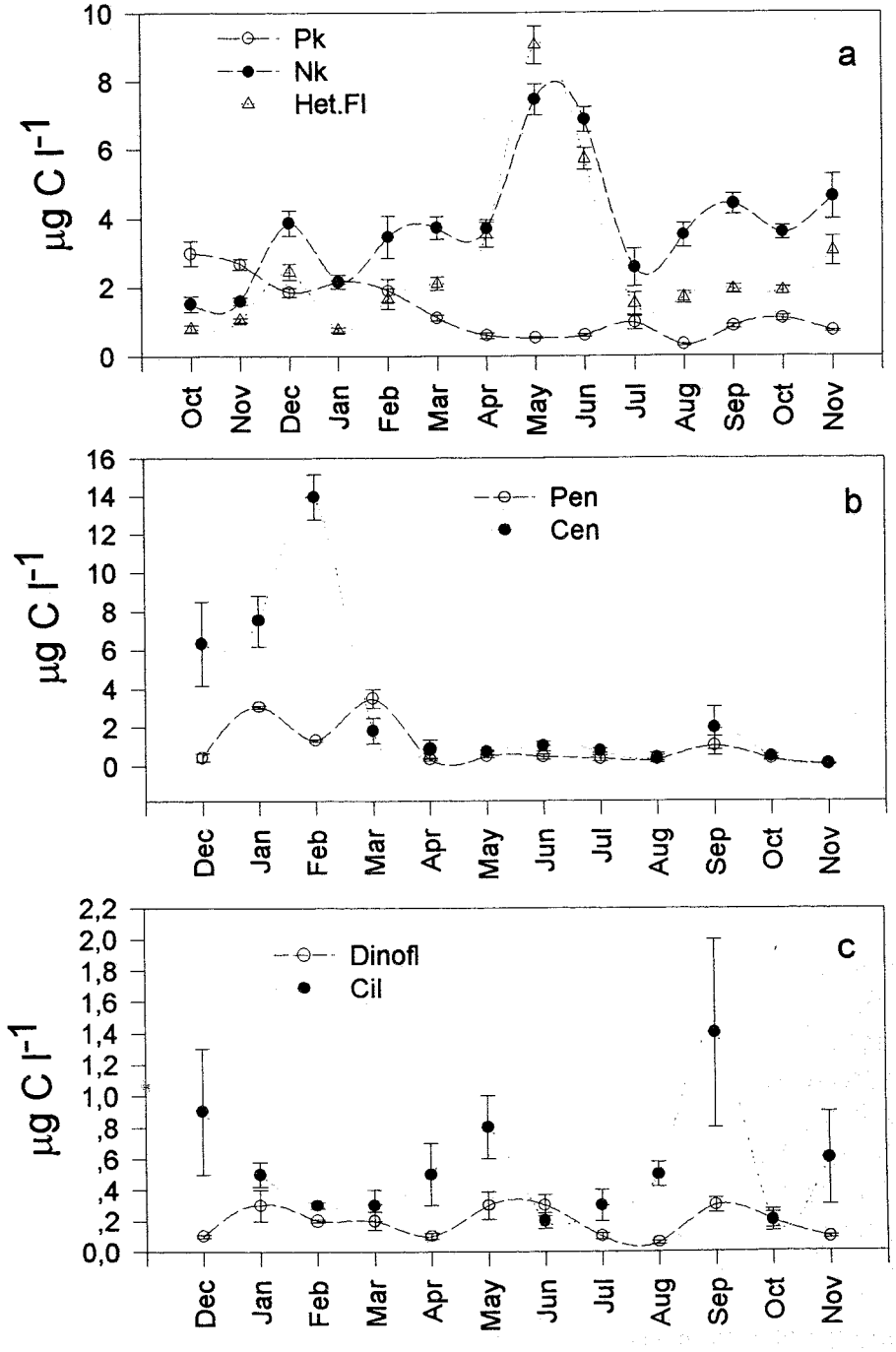

Fig. 2.4. Monthly biomass averages (± SE) in Carbon units (µg C l^{-1}) for: (a) Pk: picoeucaryotes, Nk: autotrophic nanoeucaryotes, and Het.Fl: heterotrophic flagellates; (b) Pen: pennate diatoms, Cen: centric diatoms, Din: dinoflagellates, and Cil: ciliates.

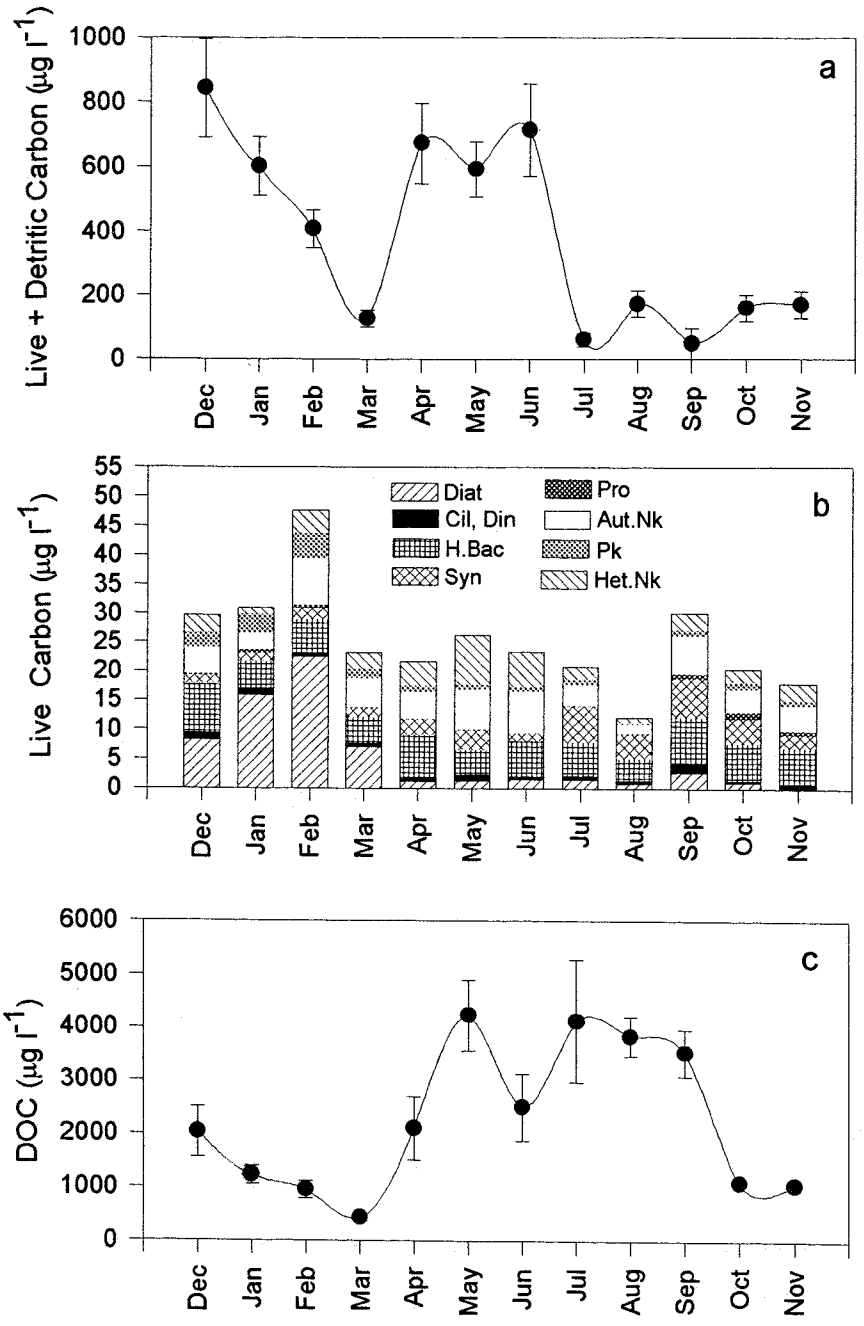

Fig.2.5. Monthly averages (± SE) expressed as □g C l^{-1} of (a) live and detrital carbon from the C:H:N analysis, (b) live carbon from cell counts with the percentage composition (Het.Nk: heterotrophic nanoeucaryotes, Pk: picoeucaryotes, Aut.Nk: autotrophic nanoeucaryotes, Pro: *Prochlorococcus*, Syn: *Synechococcus*, H.Bac: heterotrophic bacteria, Din: dinoflagellates, Cil: ciliates, and Diat: diatoms) and (c) DOC: dissolved organic carbon.

reported in kelp bed ecosystems (Moriarty and Pollard 1982). A similar fact had been previously reported by Velimirov (1986), who pointed out that despite the large amounts of DOC released by the *Posidonia oceanica* beds, the maximum bacterial abundance was never higher than 1.2 10^5 cells ml^{-1}. It remains not understood why there are not more heterotrophic bacteria if POC and DOC is not limiting. One hypothesis would be that the high values of DOC observed, mainly of phytobenthic origin (see below), would have a higher refractory fraction than phytoplankton produced DOC. An alternative hypothesis could be the controlling effect of the suspension feeders such as bivalves, sponges (Reiswig 1990, Pile et al. 1996) and ascidians (Stuart and Klumpp 1984). These organisms are known to be active predators of microorganisms and could function in a similar way as benthic suspension feeders do in controlling planktonic communities in lagoons and estuaries (Cloern 1982).

The observed decrease in bacterial cell size in winter has also been documented for bacterial communities in seagrass beds (Velimirov and Walenta-Simon 1992). These authors hypothesised that this decrease could be an adaptation to unfavourable low DOC. Our results point out that this effect could be dependent not only on DOC abundance, but also on temperature. The DOC abundance in autumn was as low as in winter and, nevertheless, the decrease in bacteria cell biovolume occurred only in winter. Furthermore, the positive relationship between bacteria biomass and water temperature suggest that the low temperature of the winter period may also be playing an important role in this process, probably by inhibiting cell growth (Marrasé 1992).

Prochlorococcus sp and *Synechococcus* sp abundance was within the low range of the abundance reported for the North Atlantic Ocean (Chisholm et al. 1988) but is in accord with the range of abundance observed in the NW Mediterranean (Vaulot et al. 1990). Sampling throughout the euphotic zone, Vaulot and co-workers (1990) documented low *Prochlorococcus sp* densities in the well-mixed nearshore water (8-12 10^3 cells ml^{-1}). These values were similar to the values observed in the present work during the same months (December and January). However, those authors did not provide data on *Prochlorococcus sp* abundance from late August to November, when we observed the highest abundance of these cells. A positive relationship between *Prochlorococcus* biomass and nutrient concentration was observed, as has been previously reported by several authors (Chisholm et al. 1988, Ferrier-Pagès and Rassoulzadegan 1994). Then, the absence of Prochlorophytes in the near-bottom community from April to August could be related to the low nutrient abundance in the water during this period. During summer, as a general trend for the Mediterranean, the nitracline is close to the deep chlorophyll maximum at a depth between 40 and 100 m (Estrada et al. 1985). Therefore, the fact that the location of the study site was shallower than the summer nitracline position may be why prochlorophytes were not observed during this period. A similar displacement of the maximum occurrence of prochlorophytes following the nitracline position has been

previously reported during late spring and summer in the North Atlantic (Olson et al. 1990).

Synechococcus abundance showed a good correlation with *Prochlorococcus* abundance during the period that *Prochlorococcus* were present in the community, as has also been observed in offshore waters (Vaulot et al. 1990). The positive relationship between *Prochlorococcus* biomass and nutrient concentration and the no relationship between *Synechococcus* biomass and nutrient concentration suggest that the presence of *Synechococcus* all the year, even when *Prochlorococcus* were absent, may be due to the *Synechococcus* higher adaptability to low nutrient levels as have been observed by other authors (Vaulot et al. 1990, Olson et al. 1990). Vaulot and co-workers (1990) suggested that *Synechococcus* and prochlorophytes may have a similar response to factors such as temperature, nutrients and light. However, our results, which include the whole year cycle, points out that, in shallow waters, the biomass of both groups exhibit a different answer to irradiance and water temperature changes. This suggest that other environmental factors may also be involve in the low abundance of *Prochlorococcus sp* during the summer period.

Synechococcus mean annual abundance was similar to that of the surface water at Villefranche sur mer (NW Mediterranean; Ferrier-Pagès and Rassoulzadegan 1994). However, the surface water values reported for Blanes bay were an order of magnitude lower, but mean biovolume per cell was approximately double (Mura et al. 1996). As a result, cyanobacteria and picoeucaryote biovolume ($\mu m^3\ l^{-1}$) in Medes Islands was similar to that of the picophytoplankton group estimated by those authors. The mean annual abundance of autotrophic nanoeucaryotes and heterotrophic nanoflagellates observed in the near-bottom planktonic community in Medes Islands agrees in abundance and biomass terms with previous values reported for surface oligotrophic waters of the North Western Mediterranean sea (Ferrier-Pagès and Rassoulzadegan 1994, Vaqué et al. 1997).

The abundance of phytoplankton cells (mean values of $2.1\ 10^4$ cells l^{-1} with a maximum of $1.2\ 10^5$ cells l^{-1}, including diatoms and dinoflagellates) was lower at the Medes Islands near-bottom community than values reported for coastal waters (mean values for an integration of 100 m depth shelf waters, maximum: $4\ 10^5$ cells l^{-1}; Margalef and Castellví 1967) and other nearby surface waters studied (two consecutive annual mean: $> 2\ 10^5$ cells l^{-1}, Mura et al. 1996). In the same way, ciliate abundance throughout the year was one order of magnitude lower (in number and in biomass) in our study site than the values reported for Blanes bay (Vaqué 1996). The lack of an autumn phytoplankton peak of abundance (despite the sporadic peak of biomass on September) could be within the normal variability of the autumn peak.

As pointed out in the introduction, there were several reasons to expect a differential dynamics in the near-bottom planktonic community with respect to that in the water column. The expected effect of all mentioned reasons (see introduction section) but one would have produced an increase in the abundance of the different groups in the near-bottom planktonic community. In this sense, the constant contribution of detritic and dissolved organic material from the benthic organisms such as algae and invertebrates, and the increase in turbulence due to the bottom effect should have enhanced production of the near-bottom planktonic communities. Also, the fact that during episodes of high primary production the main fraction of this production is directly transferred to the benthos (Kiørboe 1993) should have enhanced production of the near-bottom planktonic communities. However, the main feature that distinguished the dynamics of the planktonic communities in the studied near-bottom site from those of the water column was the relatively low abundance of the different groups. This general low abundance of the different planktonic groups together with: i) the high abundance of suspension feeders in the benthic communities of Medes Islands (suspension feeders account for over 50 % of the total benthic biomass being anthozoans among the most abundant groups; Gili and Ros 1984), ii) the recently estimated high rates of prey capture of different suspension feeders groups (Coma et al. 1995, Pile et al. 1996), iii) the high productivity of some benthic groups (Coma et al. 1998), and iv) the release of larvae by the benthos which by predation modify the abundance and composition of planktonic communities (Graff 1992), points out that benthos feeding activity might be exerting an important role in the control of the dynamics of the near-bottom planktonic communities.

Mean annual POC values (387 μg C l^{-1}) for the studied near-bottom planktonic community were within the range of POC documented for coastal waters (200-2000 μg C l^{-1}; Mann 1982), but close to the lowest values. However, POC abundance in winter and spring were within the range of values documented for near-bottom coastal systems of high productivity such as kelp beds (533-762 μg C l^{-1} in summer and winter respectively; Fielding and Davis 1989). The annual mean DOC abundance observed in this study (2560 μg C l^{-1}) was slightly higher than general values documented for coastal waters (i.e. 1000-2000 μg C l^{-1}; Fredericks and Sackett 1970). The high DOC values observed during spring and summer were within the range documented for kelp beds (Delille et al. 1997), and within the range documented for a similar depth on Mediterranean seagrass systems (Velimirov 1986). The seasonal variation of DOC and detrital POC was related with the runoff of the nearby Ter river, which is consistent with the results of Vaqué and co-workers (1997) in a nearby zone, but, it was also related with the macroalgae production. Neither, POC nor DOC variation were consistent with phytoplankton production peaks.

An approach to the POC composition was possible by the comparison between total POC (C:H:N analysis) which include live carbon and dead organic carbon in detritical form, with live organic carbon (i.e. estimated from recognisable cells). All year around,

organic carbon from detrital origin represented the main fraction of POC in the studied site and in fact, mean total live carbon (24 μgC l^{-1}) was similar to values reported for oceanic waters (Mann 1982), where the low detrital organic carbon present is mainly lost by sedimentation. The relationship between total POC and live carbon showed a seasonal variation together with the C/N ratio. In this sense, during spring total POC was about 34 times higher than live carbon with a high C/N ratio (C/N: 18); this value is consistent with the general atomic ratio of benthic marine macroalgae (C/N: 550/30; Atkinson and Smith 1983). During summer and fall total POC was 6 times higher than live carbon with a low C/N value (C/N: 4) close to the Redfield ratio for marine plankton (C/N: 106/16; Atkinson and Smith 1983), pointing out the relatively low detritical contribution of benthic macroalgae to POC during these periods in comparison to spring period.

As in other temperate seas, sublittoral phytobenthic communities from the Western Mediterranean show seasonal fluctuations in terms of biomass, diversity and production (Ballesteros 1991). Sublittoral phytobenthic communities show biomass and production maximum in spring-summer (Ballesteros 1991). In these communities, the input of organic matter from benthic macroalgae production is higher than that from phytoplankton production (Ballesteros 1989). Then, DOC peaks observed during spring and summer in the near-bottom community may come mainly from by DOC release by macrophytobenthos which explain an important fraction of the large DOC variation along the year (almost 10 folds). The POC spring peak may come from runoff, but also from the debris and decomposition of the sublittoral algae, mainly erect algae (i.e. *Rissoella, Cystoseira* and *Halimeda* species).

The differences in the DOC and POC variation along the year appears to be related to their main sources, but also to the processes that allow their maintenance in the water column. Then, first, while production of macroalgae was the main source of DOC, the main source of POC was the runoff (as indicated the partial correlation coefficients, Table 2.2). Second, the low POC values in summer appears to be due to the rapid sedimentation of the detrital material due to the low water mixing during this period (reflected by the stratification of the water column, Fig. 2.2a). In the same sense, the increase of POC in winter appears to come from the runoff and its maintenance in the water column may be favoured by the hydrodinamic conditions of the winter period (i.e. the mixing of the water column). Then, the differences in the sources as well as the differential effect of sedimentation on POC and DOC may be among the main factors that influence and differentiate the variation in abundance of POC and DOC along the year.

As a conclusion, we wish to point out that the detrital fraction was the dominant component of the total organic carbon in the near-bottom planktonic community along the year. Microbial communities (heterotrophic bacteria, *Synechococcus* and *Prochlorococcus*) contributed a low percentage to the total organic carbon. This could point out the coastal

origin (runoff and benthic macroalgae) of the high concentration of DOC and POC, which represent more substrate for microorganism activity and is one of the features that differentiate near-bottom plantonic communities from those on the water column. However, the high DOC and POC values contrast with the relatively low abundance of the different live carbon groups. The low abundance and biomass of microbial organisms in the near-bottom community could be explained by the activity of benthic organisms, probably by the predation of suspension feeders. At the same time, in shallow waters, because near-bottom layers are mixed by waves and currents, the effects of benthic organisms could be extended to the water column by hydrographic processes. Then, a general approach of the results raised out in this work could be to hypothesise that the structure and dynamic of the near-bottom planktonic communities might follow a pattern different from that exhibited by the water column planktonic communities. This pattern would be characterised by the low abundance and high temporal heterogeneity of the different planktonic communities in which the activity of benthic organisms may play an important role by seasonally releasing or removing organic components.

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3

HETEROGENEOUS FEEDING MEDIATES SUCCESSFUL SPONGE COMMUNITIES IN THE MEDITERRANEAN: THE NATURAL DIET OF THE TEMPERATE SPONGE *DYSIDEA AVARA* (DENDROCERATIDA)

I. ABSTRACT

Sponges are one of the major invertebrate groups of worldwide hard-bottom communities. In this study, we seasonally measured *in situ* rates of grazing on DOC, POC, pico-, nano- and microplankton for the common temperate sponge *Dysidea avara* along a year cycle. The natural diet of the species was highly heterogeneous including procaryotes (heterotrophic bacteria, *Prochlorococcus* sp, *Synechococcus* sp) and eucaryotes (protozoa, phytoplankton and ciliates) with a size range from 0.5 ± 0.3 μm (heterotrophic bacteria) to 70 ± 0.3 μm (pennate diatoms). Procaryotes cells clearance rate was higher than that of the other groups, suggesting a higher grazing efficiency upon these prey types. Specific clearance rate showed a pattern of decrease with sponge size increase, although it did not varied with prey concentration, nor with temperature. Overall, procaryotes contributed to 74 ± 14 % of the total ingested carbon, pico- and nanoeucaryotes contributed to 11 ± 3 %, and phytoplankton contributed to 11 ± 10 %. Therefore, *Dysidea avara* obtained 85 % of the ingested carbon from the fraction smaller than 5 μm whereas the fraction larger than 5 μm contributed 15 %. However, partial contribution of the different groups varied along the year following the planktonic composition of the water column. During winter, phytoplankton was an important component of the total uptake (26 %), whereas the rest of the year it contributed less to 7 % of the total uptake. The heterogeneity of this sponge species diet and its capacity to feed on a broad size of prey allowed the species to maintain a rather constant food uptake throughout the year. These results have shown the role of particle type (size) for selective uptake in sponges, as well as the relevance of phytoplankton in the sponge diet. This feeding plasticity may represent an advantage for the species because it attenuates the effects of seasonal fluctuations in planktonic communities. The plasticity of sponges trophic ecology could be among the main factors that contribute to the worldwide abundance and distribution of sponges.

II. INTRODUCTION

Recent studies are showing that some benthic suspension feeders play a paramount role in the energy transfer processes in littoral ecosystems (e.g. Cloern 1982; Officer et al. 1982; Kimmerer et al. 1994; Gili and Coma in press). In this sense, attention should be paid to those suspension-feeders responsible of the coupling and energy transfer between pelagic and benthic systems. However, most of this work has been carried out on molluscan populations dwelling in soft bottoms and little is known about dense suspension feeders populations on hard substrates (but see Pile et al. 1997).

Sponges are one of the major invertebrate groups of worldwide hard-bottom communities. They filter large volumes of water (up to 1 liter h^{-1} cm^{-3}, Reiswig 1971) with retention efficiencies between 75 and 99 % (Reiswig 1971, 1975; Wilkinson 1978; Pile et al. 1996). Past and present studies have shown that grazing by some sponges can be on the same order of magnitude (29-1970 mg C m^{-2} d^{-1}, Reiswig 1974, 1981; Pile et al. 1996; Pile et al. 1997) as some molluscan species (9-3621 mg C m^{-2} d^{-1}, see Griffiths and Griffiths 1987 for review). Therefore, on habitats with abundant sponges populations, they may exert an important grazing impact. Despite the importance of sponges in a number of ecosystems and the diversity of forms and habitats they occupy, diet and grazing rates of sponges species under natural conditions are still poorly known.

Laboratory experiments, indirect measurements of particles uptake, and sampling of ambient and exhalant water samples collected *in situ* have shown that sponges can feed on a wide spectrum of food sources (Reiswig 1971; Frost 1987; Pile et al. 1996). This variety of food sources ranges from dissolved organic carbon (DOC) to phytoplankton (Schmidt 1970; Frost 1987), and in extremely food-poor environments even zooplankton can be captured (Vacelet and Boury-Esnault 1995). However, the main research effort on sponge feeding has been conducted on plankton < 2 μm, both in laboratory studies (van der Vyver et al. 1990; Riisgård et al. 1993; Turon et al. 1997) with artificial food, and in field studies with natural diet (Reiswig 1971, 1975; Pile et al. 1996; Pile et al. 1997). This is due to the morphological characteristics of the filtration mechanisms which, based on fine canals and groups of choanocytes that are working as flagellates, are able to capture particles smaller than 2 μm very efficiently (Simpson 1984). But, as previously pointed out, sponges can feed on plankton larger than 2 μm. This is possible, on one hand, by the existence of pinacocytes along the extensive canal system which allows sponges to ingest sestonic particles as big as the diameter of the species ostia (Reiswig 1971; Frost 1981; Gaino et al. 1994). On the other hand, particles larger than the diameter of the ostia can be retained and picked up by the surface epithelium (Simpson 1984; Vacelet and Boury-Esnault 1995).

Therefore, due to the observed wide range of food sources that sponges can potentially feed on, an accurate evaluation of the natural diet of sponge species should account for the study of grazing upon the entire available food spectrum. In the water column the available food is a continuum from DOC to the larger forms of particulate organic carbon including both, live carbon (bacteria, *Prochlorococcus*, Cyanobacteria, protozoa, phytoplankton and zooplankton) and detritic organic carbon, although under normal circumstances zooplankton could be neglected. Furthermore, because plankton communities exhibit an important temporal variability, especially during the year in temperate seas, feeding studies should be carried out seasonally.

In the Mediterranean, as an example of temperate sea, the seasonal variation of environmental factors such as temperature, food availability (Chapter 2) and photoperiod (Zabala and Ballesteros 1989) can cause important shifts in resource allocation of benthic modular organisms. In this sense, a summer paucity of seasonal species such as hydroids (Boero et al. 1986, Llobet et al. 1991) and regression in the activity of perennial species such as tunicates (Turon and Becerro 1992), bryozoans (Zabala 1983), and gorgonians (Coma et al. 1998) has been observed in the Mediterranean. The worsening of feeding conditions during the summer period due to a decline in phyto- and zoo-plankton abundance (Valentin 1972, Coma et al. 1994, Chapter 2), as well as, in flow speed (Pasqual unpublished data) has been suggested by several authors as the main reason for the regression or inactivity of the species during this time period. Several reasons points out that the knowledge of the diet of sponges and its seasonal variation could importantly contribute to the general hypothesis that a trophic-energetic phenomenon may be underlying the summer regression of an important number of Mediterranean species. First, because pico- and nano-plankton has been documented to be the main source of POC for some sponge species (Reiswig 1971, Pile et al. 1996). Second, because the seasonal variation of these plankton groups near the bottom has been observed to follow a rather different pattern from that of phyto- and zooplankton, been the lowest biomass during the winter period (Chapter 2). Then, if the trophic-energetic phenomenon is true for the above mentioned groups, and phyto- and zooplankton are not the main contributor to sponges diet, it could be hypothetized that that the species should not be suffering the summer regression or inactivity.

In order to contribute to this hypothesis we examined the trophic ecology of the Dendroceratida sponge *Dysidea avara* (Schmidt), a common and widely distributed temperate sponge species on sciaphylous sublittoral hard bottoms (Atlantic: Lombas 1982; Mediterranean: Uriz et al. 1992). Grazing rate of this species on the different natural food sources (DOC, pico-, nano-, micro-plankton and detritus) was studied with *in situ* incubations. Feeding experiments on these potential food sources were conducted seasonally in order to cover the whole natural range of food concentration in the water column.

III. METHODS

This study was conducted at the Medes Islands Marine Reserve (NW Mediterranean Sea, see map in Chapter 1) from October 1995 to November 1996. Incubations were conducted in hemispherical U.V.-transparent Plexiglas approximately 3 L in volume as described in Chapter 1.

Scanning electron microscope (SEM) observations of the sponge tissue and of filters from both initial and final incubation water samples were carried out in order to look for direct evidences of the captured prey items. Ten tissue samples from different non incubated sponges and the initial and final water samples filters of 5 experiments were dehydrated in graded ethanol. Afterwards, the tissue and the filters were dried by the critical point method (using CO_2 as transition fluid), mounted on aluminum stubs and coated with gold in a sputter coater. Observation were done with a Hitachi S-570 SEM.

Dysidea avara dry weight was determined by drying at 100 °C for 24 hours and ash free dry weight was determined by combustion at 500 °C for 6 hours. Previously to the combustion, sponges were rinsed to remove any salts and dissected to remove any associated macrofauna.

Multiple regression analysis was used in order to establish the percentage of the variance in the estimated clearance rates, that could be explained by three independent factors which were controlled during each experiment: water temperature (°C, recorded using a WTW oxygen electrode model EOT 196), initial prey concentration (mg C l^{-1}) and sponge size (g AFDW). A backward stepwise procedure was used to exclude variables not relevant (Sokal and Rohlf 1981). Variables were square root transformed when normality (Kolmogorov-Smirnov test) and/or heterosdacity (Levene's test) requirements were not fulfilled.

We examined whether or not the species was selectively grazing on the any proportion of the plankton community through two approaches. First, we compared the CR_{AFDW} equations estimated for each prey type through a t-student test. Second, the percent of carbon in the diet of sponges was compared to the percent of carbon in the plankton component using a X^2 test (Sokal and Rohlf 1981).

IV. RESULTS

The whole organic carbon potentially available as a food resource for *Dysidea*

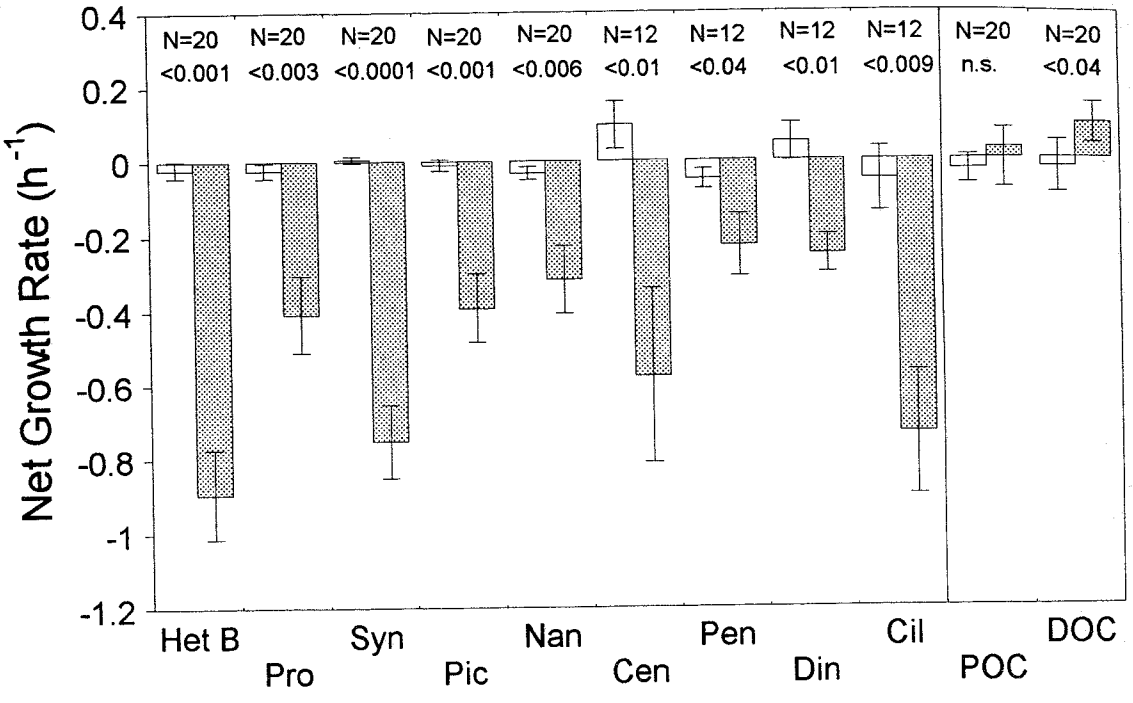


Fig. 3.1. Net growth rates of prey (mean ± SE) in the experimental (k_o, dotted bars) and control chambers (k_c , empty bars). Data are presented for each plankton group: Het B - heterotrophic bacteria, Pro - *Prochlorococcus* sp, Syn - *Synechococcus* sp, Pic - autotrophic picoeucaryotes, Nan - autotrophic nanoeucaryotes, Cen - centric diatoms, Pen - pennate diatoms, Din - dinoflagellates, Cil - ciliates, POC - detritic particulate organic carbon, DOC - dissolved organic carbon. Number of experiments and significance degree from two-tailed Wilcoxon test also shown.

avara at the study site was examined. The involved food sources included DOC, detritic organic carbon and live carbon (i.e. recognizable cells). Overall for all experiments, the growth rates calculated in the control chamber and in the experimental chamber showed that *Dysidea avara* significantly decrease all live carbon groups and exhibited a net production of DOC (mean ± SE: 0.33 ± 0.15 mg C g $AFDW^{-1}$ h^{-1}, Fig. 3.1).

Ash free dry weight specific clearance rate values [CR_{AFDW}, ml swept clear g $AFDW^{-1}$ h^{-1}, hereafter specific clearance rate] (i.e. the volume of water filtered by the sponge to retain the observed decrease in number of cells assuming a 100 percent efficiency

Table 3.1. Initial prey concentration values for the five experiments in each season (Mean ± SD). (Het B, Syn, Pic, Nan: 10^3 cells ml^{-1}) (Diatoms, Din, Cil: 10^3 cells l^{-1}). % Change: percentage of decrease in prey concentration of the final water samples with respect to initial concentration. Prey abbreviations as in figure 3.1.

	SUMMER		FALL		WINTER		SPRING	
Prey	Concentration	% Change	Concentration	% Change	Concentration	% Change	Concentration	% Change
Het B	339 ± 88	34 ± 14	349 ± 128	41 ± 16	465 ± 139	27 ± 3	361 ± 107	42 ± 7
Pro	2.69 ± 1.94	26 ± 15	23.06 ± 15.11	24 ± 17	1.68 ± 0.36	16 ± 7	1.54 ± 0.61	36 ± 4
Syn	16.45 ± 5.73	33 ± 12	30.06 ± 18.50	31 ± 15	9.20 ± 3.54	29 ± 4	10.45 ± 6.11	42 ± 7
Pic	0.79 ± 0.51	11 ± 15	1.13 ± 0.34	29 ± 17	1.93 ± 0.81	27 ± 3	0.99 ± 0.337	33 ± 7
Nan	0.30 ± 0.25	11 ± 14	0.36 ± 0.17	21 ± 14	0.40 ± 0.17	18 ± 4	1.05 ± 0.58	37 ± 5
Diatoms	10.01 ± 0.20	14 ± 3	14.70 ± 0.46	17 ± 13	100 ± 20	16 ± 2	43.02 ± 15.70	22 ± 18
Din	1.79 ± 0.70	2 ± 1	1.78 ± 0.74	13 ± 9	2.57 ± 0.44	12 ± 9	3.51 ± 2.64	13 ± 10
Cil	0.14 ± 0.06	17 ± 14	0.22 ± 0.07	26 ± 21	0.21 ± 0.04	29 ± 2	0.27 ± 0.08	45 ± 2

in particle retention) was seasonally calculated as the mean of the five experiments. For all experiments and prey types, the percentage of decrease in prey concentration during the incubation was always below 45 % (Table 3.1). Oxygen concentration at the beginning of each experiment was always slightly supersaturated. The drop in oxygen concentration during the experiments was never above 15 % of the initial oxygen concentration which have been observed to not affect animal's behavior (Crisp 1984, Ribes unpublished data). The CR_{AFDW} for each season and prey type are showed in Figure 3.2.

The experiments, carried out seasonally, allowed to cover the natural range of prey concentration in the study site along the year (10-50 mg live carbon l^{-1}) as well as the temperature range (12-23 °C; Chapter 2). Within this range, the study permitted the inspection of the mean CR_{AFDW} for all prey type in relation to sponge size (weight), prey concentration and temperature. CR_{AFDW} did not varied with prey concentration (mg live Carbon l^{-1}) and nor with water temperature (multiple regression analysis, n=20, $p > 0.5$ for both variables and all prey types). For all prey types, size was the only variable which explained a significant amount of the CR_{AFDW} variance (Table 3.2). CR_{AFDW} showed a pattern of decrease with sponge size increase with a negative power function as a best fit (Table 3.2). This effect was highly pronounced for sponges below about 1 g AFDW, but the pattern was attenuated for sponges above this size (Fig. 3.3). Then, the effects of prey concentration and temperature were examined for sponges larger than 1 g AFDW. CR_{AFDW} did not varied within the natural range of prey concentration and temperature of the study site (multiple regression analysis, n=11, $p > 0.2$ for both variables).

In order to examine whether or not CR_{AFDW} differed for the different prey groups, the parameters "a" and "b" from the relationship between prey size (S, g AFDW) and CR_{AFDW} ($CR_{AFDW} = a\ S^b$) were compared between each group (Table 3.3). CR_{AFDW} significantly varied between some prey groups. Although not statistically different from each other, CR_{AFDW} for heterotrophic bacteria, *Prochlorococcus* sp. and *Synechococcus* sp., were significantly higher than the CR_{AFDW} on pico- and nanoeucaryotes. CR_{AFDW} on pico- and nanoeucaryotes did not differ from each other, but were significantly higher than CR_{AFDW} on diatoms dinoflagellates and ciliates (Table 3.3). Overall, the variation on CR_{AFDW} among prey types suggest a pattern of inverse relationship between prey size and CR_{AFDW}.

Ingestion rate (I) was calculated as a function of clearance rate (CR) and prey concentration (C) (I= CR x C; see Chapter 1). Due to the clearance rate dependence on sponge size, the amount of carbon seasonally ingested was estimated for the two extreme size examined during the experiments (0.2 and 1.6 g AFDW). Seasonal ingested carbon by the species was calculated by applying the specific clearance rate for each prey type (Table 3.2) to the seasonal mean prey concentration values for the study area (Chapter 2). Then, a *Dysidea avara* colony of 0.2 g AFDW would ingests between 169-183 mg C g $AFDW^{-1}$ h^{-1}

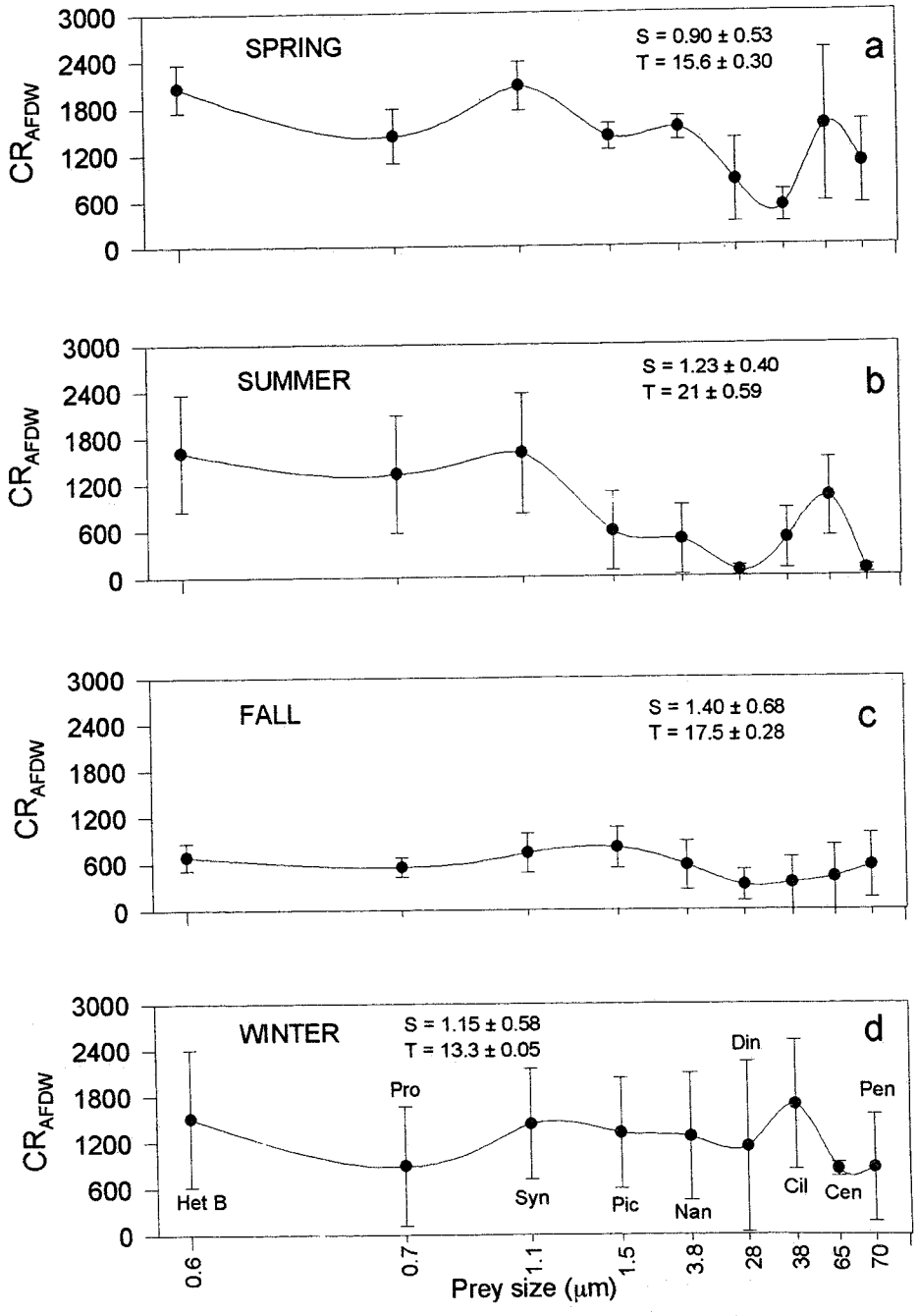


Fig. 3.2. *Dysidea avara* seasonal specific clearance rate (CR_{ADFW}; ml swept clear g $AFDW^{-1}$ h^{-1}, mean ± SD) as a function of the prey size (mm, prey size in log scale). S: sponge size used in the five experiments in g AFDW (mean ± SD). T: water temperature in °C (mean ± SD). Prey abbreviations as in figure 3.1

Table 3.2. Relationship between *Dysidea avara* specific clearance rate (CR_{AFDW}, ml swept clear g $AFDW^{-1}$ h^{-1}) and sponge size (S, g AFDW). r: correlation coefficient, p: probability, n: number of cases).

Prey	Power function	r	p	n
Heterotrophic bacteria	$CR_{AFDW} = 1276 \times S^{-1.04}$	0.84	< 0.0001	20
Prochlorococcus sp	$CR_{AFDW} = 1157 \times S^{-1.16}$	0.83	< 0.0001	20
Synechococcus sp	$CR_{AFDW} = 1321 \times S^{-0.95}$	0.83	< 0.0001	20
Picoeucaryotes	$CR_{AFDW} = 854 \times S^{-1.15}$	0.91	< 0.0001	20
Nanoeucaryotes	$CR_{AFDW} = 770 \times S^{-1.29}$	0.92	< 0.0001	20
Diatoms	$CR_{AFDW} = 537 \times S^{-0.96}$	0.51	0.023	12
Dinoflagellates	$CR_{AFDW} = 176 \times S^{-2.29}$	0.73	0.012	12
Ciliates	$CR_{AFDW} = 676 \times S^{-1.03}$	0.54	0.013	12

depending on the season (Table 3.4). A colony of 1.6 g AFDW would ingest one order of magnitude lower of carbon per unit of biomass and time (10-12 mg C g $AFDW^{-1}$ h^{-1}; Table 3.4). Overall, procariotes (i.e. heterotrophic bacteria, *Prochlorococcus* sp. and *Synechococcus* sp.) contributed to 74 ± 14 % of the total ingested carbon, pico- and nanoeucaryotes contributed to 11 ± 3 %, and phytoplankton contributed to 11 ± 10 %. Then, *Dysidea avara* obtained 85 % of the ingested carbon from the fraction smaller than 5 µm whereas the fraction larger than 5 µm contributed 15%. However, partial contribution of the different groups varied along the year. During the winter period, phytoplankton contribution to the carbon ingested by the sponge, mainly diatoms, was similar to that of procariotes (phytoplankton: 26 %, procariotes: 57 %, Table 3.4). Overall, the heterogeneous diet of the species and its capacity to feed on a wide range of prey size, allowed the species to maintain a rather constant food uptake along the year (Table 3.4).

The proportion of ingested carbon from the different prey type was statistically different from those of the water column community (summer: X^2=62.24, fall: X^2=103.84, winter: X^2=215, spring: X^2=135.4; df=7, p<0.0001 for all seasons). These differences were due to the fact that procaryotes were captured at a higher percentage than their frequency in the plankton. Furthermore, the increase in the phytoplankton contribution to the pool of live carbon in the water column during the winter period was not ingested in the same proportion by the species (Table 3.4).

SEM observations of the sponge tissue showed many trapped phytoplankton in different degrees of digestion. None of the phytoplankton remains were larger than 30 mm. SEM observations of the filters from the final incubation water samples showed an

Table 3.3. Differences between the parameters "a" and "b" from the equation $CR_{AFDW}=a\ S^b$ (Table 3.2). t - a and t - b are the t-values for the t-student test. * p<0.05, ** p<0.001, *** p<0.0001. Values without asterisck exhibited non significant differences. Prey abbreviations as in figure 3.1.

	Pro		Syn		Pic		Nan		Diat		Din		Cil	
	t - a	t - b	t - a	t - b	t - a	t - b	t - a	t - b	t - a	t - b	t - a	t - a	t - a	t - b
Het B	-2.01	-0.86	0.24	0.56	-2.28*	-0.68	-2.74*	-1.54	-3.99**	0.62	-5.95***	-7.72***	0.54	3.46**
Pro			0.337	0.65	-2.30*	0.16	-2.51*	-0.58	-2.22*	1.16	-4.41**	-5.87***	2.86*	3.70**
Syn					-2.70*	-1.34	-3.18*	-2.28*	-4.53**	-0.07	-6.62**	-8.99***	0.32	3.15**
Pic							-0.82	-1.09	-3.08**	1.47	-6.58***	-8.84***	5.07***	5.19***
Nan									-2.26**	2.44***	-5.77***	-7.41***	5.88***	6.00***
Diat											-2.20**	-3.45**	5.12***	1.25
Din													7.19***	2.22**

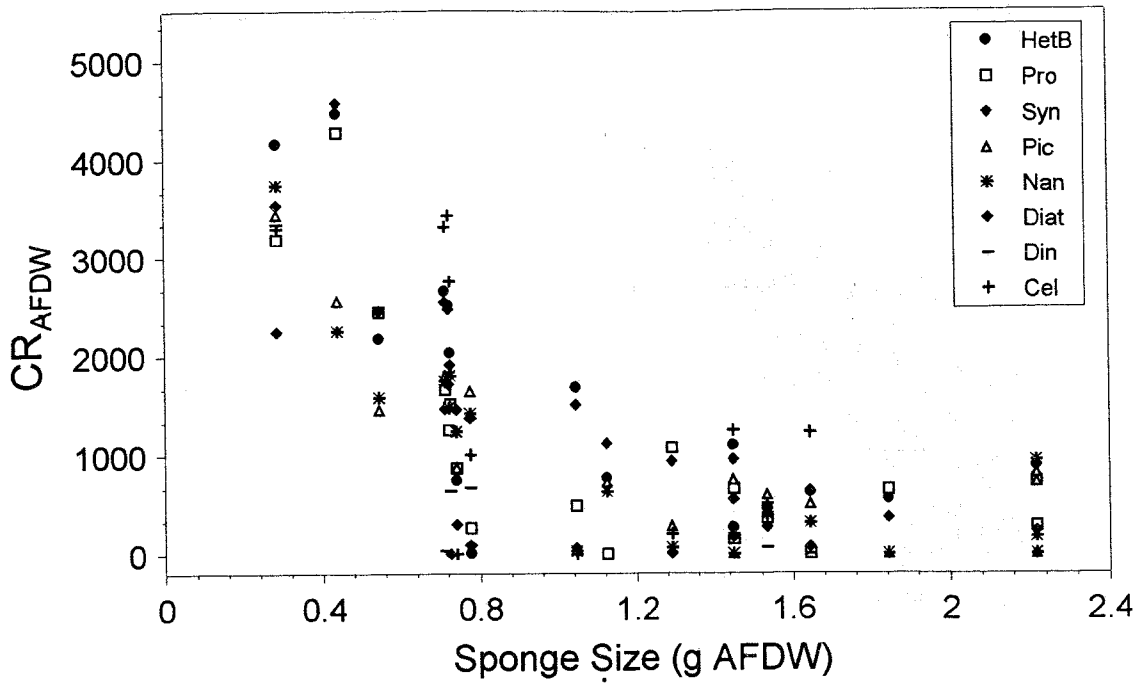


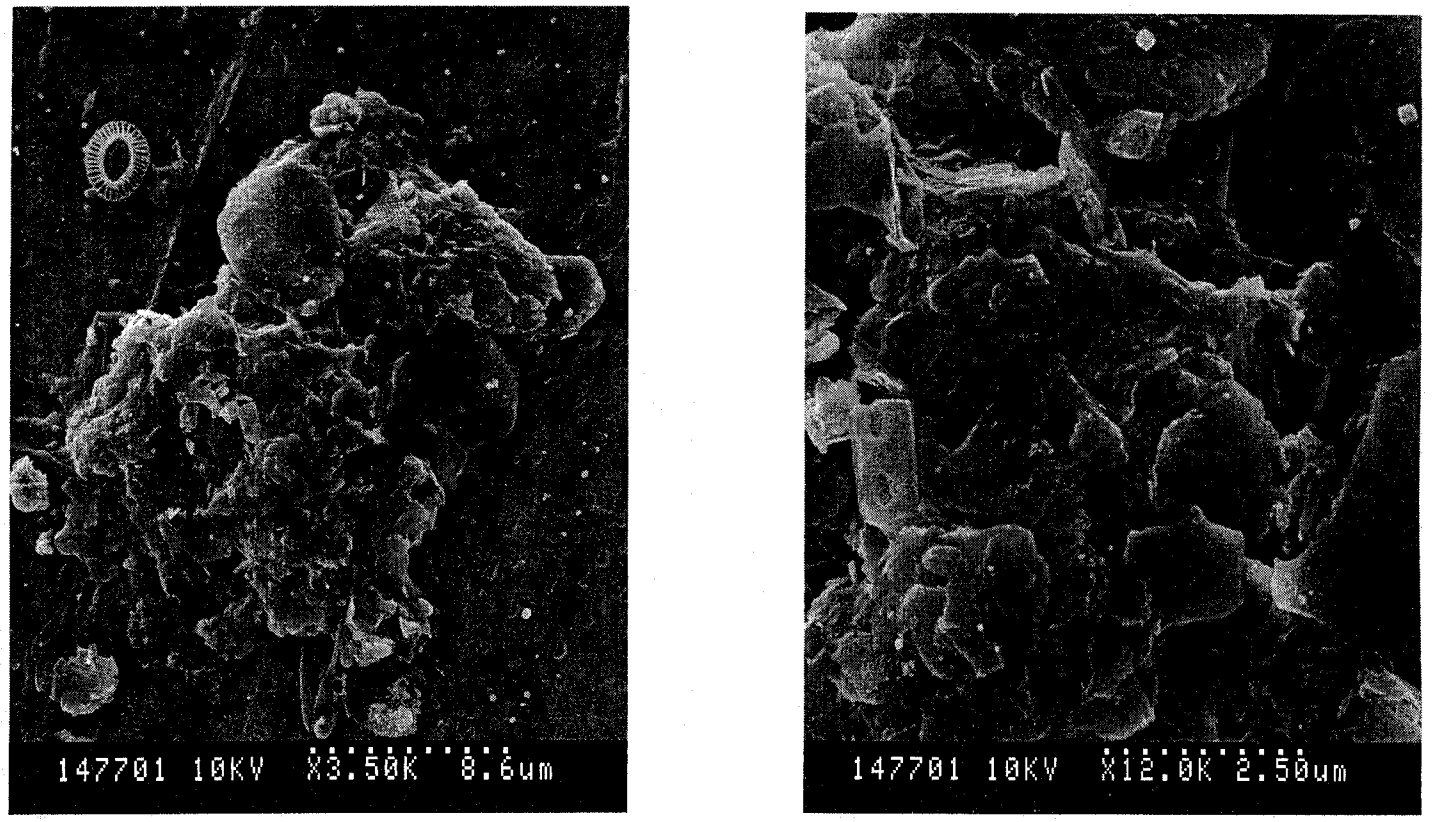
Fig. 3.3. Relationship between *Dysidea avara* size (g AFDW) and specific clearance rate (CR_{ADFW}; ml swept clear g $AFDW^{-1}$ h^{-1}) for each prey type. Prey abbreviations as in figure 3.1.

important number of pseudopellets (between 5 and 50 μm) (Fig. 3.4a), where different phytoplankton remains such as diatoms and cocolitophorals were identified (Fig. 3.4b). None of the filters from the initial incubation water samples contained such pseudopellets.

V. DISCUSSION

The diet of *Dysidea avara* was highly heterogeneous including heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*, pico-, nano- eucaryotes and microplankton. This heterogeneous diet include the capture of a broad size range of the food sources, from values of 0.5 ± 0.3 μm (heterotrophic bacteria) to 70 ± 22 μm (pennate diatoms). The ability of the sponges to efficiently capture picoplankton (plankton < 2 μm) have been well documented (e.g. Reiswig 1971; Pile et al. 1996), but less data is available about the capture of large cells such as phytoplankton (but see Reiswig 1971; Frost 1981).

Estimated clearance rate varied among some prey types, suggesting a pattern of inverse relationship between mean prey size and clearance rate. This result indicate that the smallest prey types were retained with higher efficiency showing the ability of the species to



a b

Figure 3.4. SEM observation of the filters from the final incubation water samples. a) A whole pseudopellet. b) A detail.

Table 3.4. Seasonal estimated ingestion rate (μg C g AFDW^{-1} h^{-1}) of *Dysidea avara*. Values have been estimated for the extreme. size examined during the experiments (0.2 - 1.6 g AFDW). % diet was the prey composition of the ingested carbon and % plankton was the proportion of total living carbon in the water column. Prey abbreviations as in figure 3.1.

	SUMMER			FALL			WINTER			SPRING		
	Ingestion	% diet	% plankton	Ingestion	% diet	% plankton	Ingestion	% diet	% plankton	Ingestion	% diet	% plankton
g AFDW	0.2 - 1.6			0.2 - 1.6			0.2 - 1.6			0.2 - 1.6		
Het B	76 - 4.30	43	32	60 - 3.96	33	33	59 - 3.90	35	19	63 - 3.50	35	24
Pro	2 – 0.10	1	1	9 - 0.60	5	4	1 - 0.04	0,4	1	1 - 0.02	0,2	1
Syn	74 - 4.20	42	25	86 - 5.64	47	20	37 - 2.42	22	4	57 - 3.20	32	14
Pic	2 - 0.10	1	4	2 - 0.12	1	5	3 - 0.22	2	9	2 - 0.10	1	5
Nan	11 - 0.60	6	28	15 - 0.96	8	25	20 - 1.32	12	14	22 - 1.20	12	33
Diatoms	12 - 0.70	7	7	7 - 0.48	4	7	44 - 2.86	26	49	11 - 0.60	6	18
Din	0.2 - 0.01	0,1	1	1 - 0.04	0,3	1	1 - 0.04	0,4	1	2 - 0.10	1	1
Cil	2 - 0.10	1	2	2 - 0.12	1	4	2 - 0.11	1	3	23 - 1.30	13	4
TOTAL	176 - 11			183- 12			169 - 11			180 - 10		

Table 3.5. Specific Clearance rate for various marine sponge species, range and mean values. DW: dry weight, AFDW: ash free dry weight.

Specie	Study site	Clearance rate		Reference
		ml g DW^{-1} h^{-1}	ml g $AFDW^{-1}$ h^{-1}	
Dysidea avara	Mediterranean	104 - 2046	264 - 4470	This study
			1539 ± 1241 (SD)	"
Dysidea avara	Mediterranean	1391 - 3806	--	Turon et al. 1997
Halicondria panicea	North Sea	--	1700 - 5200	Riisgård et al. 1993
Therea muricata	Norwegian-Greenland Sea	--	5000 - 9000	Witte et al. 1997
Haliclona anonyma	South Africa	5660		Stuart and Klumpp 1984
Mycale sp	Tropical	--	12360	Reiswig 1974
Tethya crypta	Tropical	--	5353	Reiswig 1973, 1974
Verongula sp	Tropical	--	3054	Reiswig 1974
Verongia fistularis	Tropical	--	3150	Reiswig 1981

select particles. The mean diameter of the ostia for *D. avara* (30 μm, Turon et al. 1997) allows the capture of all studied prey items but diatoms. Recent laboratory experiments with *D. avara* carried out with latex beads have shown that particles from 0.2 to 4 μm are retained in the choanocytes and that particles larger than 6 μm are captured by the pinacocytes; prey types about 1 μm were the sizes more efficiently captured (Turon et al. 1997). Our results agree with the results of this recent study.

Pinacocytes are found on the inhalant aquiferous systems but also on the epidermis of the sponge. Diatoms or in this case cells > 30 μm can be phagocytised by pinacocytes upon contact with virtually any surface of the sponge (Conover 1981). As pointed out before, part of the differences between ingested carbon from the different groups and their proportion in the water column appears to be because phytoplankton cells are captured less efficiently than the other prey groups in the water column. This is probably due to a difference in the process of handling and particle incorporation carried out by the pinacocytes, which can phagocytate large particles (Reiswig 1971). The lower efficiency on these groups may also be due to cell resistance to digestive enzymes (i.e. cellulose and siliceous walls), because these cells require a longer exposure to digestive enzymes (Reiswig 1971).

Several previous studies have reported the ability of sponges to capture diatoms (Reiswig 1971; Frost 1981; Simpson 1984; Witte et al. 1997). However, our knowledge is still scarce about their importance as a food source. It has been reported that digestible particles are egested as fecal pellets while indigestible particles are egested as single particles (Wolfrath and Barthel 1989). Then, the diatom remains observed inside the sponge and the large amount of diatom and cocolitophoral fragments found in the pseudopellets suggest that the species is digesting these cells.

As a general feature of the sponge feeding system, pico- and nanoplankton are captured in the choanocytes, the specific particle capture system of sponges (Kilian 1952, Simpson 1984). Our results showed a higher clearance rate for picoplankton than that for nanoplankton, suggesting a higher retention efficiency for picoplankton. Selectivity in sponge feeding has some controversial points. From a theoretical approach, the filter-pump design implies that all water being pumped runs through the collar-filter (Larsen and Riisgard 1994). Then, if all flow through the sponge have to pass through the collar-filter, it is difficult to postulate differences in retention rate. However, several studies which have estimated clearance rate based on the uptake of different prey sizes have observed that clearance rate varied among size particles, indicating different retention efficiencies. This fact has been reported for tropical, temperate and deep sea sponges (Reiswig 1971, Pile et al. 1996, Turon et al. 1997, Witte et al. 1997) showing that sponges are actively selecting particles. This selection appears to be due to a substantial selection in the process of handling and incorporation of particles (Frost 1980, Frost 1987).

Our experiments suggest that *Dysidea avara* do not significantly feed on POC of detritic origin neither on DOC. Instead, it seems that the species was a net source of DOC. The ability to uptake DOC by sponges is clear when species have symbiotic bacteria but is not clear in non-symbiotic species (Frost 1987). Our results agree with this general pattern because *Dysidea avara* is practically free of bacteria symbionts (Turon et al. 1997).

Specific clearance rate exhibited a pattern of decrease with sponge size increase. Although, the same trend has been previously observed in other sponge species (Reiswig 1974; Frost 1980; Riisgård et al. 1993), this metazoan characteristic, has been cautiously accepted for sponges (Riisgård et al. 1993). Despite differing opinions about sponges as colonial organisms (Hartman and Reiswig 1973; Simpson 1984), sponges are usually considered as a colonial animals (Jackson 1977; Frost et al. 1982) which should imply that their physiological capabilities should not change with size. However, the sponges modules (canal system) are not clearly delimited, they change continuously during the life of the sponge and probably the rate of change depends on the species. This plasticity makes the sponge shape variable overtime (Becerro et al. 1994). It has been suggested that the CR_{AFDW} decrease with sponge size increase could be the result of fewer living choanocytes per weight unit in large sponges (Riisgård et al. 1993). However, the causes underlying this effect are still not well understood.

We did not observe any significant relationship between clearance rate and temperature within the range of temperatures of this study (13°C to 22°C), which follows very close the annual temperature range of the study area (12°C to 23°C). This is in contrast to the results reached by other authors which observed a clearance rate increase with temperature increase (Riisgård et al. 1993: temperature range from 6 °C to 12 °C; Frost 1980: temperature range from 14 °C to 27 °C). The ability for constriction-dilation of inhalant canals and/or choanocyte chambers has been proposed as a possible regulatory mechanism (Riisgård et al. 1993 and references therein). However, it remains unclear how this physical factor can affect the sponge filtering activity. Most of those studies examined the effect of temperature on clearance rate under laboratory conditions and with rather fast temperature changes (i.e., hours or few days). Our experiments represent a different approach to the effect of temperature on clearance rate because they were carried out with specimens that were always keep in their natural environment. Then, they had an slowly and natural acclimation through the seasons to each temperature. Although our experimental setup was not designed to test for the effect of temperature (a size effect may be interfering with a potential temperature effect), our results suggest that under natural circumstances of gradual temperature change, clearance rate might become low affected by temperature.

Pumping activity has been shown to not be always constant for all species (Reiswig 1971, Savarese et al. 1997). Several diel cycles carried out along the year to study

respiration rate of *Dysidea avara* have shown no significant differences in respiration rate within diel cycles (Annexe II). This would suggest a rather constant level of activity as observed in the tropical demosponge *Mycale sp.* by Reiswig (1971). However, pumping without filtering has also been observed (Wilkinson et al. 1984). Then, from the incubation experiments it can not be determined whether or not filtering was constant, and the estimated clearance rates have to be regarded as average values.

Mean specific clearance rate estimated in this study was within the range of values reported for *Dysidea avara* with latex beads of 0.2, 0.5 and 1 μm in the laboratory (Turon et al. 1997). The comparison of the clearance rate values between species is difficult due to the variability within each species and the effect of sponge size. Despite this, mean clearance rate values observed for this Mediterranean species was in the same order of magnitude, although among the lowest values, to those estimated for the North Sea species *Halicondria panicea*, for the Norwegian-Greenland Sea species *Therea muricata*, as well as for most tropical species but *Mycale sp.* (Table 3.5). The high clearance rate value of the tropical species *Mycale sp.* appears to be related to the fact this species does not have symbionts. Then, because quantity of food in tropical species is lower than in temperate zones, tropical species without symbionts might require to process larger volumes of water to obtain their energy requirements than those species living in temperate seas.

Our results showed that the composition of the ingested carbon by *Dysidea avara* mainly varied according to the availability of the different prey types in the water column. During the winter months, the low values of grazing on heterotrophic bacteria and *Synechococcus* were due to a drop off of the bacteria biomass during the winter period (Chapter 2) and, the high values of grazing on phytoplankton were due to the phytoplankton winter bloom (Estrada et al. 1985). However, although highest live carbon abundance occurs during the winter period (Chapter 2), food uptake during this period was not significantly highest (Table 3.4). This was probably due to the fact that during this period about 50 % of live carbon are phytoplankton cells (Chapter 2) and, because of the lower retention efficiency of the phytoplankton cells in comparison to that of the other groups. The high values of grazing on pico- and nanoeucaryotes in May-June were due to the increase in abundance of these groups during this period but also to the higher retention efficiency of the species upon these groups with respect to that of the phytoplankton cells. Then, pico- and nanoplankton provided a stable baseline of food for the species. However, the contribution of large cells such as phytoplankton was important in the energy budget of the sponge in some periods.

Resources have usually been considered as a non-limiting factor for sublittoral sponges (Reiswig 1974; Becerro et al. 1994). They survive in very food limited environments like submarine caves and deep sea communities where they are also one of the dominant benthic groups (Riedl 1966; Gili et al. 1986; Gage and Tyler 1991).

Table 3.5. Specific Clearance rate for various marine sponge species, range and mean values. DW: dry weight, AFDW: ash free dry weight.

Specie	Study site	Clearance rate		Reference
		ml g DW^{-1} h^{-1}	ml g $AFDW^{-1}$ h^{-1}	
Dysidea avara	Mediterranean	104 - 2046	264 - 4470	This study
			1539 ± 1241 (SD)	"
Dysidea avara	Mediterranean	1391 - 3806	--	Turon et al. 1997
Halicondria panicea	North Sea	--	1700 - 5200	Riisgård et al. 1993
Therea muricata	Norwegian-Greenland Sea	--	5000 - 9000	Witte et al. 1997
Haliclona anonyma	South Africa	5660		Stuart and Klumpp 1984
Mycale sp	Tropical	--	12360	Reiswig 1974
Tethya crypta	Tropical	--	5353	Reiswig 1973, 1974
Verongula sp	Tropical	--	3054	Reiswig 1974
Verongia fistularis	Tropical	--	3150	Reiswig 1981

Nevertheless, a linkage have been shown between food availability and new production of sponges. Seasonal inputs of POC to the water have been suggested to trigger sexual reproduction in deep-sea sponges (Witte 1996), as well as lower energy regimes and reduced food availability resulted in a reduction on the sponges growth (Wilkinson and Vacelet 1979; Huysecom et al. 1988). Seasonal changes on toxicity observed in some temperate sponges has also been related to seasonal changes in food availability (Turon et al. 1996). However, whether sponges are food limited or not, the heterogeneous diet of this species diet and its capacity to feed on a broad size range of food sources allowed the species to maintain a rather constant food uptake throughout the year. This feeding plasticity may represent an advantage for the species and might distinguish its dynamics from that of other temperate suspension feeders because it attenuates the effects of the seasonal fluctuations in planktonic communities which have been pointed out to play an important role in determining the dynamic of suspension feeders in benthic communities of temperate seas (e.g. Bayne and Newell 1983; Hawkins et al. 1985, Coma et al. in press). The plasticity of the trophic ecology of this species maybe among the factors that allow this species to be one of the most widespread temperate sponges (Atlantic: Lombas 1982, Mediterranean: Uriz et al. 1992), as well as to maintain high growth rates (Uriz et al. 1996). The plasticity of sponges trophic ecology maybe among the main factors that contribute to the worldwide abundance and distribution of sponges. Feeding plasticity may represent an important strategy for benthic suspension feeders that face spatial and temporal variations in food sources.

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4

SEASONAL VARIATION OF *IN SITU* FEEDING RATES BY THE TEMPERATE ASCIDIAN *HALOCYNTHIA PAPILLOSA*

I. ABSTRACT

The natural diet and prey capture rate of the temperate ascidian *Halocynthia papillosa* were studied in the field in a western Mediterranean population. *In situ* grazing rates on DOC, detrital POC, pico-, nano- and microplankton were examined through a year cycle. The natural diet of the species included detrital organic matter, heterotrophic bacteria, *Prochlorococcus*, *Synechococcus*, protozoa and phytoplankton with a mean size range from 0.6 ± 0.3 μm (heterotrophic bacteria) to 70 ± 22 μm (pennate diatoms). Specific clearance rates varied seasonally and exhibited a pattern of increase with temperature increase, in which temperature explained 55 % of the variance in clearance rate throughout the year. Annual variation in prey concentration did not affect specific clearance rate. Overall, a mean size *H. papillosa* specimen (0.25 g AFDW) was estimated to ingest an annual mean of 1305 ± 496 μg C g AFDW^{-1} h^{-1} and 84 ± 16 μg N g AFDW^{-1} h^{-1}. Carbon from detrital origin accounted for 92 ± 2 % of the total ingested carbon with the highest values in spring, while ingestion of live carbon accounted for 8 ± 2 % of the total ingested carbon with the highest values occurring during summer and fall. However, the seasonal variation of ingested nitrogen from live particles explained 91 % of the gonadal development variance along the year, suggesting that live particles are likely to be of more significance in the diet of the species than particles from detrital origin. Feeding rates are discussed in relation to seasonal changes in food availability and production of the species (i.e., growth and reproduction). The results suggest that other factors than food availability appears to be determining the seasonal dynamics of the species.

II. INTRODUCTION

Benthic suspension feeders account for an important fraction of the biomass and production of consumers in coastal marine ecosystems (Cloern 1982, Officer et al. 1982, Hily 1991). Recently, several studies have indicated that these organisms play an important role in plankton-benthos coupling (e.g., Jørgensen 1990, Gili and Coma, in press). Ascidians, together with sponges and molluscs, are among the groups better represented in hard substrate communities in littoral zones such as tropical areas (Koike and Suzuki 1996), temperate seas (Ramos 1988, Turon 1990a, Petersen and Riisgård 1992), kelp beds (Newell et al. 1982), and Antarctic waters (Gerdes et al. 1992).

In general, growth and reproduction of benthic suspension feeders in tropical, temperate and polar seas are characterized by strong seasonality (e.g., Millar 1971, Clarke 1987, Harrison and Wallace 1990). In the case of ascidians in the Western Mediterranean, two characteristic patterns have been distinguished. First, several colonial ascidians have been documented to concentrate growth and reproduction around the spring season (Turon 1988, Turon and Becerro 1992) with non-feeding periods exhibited by some species during the warm period (Turon 1988, Turon 1992). Second the few solitary ascidians which have been studied, in contrast show maximum reproductive activity during or at the end of the summer period (Becerro and Turon 1992). This fact is surprising because seasonality in new production (i.e. growth and reproduction) usually occurs in the periods of higher food availability, and the Western Mediterranean in summer is characterized by oligotrophy and low seston concentration (Zabala and Ballesteros 1989). However, the lack of knowledge about the diet and grazing rate in nature has become a limiting step in understanding the factors that determine the dynamics of these species.

Ascidians, as sessile suspension feeders, are nutritionally dependent on the surrounding water which provides them with their food requirements. As a group, they are considered to be non-selective filter feeders able to capture particles from 0.5 to 100 μm (Fiala-Médioni 1987), with high retention efficiency for particles larger than 0.6 μm (Fiala-Médioni 1978a, Randløv and Riisgård 1979, Stuart and Klumpp 1984). The filtration rates of ascidians have been shown to be dependent on external factors such as temperature, oxygen tension, and food concentration, as well as on morphological factors such as body size (Fiala-Médioni 1978c, 1979a,b, Robbins 1983, Klumpp 1984, Petersen and Riisgård 1992). But, most of these studies have been carried out under laboratory conditions and with simplified diets (Fiala-Médioni 1978c, Randløv and Riisgård 1979, Robbins 1983, Petersen and Riisgård 1992, Petersen et al. 1995). These works provided understanding of the physical and biological factors that affect feeding activity of ascidians. However, the difficulty of estimating feeding in nature and its seasonal variation from laboratory results

(Jørgensen 1975, Okamura 1990, Vogel 1994) points to the need for studies focused on the examination of natural feeding rates.

Halocynthia papillosa (Linnaeus) is one of the most common solitary species on the rocky littoral of the North Western Mediterranean (Fiala-Médioni 1972, Turon 1990a). *H. papillosa* is an hermaphrodite species that reproduces once per year in late summer. Although it has continuous vitellogenesis throughout the year, ovogenesis occurs from March to September, and spermatogenesis from July to September (Becerro and Turon 1992). The main objectives of this study were to determine the role of DOC, detrital POC and live carbon from different taxa as food sources for the ascidian, and to contribute to knowledge of factors that determine the temporal variability in production of benthic suspension feeders living in seasonal environments. Thus natural diet and grazing rates of *H. papillosa* were examined by *in situ* incubations with the whole natural spectrum of food sources (DOC, detrital POC, pico-, nano- and microplankton). Feeding on these potential food sources was assessed seasonally in order to cover the natural range of food abundance and composition.

III. METHODS

Feeding experiments were conduced *in situ* using continuous flow incubation chambers (see Chapter 1) at the Medes Islands Marine Reserve (NW Mediterranean Sea, 42° 3' N, 3° 13' E, see map in Chapter 1) from October 1995 to November 1996. All specimens were selected to have a similar size (0.25 ± 0.08 g ash free dry weight) in order to avoid size effect on the feeding behavior (Robbins 1983, Klumpp 1984, Petersen and Riisgård 1992).

Feces were obtained from inside the chamber in several experiments. They were collected with a Pasteur pipette and frozen in liquid Nitrogen. Ten pieces of feces from different days were dehydrated in graded ethanol. Afterwards, they were dried by the critical point method (using CO_2 as transition fluid), mounted on aluminum stubs and coated with gold in a sputter coater. Observation were done with a Hitachi S-570 scanning electron microscope (SEM). *Halocynthia papillosa* dry weight was determined for all incubated specimens by drying at 90 °C for 24 hours and ash free dry weight was determined by combustion at 450 °C for 5 hours. The relationship between total dry weight and dry weight without test was established by dissecting 10 specimens and drying separately the test and the mantle at 90 °C for 24 hours. In average, dry weight without test was a 27 ± 6 % of total dry weight.

Multiple regression analysis was used in order to establish the percentage of the variance of the estimated clearance rates that could be explained by the independent factors which were controlled during each experiment: water temperature (°C, recorded using a

WTW oxygen electrode model EOT 196) and initial food concentration (cells ml^{-1} by the live particles and $\mu g\ C\ l^{-1}$ by detrital particles). A backward stepwise procedure was used to exclude variables not relevant (Sokal and Rohlf 1981). Variables were square root transformed when normality (Kolmogorov-Smirnov test) and/or the heteroscedasticity (Levene's test) requirements were not fulfilled.

In order to determine whether or not the species exhibits seasonality in growth, *H. papillosa* growth rate was estimated from changes in area of the individuals over time based on photographic monitoring. Large *H. papillosa* specimens are highly sensitive to small perturbations such as the approach of a camera which produce a reaction of contraction. Thus it is difficult to photograph specimens in a state of full expansion. Small specimens of the species were observed to be much less sensitive to the same kind of perturbations, to which they remain fully expanded (authors unpublished data). Therefore, eleven *H. papillosa* individuals smaller than 1 cm were mapped in the studied area. These individuals were photographed every 3-4 months: March'97, June'97, October'97 and February'98. Size (area, mm^2) of the individuals was determined by an image analysis software (NIH-image). The instantaneous growth rates (on a daily basis) were calculated as

$$Gr = \frac{\ln(A_1 - A_0)}{t_1 - t_0} \qquad (6)$$

where Gr is growth rate ($days^{-1}$), A_1 and A_0 are the ascidians area at the initial time t_0 and at the final time t_1.

IV. RESULTS

All sources of particulate organic carbon < 100 μm, both live (i.e. recognizable cells) and detrital, that were potentially available as food as well as DOC, were considered in this study. The taxa distinguished within the live carbon category were: heterotrophic bacteria, *Synechococcus* sp., *Prochlorococcus* sp., pico- and nanoeucaryotes, phytoplankton (diatoms and dinoflagellates) and ciliates. Overall, the prey growth rates calculated in the control and the experimental chamber showed that *Halocynthia papillosa* significantly fed on all live carbon groups as well as detrital organic carbon (Fig. 4.1); but no significant decrease in DOC was detected.

Mean seasonal ash free dry weight specific clearance rate values [CR_{AFDW}, ml swept clear g $AFDW^{-1}$ h^{-1}, hereafter specific clearance rate] (i.e. the volume of water filtered by the ascidian to retain the observed decrease in number of cells assuming a 100 %

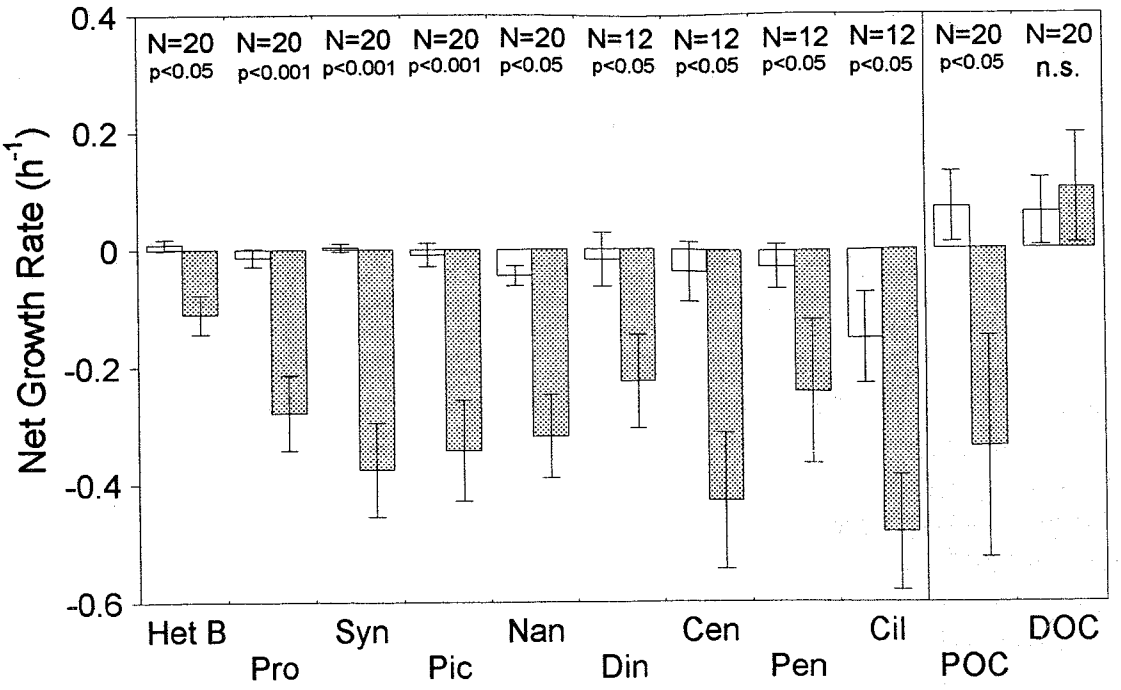


Fig. 4.1. Net growth rate of prey (mean ± SE) in the experimental (k_o, dotted bars) and control chambers (k_c , empty bars). Data are presented for each plankton group: Het B - heterotrophic bacteria, Pro - *Prochlorococcus* sp., Syn - *Synechococcus* sp., Pic - autotrophic picoeucaryotes, Nan - autotrophic nanoeucaryotes, Cen - centric diatoms, Pen - pennate diatoms, Din - dinoflagellates, Cil - ciliates, POC - detritic particulate organic carbon, DOC - dissolved organic carbon. Number of experiments and significance degree from two-tailed Wilcoxon test also shown. n.s. non significant.

retention efficiency) for each type of prey are showed in Figure 4.2. CR_{AFDW} significantly varied among seasons (Table 4.1), with the greatest values during summer (Duncan Post-Hoc test, $p < 0.005$). However, no significant differences were found in the CR_{AFDW} values between prey types (Table 4.1, Fig. 4.2).

The experiments, carried out seasonally, allowed coverage of the natural range of prey concentration (10-50 µg live carbon l^{-1}; 19-813 µg C l^{-1} from detrital origin) and almost the entire annual temperature range (12-23 °C) of the study site (Chapter 2). The effect of prey concentration (both from cells and detritus) and water temperature on CR_{AFDW} of the species was tested by mean CR_{AFDW} values of all prey groups in each experiment. CR_{AFDW} did not vary with food concentration within the natural range of cell and detritus concentration (multiple regression analysis, $p > 0.5$ for

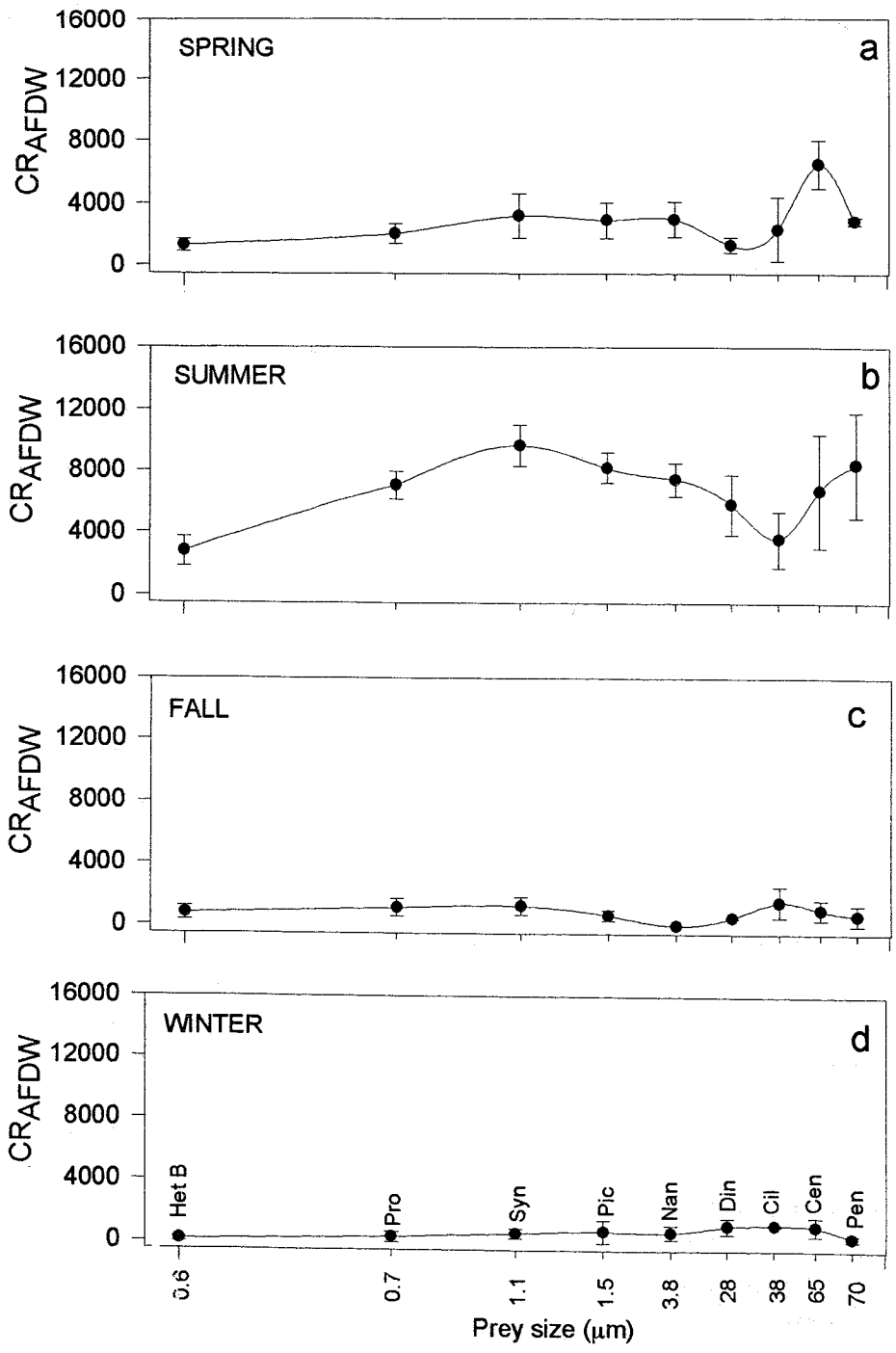


Fig. 4.2. *Halocynthia papillosa* seasonal specific clearance rate (CR_{ADFW}; ml swept clear g AFDW^{-1} h^{-1}, mean ± SE) as a function of the prey size (µm). Prey size in log scale.

Table 4.1. Analysis of variance for specific clearance rates of *Halocynthia papillosa* between prey types and among seasons. df: degrees of freedom, SS: sums of squares; MS: mean square; F: F ratio; p: probability.

Source	df	SS	MS	F	p
Prey type	9	3.01	0.329	1.119	0.358
Season	3	22.7	8.666	29.46	<0.0001
Interaction	27	6.93	0.347	1.179	0.277
Error	88	21.91	0.294		

both variables). There was a marked pattern of CR_{AFDW} increase with temperature increase (Fig. 4.3) and temperature variation explained 55 % of the CR_{AFDW} variance (multiple regression analysis, n=20, $p < 0.0001$). This pattern has a power function as best fit ($CR_{AFDW} = 0.014 \times T^{4.33}$; T: water temperature in °C). The wide range of temperatures over which the grazing experiments were carried out (13.7 to 22.6 °C) allowed the calculation of a Q_{10} (sensu Fiala-Médioni 1978c) for the clearance rate. Because the temperatures did not differ by 10 °C, Van't Hoff's formula was applied (Lucas 1996):

$$Q_{10} = (CR_{AFDWt2}/CR_{AFDWt1})/10/t_2\text{-}t_1$$

A Q_{10} value of 4.88 was estimated for the species.

In order to estimate the annual amount of carbon ingested by the species, monthly ingested carbon was calculated by applying the relationship between the specific clearance rate and temperature for each prey type, considering the mean monthly temperature and prey concentration values (Chapter 2). Ingested organic carbon came from two main sources: carbon of detrital origin and carbon from live cells. An individual of *H. papillosa* of the studied size (mean 0.4 g AFDW) ingested an annual mean (± SE) of 1305 ± 496 µg C g $AFDW^{-1}$ h^{-1} (Fig. 4.4a). Carbon of detrital origin accounted for 92 ± 2 % of the total ingested carbon and showed a marked seasonal pattern in which spring was the season with the highest values of ingestion (4059 ± 350 µg C g $AFDW^{-1}$ h^{-1}). Ingestion of live carbon accounted for 8 ± 2 % of the total carbon ingested, ranging from 0.8 ± 0.3 % in spring to 8 ± 4 % in winter (Fig. 4.4a). The highest values of live carbon ingestion occurred during summer and fall (70 ± 14 and 57 ± 19 µg C g $AFDW^{-1}$ h^{-1}, respectively) which were about 4 times the ingestion during the winter and spring period (15 ± 1 and 15 ± 7 µg C g $AFDW^{-1}$ h^{-1}, respectively). In summer and fall, bacteria accounted for about 45 % of the total ingested live carbon. During winter phytoplankton accounted for over half of the total ingested live carbon and flagellates accounted for 50% of the total live carbon during spring (Fig. 4.4b).

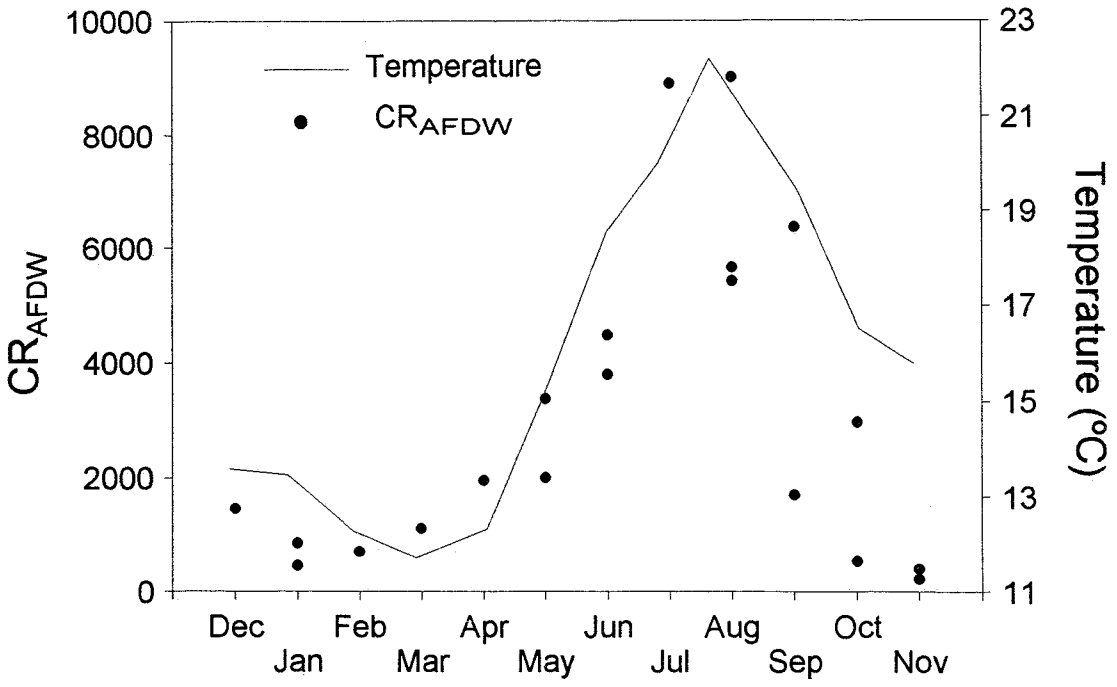


Fig. 4.3. Relationship between *Halocynthia papillosa* specific clearance rate (CR_{ADFW} ; ml swept clear g $AFDW^{-1}$ h^{-1}) and temperature (ºC). Clearance rate calculated from *Synechococcus* sp cell type.

The annual amount of nitrogen ingested by the species has been calculated from the C:N ratios reported in the chapter 2. An average C:N of 4 has been applied for the ingested live carbon and a C:N of 14 for the ingested detrital carbon. There was strong seasonality in the contribution of live particles and detritus as nitrogen source (Fig. 4.5). During winter and fall, most nitrogen came from the live fraction (between 32 and 69 %). This pattern changed during spring and summer, when detrital particles were the main source of nitrogen for the species (98 and 76 %, respectively).

In four occasions, during the experiments carried out throughout the year, we observed the production of feces. Feces were about 3 cm long and width approx. 225 µm (Fig 4.6a). Scanning electronic microscopy (SEM) observations of them showed a highly compacted structure, without a defined outer layer, within which it was possible to distinguish a large amount of diatoms and dinoflagellates fragments together with non identifiable material (Fig 4.6b). The examination of the gut contents of 20 freshly collected individuals showed similar results. In no occasion were zooplankton prey items or remains observed either in the feces or in the gut contents.

The growth rate of *H. papillosa* estimated from changes in the individuals area over

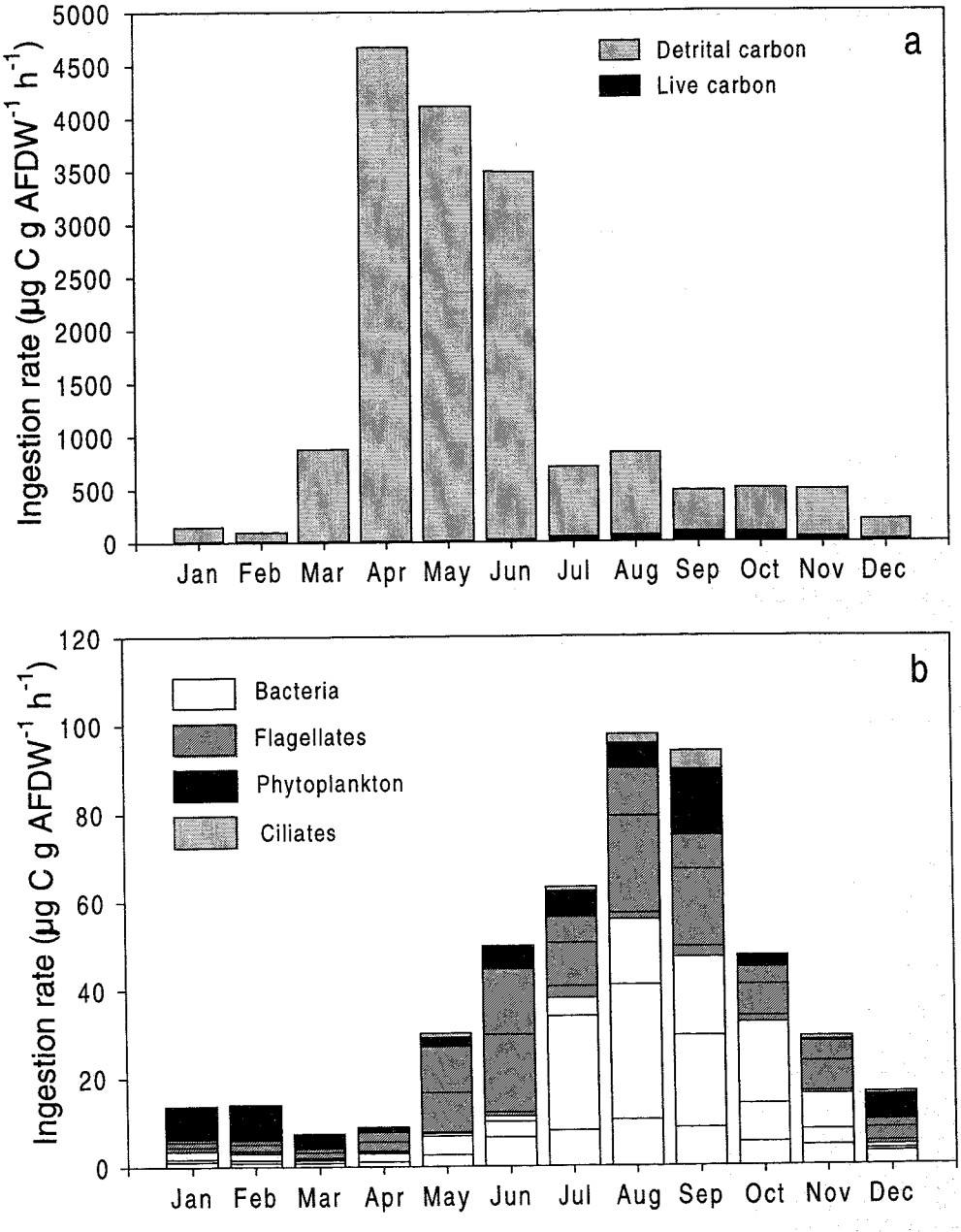


Fig. 4.4. Monthly estimation of total ingested organic carbon (µg C g AFDW^{-1} h^{-1}) by *Halocynthia papillosa* through an annual cycle. a) Total amount divided in the two main sources: live carbon and carbon from detrital origin. b) Composition of the ingested live carbon. Bacteria include heterotrophic bacteria, *Synechococcus* sp and *Prochlorococcus* sp; flagellates include picoeucaryotes, autotrophic and heterotrophic nanoeucaryotes and phytoplankton include diatoms and dinoflagellates.

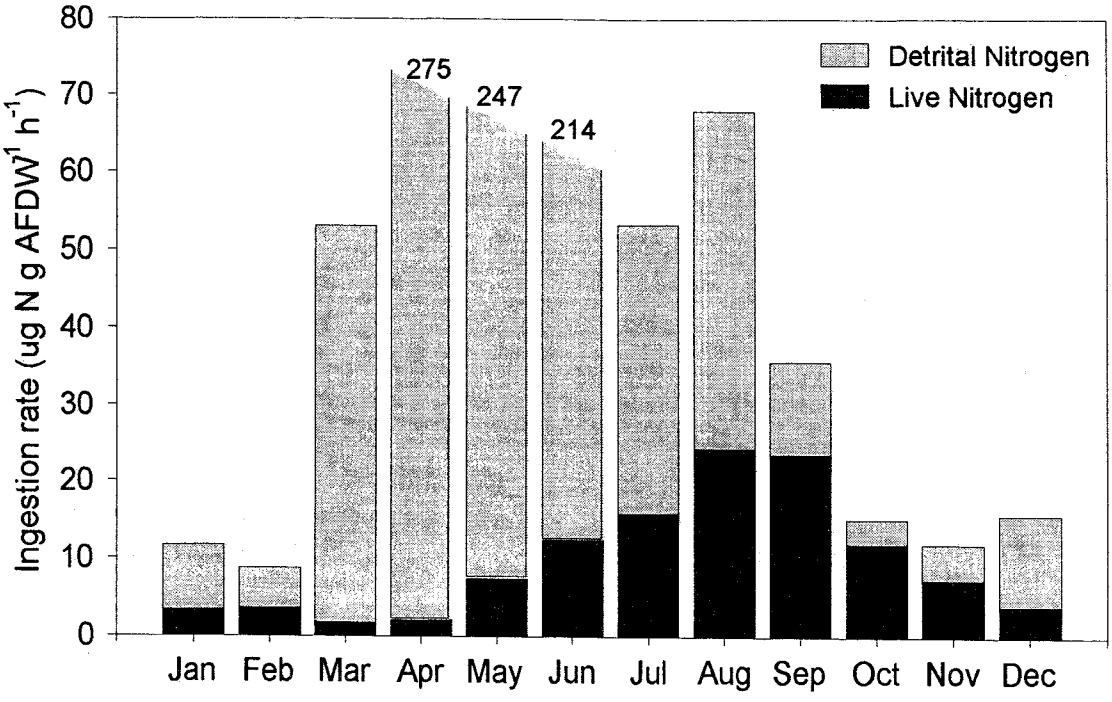
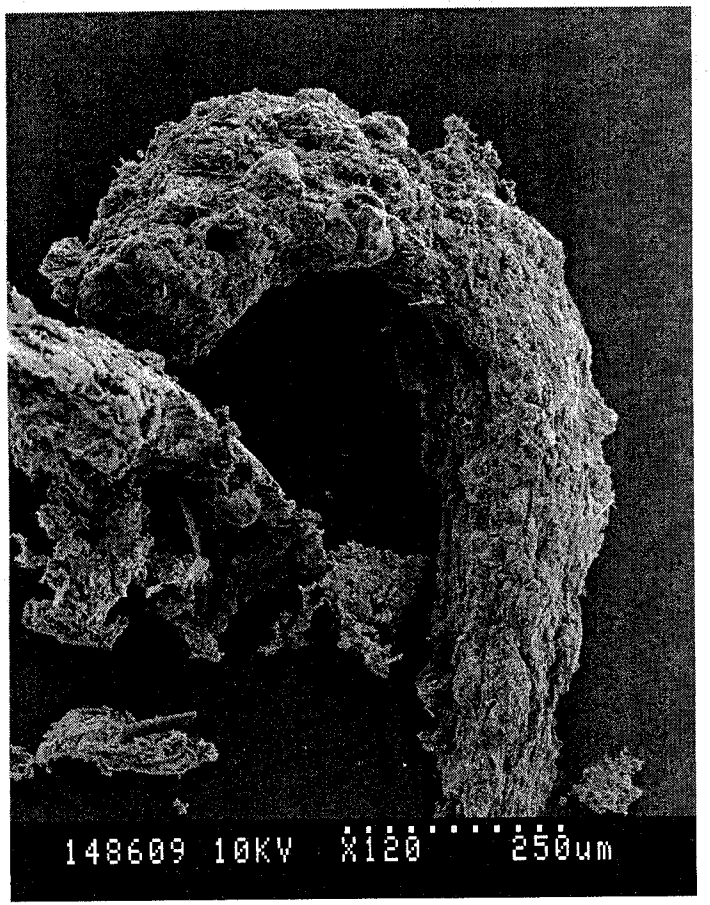


Fig. 4.5. Monthly estimate of total ingested nitrogen (μg N g AFDW^{-1} h^{-1}) by *Halocynthia papillosa* through an annual cycle.

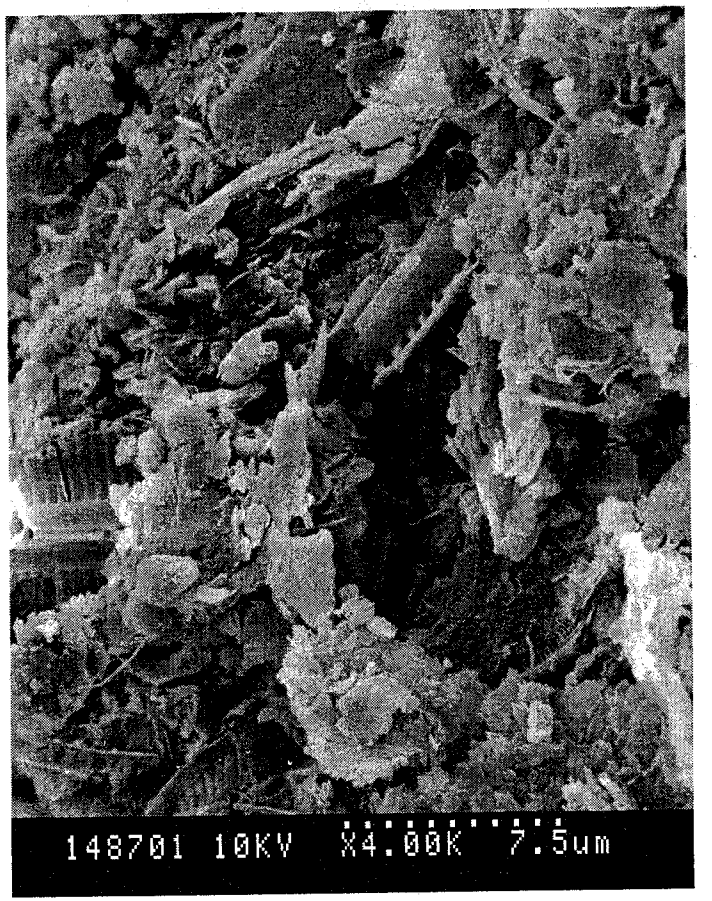
time, showed significant differences between seasons (Kruskal-Wallis test H = 7.25, df = 2, p = 0.026). Maximum growth rate values were recorded in spring and summer and minimum in fall and winter (Fig 4.7). Then, the period with higher somatic growth in *H. papillosa* agree with the period of higher gonadal development indicated by the gonad index (i.e., gonad weight/mantle weight; Becerro and Turon 1992) of the species (Fig 4.7).

V. Discussion

The natural diet of *Halocynthia papillosa* was highly heterogeneous, including live and detrital organic carbon. In size terms, this heterogeneous diet included the capture of a broad size range of food sources, from 0.6 (heterotrophic bacteria) to 70 μm (pennate diatoms). The smallest particle size captured by *Halocynthia papillosa* agrees with the size of the branchial filters (0.36-1.16 μm in diameter, Turon 1990b). In general, ascidians have been considered as nonselective suspension feeders for particles between 0.5 and 100 μm (Fiala-Médioni 1987). These potential food sources are retained by the mucous net secreted by the endostyle in the pharyngeal cavity and afterwards transported as a food cord to the esophagus (Fiala-Médioni 1978b). It has been pointed out that large particles (>100 mm) usually do not reach the branchial cavity because of retention by the oral



a



b

Figure 4.6. a) SEM observation of the *Halocynthia papillosa* feces. b) Detail of the feces composition showing several diatoms pieces.

tentacles (Millar 1971, Klumpp 1984). However, some studies which examined the gut contents of ascidians have shown that some species consume a variety of invertebrate larvae and eggs (Young 1988, Bingham and Walters 1989). Although this work was mainly focused on the study of prey items smaller than 100 mm, the examination of the feces of the species showed a large amount of phytoplankton remains as well as non identifiable material, but no zooplankton remains. Furthermore, no invertebrate larvae or egg remains have not been observed in the examination of the gut contents of the individuals examined in this study. However, these observations do not disprove that *H. papillosa* could be feeding on zooplankton to some extent.

The marked seasonal variation in the CR_{AFDW} of the species prompted the question of what environmental cues might influence its variability. As mentioned in the introduction

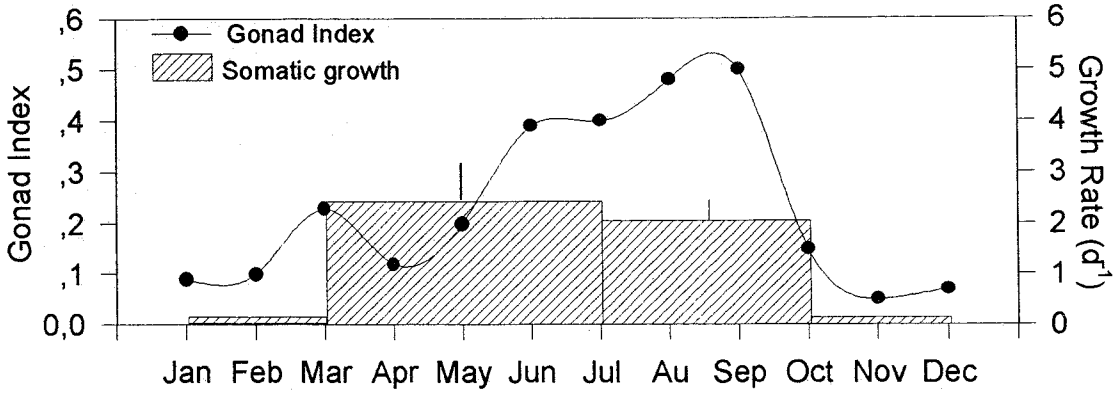


Fig. 4.7. Monthly evolution through a year cycle of the *Halocynthia papillosa* gonad index (data from Becerro and Turon 1988) and somatic growth rate (x 1000 days $^{-1}$).

introduction, temperature, food concentration, body size and oxygen tension have been suggested as the main factors that affect filtration rate (Fiala-Médioni 1978a,b,c, Robbins 1983, Klumpp 1984, Petersen and Riisgård 1992). We did not examine the effect of oxygen tension on filtration rate of *H. papillosa* because all experiments were performed in the field where oxygen concentration was always fully saturated or slightly supersaturated. Oxygen concentration during the incubation experiments was never allowed to decrease by more than 10 % of the initial oxygen concentration, a fall magnitude which appears not to affect the behavior of the animal (Fiala-Médioni 1979a, Crisp 1984). Because specimens of similar size (0.4 ± 0.1 g AFDW) were used in the experiments, the effect of body size on filtration rate will not be examined. The experiments covered the natural range of prey concentration (10-50 µg live carbon l^{-1}; 19-813 µg C l^{-1} from detrital origin, Chapter 2) and within this range, *H. papillosa* CR_{AFDW} was not significantly affected by food concentration. These results agree with previous studies carried out with graphite

Table 4.2. Specific clearance rate values in milliliters reported for different species of ascidians. DW: total dry weight, weight without tunic, Indirect: results obtained from clearance experiments, DW wt: dry Direct: results obtained from flow measurements, Lab: laboratory studies, In situ: field studies.

Specie	ml g DW^{-1} h^{-1}	ml g DW_{wt}^{-1} h^{-1}	Collection site	Observations	Reference
Clavelina lepadiformis		2489	Mediterranean	Indirect, Lab	Fiala-Médioni 1974
Ciona intestinalis		3517	Mediterranean		
Halocynthia papillosa		6349	Mediterranean		
Microcosmus sabatieri		6909	Mediterranean		
Phallusia mammillata		4380	Mediterranean	Indirect, Lab	Fiala-Médioni 1973
		352 - 4386	Mediterranean	Indirect, Lab	Fiala-Médioni 1978a
Ascidia mentula		3422	Mediterranean	Direct, In situ	Fiala-Médioni 1978b
Phallusia mammillata		2070	Mediterranean		
Styela plicata		4892	Mediterranean		
Microcosmus sabatieri		3084	Mediterranean		
Ciona intestinalis		4331	Mediterranean	Indirect, Lab	Fiala-Médioni 1978c
		5906	Mediterranean	direct, Lab	
Phallusia mammillata		4779	Mediterranean	Indirect, Lab	
		6312	Mediterranean	direct, Lab	
Styela plicata		8760	Mediterranean	Indirect, Lab	
		10708	Mediterranean	direct, Lab	
Ciona intestinalis	5529 - 17678		Danish fjord	Indirect, Lab	Petersen & Riisgård 1992
Styela clava		1320	Southampton estuary	Indirect, Lab, 15°	Holmes 1973
Ascidiella aspersa		2580	Southampton estuary	Indirect	
		3240	Southampton estuary	Indirect	
		5340	Baltic	Indirect & direct	Randløv & Riisgård 1979
Pyura stolonifera		480 - 1260	South-Africa	Indirect, 14°C	Klumpp 1984
Didemnum molle	2000		Fijjian seagrass	Indirect, Lab	Koike and Suzuki 1996
Lissoclinum bistratum	180				
Lissoclinum voeltzkowi	70				
Didemnum cf	3600				
Aplidium altarium	2600				
Halocynthia papillosa	1445 - 6244	5351 - 22848	Mediterranean	Indirect, In situ	This study

particles (Jørgensen 1955) and with food that resembles the composition of natural seston (Klumpp 1984). However, in laboratory studies, some authors have reported that food concentration affects filtration rate with a switching off of filtering at very low food concentration, and a satiation point at very high food concentrations (Fiala-Médioni 1979b, Robbins 1983, Klumpp 1984, Petersen and Riisgård 1992). Our results indicate that, in nature, there is a wide range of food concentration over which filtration rate remains constant, although *H. papillosa* may exhibit different feeding behavior at extreme food concentrations. Temperature appears to be the factor that best explains CR_{AFDW} seasonality. This result agrees with several previous laboratory studies (Holmes 1973, Fiala-Médioni 1978c, Robbins 1983, Petersen and Riisgård 1992). The Q_{10} estimated for *H. papillosa* represents a 5-fold increase in clearance rate over the temperature range of 13.7 to 22.6°C. This value is within the spread of values described for ascidian species studied to date which range from 0.36 to 8.34 (Holmes 1973, Fiala-Médioni 1978c, Robbins 1983, Petersen and Riisgård 1992). Our data suggest that the optimal temperature value for filtration activity of *H. papillosa* corresponds with the maximum temperature of the water through the annual cycle at the study site (23° C). However, temperatures associated with maximum filtration activity may greatly differ between ascidian species (Fiala-Médioni 1978c).

Although the positive effect of temperature on filtration rate appears to be a widespread characteristic of ascidian species, the mechanism that produces it is unclear. Some authors conducting laboratory experiments with other suspension feeders have interpreted low filtration rates at low temperatures as an energy-saving adjustment. This serves to reduce high costs of filter feeding during winter in temperate seas, when food concentrations are low (Newell and Bayne 1980). This appears to not be the case in *H. papillosa*, because although low filtration rates have been observed at low temperatures, the concentration of phytoplankton and detritus were high during the winter period (Chapter 2). It has also been pointed out that the ascidian ciliary pharyngeal pump operates in the absence of physiological mechanisms for regulating water pumping as happens with suspension-feeding bivalves (Jørgersen et al. 1990). In this sense, Petersen and Riisgård (1992) suggested that the increase of clearance rate in ascidians with temperature increase could correspond to a decrease in water viscosity.

Halocynthia papillosa ingestion rate also varied seasonally. The positive correlation between temperature and clearance rates together with the variation in food concentration over the year (Chapter 2) may to explain the marked seasonality observed in the ingestion rate of the species. Maximum ingestion rates observed in spring were associated with the relatively high clearance rate and the high abundance of seaweed detrital POC in the water column during this period (Ballesteros 1991, Chapter 2). During the summer period, clearance rate increased but available POC decreased due to sedimentation (Chapter 2). Thus, total ingestion rate decreased, but the quality of the ingested material increased

because sedimentation is more important for detrital POC than for live carbon. The low ingestion rate values during the winter months were mainly due to the decrease in clearance rate.

Table 4.2 shows CR_{AFDW} values for different species and habitats. The wide range of CR_{AFDW} reported for *H. papillosa* in this study was mainly due to variation in CR_{AFDW} over the year. CR_{AFDW} depends on variables such as body size (Klumpp 1984), temperature (Fiala-Médioni 1978a) and the methods used to obtain the CR_{AFDW} values (Fiala-Médioni 1978c), making the comparison between species difficult. However, in general, Mediterranean species appears to exhibit higher CR_{AFDW} values than estuarine, kelp bed or tropical species (Table 4.2). The lower CR_{AFDW} values reported by ascidians in estuarine and kelp bed environments could be related to the richness in suspended organic materials of these habitats. In this sense, ascidian species living on these conditions may need to process a lower water volume than those living in areas with less suspended organic materials such as the Mediterranean. The lower CR_{AFDW} reported for tropical species may be related with the fact that they are harboring symbiotic algae. These symbiotic ascidian species obtain an important portion of their carbon gain from the photosynthetically-fixed carbon (Koike et al 1993, Koike and Suzuki 1996). Thus, they may need to process lower water volume in order to cover their requirements.

Although the role of detritus in benthic invertebrate nutrition has been widely debated (Levinton et al. 1984), the fate and role of detritus derived from benthic marine macrophytes is an issue of considerable importance in coastal trophic dynamics (Tenore et al. 1982). Models of energy flow derived from estuarine systems suggest that detrital pathways are, by far, the dominant route by which seagrass production is utilized by higher trophic levels (e.g., Phillips 1984, Thayer et al 1984). In general, attention has been focused on detritus produced by marine angiosperms (such as marsh and eel grasses) and utilized by deposit-feeding invertebrates (Valiela 1984 and citations therein). However, detritus of macroalgal origin constitutes one of the main sources of the organic carbon pool in the study area (Ballesteros 1991, Chapter 2), as has been reported in general for coastal waters (Mann 1982). *H. papillosa* filtered all items at equal rates, in accord with previous results obtained by Robbins (1983, 1984) that ascidian diets mirror suspended food composition. Because detrital POC was always about an order of magnitude higher than live POC (Chapter 2), organic carbon from detrital origin was the main source of ingested carbon throughout the year. Several studies have shown the importance of the origin of detritus for macroconsumers (Tenore et al. 1982, Crosby et al. 1989, Charles et al. 1996). In contrast with fecal pellets and vascular plant detritus, much of the potential energy from seaweed detritus is readily available (i.e. assimilable) to macroconsumers without the degradation of microbial organisms. Thus, although assimilation rates in ascidians have been shown to be much higher for phytoplankton cells (90 %, Fiala-Médioni 1973; 75 %, Klumpp 1984) than

for organic carbon of detrital origin (42 %, Klumpp 1984), using the assimilation rates from Klumpp (1984) for both carbon sources, the annual mean organic carbon from detrital origin assimilated by *H. papillosa* was an order of magnitude higher than that obtained from live carbon (detrital origin: 714 mg C g AFDW^{-1} h^{-1}, live carbon: 21 mg C g AFDW^{-1} h^{-1}). Nevertheless, because of its high C:N ratio, the detrital pool is likely to represent a relatively poor nitrogen source for the species in comparison with that from the live carbon (Tenore et al 1982, Seiderer and Newell 1985). In this sense, the annual mean consumption of nitrogen from live particles ingested by *H. papillosa* was of the same order of magnitude as that obtained from detrital particles (live particles: 10 ± 2 SE mg N g AFDW^{-1} h^{-1}, detrital particles: 74 ± 29 SE mg N g AFDW^{-1} h^{-1}). Furthermore, in some periods such as fall, the importance of live particles as a source of nitrogen was higher than that of detrital particles. Thus in the feeding ecology of *H. papillosa*, carbon obtained from pico- nano- and microplanktonic organisms may be an essential source of nitrogen and others nutrients necessary for growth and reproduction, especially in some periods of the year.

Annual variation in the growth rate suggests that periods of highest growth correlate with those of highest gonadal development. In order to assess to the qualitative importance of the detrital and live particles, the seasonal pattern of both resources as a source of nitrogen was compared to that of gonadal development (but not to growth due to the wide sampling intervals). The seasonal variation of nitrogen from detrital origin did not significantly correlate with gonadal development during the year (multiple regression analysis, $p = 0.177$). However, the seasonal variation of nitrogen from live particles explained 91 % of gonadal development variance (multiple regression analysis, $p < 0.0001$). These results suggest that live particles are likely to be of more significance in the diet of the species than particles from detrital origin. These results agree with those obtained by Seiderer and Newell (1988) for the ascidian *Pyura stolonifera* in kelp beds. Thus, the main fraction of the particulate material present in the water column appears to be relatively poorly exploited by some ascidian species, which has been suggested to be due to the lack of the necessary digestive enzymes (Seiderer and Newell 1988).

This study has reported the diet and natural grazing rate of *H. papillosa* over an annual cycle, and has shown that growth and reproduction of the species mainly occur during spring and summer. This fact is surprising because seasonality in new production (i.e. growth and reproduction) usually occurs in the periods of higher food availability, and the Western Mediterranean in summer is characterized by oligotrophy and low seston concentration (Zabala and Ballesteros 1989). However, different reasons suggest that food availability does not seem to be limiting. First, it has been recently shown that oligotrophy and low seston during the summer period in the Mediterranean refers mainly detrital POC, but the abundance of live particles remains rather constant through the year (Chapter 2). Because the ingestion of live particles appears to be the main determinant of growth and

reproduction, food availability should not significantly affect the species new production. Furthermore, because food intake of the species was mainly dependent on CR_{AFDW} seasonality (i.e., maximum CR_{AFDW} during spring and summer), our work suggests that other factors (such as larval survival, competition, predation, etc...) rather than food concentration appear to be determining the seasonal dynamic in growth and reproduction of the species.

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5

HETEROGENEOUS FEEDING IN BENTHIC SUSPENSION FEEDEERS: THE NATURAL DIET OF THE TEMPERATE GORGONIAN *PARAMURICEA CLAVATA* AND ITS PREDATION IMPACT ON MICROBIAL COMMUNITIES

I. ABSTRACT

Paramuricea clavata Risso, 1826 (Coelenterata: Octocorallia) is one of the most characteristic members of the benthic Mediterranean fauna. The natural diet and prey capture rate of the species were studied in the field in a western Mediterranean population. *In situ* grazing rates on DOC, detrital POC, pico- nano- and microplankton were examined through a year cycle. *P. clavata* significantly capture nano-eucaryotes, phytoplankton and ciliates as well as detrital POC. In carbon units, the species ingested an annual mean of 0.192 ± 0.175 μg C μg C^{-1} d^{-1} from these food sources. No significant capture of DOC and picoplankton (heterotrophic bacteria, *Prochlorococcus*, *Synechococcus* and picoeucaryotes) was observed. Carbon from detrital origin accounted for 86 ± 14 % of the total ingested carbon and showed a marked seasonal pattern in which winter and spring were the seasons with the highest values of food intake. Ingestion of the examined live carbon also exhibited a marked seasonal pattern with the highest values occurring in winter. The results have shown that *P. clavata* has a broad and heterogeneous diet that range from 3.8 μm (nanoeucaryotes) to 700 μm (copepods). The seasonal pattern of prey capture of detrital and live POC showed minimum values during the summer period, which support the hypothesis that a trophic-energetic phenomenon may be underlying the summer regression in activity of the species. It was calculated that the species daily captured between 1-22 % of diatoms, 1-9 % of nanoeucaryotes, 1-26 % of dinoflagellates, and 2-99 % of ciliates per m^3 of the water adjacent to the bottom. The estimated grazing rate of the species suggest that, in littoral ecosystems, predation effect of macroinvertebrates on microorganisms can not be disregarded.

II. INTRODUCTION

Microbial communities are the main contributor to pelagic planktonic communities in terms of biomass (Stoeckner and Antia 1986) and production (Platt et al. 1983, Burkill et al. 1993). As a consequence of this relevance, an important amount of research has been conducted to study the dynamic of these planktonic communities in the water column and their trophic interactions with other groups of plankters (Azam et al. 1983, Sherr and Sherr 1991, Van Wambeke et al. 1996). In littoral ecosystems, little work have been carried out about the trophic interactions between microbial communities and benthic macroinvertebrates. However, recent studies have shown that the role of bacteria, protozoa and phytoplankton in the diet of benthic suspension feeders other than bivalves appears to be higher than previously thought (Fabricius et al. 1995, Pile et al. 1996, Pile et al. 1997).

Earlier studies about feeding by suspension feeders distinguished between those that captured small particles (i.e. microphagous) and those that captured zooplankton (i.e. macrophagous) (Jorgensen 1975, Fauchald and Jumars 1979). However, it has been shown that species with the former strategy capture a variety of particulate matter that range from detritus to small organisms such as bacteria (Reiswig 1975, Leonard 1989, Gaino et al. 1994). It has also been shown that species which present the zooplankton feeding strategy can capture and assimilate a wide range of prey types that range from bacteria to phyto- and zoo-plankton (Coffroth 1984, Coma et al. 1994, Fabricius et al. 1995), suggesting that, in general, the diet of benthic suspension feeders may be broader then previously though.

Anthozoans are among the more conspicuous components in littoral benthic communities in tropical and temperate areas (e.g., True 1970, Loya 1972). Feeding studies providing natural prey capture rates are available for an small number of hexacorallian and octocorallian species (e.g., Porter 1974, Lewis 1982, Johnson and Sebens 1993, Coma et al. 1994, Sebens et al. 1996). But, these studies have mainly been focused on zooplankton as the principal prey item. Gorgonians are an special case because feeding studies have rarely shown the capture of prey items (Kinzie 1973, Lasker 1981, Lasker et al. 1983). Then, alternative food sources such as suspended particulate matter (Lasker 1981), mucus produced by corals (Coffroth 1984), dissolved organic matter (Murdock 1978), and microplankton (Annexe I) have been proposed. In fact, significant capture of naturally occurring zooplankton prey *in situ* have only been shown in the Mediterranean species *Paramuricea clavata* Risso (Coma et al. 1994). But, even in this species, a recent study about its energetic requirements has shown that the capture of zooplankton can only account for about 50 % of its energetic requirements, suggesting that also in this species other food sources should be explored (Coma et al. 1998).

The gorgonian *Paramuricea clavata* is one of the most characteristics members of the benthic Mediterranean fauna, presenting the highest biomass (True 1970), and

structural complexity (Gili and Ballesteros 1991) of some sublittoral communities. Several previous studies have shown a marked seasonal pattern in the new production of the species along the year which is characterized by the low activity during the summer period (i.e., non reproductive investment, Coma et al. 1995a,b; low growth investment, Coma et al. in press; higher percentage of colonies with contracted polyps, Coma et al. 1994). In a previous study we suggested that the low ingestion during the summer may underlie the low activity of the species during this time period (Coma et al. 1998). However, because the zooplankton ingestion could not fulfill the energy requirements of the species, the exploration of other food sources became a crucial point in order to support or reject the hypothesis.

The lack of prey capture observed by some feeding studies (e.g., Kinzie 1973, Lasker 1981) might have been an artifact due to the methodology (i.e., examination of gut contents), which underestimates small soft-bodied prey because they leave no recognizable remains. Therefore, in this study, we expand our observations about the feeding of *Paramuricea clavata* by examining the grazing rate of the species on DOC, detrital POC, pico-, nano-, and microplakton through *in situ* incubation chambers. We focused on four main goals: (1) what sestonic particles the species feed on?, (2) what is the grazing rate upon these particles?, (3) is there seasonal variation in grazing rate?, and (4) what is the effect of the species predation on the plankton community?

III. METHODS

Feeding experiments were carried out in a station located at the Medes Islands Marine Reserve (NW Mediterranean Sea, 42° 3' N, 3° 13' E) from October 1995 to November 1996. Incubations were conduced in hemispherical U.V.- transparent Plexiglas chambers approximately 3L in volume (see Chapter 1). Whole *Paramuricea clavata* specimens between 12-15 cm in colony height, were removed from their substrate and cleaned from macroepibionts. Removed specimens were attached to PVC posts. These colonies were kept in their natural environment and with conspecifics until used in incubation experiments performed *in situ*. At the beginning of each experiment, a *P. clavata* colony on a PVC post was placed on the base of the experimental chamber. Colonies were allowed to fully expand before the experiment started. For sampling procedure and samples preservation and analysis see Chapter 1.

Paramuricea clavata colonies were rinsed to remove any salts and any associated macrofauna in order to determine their weight. Dry weight was determined by drying at 90 °C for 24 hours. In order to determine ash free dry weight (AFDW), tissue and axis of the colonies were separated. Then, the combustion was separately performed for both tissue and axis at 450 °C for 5 hours.

Multiple regression analysis was used to establish the percentage of the variance of the estimated filtration rates that could be explained by the independent factors which were controlled during each experiment: water temperature (°C, recorded using a WTW oxygen electrode model EOT 196), food concentration (mg C l^{-1}), and colony size (g AFDW). A backward stepwise procedure was used to exclude variables not relevant (Sokal and Rohlf 1981). Variables were square root transformed when normality (Kolmogorov-Smirnov test) and/or the heterosdacity (Levene's test) requirements were not fulfilled.

Daily ingestion rates were estimated based on the ingestion rates calculated from the feeding experiments and corrected by the activity rhythm (i.e., mean percentage of hours that the colonies have the polyps expanded) during each month along the year (Coma et al. 1994 and unpublished data). Ingestion have been expressed in terms of number of cells per polyp and time and in terms of carbon as a proportion of organic carbon weight of tissue (devoid of spicules and skeletal axis) and time.

IV. RESULTS

All sources of particulate organic carbon < 100 μm, live (i.e. recognizable cells), detrital and DOC were considered in this study as potential food sources. Live carbon included: procaryotes, pico- and nanoeucaryotes, phytoplankton (diatoms and dinoflagellates) and ciliates. Fig. 5.1 shows the comparison between growth rates in the control and the experimental chambers for the different food sources. *Paramuricea clavata* significantly captured nanoeucaryotes, diatoms, dinoflagellates and ciliates as well as detrital particulate organic carbon (detrital POC) (Fig. 5.1). No significant decrease in prey items smaller than nanoeucaryotes (mean ± SE: 3.8 ± 1.4 μm) was observed.

Mean seasonal specific clearance rates (CR_{AFDW}, ml swept clear g $AFDW^{-1}$ h^{-1}) for each prey type are showed in Figure 5.2. There was significant differences in the CR_{AFDW} between prey types (Table 5.1). These differences were due to the fact that the species did not significantly captured prey items smaller than nanoeucaryotes (see above). However, CR_{AFDW} did not varied between nanoeucaryotes, phytoplankton and ciliates (Scheffé's Post-hoc test, $p>0.05$). CR_{AFDW} significantly varied among seasons (Table 5.1), due to the high CR_{AFDW} values during winter (Scheffé's Post-hoc test, $p<0.005$). The effects of water temperature, food concentration and colony size on CR_{AFDW} over the year was tested. Food concentration (mg C l^{-1}) variation along the year explained 50 % of the CR_{AFDW} variance (multiple regression analysis, $n=20$, $r^2=0.50$, $p<0.001$) and showed a marked pattern of CR_{AFDW} increase with food concentration increase (Fig. 5.3). Neither temperature or colony size did explain any significant amount of the CR_{AFDW} variance through the year (multiple regression analysis, $p>0.5$ for both variables).

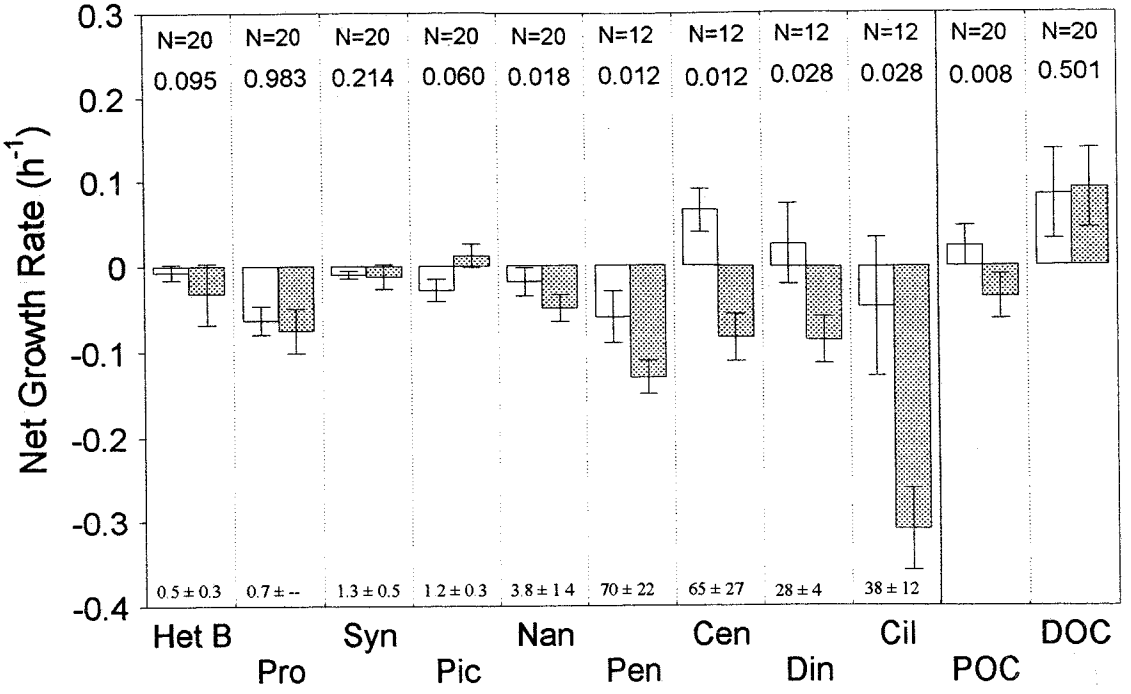

Fig. 5.1. Net growth rates of prey (mean ± SE) at the gorgonian chamber (in dotted bars) and in the control chamber (in empty bars) for each plankton group. Mean maximum length (μm) of each group (mean ± SE) at the figure bottom. Het B - heterotrophic bacteria, Pro - *Prochlorococcus sp*, Syn - *Synechococcus sp*, Pic - autotrophic picoeucaryotes, Nan - autotrophic nanoeucaryotes, Pen - pennate diatoms, Cen - centric diatoms, Din - dinoflagellates, Cil - ciliates, POC - detrital particulate organic carbon, DOC - dissolved organic carbon. The number of experiments (N) and the significance degree from two-tailed Wilcoxon test are also shown.

The seasonal variation of nano- and microplankton ingestion rate by the species is presented in Table 5.2. All year long, the highest ingestion rate in number of cells was for nanoeucaryotes. However, in biomass terms, the large size cells of phytoplankton accounted for the greatest contribution to the ingesta. The highest values of ingesta were observed during winter (Table 5.2), mainly due to the high biomass values of phytoplankton during this time period (Chapter 2).

The CR_{AFDW} was always estimated for colonies with expanded polyps. However, the species exhibits a pattern of expansion and contraction that seems to have a seasonal pattern (Coma et al. 1994, and unpublished data). Then, in order to calculate the annual amount of carbon ingested from predation on nanoeucaryotes, dinoflagellates, diatoms and ciliates, three factors were considered. First, the seasonal variation observed in CR_{AFDW} of the species along the year (which have been observed to be related to prey concentration, Fig. 5.3). Second, the monthly mean prey concentration value for each prey type along the

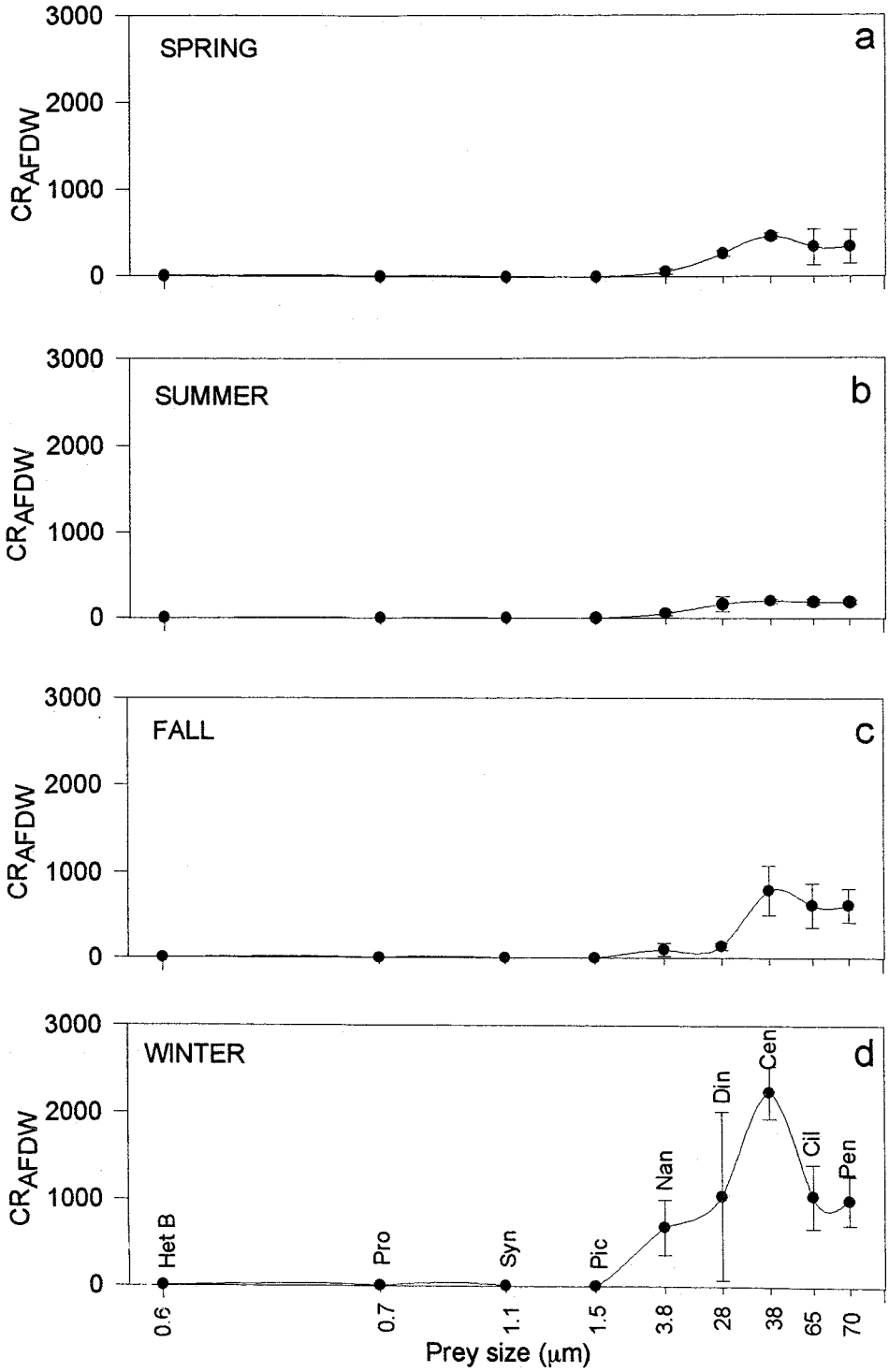

Fig. 5.2. Seasonal specific clearance rate (CR_{ADFW}; ml swept clear g $AFDW^{-1}$ h^{-1}, mean ± SE) of *Paramuricea clavata* as a function of the prey size (µm). Prey size in log scale. Abbreviations as in figure 5.1.

Table 5.1. Analysis of variance for *Paramuricea clavata* specific clearance rate between captured prey types (i.e. Heterotrophic bacteria, *Synechococcus*, *Prochlorococcus*, pico and nanoeucaryotes, diatoms, dinoflagellates, ciliates and detritus) and among seasons.
df: degrees of freedom, SS: sums of squares, p: probability.

Source	df	SS	MS	F	p
Prey type	8	6904989	863123	5.7	< 0.0001
Season	3	51435442	1714514	11.32	< 0.0001
Interaction	24	6883629	336952	1.89	0.015
Error	96	14540300	151461		

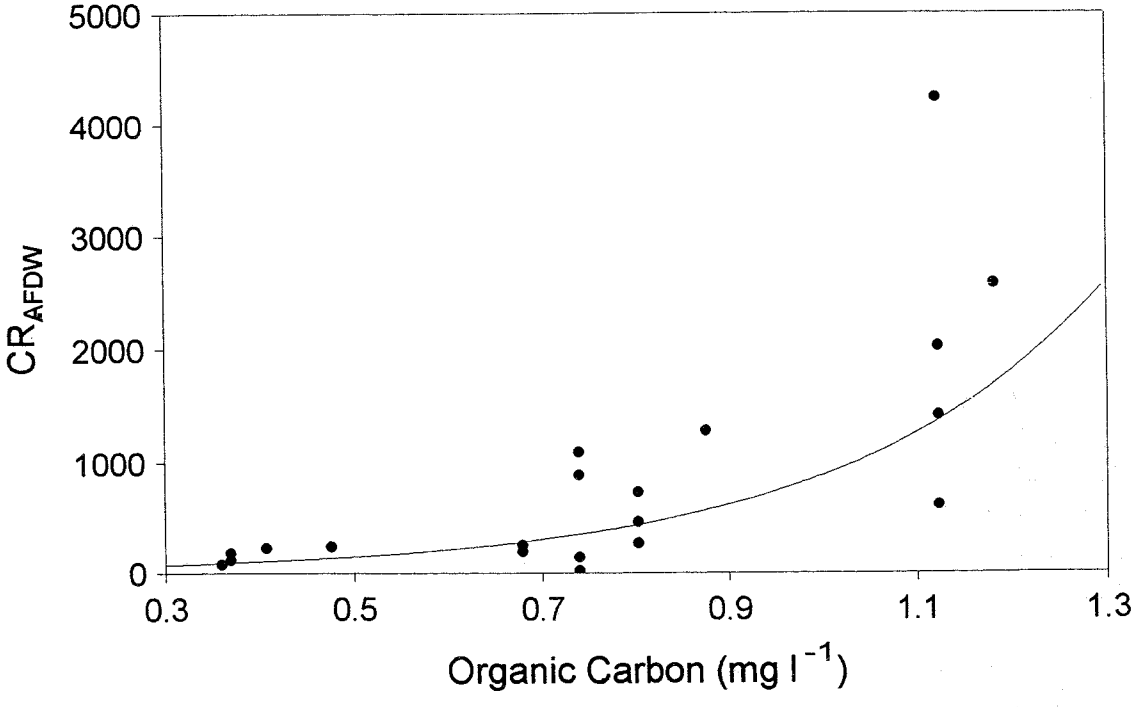

Fig. 5.3. Relationship between *Paramuricea clavata* specific clearance rate (CR_{ADFW} ; ml swept clear g $AFDW^{-1}$ h^{-1}) and total organic Carbon.

year (data from Chapter 2). This two factors allowed an estimation of the monthly number of cells ingested per polyp and hour. The third factor was an estimation of the variation through the year of the number of hours with expanded polyps (activity rhythm; data from Coma et al. 1994 and unpublished data), which allowed the calculation of the daily ingestion rate through the year. These calculations are presented in Table 5.3. According to these estimations, the species ingested between 31-794 cells $polyp^{-1}$ d^{-1} (mean ± SD, 276 ± 225) from feeding on nanoeucaryotes, dinoflagellates, diatoms and ciliates depending on the season.

Table 5.2. Seasonal variation of nano- and microplankton ingestion rate (mean ± SD) by *Paramuricea clavata*. Data expressed in number of cells and carbon units.

Prey	Summer	Spring	Fall	Winter
		Cells $polyp^{-1}$ h^{-1}		
Nanoeucaryotes	29 ± 14	26 ± 12	19 ±15	43 ± 33
Diatoms	0.1 ± 0.04	3 ± 1	0.04 ± 0.02	1 ± 0.4
Dinoflagellates	0.2 ± 0.1	1 ± 0.1	0.2 ± 0.04	0.3 ± 0.2
Ciliates	0.04 ± 0.02	0.1 ± 0.04	0.1 ± 0.04	0.3 ± 0.1
		pgC µg C $tissue^{-1}$ h^{-1}		
Nanoeucaryotes	214 ± 104	234 ± 114	276 ± 230	829 ± 464
Diatoms	117 ± 65	225 ± 113	362 ± 115	1377 ± 688
Dinoflagellates	63 ± 33	56 ± 25	39 ± 15	33 ± 27
Ciliates	221 ± 115	207 ± 98	210 ± 96	195 ± 115

In terms of carbon, *P. clavata* ingested an annual (mean ± SD) of 0.192 ± 0.175 µg C µg C^{-1} d^{-1} from the particles < 100 µm (Fig. 5.4a). Carbon from detrital origin accounted for 86 ± 14 % of the total ingested carbon and showed a marked seasonal pattern in which winter and spring were the seasons with the highest values of ingesta (Fig. 5.4a). Ingestion of live carbon also exhibited a marked seasonal pattern with the highest values occurring during winter (Fig. 5.4b). Phytoplankton (diatoms and dinoflagellates) was the main contributor to the live particles diet accounting for over 48 ± 6 % of the total ingested live carbon.

The contribution of carbon ingested from detrital POC, nanoeucaryotes, phytoplankton, and ciliates observed in this study was compared to that of zooplankton prey items previously reported (Coma et al. 1994). Then, it can be observed that, in carbon

Table 5.3. *Paramuricea clavata* daily capture rates estimated from the ingestion rate and the rhythm of activity. Dia: diatoms, h d^{-1}: daily number of hours with expanded polyps. The other abbreviations as in figure 5.1.

		Cel pol^{-1} h^{-1}			Rhythm		Cel pol^{-1} d^{-1}			
Month	Nk	Dia	Din	Cil	h d^{-1}	Nk	Dia	Din	Cil	Total
Dec	78	0.49	0.29	0.30	10	783	4.88	2.86	2.99	794
Jan	38	0.59	0.43	0.32	8.2	314	4.83	3.56	2.60	325
Feb	33	1.16	0.44	0.60	9.4	312	10.91	4.15	5.63	332
Mar	13	1.51	0.23	0.04	8.9	119	13.45	2.03	0.33	135
Apr	22	0.84	0.21	0.04	8.9	198	7.43	1.85	0.40	208
May	62	1.18	0.41	0.04	7.4	459	8.73	3.05	0.31	471
Jun	55	1.33	0.19	0.02	8.8	482	11.70	1.64	0.20	495
Jul	18	0.02	0.14	0.03	1.7	31	0.04	0.23	0.05	31
Aug	21	0.01	0.10	0.01	3.1	65	0.03	0.31	0.04	65
Sep	20	0.04	0.09	0.10	7.7	156	0.33	0.66	0.74	158
Oct	20	0.04	0.08	0.07	8.6	175	0.35	0.70	0.58	176
Nov	21	0.00	0.06	0.05	6	124	0.03	0.35	0.31	125
Mean						268	5.22	1.78	1.18	276
SD						217	5.12	1.37	1.71	225

units, ingestion of detrital POC was about half of that of the zooplankton (46 ± 39 %; mean ± SD; Table 5.4). As previously observed, the contribution of detrital POC exhibited a marked seasonal pattern with the highest values observed during winter and spring, when it accounted for 73 ± 28 % of the zooplankton ingestion. The ingestion of nanoeucaryotes, phytoplankton, and ciliates accounted for a mean value of 2 ± 2 % of the zooplankton ingestion

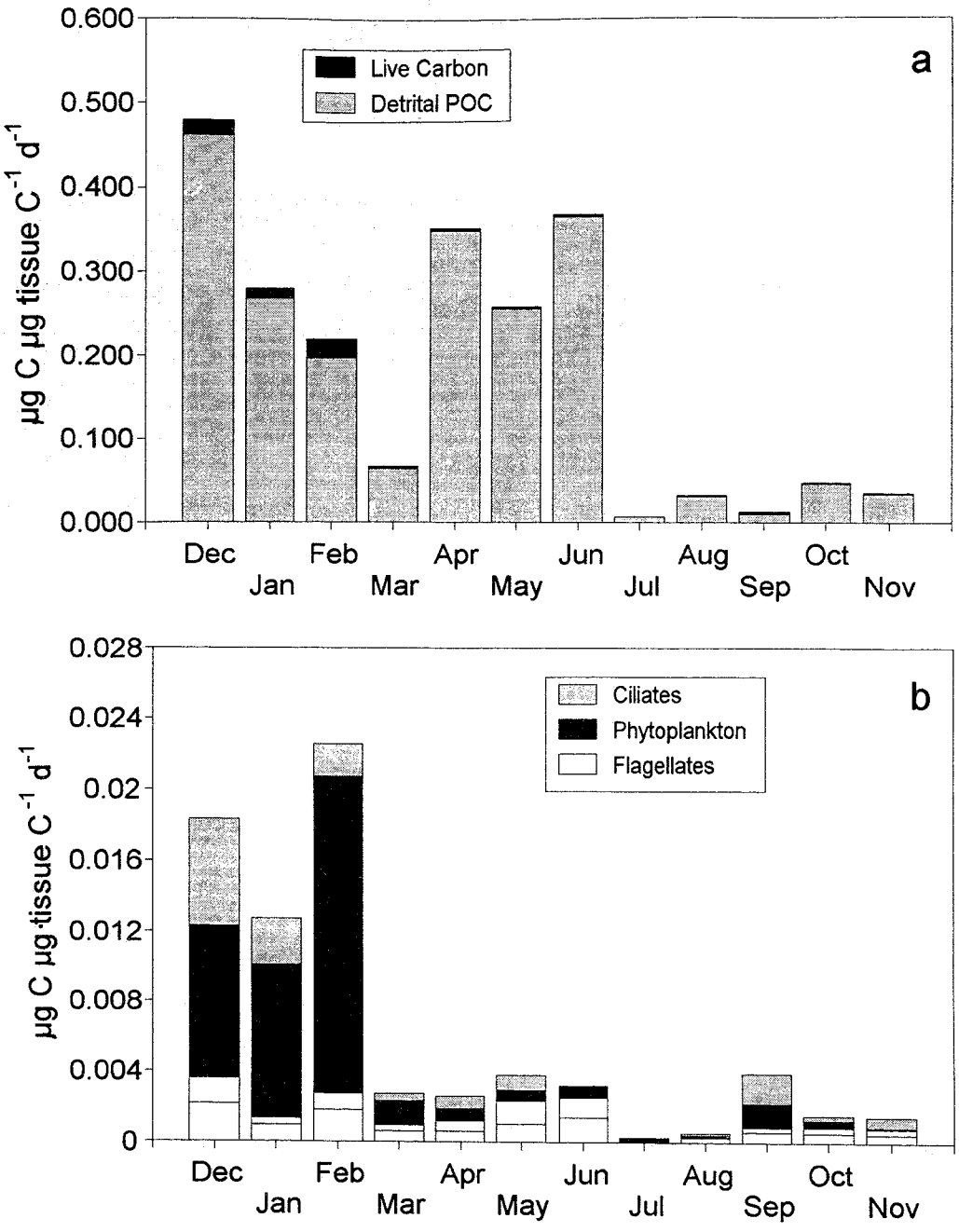

Fig 5.4 Monthly estimations of the total ingested organic carbon (µg C µg tissue C^{-1} h^{-1}) by *Paramuricea clavata* over an annual cycle. a) Total amount divided in the two main food sources: live carbon and carbon from detrital origin. b) Composition of the ingested live carbon. Flagellates include autotrophic and heterotrophic nanoeucaryotes and phytoplankton include diatoms and dinoflagellates.

Table 5.4. Monthly variation of *Paramuricea clavata* capture rates (μg C $polyp^{-1}$ d^{-1}) x 100 of particles <100 μ (live and detritus), and zooplankton (data from Coma et al. 1994). Det: detritus, Zoop: zooplankton.

Month	Live < 100 μ	Detritus < 100 μ	Zooplankton	% Det/Zoop	% Live/Zoop
Dec	1.84	48.1	50	97	4
Jan	1.27	28.1	29	98	4
Feb	2.25	21.8	37	59	6
Mar	0.28	6.6	19	35	1
Apr	0.26	35.2	44	80	1
May	0.38	25.8	62	41	1
Jun	0.32	37.0	38	98	1
Jul	0.03	0.6	25	2	0
Aug	0.06	3.1	30	10	0
Sep	0.39	1.3	26	5	2
Oct	0.15	4.6	29	16	1
Nov	0.15	3.4	63	5	0
Mean	0.62	18.0	38	46	2
SD	0.75	16.7	14	39	2

V. DISCUSSION

Natural diet and feeding mechanisms

The study, carried out seasonally over an annual cycle, has covered the entire natural range of potential prey items but zooplankton, which included DOC, detrital POC, pico-, nano-, and microplankton. The results have shown that the species regularly feeds on detrital POC, nanoeucaryotes, phytoplankton and ciliates, but not on items of size smaller than 3 μm, neither on DOC. In a previous study we observed that nauplii, copepod eggs, other invertebrate larvae, and other prey items between 100 and 200 μm account for the bulk of the zooplankton prey captured by the species, although adult copepods up to 600-700 μm were also captured (Coma et al. 1994). Therefore, the range of prey items captured by *Paramuricea clavata* appears to be between 3.8 μm (nanoeucaryotes) and 700 μm (adult copepods). This broad size range of food sources is similar to that recently obtained through both gut contents and *in situ* feeding experiments on tropical gorgonians

Table 5.5. *Paramuricea clavata.* Estimated size and variability of the ecological predatory impact of a 1 m^2 population (at 32 colonies m-2) in number of cells and in carbon units. Percentage (%) of cleared cells estimated using cells abundances from Chapter 2. Abbreviations as in figure 5.1.

	Ingestion Cells m^{-2} d^{-1}				% Cleared Cells				Ingestion µg C m^{-2} d^{-1}			
Month	Nan	Dia	Din	Cil	Nan	Dia	Din	Cil	Nan	Dia	Din	Cil
Dec	1,25E+08	7,80E+05	4,57E+05	4,79E+05	9	22	26	99	594	5907	15	1195
Jan	5,02E+14	7,72E+05	5,69E+05	4,16E+05	7	18	21	85	238	5842	19	1039
Feb	4,99E+13	1,74E+06	6,63E+05	9,00E+05	9	20	24	98	236	13202	22	2247
Mar	1,91E+14	2,15E+06	3,24E+05	5,33E+04	3	10	13	22	114	121	18	12
Apr	3,17E+13	1,19E+06	2,95E+05	6,33E+04	3	10	13	22	190	67	16	15
May	7,34E+13	1,40E+06	4,88E+05	4,89E+04	2	9	11	18	440	79	27	11
Jun	7,70E+14	1,87E+06	2,62E+05	3,26E+04	3	7	7	11	499	243	18	156
Jul	4,93E+14	6,42E+03	3,74E+04	8,53E+03	1	1	1	2	32	1	3	41
Aug	1,03E+14	4,72E+03	5,01E+04	6,20E+03	1	3	3	4	67	1	4	30
Sep	2,49E+14	5,23E+04	1,05E+05	1,19E+05	2	10	3	19	143	21	5	69
Oct	2,79E+12	5,52E+04	1,11E+05	9,25E+04	3	11	4	22	160	22	5	53
Nov	1,98E+14	4,18E+03	5,65E+04	4,90E+04	2	8	3	15	114	2	2	28
				Mean	4	11	11	35	236	2126	13	408
				SD	3	6	9	37	180	4147	9	713
				Max	9	22	26	99	594	13202	27	2247
				Min	1	1	1	2	32	1	2	11

which have been observed to capture, although at low rates, from prey items larger than about 5 μm to zooplankton prey items about 700 μm (Annexe I). The results are also consistent with previous gut contents studies (e.g., Leversee 1976, Lasker 1981, Lasker et al. 1983) as well as with feeding experiments carried out in the laboratory (Sorokin 1991), suggesting that detrital POC, nano-, and microplankton may constitute a regular feeding source for many gorgonian species. In general, benthic suspension feeders face a wide spectrum of potential prey that include DOC, detritical POC and live POC and, even those cnidarian species that appear to rely almost exclusively on zooplankton exhibit a important diet variety (Sebens and Koehl 1984, Barangé and Gili 1988). Although, in some species a single prey type can provide the energetic requirements (Klumpp 1984, Asmus and Asmus 1991), it appears that wide and heterogeneous diets are the most common feeding strategy in littoral benthic suspension feeders (e.g., Stuart and Klumpp 1984, Coma al. 1995c, Chapter 3 and 4).

The capture of a wide spectrum of prey items by *P. clavata* appears to be mainly carried out through tentacular feeding by using some of the capture mechanisms described by the aerosol filtration theory (Rubenstein and Koehl 1977). As in scleractinian corals (Sebens et al. 1996), the mechanism of direct interception is likely to be the main mode of nano- and microplankton capture. But, aside from the aerosol filtration theory, the capture of small organisms and particles is also facilitated by a morphologic characteristic of the octocorals: the siphonoglyph. The siphonopglyph drives a constant water current through the gastrovascular cavity mainly for respiratory and excretory reasons (Hyman 1940), but it also allows particles to come in and out of the gastrovascular cavity (Grasse 1987). As suggested by Pratt (1905), the constant flow through the gastrovascular cavity may facilitate the capture of microorganisms by the mesenterial filaments. The ingestion of mucus is another mechanism of capture of suspended detrital and live particles (Coffroth 1984) which, in areas where an important number of species produce mucus such as in tropical areas, may even allow to capture a significant amount of organisms of size smaller than 3 μm such as bacteria.

Heterogeneous diet from an optimality approach

It has long been postulated that most suspension feeders are non-selective (Jørgensen 1966), and that capture items will be mainly limited due to structural constraints (Rubenstein and Koehl 1977). Then, within morphological limitations, the composition of the ingested material should be similar to that of suspended material in the surrounding water. However, because most studies have been carried out with restricted or artificial diets (e.g. Leversee 1976, Lasker 1981), or through gut contents analysis which potentially underestimates small soft-bodied organisms because they leave no recognizable remains (e.g. Coma et al. 1994), they do not reveal which organic matter fractions are been used as food sources. The results of the present study together with those from the previous gut

content analysis of the species (Coma et al. 1994) have shown that the diet of the gorgonian *Paramuricea clavata* is highly heterogeneous and that the species ingest particles and organisms ranging in size from 3 to 700 μm. The broad and varied diet observed in this species is consistent with past and present studies on this (Sorokin 1991, Annexe I) and on other suspension feeders macroinvertebrates groups such as sponges (Reiswig 1971, Chapter 3), ascidians (Randlov and Riisgard 1979, Klumpp 1984, Chapter 4), hydrozoans (Coma et al. 1995c, Gili et al. 1996).

Benthic suspension feeders live in an environment where resources are heterogeneous and stochastic where potential preys are diluted and patchy distributed. Actually, the optimal foraging theory developed for plankton organisms (Lehmann 1976) has experienced a comeback with its application to benthic organisms and in particular to suspension feeders (Hughes 1980, Okamura 1990). From an optimality approach, an important strategy of benthic suspension feeders is that they invest little effort in food capture. The cost is virtually nil in passive suspension feeders while pumping in active suspension feeders may account for up to 4 % of the energy demand (Riisgård and Larsen 1995). Then, the persistence of suspension feeders depend on the constancy of a moderate hydrodynamic conditions and bursts of pelagic production in the area. In the framework of the optimal foraging theory (Stephens and Krebs 1986), species that expend low levels of energy in foraging are considered as ecologically highly successful (Hughes 1979).

In an heterogeneous and stochastic environment and from an optimality point of view, mobile consumers solve the problem to locate food patches by moving around and some have the capacity to change prey type by changing the capture technique (Akre and Johnson 1979) which maximize the profitability of the search and capture activities (Stephens and Krebs 1986). However, sessile consumers, represented by a large amount of benthic invertebrates, have to develop a completely different approach to survive and success in the same environment. Some sessile suspension feeders also have the capacity to alternate modes or feeding techniques (see Okamura 1990 for review). However, benthic suspension feeders such as gorgonians, corals, hydrozoans, ... have a highly reduced capacity to modify its feeding technique, although the percentage of success of the different mechanisms of the aerosol filtration theory may change as a function of the hydrodynamic regime (e.g. LaBarbera, 1984). The heterogeneity in the diet of benthic suspension feeders allows them to opportunistically exploit a wide range of resources which are highly variable both in space and time. In this sense, as it has been pointed out by Okamura (1990), the foraging strategy followed by sessile consumers appears to represent a different approach to that described by mobile consumers more traditionally studied such as the zooplankton (DeMott 1990). But, even in zooplankton organisms for which an important selectivity capacity have been observed (e.g., Starkweather and Bogdan 1980, DeMott 1989, Huntley et al. 1986), analysis of gut contents and fecal pellets from natural populations of several groups such as copepods and salps provide evidence of rather broad diets (Turner 1984,

Hart 1987, Purcell and Madin 1991). The heterogeneity observed in the diet of several benthic suspension feeder species from different taxa may represent one of the most important characteristics that have allowed the success of the suspension feeding in benthic invertebrates.

Grazing rate and predation impact on the microbial community

Mean annual capture rates on phytoplankton estimated for *P. clavata* (5 diatoms polyp^{-1} d^{-1}) was two times higher than that estimated for the tropical gorgonian *Plexaura flexuosa* (2 diatoms polyp^{-1} d^{-1}) using the same methodology (Annexe I). Moreover, during winter and spring months, *P. clavata* ingested a number of diatoms up to five times that of *P. flexuosa*. The total number of cells of nano- and microplankton captured by *P. clavata* (276 cells polyp^{-1} d^{-1}) was two orders of magnitude higher than the estimated for *P. flexuosa* (7.2 cells polyp^{-1} d^{-1}). Furthermore, we previously showed that the capture rate on zooplankton of *P. clavata* was also an order of magnitude higher than that of tropical gorgonians (Annexe I). The higher prey capture on microplankton appears to be reflecting the same trend previously observed in zooplankton capture rate. As pointed out in the latter study, the higher heterotrophic activity of the Mediterranean species appears to be related with the fact that it is exclusively heterotrophic while the tropical species have symbiotic zooxanthellae, as do most tropical shallow water gorgonians (Johannes et al 1970, McCloskey and Muscatine 1984).

Our results have pointed out that *P. clavata* captured an important amount of POC from detrital origin. The importance of detrital POC as a food source for benthic organisms have long been recognized (e.g., Tenore et al. 1982). Evidences of detrital POC utilization have been usually been reported for active suspension feeders (e.g., Charles et al. 1996). In cnidarians, there have been few direct evidences of detrital POC utilization (Coma et al. 1995c). However, the results of experimental studies carried out with artemia cysts o sephadex beads (e.g., Leversee 1976, Lasker 1981, Lasker et al. 1983) showed that gorgonians are able to capture non-motile prey items and to incorporate POC (Coffroth 1984), therefore suggesting the potential utilization of detrital POC in nature. Then, our results together with those experimental studies previously carried out in different gorgonian species, suggest that the capture of detrital POC might be a common feature among gorgonians species. Nevertheless, it is difficult to quantify the importance of detrital POC as a food source (Tenore et al. 1982). At the study site, there is a broad change in quality of the detrital POC over the annual cycle with the C/N ratio ranging from 18 during spring to 4 during summer and fall (Chapter 2). Then, because the highest capture of detrital POC occurs during winter and spring (Fig. 5.4a) and because the assimilation efficiency of detrital POC is likely to be much lower than that of nano-, micro-, and zooplankton, the importance of detrital POC appears to be mainly as a source of carbon.

The results obtained in the present study have shown a marked seasonality in the prey capture. The highest detrital POC capture rates occurred during winter and spring while the highest values of live particles uptake were observed during the winter period. This seasonal variation on capture rate of both prey types was mainly depended on the abundance of food concentration on the water column. This pattern is similar to that previously observed with the capture of zooplankton prey items (Coma et al. 1994). In a previous study we suggested that the low ingesta during the summer may underlie the low activity of the species during this time period (Coma et al. 1998). The results support the hypothesis that a trophic-energetic phenomenon may be underlying the summer regression in activity of the species because the seasonal pattern of prey capture of detrital and live POC also showed minimum values during the summer period.

Density of *P. clavata* at the study site was 32 colonies m^{-2}, with a mean height of 30 cm (study area: 10 m^2). Coma et al. (1994) estimated a total of 159,916 polyps per square meter for the area, based on the colony density, polyps density and demographic structure of the *P. clavata* population (Coma 1994). At the estimated capture rates, *P. clavata* polyps captured between 4 - 1870 10^3 diatoms, 5 - 125 10^6 nanoeucaryotes, 4 - 70 10^4 dinoflagellates, and 6 - 900 10^3 ciliates per m^3 and day depending on the time period along the year (Table 5.5). Overall, this capture was the equivalent of removing between 76 and 15708 (mean ± SD, 2782 ± 4915) µg C per m^2 per day. Ambient concentrations of these microorganisms along the year cycle are presented in Table 5.5 (data from Chapter 2). This predation implies that the *P. clavata* population is daily removing between 1 - 22 % of diatoms, 1 - 9 % of nanoeucaryotes, 1 - 26 % of the dinoflagellates, and 2 - 99 % of ciliates per m^3 of the water adjacent to the bottom.

The capacity of gorgonians to feed on microorganisms has also been shown in several other species (Sorokin 1991, Annexe I). Therefore, because gorgonians are among the more conspicuous components in littoral benthic communities in tropical (Bayer 1961, Tursch and Tursch 1982) and in temperate seas (Pérès and Picard 1964, Gili and Ros 1985), the estimated impact of *P. clavata* and likely of other gorgonian species, on the microbial assemblages appears to not be negligible. Furthermore, several recent studies on different macroinvertebrates groups such as sponges (Pile et al. 1996, Pile et al. 1997), ascidians (Klumpp 1984, Riisgård et al. 1996a), polichaetes (Riisgård et al. 1996b, Vedel 1998) and hydroids (Barange and Gili 1988, Coma et al. 1995c) are also showing an important predation impact on microorganisms. Then, in littoral ecosystems, the predation effect of macroinvertebrates on microorganisms can not be disregarded.

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6

RESPIRATION AND ENERGY SURPLUS OF THREE SPECIES OF BENTHIC SUSPENSION FEEDERS: ARE THERE ADAPTIVE PATTERNS TO A TROPHIC SEASONAL CRISIS ?

II. INTRODUCTION

Studies on the dynamic of Mediterranean suspension feeders show that both reproduction and growth usually exhibit a marked seasonal pattern and, this two processes may coincide over some periods (Llobet et al 1991, Becerro and Turon 1992, Coma et al 1998). Some of these studies have hypothesized that food availability could be the main reason for the seasonal dynamic of those species. This hypothesis is based on the fact that the western Mediterranean is characterized by two peaks of plankton production and biomas during the year related to mesoscale hydrographic structures (Margalef 1969). An autumn peak which develops after the disappearance of the thermocline by wind driven forces, and a late winter-early spring peak that develops after nutrients have been supplied in the water column by upwelling of deep waters, by coastal runoff or by wind forces which mix surface and intermediate water. These nutrient supplies enhances phytoplankton development (Estrada et al 1985). Within this framework, summer months are a period of stratification of the water column and low productivity. The most characteristic feature of the seasonal dynamic of benthic suspension feeders previously cited in the Mediterranean appears to be related with the regression or inactivity of the organisms during the summer period. A form of estivation has been described for some species (e.g. Turon 1992, Coma et al. 1998) comparable to the phenomenon of hibernation described in colder seas (Hughes 1989).

If a trophic-energetic phenomenon would be underlying the regression or inactivity of the organisms during the summer periods, it could be hypothesized that this phenomenon should be reflected by a rough approximation to the bioenergetics of the species. Energetic studies can examine how matter and energy are invested in reproduction, growth and maintenance throughout the year. However, the study of all the parameters of an energy budget is highly laborious, and limit the number of species that can be examined. Then, the estimation of a ratio between the energy gain and the cost of respiratory processes (G/C ratio) may represent an alternative approach to examine whether or not the seasonal dynamic of some Mediterranean species is regulated by trophic constraints. Respiratory processes included basal metabolism or maintenance, cost of somatic and gonads synthesis, and cost of activity (Clarke 1987a).

In general, respiration of an organism is quantified by measuring oxygen consumption per unit of time (Lucas 1996). Focusing the attention on metazoan, oxygen, together with food (including nutrients) and light, can be considered as the fuel that makes their life go. There is a continuous energetic demand for staying alive (i.e. basal metabolism), which in terms of respiration demand it can be considered as an oxygen consumption background more or less affected in poikilotherm organisms by changes in the ambient temperature. During periods of synthesis of somatic and/or gonad tissue, there is an increase in oxygen consumption due to the respiratory costs of synthesis (Clarke 1987a).

The obtention of both sources (oxygen and food) by the marine invertebrates is widely known to be affected by the water temperature. This fact has been explained as the effect of temperature to enzyme catalyzed reactions and also to the rate of supply of metabolites such as oxygen to the tissues (see Clarke 1983 for review). In this sense, Parry (1983) showed that seasonal variations in growth may explain most of the observed seasonal patterns of respiration in marine invertebrates, including some previously interpreted as seasonal acclimation to temperature. Then, it is important to seasonaly carry out the examination of the respiration rate.

In order to examine the above mentioned hypothesis we will seasonaly estimate the Gain/Cost (G/C) ratio for three taxonomically unrelated species (different phylum) that all dwell in the same community: the gorgonian *Paramuricea clavata*, the ascidian *Halocynthia papillosa* and the sponge *Dysidea avara*. The three species are characteristic members of the Mediterranean coraligenous community (after Laubier 1966). An important data set is already available for the gorgonian species (Coma et al. 1994, 1995a, 1995b, in press), for which a preliminary energy budget has been carried out (Coma et al. 1998). This work pointed out that zooplankton prey items present in the stomach contents could not be the only source of food for the species, as it has been recently shown (Chapter 5). Coma and coworkers also showed that respiration appears to share for the largest fraction of the energy demand and that a detailed seasonal respiratory study was necessary to better understand the dynamic of the species.

The aim of this study was to evaluate *in situ* the seasonal respiration rates of three species of benthic suspension feeders (the sponge *Dysidea avara*, the ascidian *Halocynthia papillosa* and the gorgonian *Paramuricea clavata*) in relation to changes in water temperature. Respiration together with feeding rates of the species were used to calculated the variation of the G/C ratio through the year. For those species for which data are available, the G/C ratio will be compared with the variation of the production of the species through the year. In the other cases, we will predict the best time to invest in growth and reproduction. The results are discussed as different life history adaptations to the same environment.

III. METHODS

The study was conducted at the Medes Islands Marine Reserve (NW Mediterranean Sea, 42° 3' N, 3° 13' E, see map in Chapter 1) from October 1995 to November 1996. Specimens of the sponge *Dysidea avara*, the ascidian *Halocynthia papillosa*, and the gorgonian *Paramuricea clavata* were selected to have a similar size (*D. avara*: 1.67 ± 0.34 SD g ash free dry weight (AFDW); *H. papillosa*: 0.51 ± 0.14 SD g AFDW; *P. clavata*: 0.95 ± 0.19 SD g AFDW) in order to avoid a size effect on respiration rate (Lucas 1996).

About a month before the experiments, several specimens from the three species were removed with a piece of substrate, cleaned from macroepibionts, and placed to artificial supports using an inert cement. These specimens were kept in their natural environment with conspecifics. This allowed to highly reduce the disturbance of the incubated specimens. Respiration experiments were conducted in hemispherical U.V.- transparent Plexiglas approximately 3 L in volume. The chamber (one experimental -with organism-, and one control) were placed at 15 m depth and water was recirculated with a pump at a speed of 1.2 cm s^{-1}. At the beginning of each experiment, one specimen was placed on the base of the experimental chamber and both incubation chambers were closed. However, the inlet and outlet apertures of the chambers were not connected, so that the system worked as an open-flow one. After an acclimation period of 1 hour, inlet and outlet apertures were connected and the system started to run as a closed-flow. In both chambers, oxygen concentration was recorded continuously using WTW oxygen electrodes model EOT 196. For more details about the system see Annex II. Respiration was estimated from 24 hours cycles during which oxygen concentration was recorded in both chambers every 2 minutes by a data-logger. In some occasions, the 24 h cycle could not be completed due to bad weather. The water inside both chambers was totally renewed by means of a flushing pump when oxygen concentration changed 20 % from its initial level (Crisp 1984). The control chamber was used to detect oxygen variations not due to the studied specimen. The behavior of the incubated specimens as well as that of the conspecifics was periodically monitored by direct observation.

Every two-three weeks, a daily cycle with different individuals for each species was carried out. Respiration rates (mg O_2 $weight^{-1}$ $hour^{-1}$) were estimated from each 0.4 mg O_2 l^{-1} decrease in oxygen concentration in the organism chamber during each experimental period. *D. avara* dry weight (DW) was determined by drying at 100 °C for 24 hours, and ash free dry weight (AFDW) was determined by combustion at 500 °C for 6 hours. *H. papillosa* and *P. clavata* dry weight was determined by drying at 90 °C for 24 hours, and ash free dry weight by combustion at 450°C for 5 hours.

The oxygen concentration range within which the species behavior (assessed by direct observation) and respiration rate of the species were not affected was assessed throughout several experiments carried out without the flushing pump in which oxygen concentration decreased to about 50 % from the initial values. From these experiments, it was determined that a 20 % decrease in oxygen concentration from the initial concentration did not affect the species behavior, nor respiration rate. For the three species, oxygen consumption through the daily cycle included several renewals of the water inside the chamber. Then, each daily cycle provided a number of pseudoreplications that allowed to examine again whether or not the 20% drop in initial oxygen concentration affected respiration rate (see Fig.6.1 as an example). This was tested by comparing within a daily cycle and for each experiment (pesudoreplicate), the first respiration rate estimate

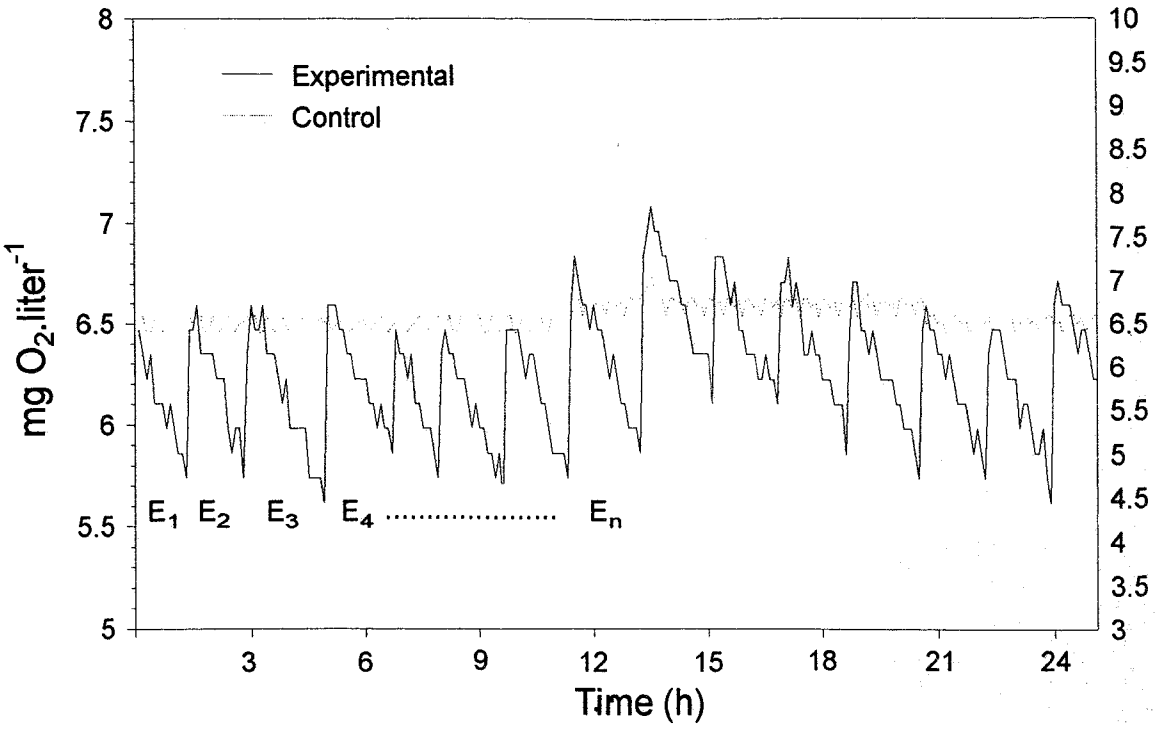

Fig. 6.1. *Dysidea avara*. Oxygen concentration (mg O_2 litter^{-1}) inside the chambers during a daily cycle. Oxygen values were recorded every 2 minutes in both chambers and water inside both chambers were renewed when oxygen concentration changed 15 % from its initial value. Experimental: chamber with sponge. Control: chamber without sponge.

(organism under the initial oxygen concentration) with the last one (organism under almost 20 % drop in oxygen concentration; Fig. 6.1) through a t-student for dependent samples (Sokal and Rohlf 1981).

Variation in respiration rate was examined at three levels: within a daily cycle, between days within a season, and between seasons. Variation within a daily cycle was exanimate for the three species by comparing measurements within pseudoreplication (Fig. 6.1) with those from others experiments within the same daily cycle using a one-way ANOVA. Variation between days within a season and between seasons was tested using data from all daily cycles through a two-way nested ANOVA (days nested in season).

Multiple regression analysis was used to establish the percentage of the variance of the estimated respiration rates through the year that could be explained by the independent factors which were controlled during each experiment: water temperature and organism weight. (Sokal and Rohlf 1981). Because reproductive data for both the ascidian species (Becerro and Turon 1992) and the gorgonian species (Coma et al. 1995a) was available as

monthly means, the relationship between respiration and reproductive investment was examined by using a regression analysis but with the monthly mean values of respiration rates and reproductive investment. Variables were square root transformed when the normality (Kolmogorov-Smirnov test) and/or the heterosdacity (Levene´s test) requirements were not fulfilled (Sokal and Rohlf 1981).

The ratio between energy gain (G: estimated as ingested carbon in μg C g $AFDW^{-1}$ d^{-1} for the sponge and the ascidian species, and in μg C $polyp^{-1}$ d^{-1} for the gorgonian) and the cost of the respiration (C: estimated in the same units than ingestion), hereafter G/C ratio, was calculated to examine the dynamic of the species along the year from an energetically point of view. G/C values above 1 reflect a positive energy surplus, the species can invest in somatic growth, reproduction and/or store energy (reserves). G/C values below 1 reflect a that the species is under an energetically unfavorable period.

Ingestion rate data for each species come from previous studies (Chapters 3, 4 and 5). A respiratory quotient RQ of 0.75 has been used as the mean value reported for several benthic marine invertebrates species (Hatcher 1989). Oxygen consumption (mg O_2 g $AFDW^{-1}$) was expressed in terms of carbon through the oxygen conversion factor 0.281 calculated from the equations described by McCloskey et al (1978). Q_{10} values were estimated by applying the Van´t Hoff's formula:

$$[Q_{10} = (CR_{AFDWt2}/CR_{AFDWt1})/10/t_2\text{-}t_1\text{; Lucas 1996}] \quad (7)$$

because temperatures differed by less than 10 °C.

Ten apical fragments from 10 different *P. clavata* colonies smaller than 10 cm in height (non reproductive young colonies; Coma et al. 1995a) were seasonally collected from the same area to examine lipids content variation of the species tissue along the year. The fragments for biochemical analysis were frozen immediately in liquid nitrogen and stored at -80 °C until analyzed. Lipid content was analyzed following the method of Barnes and Blastock (1973).

IV. RESULTS

Respiration rate was estimated from the decrease in oxygen concentration in the chamber with the organism between renewals, because none of the controls exhibited significant changes in oxygen concentration along the experiments. In none of the three species, respiration rates were significantly affected by the 20 % drop of the initial oxygen concentration (t-student for dependent samples; *Dysidea avara*: t = 1.1139, df = 28, p = 0.265; *Halocynthia papillosa*: t = 0.995, df = 19, p = 0.3322; *Paramuricea clavata*: t = -

0.56, df= 13, p= 0.5829). Because, initial oxygen concentration was always saturated or slightly supersaturated, the 20 % drop of the initial oxygen concentration was always above 80 % saturation.

For all species, respiration rate did not vary significantly vary through the daily cycle (one-way ANOVA, Table 6.1, see Fig. 6.1 as an example). Then, none of the three species exhibited a daily pattern of respiration rate. Respiration rate neither varied among days within months (two-way nested ANOVA, Table 6.2), however, it showed a significant variation between months (Table 6.2).

Table 6.1. Analysis of variance of respiration rates between experiments (slope) in a dial cycle. df: degrees of freedom, SS: sums of squares, MS: mean square, F: F ratio, P: probability.

Specie	df	SS	MS	F	p
D. avara	28	0.001	0.000043	0.489	0.978
error	53	0.005	0.000088		
H. papillosa	21	0.0003	0.000012	1.132	0.361
error	36	0.0004	0.00001		
P. clavata	9	0.00035	0.000039	0.652	0.747
error	52	0.0031	0.000059		

Temperature is one of the most important factors that drive metabolic activity in marine organisms (Kinne 1963). Then, we examined the effects of temperature and size (weight) on respiration rate for all species. It showed that temperature explained a significant amount of variance (multiple regression analysis) for *D. avara* ($r^2 = 0.91$, n = 142, $p < 0.0001$) and for *H. papillosa* ($r^2 = 0.66$, n = 140, $p < 0.0001$) but not for *P. clavata*. In none of the three species, respiration rate varied within the studied range of specimen sizes (*D. avara*: 1.67 ± 0.34 SD g AFDW; *H. papillosa*: 0.51 ± 0.14 SD g AFDW; *P. clavata*: 0.95 ± 0.19 SD g AFDW). Respiration rate of both the sponge species and the ascidian species linearly increased with temperature within the temperature range at the study site (Fig. 6.2a). From 12 to 22° C, a Q_{10} of 3.56 was estimated for the sponge species, and a Q_{10} of 2.2 was estimated for the ascidian species.

The comparison of the gonadal development for the gorgonian species (data from Coma et al. 1995a) with the mean monthly values of respiration rates showed that reproductive investment explained 88 % of the variance in respiration rate ($r^2 = 0.78$, n = 12, p = 0.0001; Fig. 6.2c). The importance of the period of production on the respiration rate of the gorgonian species in contrast to that of temperature was also clearly shown by

the comparison of two months with similar temperature but with important differences in production. Then, the gorgonian respiration rate in June (about 17 °C and high production period) was 33 % higher than that in October (about 17 °C and low production period) (one-way ANOVA, $F_{(1,83)} = 15.88$, $p<0.0001$; Fig. 6.2c). Monthly gonadal development for the ascidian species (gonad index; data from Becerro and Turon 1992) was also compared to the mean monthly values of respiration rates. It showed that gonadal development accounted for a 87 % of the variance in respiration rate of the ascidian species ($r^2 = 0.77$, $n = 12$, $p = 0.0002$; Fig. 6.2b). Whether or not gonadal development affected respiration rate of the sponge *Dysidea avara* was not examined because, to date, no data is available for the gonadal development of the species.

Table 6.2. Analysis of variance of respiration rates between days and months. Days nested in months. df: degrees of freedom, SS: sums of squares, MS: mean square, F: F ratio, p: probability.

Especie	df	SS	MS	F	p
D. avara					
Day	17	0.203	0.169	0.289	0.99
Month	5	10.177	3.392	58.03	< 0.0001
error	452	7.365	0.058		
H. papillosa					
Day	17	0.055	0.027	0.224	0.799
Month	5	2.487	0.622	5.096	0.0008
error	321	14.275	0.122		
P. clavata					
Day	17	0.239	0.119	0.184	0.832
Month	5	13.410	1.118	5.719	0.04
error	254	115.080	0.650		

The effect of expansion and contraction of the polyps of the gorgonian species on respiration rate of the colony was examined in several occasions. The respiration rate of colonies with expanded polyps was significantly different from that of colonies with contracted polyps (ANOVA $F_{(1, 46)} = 4.03$, $p < 0.05$). On average, respiration rate of colonies with expanded polyps was 26 % higher than that of colonies with contracted polyps.

We calculated the variation of the Gain/Cost ratio (G/C) for the three species using the respiration rate values through the year as well as ingestion rate data from previous

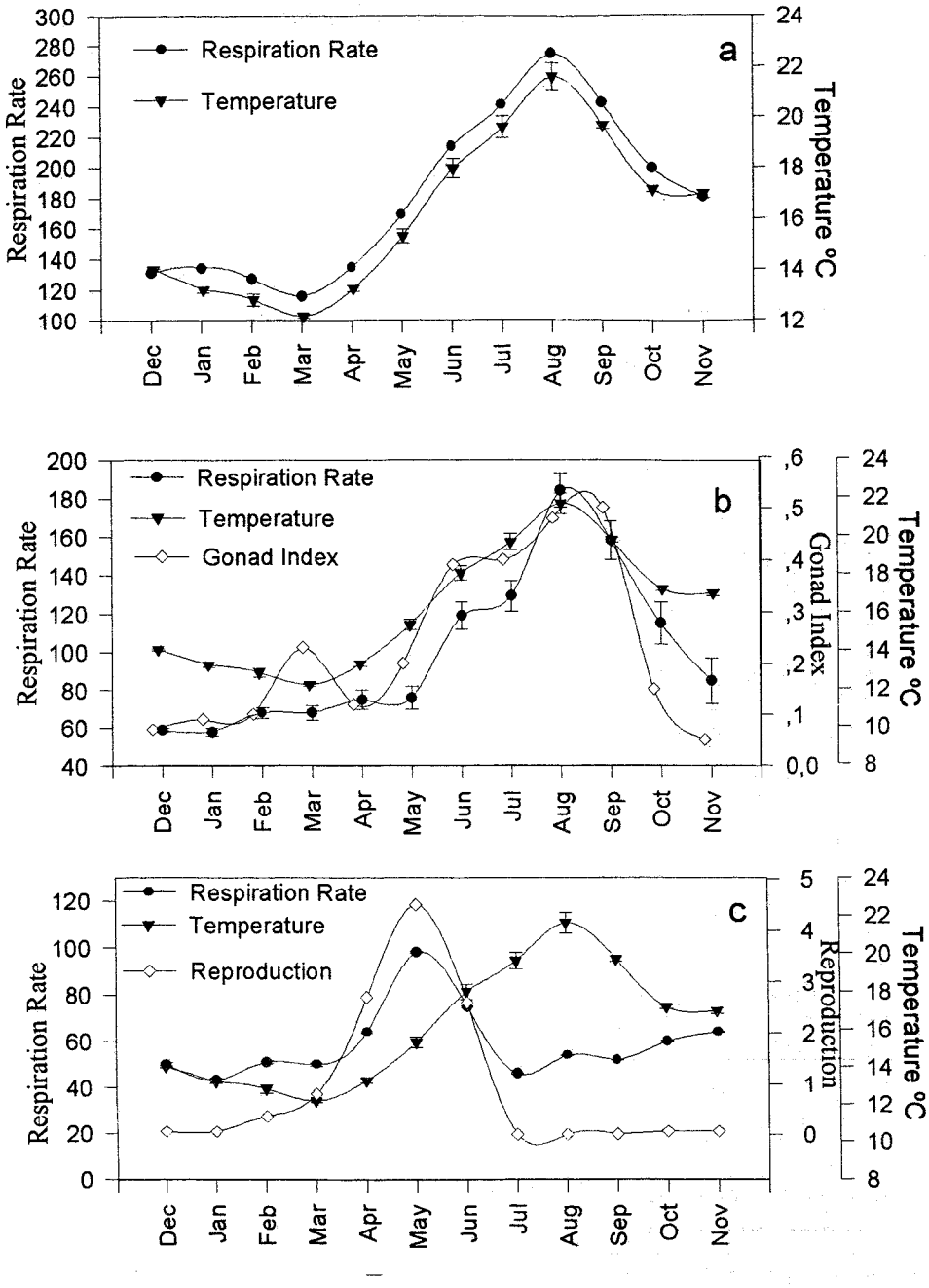

Fig. 6.2. a) *D. avara*, variation of respiration rate (μg C g AFDW^{-1} h^{-1}) through the year, b) *H. papillosa*, variation of respiration rate (μg C g AFDW^{-1} h^{-1}) and gonad index (i.e. gonads proportion of the dry weight of the mantle (Becerro and Turon 1992) through the year, c) *P. clavata*, variation of respiration rate and gonadal development both in (μg pol^{-1} d^{-1}) x 100 (Coma et al 1995a). The temperature variation over the annual cycle at the study site is included in each species graph.

feeding studies on the diet and its seasonal variation (Chapters 3, 4 and 5). From an energy budget approach, this ratio provides a parameter which allows to detect the energetically best and worst periods throughout the year (Fig. 6.3). The sponge species showed maximum G/C values during the winter and spring, while minimum values occurred during summer. The low G/C values observed during the summer months were due to the increase in respiration rate during this time period (Fig 6.4a). The gorgonian species showed a similar pattern to that of the sponge with maximum G/C values during the winter period and minimum values during summer (Fig. 6.3). However, for this species, the low G/C values during the warm months were related to the low ingestion rate during this time period (Fig. 6.4c). To estimate ingestion rate both zooplankton (Coma et al. 1994) and pico-nano- and micro-seston (Chapter 5) were considered.

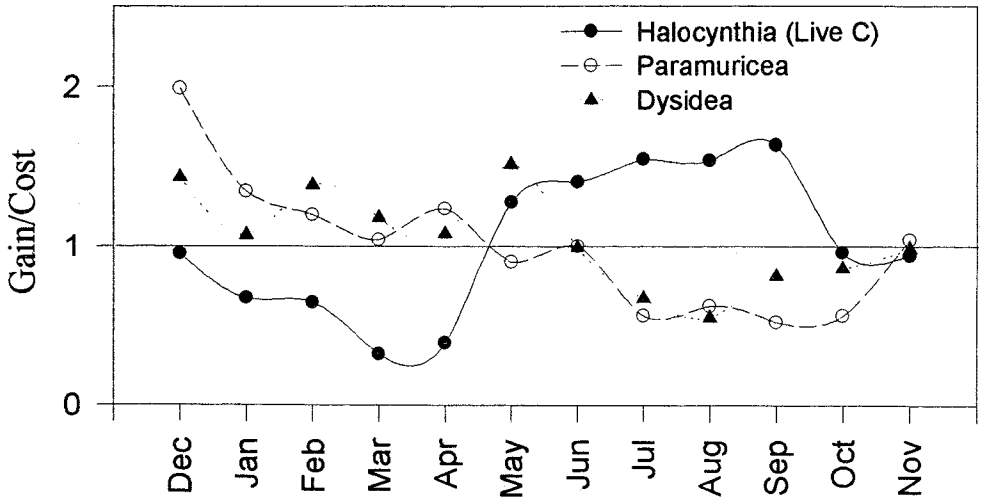

Fig. 6.3 Variation of the Gain/Cost index (i.e. proportion between ingested carbon and loses by respiration, both in carbon units) through the year for *Dysidea avara*, *Halocynthica papillosa* and *Paramuricea clavata*

In contrast to the pattern observed in the other two species, the ascidian species showed minimum G/C values during winter, G/C values gradually increased during spring, and the highest values were observed during the summer period (Fig. 6.3). The high G/C values during the summer period were due to an important increase in ingestion rate, because respiration rate also increased during this time period (Fig. 6.4b). For the ascidian species, if ingestion of carbon from detrital origin would had been considered, G/C values would had been high all year long. However, for the estimation of the G/C values only ingestion of live carbon was considered because new production of the species has

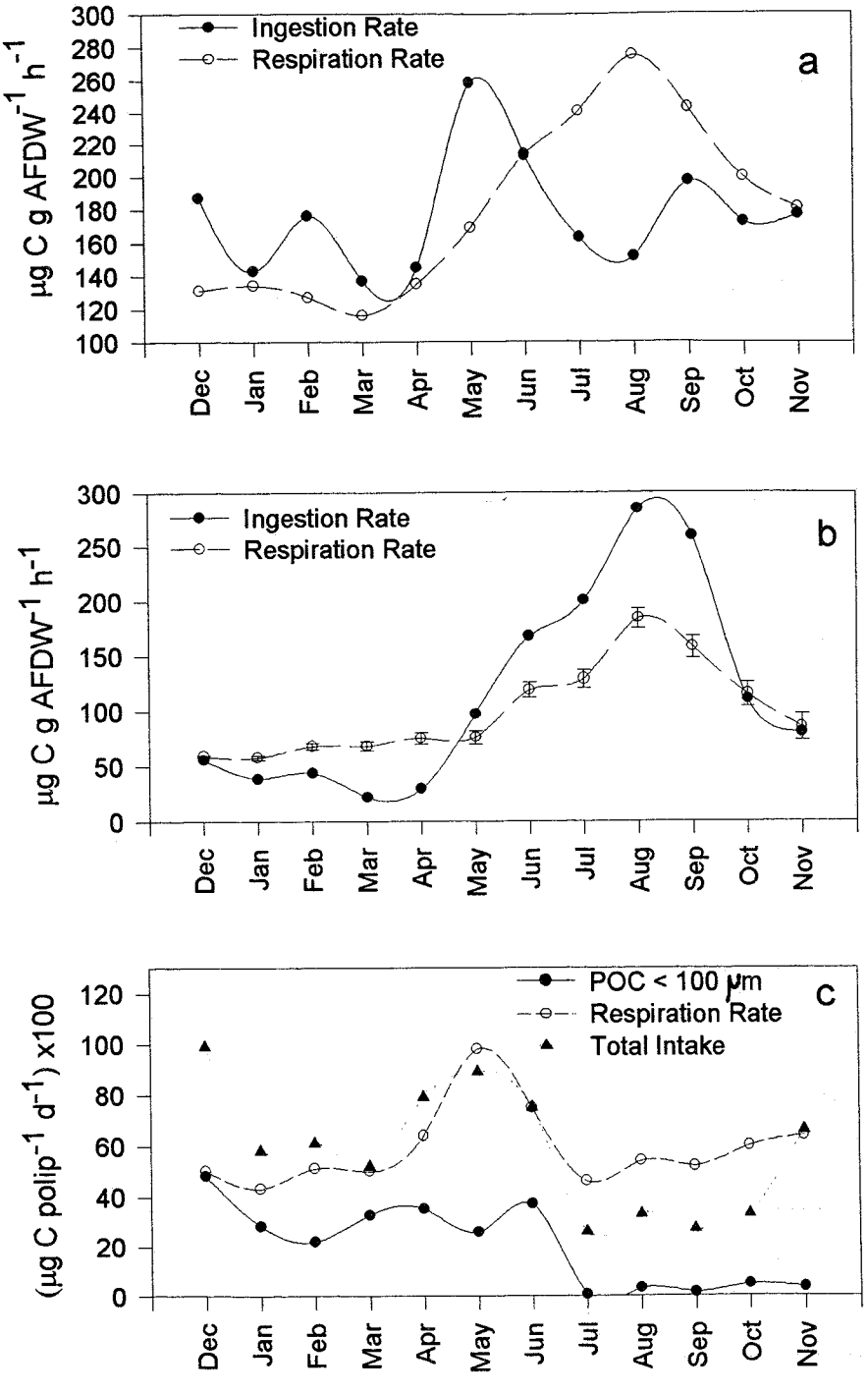

Fig. 6.4 Variation of the ingestion and respiration rates through the year for a) *D. avara*, b) *H. papillosa*, c) *P. clavata*. *P. clavata* ingestion separated between total uptake and uptake of POC < 100 µm.

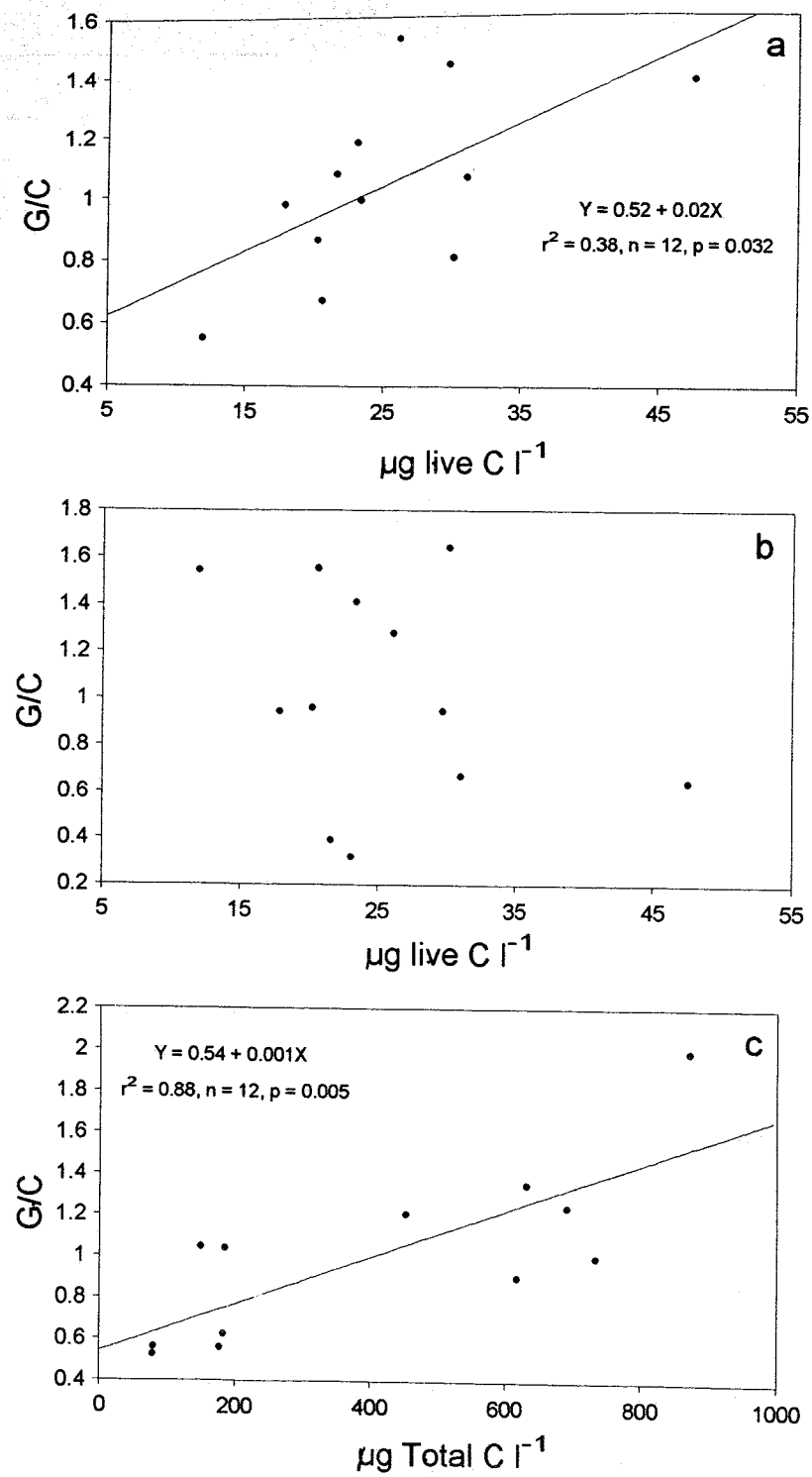

Fig. 6.5. Variation of the Gain/Cost index as a function of food concentration in µg C l^{-1} for a) *D. avara*, b) *H. papillosa*, c) *P. clavata*.

been observed to highly correlate with the ingestion of live carbon, but not with that of detrital particulate organic matter (Chapter 4).

Gain/Cost values of the sponge *D. avara* and of the gorgonian *P. clavata* species exhibited a positive relationship with the carbon content of the water (Chapter 3 and Chapter 5) (Fig. 6.5a,c). This suggest that energy surplus of these two species was highly dependent on food availability. On the contrary, the G/C values of the ascidian *H. papillosa* were not dependent on food availability (Fig 6.5b).

A seasonal estimation of the lipid content of the *P. clavata* tissue showed significant differences among seasons (one-way ANOVA $F_{(3,52)} = 21.6$, $p < 0.0001$; Fig. 6.6). These differences were due to the marked decrease in lipids content during summer and fall (Scheffé's Post-Hoc test, $p< 0.001$).

V. DISCUSSION

Seasonality in respiration rate

The respiration rates values reported in this study for the sponge species *D. avara* as well as those reported for the ascidian species *H. papillosa* were within the range of values reported in the literature for each taxa (Table 6.3). The respiration rates values reported for the gorgonian species *P. clavata* were also within the values reported for other octocoral species without symbionts (Table 6.3). Respiration rates data is not available for a large number of octocoral species, however, present data suggest a trend of higher respiration rate for octocoral species with symbionts in comparison to that of octocoral species without symbionts. This trend could be related to the fact that respiration rate in symbiotic species usually include both symbiont and octocoral oxygen consumption.

The bimonthly study of the respiration rates of the three species over the year showed a marked seasonal pattern. Metabolic rates of both active suspension feeder species (the sponge and the ascidian species) appeared to be markedly affected by temperature changes. Using the Q_{10} values as an index of the biological sensitivity to temperature (Clarke 1988), the values of 3.56 for the sponge and 2.2 for the ascidian were within the range accepted for unstressed organisms (1-5; Clarke 1988) and close to the range reported for most biological processes (2-3; Valiela 1995). In contrast, the metabolic rate of the passive suspension feeder species (the gorgonian *P. clavata*) did not exhibited any significant response to temperature. *P. clavata* results fit the hypothesis that there have been a general evolutionary trend of compensation for temperature in basal metabolism (Clarke 1987b). Within this trend, poikilotherms organisms, through an adaptation process, would tend to adjust the velocity of their physiological reactions and consequently to smooth, within a certain temperature range, the influence of the seasonal temperature

Table 6.3. Respiration rates for various marine sponges, ascidians and octocorals species, mean ± SD. DW: dry weight, AFDW: ash free dry weight.

SPONGES	ml O_2 g DW^{-1} h^{-1}	ml O_2 g $AFDW^{-1}$ h^{-1}	Reference
Suberites carnosus	0.79		Cotter (1978)
Spongilla lacustris	0.68		Karchenko and Lyashenko (1986)
Halichondria panicea		0.68	Barthel (1988)
Halichondria panicea	0.63	1.84	Thomassen and Riisgård (1995)
Mycale sp		1.49	Reiswig 1974
Verongia gigantea		1.08	Reiswig 1974
Tethya crypta		0.44	Reiswig 1974
Thenea abyssorum		0.50 ± 0.16	Witte and Graf (1996)
Thenea muricata		0.42 ± 0.25	Witte and Graf (1996)
Tetilla cranium		0.42 ± 0.07	Witte and Graf (1996)
Dysidea avara	0.22 ± 0.09	0.65 ± 0.25	This study
ASCIDIANS	**l O_2 g DW_{wt}^{-1} h^{-1}**		
Ciona intestinalis	1.2		Markus and Lambert (1983)
Phallusia mammillata	0.4 - 0.7		Fiala-Medioni (1978)
Styela clava	0.58		Markus and Lambert (1983)
Styela plicata	0.97		Markus and Lambert (1983)
Halocynthia papillosa	0.97 ± 0.65		This study
OCTOCORALS		**mg O_2 g $AFDW^{-1}$ h^{-1}**	
Without symbionts			
Cavernularia obesa		0.81	Mori (1968)
Pennatula rubra		0.42 - 0.95	Brafield and Chapman (1965)
Veretillium cynomorium		0.42 - 1	Brafield and Chapman (1965)
Pteroides griseum		0.42	Brafield and Chapman (1965)
Alcyonium siderium		0.27	Sebens (1987)
		0.21	Sebens (1987)
		1.81	Sebens (1987)
Paramuricea clavata		0.67 ± 0.47	This study
With symbionts			
Plexaura flexuosa		1.40 ± 0.15	Annex I
Eunicella tourneforti		0.3	Lewis and Post (1982)
Muriceopsis flavida		0.75	Lewis and Post (1982)
Gorgonia ventalina		0.76	Lewis and Post (1982)
Briareum asbestinum		0.15	Lewis and Post (1982)
Eunicella stricta		0.21 - 0.27	Chapman and Théodor (1969)
Dendronepthya dollfussi		1.3	Svoboda (1978)
Lithophyton arboreum		2.3 - 3.8	Svoboda (1978)
Heteroxenia spp.		2.1 - 3.2	Svoboda (1978)
Xenia perauensis		2.2 - 2.8	Svoboda (1978)

changes on their basal metabolism (Vernberg and Vernberg 1970, Widdows and Bayne 1971).

The seasonal pattern in respiration rate of *P. clavata* closely followed the seasonal reproduction pattern of the species (Coma et al. 1995a,b). This appears to be related to the increase in oxygen consumption due to the respiratory costs of synthesis (Clarke 1987a). The seasonal pattern in respiration rate of *H. papillosa* also followed the seasonal reproduction pattern of the species (Becerro and Turon 1992). The effects of both temperature and production of the species can be distinguished with difficulty when respiration measurements are recorded in the field. Our results point out that, although a positive relationship exist between respiration rate and temperature, the pattern of production can also explain an important fraction of the observed seasonal pattern in respiration. The respiration study on *H. papillosa* clearly points out the fact that, if no production data would be available, temperature alone would had been suggested to be the main factor related with the seasonal pattern of respiration variation along the year. This fact was previously pointed out be Parry (1983) who observed that many studies about the seasonal pattern of respiration which had been interpreted as a response to temperature, could also be explained by the pattern of production of the species. Thus, because no data is available on the pattern of activity of the sponge species, it can not be concluded that temperature is the main factor that affects the seasonal pattern in respiration rate of the species. Our results points out an important role of the pattern of production in the seasonal pattern of respiration of some benthic suspension feeders in the Mediterranean, which is in accordance with studies in other areas (e.g. Parry 1978, 1983; see Clarke 1987a for review). However, the generalization of this fact can be assessed with difficulty because data about production periods of benthic suspension feeders in the Mediterranean is only available for a few species.

G/C ratio seasonality and food availability

In temperate seas, such as the Mediterranean, important changes of the main environmental parameters such as temperature, hydrodynamism and photoperiod determine important changes in food availability along the year (Zabala and Ballesteros 1989, Coma et al. 1994, Chapter 2). Studies about the dynamics of Mediterranean suspension feeders have shown that both reproduction and growth exhibit a clear seasonal pattern (Llobet et al. 1991, Becerro and Turon 1992, Turon and Becerro 1992, Coma et al. 1995a, Coma et al. in press). Some of these studies have suggested the hypothesis that a trophic-energetic phenomenon may be underlying the seasonal dynamic of benthic suspension feeders, especially characterized by the summer regression or inactivity of the organisms (see introduction). The estimated G/C ratio allowed us to examine whether or not the seasonal dynamic of these Mediterranean species was regulated by trophic constraints.

The G/C ratio exhibited a clear seasonal pattern for the three species. As we have discussed before the lack of data about the pattern of production of the sponge species does not allow to assess whether the increase in basal metabolism during the summer period was mainly a response to temperature increase or if production of the species also concentrate during this time period. However, the negative G/C values for the sponge *D. avara* observed in the summer period, which were driven by an increase in respiration rate and not by a decrease in ingestion, suggest that this species might concentrate its production period in summer, probably using reserves build up during winter and spring (when G/C values were positive). There is evidence that other sponge species are able to accumulate reserves (Barthel 1986). Data on the pattern of production of the species, as well as, a seasonal monitoring of the biochemical composition of the species are suggested as necessary in order to understand the dynamic of this sponge species.

In a preliminary approach to the energy budget of the gorgonian species *P. clavata* (Coma et al. 1998) we pointed out that the zooplankton prey items could not be the only source of food for the species as well as that a detailed seasonal respiratory study should be carried out. Recently, other sources of food have been observed to account for an important share of the species diet (Chapter 5). In this work, we have examined the respiration rate of the species through the year. This two aspects have allowed to complete the energy budget of the species and to estimate the G/C ratio. The negative G/C values for the gorgonian species were driven by a decrease in ingestion rate, in contrast to what was observed for the sponge species. Then, the variation along the year of the G/C values clearly suggest that the trophic-energetic hypothesis becomes the most reliable explanation for the summer decrease of the species activity. Moreover, preliminary data on the lipid content of the species over the year showed an important decrease in lipids content during summer and fall, which is consistent with the negative values of the G/C ratio during this time periods (Fig. 6.6), and reinforce the trophic-energetic hypothesis. This results suggest that winter is the most favorable period for *P. clavata* to invest in production and/or resource storage. The ability to store lipids has been shown in other cnidarians species (Harland et al. 1992, Bouillon 1995). This reserves can be used like fuel energy demands when food is scarce, as the seasonal variation of the lipid content suggest.

In contrast to the sponge and gorgonian species, the estimated G/C values for the ascidian species *H. papillosa* over the year showed a pattern of negative values during winter and early spring, and positive values from late spring to fall. This pattern was due to the filtration rate increase with temperature (Chapter 4), which, in carbon units, provided a higher ingestion rate than the increase of losses due to the respiration rate increase with temperature. The pattern of temperature response of the metabolic rate of *H. papillosa* was similar to that observed in other temperate ascidian species such as *Styela plicata* (Fisher 1976). The increase in ingestion rate with temperature increase has also been observed in

other ascidian species (Fisher 1976, 1977) as well as in bivalves (Seiderer and Newell 1979).

The relationship between the G/C ratio and the abundance of resources exhibited a clear pattern, although it differed among the species. The G/C index of the ascidian species *H. papillosa* did not exhibited any significant relationship with resource abundance. Because the ingestion of live carbon appears to be the main determinant of growth and reproduction (Chapter 4), and the abundance of live carbon remains rather constant through the year (Chapter 2), other factors rather than food concentration appears to be determining the seasonal dynamic of the species. *H. papillosa* followed a similar pattern to that observed in previously observed for the solitary ascidian *Ciona intestinalis* (Petersen et al. 1995), maintaining high growth rates even during periods of low food concentration. Ascidians in the same way as bivalve molluscs (Jørgensen 1990), can increase the pumping rate when they need more food, this capacity may be interpreted as a process which allow the organism a certain degree of emancipation from the environment.

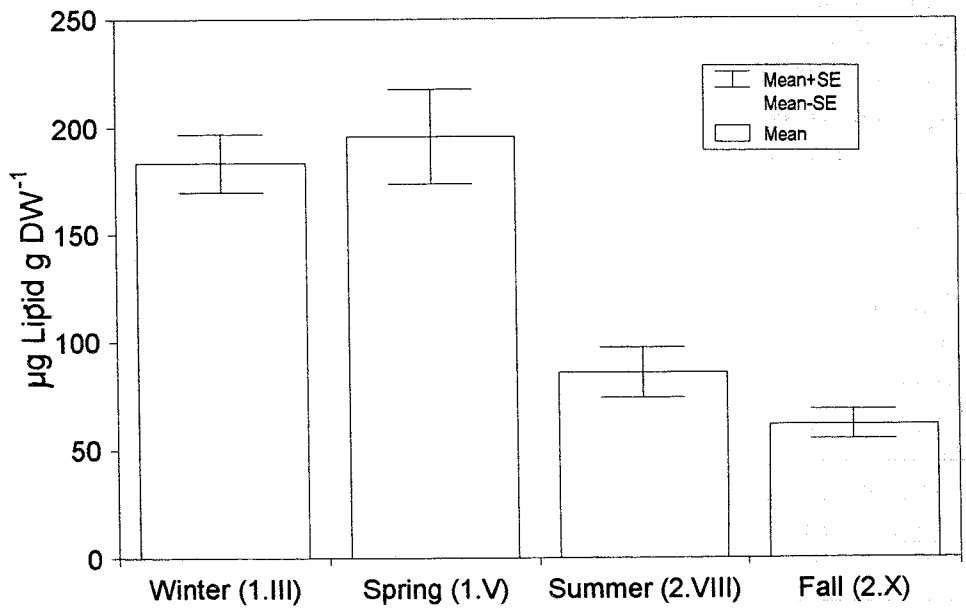

Fig. 6.6. Variation of the total lipids content in µg Lipid g DW^{-1} of *Paramuricea clavata* tissue over the year for colonies < 10 cm height. Dates of sampling are provided beside each season.

The positive relationship between the G/C ratio and resource abundance for the sponge and the gorgonian species showed that the G/C ratio was depended on resource abundance in both species. Because the G/C values were positive only during certain time periods through the year, growth, reproduction or resource storage investments appears to be only possible during the months when enough food is available. Growth and

reproduction have been directly related to food availability for some invertebrate marine species (e.g. Briscoe and Sebens 1988, Minor and Scheibling 1997). This positive relationship specially occurs in areas with a narrow temperature range such as sub-tropical areas (see Conover 1978 for review) or polar zones (Grebmeier et al 1988) were climatic constrains (mainly temperature) do not became into play. Our results suggest that both temperature and food availability are determining the seasonal dynamic of these benthic suspension feeders in the Mediterranean. The importance of both parameters has been observed to differ depending on the species. Then, the results of both the sponge and the gorgonian species are consistent with the hypothesis that a trophic-energetic phenomenon may be underlying the seasonal dynamic of benthic suspension feeders in the Mediterranean. Regression or inactivity of the colonies in summer has also been observed in the life cycles of a number of benthic suspension feeders in the Mediterranean, including hydroids (Boero et al. 1986, Llobet et al. 1991a), zoantharians (R. Coma unpublished data), octocorals (Garrabou 1997, R. Coma unpublished data), and bryozoans (Zabala 1983). The summer period has also been pointed out to be an unfavorable period for most colonial ascidian species, when a panoply of non-feeding and resistance forms has been observed for several species (Turon 1988, Turon 1992, Turon and Becerro 1992). This fact points out that the increase in the activity of this ascidian species during the summer period appears to be more an exception rather than a general pattern for the group. A negative relationship between filtration rate and detrital POC has been observed for the ascidian species (r^2= 0.41, n= 12, p = 0.0004). This fact suggest that the low filtration rate of *H. papillosa* during the late fall, winter and early spring periods could be related to a negative effect of high detrital concentrations on filtration rate.

We are aware that the in estimating the G/C ratio we have not considered several important parameters such as assimilation and excretion. These two parameters would affect the estimated absolute values. However, they are not expected to affect the general trend of the energy budget of the species through the year. Furthermore, the error terms of the respiration and ingestion rates estimations were small enough so that they would not significantly affect the observed seasonal pattern of the G/C for the species. Overall, our results suggest that food availability plays an important role in determining the seasonal dynamic of some benthic suspension feeders in the Mediterranean.

ANNEXE I

HETEROTROPHIC FEEDING BY GORGONIAN CORALS WITH SYMBIOTIC ZOOXANTHELLA

I. ABSTRACT

Gorgonians are one of the most characteristic groups in Caribbean coral reef communities. In this study, we measured *in situ* rates of grazing on pico-, nano- and microplankton, zooxanthellae release, and respiration for the ubiquitous symbiotic gorgonian coral *Plexaura flexuosa*. Zooplankton capture by *P. flexuosa* and *Pseudoplexaura porosa* was quantified by examination of stomach contents. In nature, both species captured zooplankton prey ranging from 100 to 700 μm, at a grazing rate of 0.09 and 0.23 prey polyp^{-1} d^{-1}, respectively. Because of the greater mean size of the prey and the higher mean prey capture per polyp, *P. porosa* obtained 3.4 x 10^{-5} mg C polyp^{-1} d^{-1} from zooplankton, about 4 times the grazing rate of *P. flexuosa*. On average, *P. flexuosa* captured 7.2 ± 1.9 microorganisms polyp^{-1} d^{-1} including ciliates, dinoflagellates and diatoms, but they did not appear to graze significantly on organisms smaller than 5 μm (heterotrophic bacteria, *Prochlorococcus*, *Synechococcus sp.* or picoeucaryotes). Zooplankton and microbial prey accounted for only 0.4 % of respiratory requirements in *P. flexuosa*, but they contributed 17 % of nitrogen required annually for new production. Although the contribution of microbial prey to gorgonian energetics was low, dense gorgonian populations found on many Caribbean reefs may be important grazers of plankton communities.

II. INTRODUCTION

The role of food as a constraining factor in population and community ecology has been widely debated (Hairston et al. 1960; Schoener 1974; Olson and Olson 1989). Depletion of planktonic microbial (Linley and Koop 1986; Ayukai 1995), phytoplankton and zooplankton communities (Glynn 1973; Buss and Jackson 1981) has been observed on coral reefs, suggesting an important role for nutrient limitation in the distribution and abundance of suspension feeders (Schoener 1974). However, the natural diets of most suspension feeders are poorly known, and our lack of knowledge about their feeding habits has become a limiting step in understanding the factors that constrain populations.

The coral-zooxanthella symbiosis makes feeding biology in reef anthozoans particularly complex. Reef anthozoans obtain high energy compounds (mainly carbohydrates) from the symbiotic algae through translocation (Johannes et al. 1970; McCloskey and Muscatine 1984; Muscatine et al. 1984). The translocated products are used mostly for respiration and only a small proportion is destined for new growth of the colony (Davies 1984; Falkowski et al. 1984), probably because the photosynthetic products are deficient in nutrients such as nitrogen and phosphorous (Muscatine 1967; Battey and Patton 1986). Therefore, heterotrophic feeding might be required to provide indispensable elements for growth and reproduction of the coral (Muscatine 1973; Muscatine and Porter 1977; Sebens 1987).

In corals (Hexacorallia), zooplankton capture seems to be the main source of heterotrophic feeding (Muscatine and Porter 1977; Sebens 1987), although it has been quantified for only a small number of hard corals (Porter 1974; Johnson and Sebens 1993; Sebens et al. 1996) and zoanthids (Koehl 1977; Sebens 1977). Among the soft corals (Alcyonacea), zooplankton capture has been quantified for several species (Lewis 1982; Sebens and Koehl 1984), and recently, phytoplankton has been documented also to be an important component of the diets of some asymbiotic species (Fabricius et al. 1995a, b). However, although gorgonians are one of the most characteristic components of tropical seas (Bayer 1961; Tursch and Tursch 1982), their natural diets are still poorly understood.

Although it has been shown that gorgonians are able to ingest particulate matter (Leversee 1976, Lasker et al. 1983; Sponaugle and LaBarbera 1991), field studies have rarely observed grazing on natural prey (Kinzie 1973; Lasker 1981; Lasker et al. 1983). This apparent lack of grazing might be an artifact due to the methodology, gut content analysis, used by previous studies. This method is useful in the study of prey organisms with hard parts, but potentially underestimates small soft-bodied prey because they leave no recognizable remains. Therefore, the role of these small and soft-bodied organisms in the diet of benthic suspension feeders appears to be largely unknown (but see Pile et al. 1996; Pile 1997; Pile et al. 1997), although it could be relevant due to the fact that pico- and

nanoplankton are major contributors to biomass and productivity of the water column (Platt et al. 1983).

In this study, we examine the natural diets of the ubiquitous symbiotic gorgonian corals *Plexaura flexuosa* and *Pseudoplexura porosa*. We focused on three main goals: 1) to determine which planktonic taxa grazed on by gorgonians; 2) to estimate grazing rates on these taxa; and 3) to examine the role of heterotrophic feeding in symbiotic gorgonians. We found that gorgonians could grazing on some microorganisms, which may have a substantial impact on the plankton community over coral reefs.

III. METHODS

This study was mainly conducted in House and Korbiski reefs, San Blas Islands (Panamá), close to the field station of the Smithsonian Tropical Research Institute (see map in Brazeau and Lasker 1992). Additional samples for the stomach contents study were collected also in the Florida Keys (Pickles reef: 24° 59' N; 80° 24' W and Conch reefs: 24° 57' N; 80° 27' W). *Plexaura flexuosa* is among the most common and widely distributed anthozoans of the shallow Caribbean reefs (Kinzie 1973; Opresko 1973).

Gut content analysis were used to quantify predation on zooplankton prey larger than 50 μm which left recognizable remains. *Pseudoplexaura porosa* samples were collected at Korbiski reef every three hours from 08:00 on August 13 to 08:00 h on August 14, 1993. For each sampling, one terminal branch from each of 5 different colonies was collected and immediately fixed in 10 % formalin. Samples were rinsed thoroughly to remove any plankton remaining on the colony surface. The gut contents of 20 randomly chosen polyps from each sample were examined under a microscope at 400x. Thus, for each sample 100 polyps were examined, yielding a total of 900 polyps of *P. porosa* for the entire 24 hour cycle. For *Plexaura flexuosa*, terminal branches from 40 colonies were collected on various dates in 1994 and 1995 in the Florida Keys (Pickles and Conch reefs) and Panamá (Korbiski reef). Ten polyps were dissected per branch, giving a total of 400 *P. flexuosa* polyps. The other procedures were the same as used for *P. porosa*. In both cases, prey were identified to the level of major taxonomic group; length and width of all prey were measured under the microscope.

Grazing rate, expressed as the number of prey items captured per polyp and hour, was calculated using the equation (Coma et al. 1994):

$$C = N\left[\sum_{t=0}^{D} 1 - (t/D)\right]^{-1} \qquad (8)$$

where C = number of prey captured polyp^{-1} h^{-1}; N = prey items per polyp; t = time (in h); and D = digestion time (in h). Gut contents were extrapolated to daily rates of intake by assuming a digestion time of 6 hours (Lewis 1982).

Prey biomass was estimated from biovolumes (Sebens and Koehl 1984), using conversion factors to wet weight (1.025, Hall et al. 1970), dry weight (13 % of wet weight, Beers 1966; Murphy 1971), and carbon content (45 % of dry weight, Biswas and Biswas 1979). The nitrogen content was estimated from the carbon:nitrogen ratio of each group (Gorsky et al. 1988).

A total of 7 incubation experiments was carried out: 4 during daylight hours (between 8:00 h and 17:00 h) and 3 during the night (between 19:00 h and 24:00 h) on 7 different days. At the beginning of each experiment, a *P. flexuosa* colony on a cement flat was placed on the gorgonian chamber. Colonies were allowed to expand fully before the experiment started. Colonies that did not expand within a few minutes were eliminated from the experiment. After this acclimation time, both incubation chambers were closed and 3 replicate water samples of 200 ml were collected from both chambers and preserved for further analysis. After 3 hours, 3 replicate water samples were collected again. Predation was calculated from decreases in prey concentration in the gorgonian chamber relative to the control chamber. The potential prey items in this fraction included: heterotrophic bacteria, *Synechococcus sp*, *Prochlorococcus sp.*, eucaryotic picoplankton, ciliates, and phytoplankton (diatoms and dinoflagellates).

Respiration was estimated from two different series of experiments: a) dark feeding incubations (3 experiments), where oxygen concentration was measured at the start and end of the experiments and b) 7 hour-dark incubations (from 23:00 h to 6:00 h) during which oxygen concentration was recorded in both chambers every 2 minutes by a data-logger. During the 7 dark hours period experiments, the water inside both chambers was totally renewed when the oxygen concentration changed 20 % from its initial level (Crisp 1984). Respiration rates were estimated from the decrease in oxygen concentration in the gorgonian chamber during each experimental period. The control chamber was used to detect possible oxygen variations not due to the gorgonian colony. Metabolic rate (mg O_2 polyp^{-1} day^{-1}) of a whole colonies (animal + algal) was determined from mean nighttime hourly oxygen respiration rates extrapolated to 24 hours and divided by the number of polyps. Oxygen units were converted to carbon equivalents using the carbon to oxygen conversion factor of 0.375 (McCloskey et al. 1994).

P. flexuosa dry weight was determined by drying at 60°C and ash free dry weight was determined by combustion at 450 °C for 5 hours. The number of polyps per colony (N) was estimated from colony height (H, cm) using the regression equation: $N = 0.934\ H^{3.062}$ (Beiring 1997). For the determination of nitrogen content, 10 terminal branches of about

10 mm in length were ground and dried at 60°C. The samples were assayed on a Perkin-Elmer model 240 CHN analyzer.

The gastrovascular cavity was examined with a scanning electron microscope (Hitachi S-570). Ten polyps of *Plexaura flexuosa* from five different colonies were dissected (a total of 50 polyps) and dehydrated in graded ethanol. Afterwards, the polyps were dried by the critical point method (using CO_2 as transition fluid), mounted on aluminum stubs and coated with gold in a sputter coater.

IV. RESULTS

Feeding on zooplankton

Gut contents of *Plexaura flexuosa* and *Pseudoplexaura porosa* were dominated by zooplankton prey, particularly gastropod larvae; only on a few occasions other groups (protozoan and diatoms, Table I.1) were observed. Both species grazed zooplankton prey items ranging in size from 100 to 700 μm. The number of prey per polyp grazed by *P. porosa* over the daily cycle ranged from 0 to 0.10 prey polyp^{-1}. Mean *P. porosa* daily zooplankton grazing rate was 0.23 prey polyp^{-1} (Table I.2). *P. flexuosa* exhibited lower zooplankton grazing rates (0.09 prey polyp^{-1} day^{-1}) than *P. porosa*. Because of the greater mean size of the prey items and the higher mean prey capture per polyp, *P. porosa* obtained 0.034 mg C polyp^{-1} day^{-1} from zooplankton, almost 4 times the grazing rate of *P. flexuosa* (Table I.2).

Feeding on small plankton

Figure I.1 shows net growth rates calculated for each plankton taxa (not including zooplankton > 100 μm) in the control chamber and in the gorgonian chamber, together with the mean size of each taxa. Growth rates of *Thalassionema sp*, pennate diatoms, dinoflagellates and ciliates were significantly lower in the gorgonian chamber than in the control chamber (Fig. I.1). Ingestion rates for these organisms are presented in Table I.3. The highest ingestion rate was for dinoflagellates (3.7 prey items polyp^{-1} day^{-1}), followed by pennate diatoms (1.9), ciliates (1), and *Thalassionema sp.* (0.4). Within the range of pennate diatoms, dinoflagellates and ciliates concentrations (1-3.5 x 10^6 μm^3 liter^{-1}) present during the experiments, grazing rates of *P. flexuosa* on these groups was similar and did not vary with concentrations (Fig. I.2). Therefore, these groups appears to be close to the maximum grazing rate. Grazing on *Thalassionema sp.* was lower than on the previously mentioned groups. This could be due to the low concentration of this group or, although unlikely, to a selection against it (Fig. I.2). On average, *P.flexuosa* polyps ingested 4.0 ± 1.4 x 10^{-9} g C per polyp and day (Table I.3). Therefore, the contribution of nanoplankton

and microplankton as carbon source was about half of the zooplankton captured (Table I.2 and I.3).

Table I.1. Number and type of prey items captured by *Pseudoplexaura porosa* over the diel sampling period (13-14 August 1993, 900 dissected polyps), and *Plexaura flexuosa* from 40 colonies collected between 1994-95 (400 polyps dissected). Length and width in μm.

Pseudoplexaura porosa	Number of prey at each time									Total	Mean prey size	
	8:00	11:00	14:00	17:00	20:00	23:00	2:00	5:00	8:00		Length	Width
Gasteropod larvae	6	1	1	-	-	-	-	3	-	11	245	150
Copepod egg	1	3	1	1	-	-	-	-	-	6	158	-
Harpacticoid	2	1	-	-	1	1	1	1	-	6	311	110
Calanoid	-	-	1	-	-	-	1	-	-	2	518	163
Copepod fragment	-	-	-	-	-	-	-	1	-	1	173	134
Nauplii	-	-	-	-	-	-	-	-	-	1	250	115
Cladocera	-	-	-	-	-	-	-	1	-	1	461	192
Protozoa	1	-	-	-	-	-	-	-	-	1	240	150
Centric diatom	-	-	-	1	-	-	-	-	-	1	115	-
Total	10	5	3	2	1	1	2	6	-	30		
Plexaura flexuosa												
Gasteropod larvae										4	150	138
Protozoa (Foraminifera)										1	300	300

The mean size of the picoeucaryotes was 1.47 ± 0.30 μm (n= 307). Net growth rates of picoeucaryotes were not significantly different between the gorgonian chamber and the control chamber (Fig. I.1). Growth rates of heterotrophic and autotrophic bacteria (*Prochlorococcus* and *Synecoccocus sp.*) were also not significantly different between the gorgonian chamber and the control chamber (Fig. I.1). Therefore, *Plexaura flexuosa* did not appear to graze significantly on organisms smaller than 5 mm in our study.

The number of round cells (7-9 μm) increased during the incubations from initially < 0.1 cell ml^{-1} to final densities of $10\text{-}10^2$ cells ml^{-1}. These round cells were identified as zooxanthellae by comparison with a culture of isolated zooxanthellae (T. Goulet). To determine the origin of these zooxanthella, we examined by scanning electron microscopy (SEM) the gastrovascular walls of the polyps from incubated colonies. Apparently healthy and viable zooxanthellae were pinched off and released by host cells as described by Gates and Muscatine (1992). Examination of *Plexaura flexuosa* polyps from freshly collected

Table I.2. Zooplankton prey capture rate (prey polyp^{-1}), prey size, prey biomass, daily prey capture and daily biomass capture in carbon and nitrogen units. Mean ± SD

	P. porosa	*P. flexuosa*
Number of Polyps	900	400
Prey polyp^{-1}	0.03 ± 0.03	0.01 ± 0.005
Prey Size (μm)	261 ± 100	180 ± 68
Prey Biomass (μg C)	0.150	0.104
Prey polyp^{-1} d^{-1} (*)	0.227	0.086
μg C polyp^{-1} d^{-1}	0.034	0.009
μg N polyp^{-1} d^{-1}	0.006	0.002

(*) Daily prey capture based on a digestion time of 6 h (Lewis 1982)

colonies showed that expulsion of zooxanthellae seems to occur regularly under natural conditions. Zooxanthella release by *P. flexuosa* was greater during the daytime (mean = 878 ± 658 cells cm^{-2} hour^{-1}) than at night (mean = 289 ± 216 cells cm^{-2} hour^{-1}). The estimate of the expelletion rate for natural colonies was based only on our nighttime estimate, because it has been suggested that an increase in oxygen level could be a physiological stress leading to the expulsion of zooxanthellae (Lesser and Shick 1989), and we observed that oxygen concentrations increased during daytime incubations (due to light-dependent photosynthesis). Our estimate is that a total of 6,840 zooxanthellae cells cm^{-2} day^{-1} were released.

Respiration rates

Oxygen concentration in the control chamber did not change significantly over time for both dark feeding experiments and the monitoring through the 7 hour-dark incubation (see methods; Fig. I.3). The computed respiration rates for *P. flexuosa* were similar: 1.31 ± 0.05 mg O_2 per hour per g AFDW for the dark feeding experiments and 1.48 ± 0.25 mg O_2 per hour per g AFDW for the 7 hour-dark incubation experiment. This oxygen consumption represents a daily requirement of 12.77 ± 1.88 mg C g AFDW^{-1} day^{-1} or 3.72 x 10^{-6} ± 5.46 x 10^{-7} g C polyp^{-1} day^{-1}.

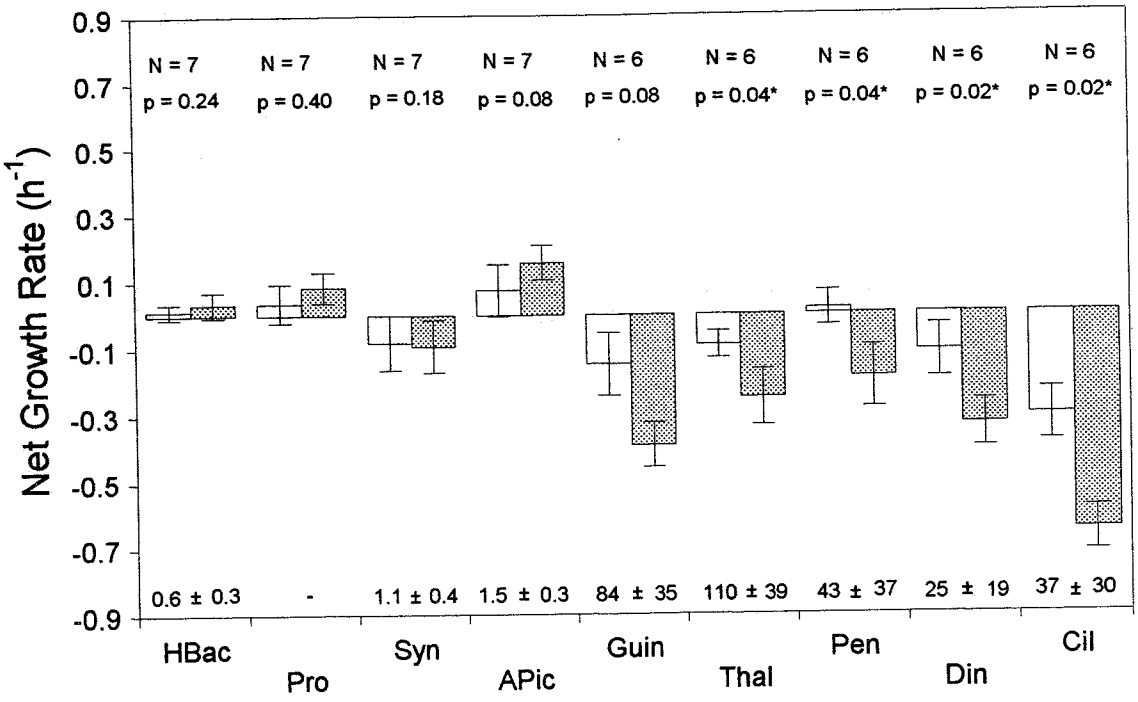

Fig. I.1. Prey growth rate (mean ± SE) at the gorgonian chamber (k_o in dotted bars) and in the control chamber (k_c in empty bars) for each plankton group. Maximum length of each group (mean ± SE) at the figure bottom. HBac - heterotrophic bacteria, Pro - *Prochlorococcus sp*, Syn - *Synechococcus sp*, APic - autotrophic picoeucariotes, Guin - *Guinardia sp*, Thal - *Thalasionema sp*, Pen - pennate diatoms, Din - dinoflagellates, Cil - ciliates. N = number of experiments, and p = significance degree from Two-tailed Wilcoxon test.

Based on data on prey size and ingestion rates, we estimated the contribution of various prey items to total carbon and nitrogen needs of the gorgonian. *Plexaura flexuosa* obtained about 8.9×10^{-3} mg C and 1.6×10^{-3} mg N polyp^{-1} day^{-1} from zooplankton (Table I.2), and 4.0×10^{-3} mg C and 0.6×10^{-3} mg N polyp^{-1} day^{-1} from feeding on other plankton (Table I.3). Ciliates contributed the most to C and N requirements (51 %), followed by diatoms (30 %) and dinoflagellates (18 %). Together, zooplankton and other plankton prey accounted for only 0.4 % of the estimated respiratory requirement in carbon units.

V. DISCUSSION

To our knowledge, this study is the first attempt to study gorgonian feeding *in situ* utilizing the entire natural range of potential prey. Previous studies had already shown the ability of octocorals to feed on particulate matter (Leversee 1976), dissolved organic matter (Schlichter 1982), mucus (Coffroth 1984), and microzooplankton (Sorokin 1991), but these

Table I.3. *Plexaura flexuosa* daily capture rates (mean ± SE) of diatoms, dinoflagellates and ciliates expressed as cell number, carbon (C) and nitrogen (N) per polyp, per dry weight (DW), and per ash free dry weight (AFDW). Total mean was estimated from averaging all experiments. All values but cell number are x 10^{-9}.

Ingestion	Diatoms		Dinoflagellates	Ciliates	Total
	Thalassionema	Pennate			
cells polyp^{-1} d^{-1}	0.39 ± 0.20	1.88 ± 0.63	3.67 ± 2.12	1.05 ± 0.31	7.20 ± 1.95
g C polyp^{-1} d^{-1}	0.02 ± 0.01	0.43 ± 0.19	0.93 ± 0.32	2.37 ± 1.30	4.00 ± 1.39
g C g DW^{-1} d^{-1}	12.80 ± 6.69	247 ± 106	531 ± 185	1350 ± 743	2280 ± 793
g C g AFDW^{-1} d^{-1}	76.70 ± 41.90	1480 ± 639	3190 ± 1110	8140 ± 4470	13700 ± 4770
g N polyp^{-1} d^{-1}	0.004 ± 0.002	0.09 ± 0.04	0.13 ± 0.05	0.31 ± 0.17	0.58 ± 0.18
g N g DW^{-1} d^{-1}	2.49 ± 1.38	50.1 ± 21.8	74.90 ± 26.0	176 ± 96.6	332 ± 104
g N g AFDW^{-1} d^{-1}	15.0 ± 8.30	301 ± 131	450 ± 157	1060 ± 581	2000 ± 625

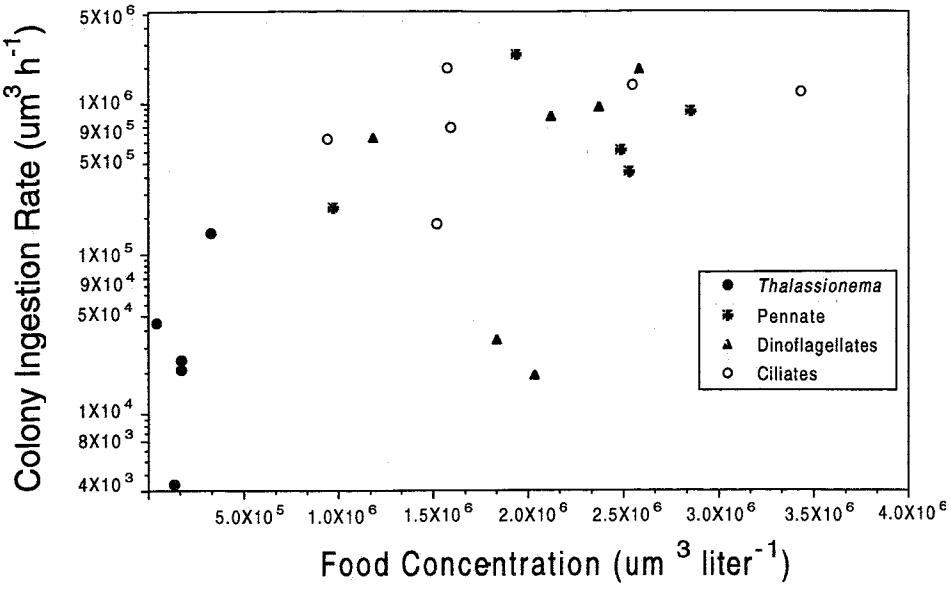

Fig. I.2. Relationship between food concentration (μm^3 $liter^{-1}$) and ingestion rates (μm^3 h^{-1}, in log scale).

studies were conducted either in the laboratory (Leversee 1976; Sorokin 1991; Dai and Lin 1993) or *in situ,* but with simplified diets (Lasker et al. 1983; Coma et al. 1994).

Gut content examinations showed that *Plexaura flexuosa* and *Pseudoplexaura porosa* captured small zooplankton, similar to the types of prey documented for other gorgonians (Coma et al. 1994) and octocorals (Lewis 1982; Sebens and Koehl 1984; Fabricius et al. 1995b). That gorgonians prey only on small zooplankton has been attributed to the low density of nematocysts in octocorals (Mariscal and Bigger 1977). The ability of gorgonians to capture this kind of plankton is also linked to the fact that such small organisms cannot out-swim most water currents and so their transport is similar of that of particulate matter (i.e. largely a function of the water movement).

Plexaura flexuosa captured also an important number of microplankton prey such as ciliates, dinoflagellates and diatoms. Although cnidarians traditionally have been considered as strictly carnivorous (Hyman 1940), more recent studies have found evidence of herbivory (Roushdy and Hansen 1961; Fabricius et al. 1995a, b), which is supported by the presence of plant-digesting carbohydrases in certain soft coral species (Elyakova et al. 1981). It was surprising that *P. flexuosa* did not significantly decrease heterotrophic and autotrophic picoplankton concentration because it has been documented that many hard corals are able to feed upon particles in this size range (Sorokin 1973). As pointed out by Sorokin (1991),

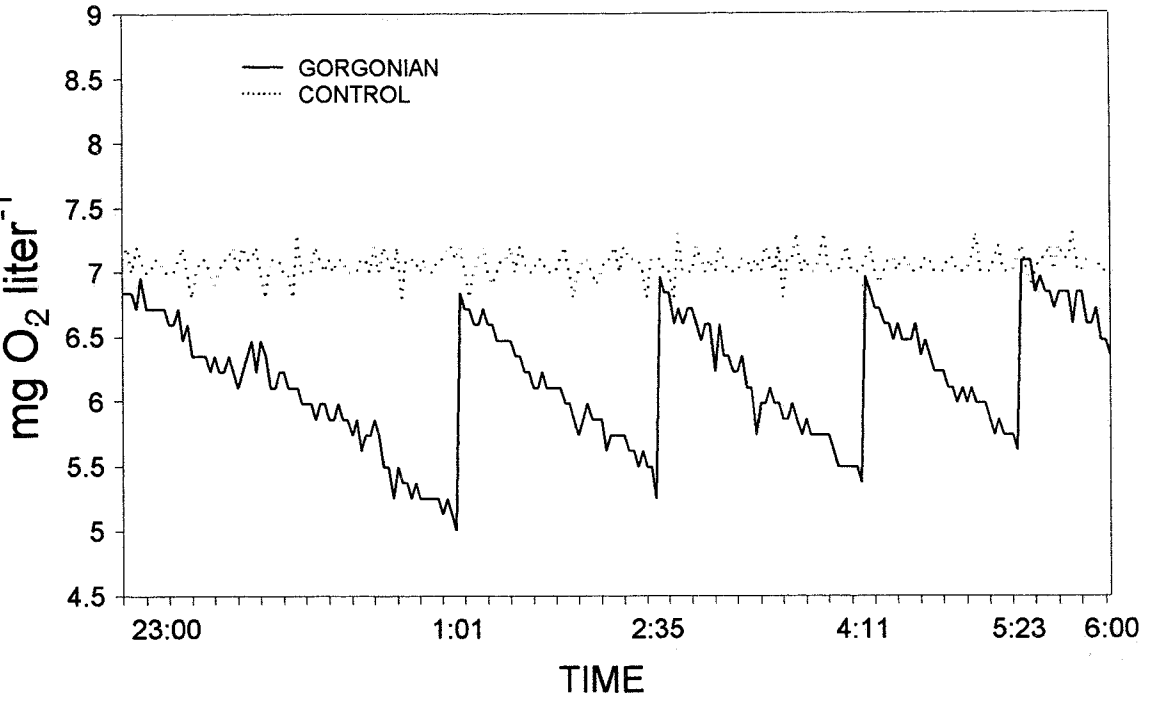

Fig. I.3. Oxygen concentration (mg O_2 liter^{-1}) inside the chambers during the 7 dark hours period (from 23:00 h to 6:00 h). Oxygen values were recorded every 2 minutes in both chambers and water inside both chambers was renewed when oxygen concentration changed 20 % from its initial level. Gorgonian: chamber with gorgonian colony. Control: chamber without gorgonian colony. Respiration by *Plexaura flexuosa* was determined from the average of the oxygen uptake slopes.

there seems to be substantial variability in the feeding spectrum of different soft corals (Octocorallia) species.

Our estimated capture rates of *Plexaura flexuosa* and *Pseudoplexaura porosa* on zooplankton agree with previous observations on tropical gorgonian feeding for other species (Kinzie 1973; Lasker 1981; Lasker et al. 1983). These low capture rates contrast with those of *Paramuricea clavata*, the only gorgonian species in which significant *in situ* capture of naturally occurring prey has been demonstrated to date (Coma et al. 1994; Table 4). This is probably because *P. clavata* is exclusively heterotrophic while *Pseudoplexaura porosa* and *Plexaura flexuosa* have symbiotic zooxanthellae, as do most tropical shallow water gorgonians. The zooplankton capture rates estimated for both species in our study were within the lower range of values observed on corals (Table I.4); some of the higher values reported in the literature could be overestimates because they were quantified after a period of starvation and with zooplankton concentrations up to 10 to 30 times higher than natural zooplankton concentration (Sebens et al. 1996).

The percent of respiration supported by heterotrophic feeding (in carbon units) was used to compare *P. flexuosa* feeding rates on plankton to those obtained for other gorgonian species by Sorokin (1991). In *P. flexuosa*, mean compensation for respiratory losses by heterotrophic feeding was 0.035 % (with a maximum of 0.07 %) on algae and 0.037 % (with a maximum of 0.09 %) on ciliates. Sorokin (1991) estimated feeding rates on algae and ciliates for three symbiotic gorgonians in the laboratory using radioactively labeled prey. In his work, algae accounted for 0.1 - 3 % of respiratory losses and ciliates for 1.7 - 3.6 %. The higher values observed by Sorokin (1991) may have been due to the high prey concentrations used in his experiments, although it may also be due to variability in feeding rates among different species.

The respiration rate estimated for *Plexaura flexuosa* was similar to those obtained for other octocoral species by Megner and Svoboda (1977; 2.5 to 3 mg O_2 g $AFDW^{-1}$ h^{-1}) and Svoboda (1978; 1.3 to 2.9 mg O_2 g $AFDW^{-1}$ h^{-1}), working with whole colonies and similar temperatures. Our respiration rates are also similar to the respiration values reported by Fabricius and Klumpp (1995) for soft coral species in expanded state and in shallow waters (0.54 to 1.21 mg O_2 g $AFDW^{-1}$ h^{-1}). However, the respiration rate we found is higher than that reported by Lewis and Post (1982); their low respiration rates might be an artifact due to incubation without continuous flow, which induces the contraction of colonies (unpublished data) and consequently lowers respiration rates (Sebens 1987; Fabricius and Klumpp 1995).

The estimated respiration rate allowed us to evaluate the quantitative importance of zooplankton and other plankton in the energy budget of the gorgonian. Zooplankton and other plankton did not appear to be important sources of energy for *Plexaura flexuosa,* accounting for < 1 % of the carbon required by basal metabolism. This result contrast with the importance of zooplankton in heterotrophic gorgonians, which has been estimated to account for 50 % of the energy demand (Coma et al. in press). Other external carbon sources, such as dissolved organic matter, have been shown to be quantitatively unimportant for gorgonians (Sorokin 1991; H. R. Lasker unpublished data). Therefore, our results suggest that carbon for *P. flexuosa* comes mainly from their symbiotic zooxanthellae.

In order to determine the importance of zooplankton and microorganisms as N source for gorgonians, we estimated the relative contribution of these prey to the N needs of *P. flexuosa*. The nitrogen requirement for new production was estimated as the requirement for annual growth. New production usually includes growth and reproduction, but in small colonies (< 20 cm) all new production is invested in growth (Beiring 1997). The average growth rate per branch of small *P. flexuosa* colonies was 2 cm yr^{-1} (Coma unpublished data), and nitrogen content of the colony tissue was 0.045 ± 0.011 mg N per

Table I.4. Prey capture rates for various hexacorallian and octocorallian species.

	Prey polyp^{-1}	Reference
Octocorallia		
Gorgonacea		
Pseudoplexaura porosa	0.03	This study
Plexaura flexuosa	0.01	"
Paramuricea clavata	0.6	Coma et al. 1994
Alcyonacea		
Xenia elongata	0.3	Lewis 1982
Sacrophyton trocheliophorum	0.7	"
Lemnalia sp.	<0.1	"
Lobophytum cristagalli	0.1	"
Sinularia densa	<0.1	"
Sinularia capillosa	0.2	"
Sinularia microclavata	0.2	"
Dendronephthya hemprichi	0.02	Fabricius et al. 1995 b
Hexacorallia		
Madreporaria		
Monastrea cavernosa	0.1-0.7	Porter 1974
Meandrina meandrites	1.8	Johnson and Sebens 1993
Monastrea cavernosa	0.23	Sebens et al. 1996
Madracis mirabilis	0.11	"
Zoanthidea		
Palythoa variabilis	0.11-0.12	Sebens 1977
Palythoa caribaeorum	0.03-0.04	"
Zoanthus solandri	0.04	"
Zoanthus sociathus	0.02-0.05	"

millimeter of terminal branch (n = 10) or 0.6 ± 0.09 % of dry weight. Therefore, a *P. flexuosa* colony of 11 cm height (total length = 57.4 cm) with 18 primary branches would require a minimum of 16.2 mg N per year. A colony of this size derives 2.0 mg N per year from the zooplankton (Table 2) and 0.7 mg N per year from other plankton (estimate based on 50 polyp cm^{-2} [Beiring 1997] and grazing rates in Table 3), which together represent 17 % of the minimum annual nitrogen needs for new production.

Our data indicate that feeding on zooplankton and other plankton had a relatively low contribution to the overall nutrient requirement of *P. flexuosa*. Plankton patchiness could produce sporadic episodes of high prey capture rates, as has been documented in another gorgonian (Coma et al. 1994). However, these sporadic events probably do not account for all the remaining N requirement. This small role of zooplankton and other plankton as food source for symbiotic gorgonians suggests that not only C but also N might come from the symbiotic zooxanthellae.

P. flexuosa colonies continuously expelled significant numbers of apparently healthy and viable symbiotic zooxanthellae. This observation supports the hypothesis that the expulsion of zooxanthellae by the host is a natural mechanism for regulating the concentration of algae in the tissues and maintaining the efficiency of the symbiosis (Steele 1976; Hoegh-Guldberg et al. 1987; Trench 1987). We examined the importance of this zooxanthellae release for the C and N budget of the coral by taking into account that the density of zooxanthellae per unit area of coral tissue seems to remain quite constant (1-2 x 10^6 cells cm^{-2}; Drew 1972; Muscatine et al. 1985). Our observed release rate of 6.8 x 10^3 cells cm^{-2} day^{-1}, which is on the same order of magnitude of that observed in *P. damicornis* by Stimson and Kinzie (1991), was < 0.5 % of the total standing stock. Therefore, zooxanthellae release was not a significant loss of fixed carbon. No significant loss of fixed carbon due to zooxanthellae release has been also observed for several taxa (Hoegh-Guldberg et al. 1987; Stimson and Kinzie 1991). At the estimated rate of zooxanthellae release, the annual loss of nitrogen through expelled zooxanthellae would account for 0.84 mg N. Since this amount represents only about 5 % of the previously estimated nitrogen requirement for new production, it appears that the expelled zooxanthellae were not a very important loss of nitrogen for the coral.

Density of *P. flexuosa* in the studied area was 0.45 colonies m^{-2}, with a mean height of 44 cm in a study area of 100 m^2 with 45 colonies. This density would give a total of 9.5 x 10^4 polyps per square meter for this gorgonian, based on the relationship between colony height and polyp number found by Beiring (1997). At the estimated capture rates (Table 3), *P. flexuosa* polyps can ingest about 1.7 x 10^5 diatoms, 2.3 x 10^5 flagellates, and 6.8 x 10^4 ciliates per square meter per day. Overall, this capture is equivalent to removing 0.15 mg C per m^2 per day from the plankton. Ambient concentrations of these prey items during the experimental period (Aug. 25 to Sept. 1, 1995) were 1.97 ± 0.46 diatoms ml^{-1} (including

only *Thalasionema sp* and pennate species [N= 12]), 2.27 ± 2.00 dinoflagellates ml^{-1} (N= 12), and 0.62 ± 0.29 ciliates ml^{-1} (N= 12). This implies a daily removal of 9 % of the diatoms, 10 % of the dinoflagellates and 11 % of the ciliates of the water mass within 1 m of the reef.

The estimated impact of *P. flexuosa* on the microbial assemblages is not negligible. *P. flexuosa* is a ubiquitous Caribbean gorgonian, and similar densities of this species have been documented on other reefs in Panama (Lasker et al. 1988) and Florida (Beiring 1997). It has also been shown that other gorgonian species can prey on microorganisms (Sorokin 1991). The high density of gorgonians species and populations in the Caribbean reefs suggests that gorgonians could be important predators on plankton communities. Furthermore, it seems that predation impact by sponges on microorganisms can be much greater than in gorgonians (Pile et al. 1996; Pile 1997; Pile et al. 1997). Therefore, in shallow water ecosystems, the effect of grazing by macroinvertebrates on water column microorganisms might be much greater than previously thought and might need further study.

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ANNEXE II

AN IMPROVED CLOSED SYSTEM RESPIROMETER FOR MEASURING *IN SITU* RESPIRATION AND OXYGEN PRODUCTION IN BENTHIC ORGANISMS

I. INTRODUCTION

Energetic studies contribute to understanding the dynamic of benthic organisms especially in areas with important seasonal variations of the main environmental factors (Coma et al. 1998). Within the framework of an energetic approach to the study of the dynamic of benthic organisms, respiration constitute one of the largest fraction of the energy demand of benthic organisms. Respiration can be defined as an energy producing process in living systems that degrades organic matter. The energy released during this degradation is used by the living system to achieve the goals of its survival strategy (Lucas 1996). Environmental parameters such as water temperature as well as biological parameters such as the physiological state (e.g. reproductive and/or growth period, ...) affect the respiration rate of benthic marine invertebrates (Clarke 1983, 1987a). Usually, respiration studies have been performed under laboratory conditions allowing to isolate the effects of the different parameters which affect respiration. However, although laboratory studies importantly contribute to the knowledge of their metabolism, it is always difficult to extrapolate laboratory results to the field. In contrasts *in situ* studies cannot isolate the effects of different parameters which are affecting respiration, but instead they are carried out with fully adapted organisms under natural conditions. Then, from an ecological point of view, reliable estimation of the natural metabolism should preferentially come from field studies.

Using a combination of oxygen electrodes, pumps and a data logger we designed a system which solve most of the problems related with closed systems. We report here a description of the system which have been used to study respiration of three benthic suspension feeder species. The system completely submersible and surface-independent, allow to measure oxygen uptake or oxygen production under natural conditions.

II. DESCRIPTION OF THE SYSTEM

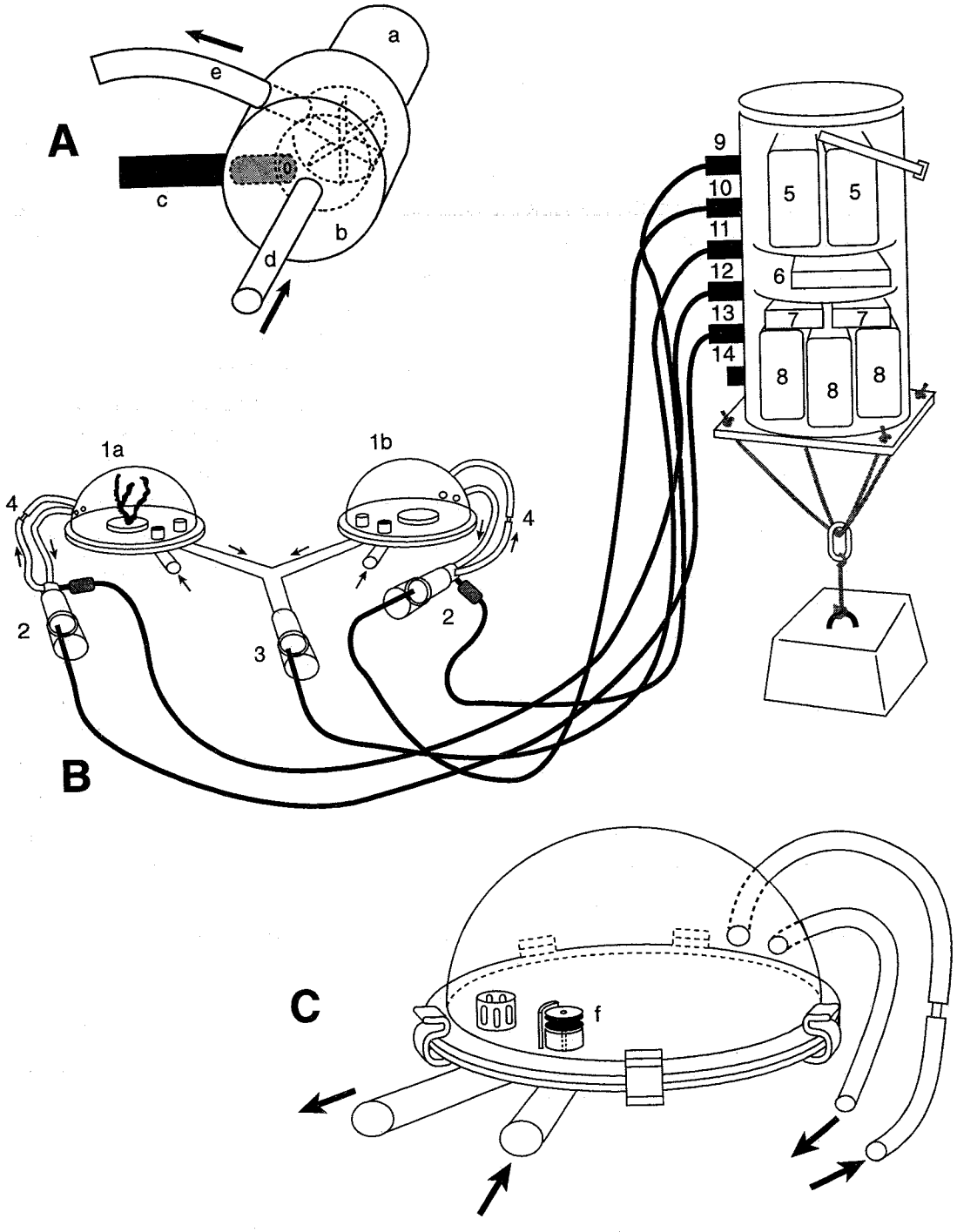
Figure II.1B shows a general view of the experimental set-up for respiration measurements. The unit has two major components, the incubation chambers and the underwater housing. Both chambers (1a and 1b in Figure II.1B) were made from hemispherical pieces of transparent Plexiglas (Svoboda 1978, Svoboda and Ott 1983), approximately 3 litters in volume and sealed to a flat base, also of transparent plexiglas, with a soft O-ring of foam rubber, glued firmly to the rim of the hemisphere. Plastic fasteners with hooks securely anchor the chambers (Fig. II.1C). The chambers have an inlet and an outlet apertures connected to a common piece of PVC and rubber tubing (not silicone rubber tubing because it is highly permeable to oxygen), so the system becomes closed. An small submersible electric pump (flow pump; Comet für Caravan und Boot,

12V) (2 in Figure II.1B) was placed at the outlet aperture of each chamber. These two pumps were set to work at 3.3 V. Then, during normal operation, sea water recirculated through the chamber at a speed of 1.2 cm s^{-1} (this flow becomes turbulent inside the chamber). Homogeneous mixing takes place within a few minutes. The submersible polarographic oxygen and temperature probe (Clark-type oxygen probe, WTW EOT-196) was pressure / temperature-compensated (Kanwisher 1959). It was located close to the flow pump propeller to avoid the formation of microgradients. The probe and the pump were connected through a piece of Plexiglas (Fig. II.1A). Both, the flow pump and the oxygen sensor were connected to the underwater housing by 2 meters of underwater cable and through waterproof electrical connectors. At the base of each chamber there is an outlet which connected to a third pump (flushing pump) (3 in Figure II.1B) that allows the water inside the chamber to be automatically changed. The flushing pump, controlled through the data-logger software, sucked out the water simultaneously from inside both chambers. Then, a second outlet at the base of each chamber provided with a directional valve, allows water to come in while the flushing pump is working. (f in Figure II.1C).

The second main component is a cylindrical underwater housing (70 x 35 cm) which contain: the power supply and converters, a data logger, and the electronics of the probes. The power supply is located at the base of the underwater housing and consist in three batteries (Hitachi Sealed led-acid, 12 V, 6.5Ah, Shin-kobe Electric Machinery Co., Ltd) which provide the power for the three pumps to work (8 in Fig. II.1B). The flushing pump (Fig. II.2) works at 12 volts and is activated when there is a change in a certain percentage of the initial concentration in the chamber were the organism is located. The percentage of change of the initial concentration that determines flushing can be selected through the data logger software.

A voltage-converter system (DC/DC converter) reduced voltage from the general power supply (12 V) to the flow pumps (3.3 V) (7 in Figure II.1B, Fig. II.2). The data logger (Tattletale Model 4A, Onset Computer Corporation) with its own battery (9 V) is located above the batteries and separated from them with a PVC platform (6 in Figure II.1B). Two oxymeters electronics (WTW microprocessor Oxymeter Oxi 196) which process the signal from the oxygen sensor are located on top of the data logger, also separated from it with a PVC platform (5 in Figure II.1B). The measurements of both oxymeters are recorded by the data logger. When the oxygen concentration change reach the determined value (in our case 20 %), the software automatically activated the flushing pump (Fig. II.2). The next measure after the 36 seconds flushing period becomes the initial concentration of reference.

The data logger, which has 8 input channels and 16 digital output channels, was connected to the electronics of both oxymeters as well as to the flushing pump (Fig II.2).


A
a
e
c
b
d
B
1a
1b
2
3
4
9
10
11
12
13
14
5
6
7
8
C
f

The analogue outputs of the oxygen and temperature meters were fed into a signal conditioner before go to the data logger where the signal is recorded (Fig. II.2). The data logger recorded the oxygen and temperature measurements of both chambers every 128 seconds. The data logger software made possible to determine the start time of measurement collection, the time interval between measurements (measuring rate), the percentage of oxygen concentration change from the initial concentration that determined the activation of the flushing pump, as well as the time period of flushing.

By means of both, a dye and controlling the oxygen values, it was estimated that the flushing pump could change the water inside both chambers in 22 seconds. In order to ensure a good renovation of all the water within the chambers, we programmed the length of the flushing in 36 seconds. This value can be modified through the software of the data logger.

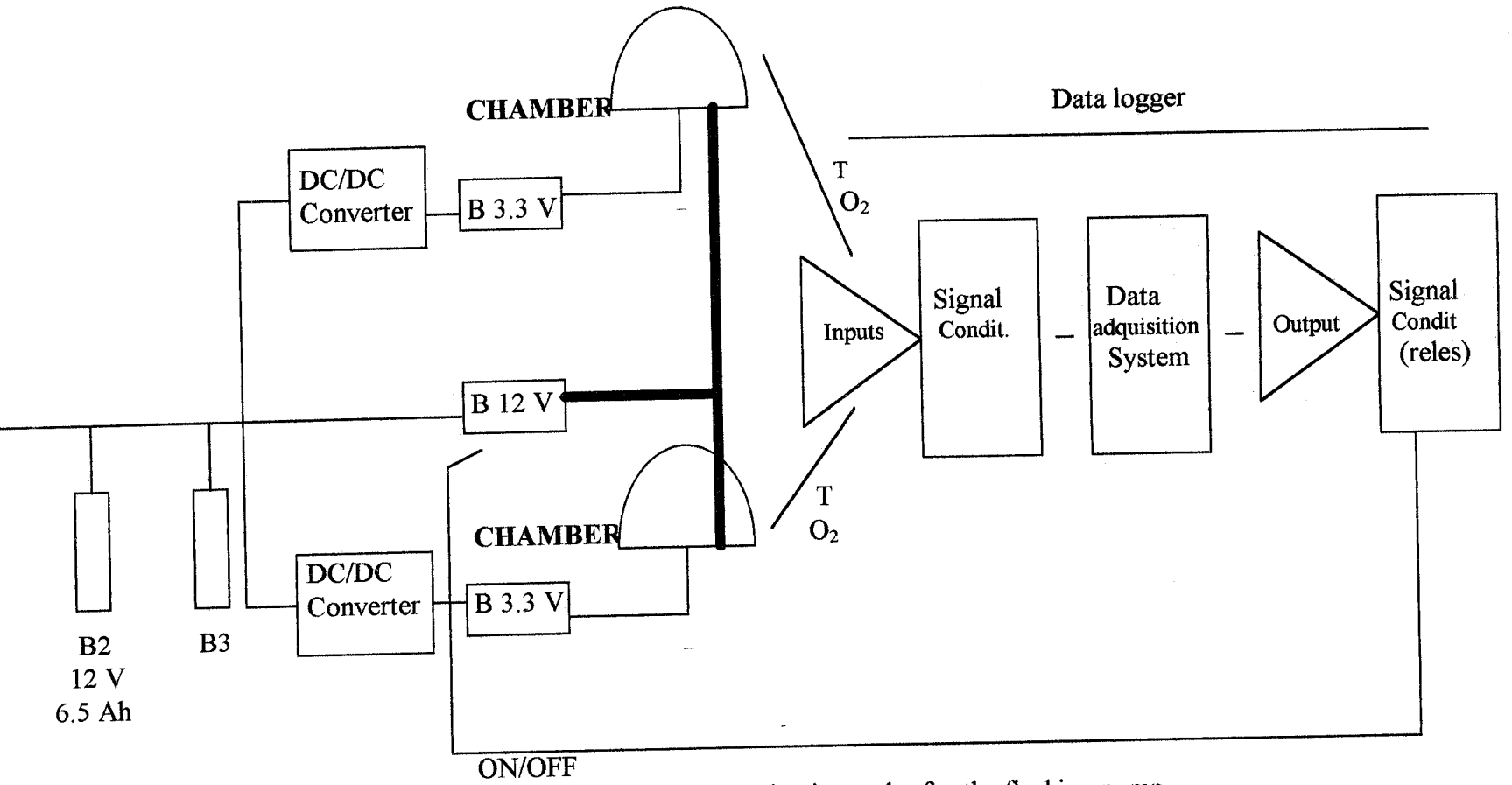
The upper side of the underwater housing is made of clear acrylic glass. Then, the panels of the oxymeter electronic units can be observed. This allowed to detect any problem in the functioning of the instrument during the experiments. The cover is sealed to the housing by a soft silicone rubber O-ring.

III. Application and Discussion

The chambers and the underwater housing were designed to be handle by scuba divers. The underwater housing with the sensors and pumps have a positive buoyancy of about 12 kg. A weight belt was used to provide an slightly negative buoyancy. Then, it was easily handle by a diver to the experimental place where it was attached to a 50 kg cement flat, allowing the *in situ* study of the organisms. The devise was also though to have an autonomy over 24 h that allowed to examine the potential variation in the respiratory activity along the daily cycle.

As application examples, the instrument was used to study respiration in three common Mediterranean benthic invertebrates species: the sponge *Dysidea avara*, the ascidian *Halocynthia papillosa* and, the gorgonian *Paramuricea clavata* (see Chapter 6 for details).

Figure II.1. A.- a: flow pump, b: Plexiglas piece, c: oxygen probe, d: comes from the outlet of the chamber, e: go to the inlet of the chamber. **B.-** 1a: experimental chamber, 1b: control chamber, 2: flow pumps (3V), 3: flushing pump (12V), 4: PVC piece connecting inlet and outlet apertures of the chambers, 5: oxymeters electronics, 6: data logger, 7: voltage-converter, 8: batteries, 9-13: waterproof electrical connectors, 14: ON/OFF switch. **C.-** f: valve.



Activation order for the flushing pump

Several problems had been described in relation to the use of closed systems to study metabolism such as the decline in oxygen concentration and the accumulation of metabolites, the formation of oxygen gradients as a result of water stagnation, and inconsistent respiration readings obtained in relation to experimental duration (Kamler 1969). However, several studies about the metabolism of benthic communities have optimized the use of closed systems providing an important tool for measuring oxygen concentration change *in situ* (Smith et al 1972, Zeitzschel and Davies 1978, Hall et al 1979, Svoboda and Ott 1983, Patterson et al 1991, Glud et al 1995). As in the mentioned works, we have described a system which solve the potential problems of a closed system. The formation of oxygen gradients have been solved with the continuous flow forced by both flow pumps which were continuously working. Oxygen concentration decrease which can affect the behavior of the organism (Herreid 1980) and the accumulation of metabolites were solved through the automatic flushing system which periodically renewed the water from both chambers, which can be modified depending of the studied species. We believe that this autonomous operating system is a useful tool to study metabolic activity of benthic organisms (both production and consumption of oxygen) under natural conditions.

We are conscious that an important parameter such as water movement which is know to affect aquatic organisms respiration (Boynton et al 1981, Patterson and Sebens 1989, Patterson et al 1991) have not been considered into account. All this work has been limited to a only flow speed which is rather low (1.2 cm s^{-1}). However, the described apparatus may allow to have several flow speeds by modifying the voltage converter. Then, with the actual set-up the system is reducing the 12 V of the general power supply to the 3 V of the flow pumps. However, the converter could easily provide a range of voltage to the pump from 3 to 12 V which will allow a range of flow speed.

Figure II.2. B1, B2, B3: batteries, DC/DC converter: voltage converter system, B 3.3V: flow pumps, B12V: flushing pump. Signal condit: conditioner of the analogue outputs.

CONCLUSIONS

CONCLUSIONS

Seasonal variation of POC and DOC

1.- All year long detrital organic carbon represented the main POC fraction, and mean live carbon was 24 ± 9 μg C l^{-1}.

2.- Winter and Spring were the seasons with maximum values of POC and Spring and Summer had the maximum values of DOC.

3.- Over the year, heterotrophic bacteria was the main contributor to live carbon (26 ± 7 %). But, seasonally the size composition of live carbon showed two clear periods: From December to March was dominated in biomass by microplankton while from April to November, pico and nanoplankton cells were dominant.

4.- The seasonal variation of water temperature and nutrient concentration were the environmental variables which better explained the changes observed in the contribution of the different planktonic groups to the pool of live carbon.

5.- The dynamics of the near-bottom planktonic communities was characterized by a low biomass in pico, nano, and microplankton components in contrast to previous water column studies. This pattern could be related to the physical and chemical characteristics of the environment as well as to the potential role that benthic organisms may be exerting in the control of the dynamic of the near-bottom planktonic communities.

Diet and feeding rates by the sponge *Dysidea avara*

6.- The diet of *D. avara* was highly heterogeneous including procaryotes (heterotrophic bacteria, *Synechococcus* sp., *Prochlorococcus* sp.) and eucaryotic cells (protozoa, phytoplankton and ciliates) with a size range from 0.5 ± 0.3 μm (heterotrophic bacteria) to 70 ± 0.3 μm (pennate diatoms).

7.- Procaryotes cells clearance rates were higher than that of the other groups, suggesting a higher grazing efficiency upon these prey types.

8.- Specific clearance rate showed a pattern of decrease with sponge size increase, although it did not varied with prey concentration, nor with temperature.

9.- Overall, procaryotes contributed to 74 ± 14 % of the total ingested carbon, pico and nanoeucaryotes contributed to 11 ± 3 %, and phytoplankton contributed to 11 ± 10 %.

10.- Partial contribution of the different groups varied along the year following the planktonic composition of the water column. Thus, during winter phytoplankton was an important component of the total uptake (26 %), whereas the rest of the year it contributed less to 7 % to the total uptake.

11.- The feeding plasticity of *D. avara* may represent an advantage for the species because it attenuates the effects of seasonal fluctuations in planktonic communities. This plasticity could be among the main factor that contribute to worldwide abundance and distribution of sponge.

Diet and feeding rates by the ascidian *Halocynthia papillosa*

12.- The natural diet of *H. papillosa* included detrital organic matter, heterotrophic bacteria, *Synechococcus* sp., *Prochlorococcus* sp., protozoa and phytoplankton with a size range from 0.6 ± 0.3 μm (heterotrophic bacteria) to 70 ± 22 μm (pennate diatoms).

13.- Specific clearances rates varied seasonally and exhibited a pattern of increase with temperature increase, in which temperature explained a 55 % of the variance in clearance rate along the year.

14.- Overall, a mean size *H. papillosa* specimen (0.3 g AFDW) was estimated to ingest an annual mean of 1305 ± 496 μg C g AFDW^{-1} h^{-1} and 84 ± 16 μg N g AFDW^{-1} h^{-1}. Carbon from detrital origin accounted for 92 ± 2 % of the total ingested carbon with the highest values in spring, while ingestion of live carbon accounted for 8 ± 2 % of the total ingested carbon with the highest values occurring during summer and fall.

15.- The seasonal variation of ingested nitrogen from live particles explained 91 % of the gonadal development variance along the year, suggesting that live particles are likely to be of more significance in the diet of the species than particles from detrital origin.

Diet and feeding rates by the gorgonian *Paramuricea clavata*

16.- The natural diet of *P. clavata* showed that the species significantly captured nanoeucaryotes, dinoflagellates and ciliates as ell as detrital particulate organic carbon. No significant decrease in prey items groups smaller than 3-4 μm was observed. This results together with a previous study of zooplankton capture rates shown that the specie face a wide spectrum of potential prey, which is in agreement with the capture mechanisms described by the aerosol filtration theory.

17.- *P. clavata* ingested an annual (mean ± SD) of 0.192 ± 0.175 μg C μg C^{-1} d^{-1} from the particles < 100 mm. Carbon from detrital origin accounted for 86 ± 14 % of the total

ingested carbon. Winter and spring were the seasons with the highest values of food intake. Phytoplankton was the main contributor to the live particles diet accounting for over 48 ± 6 % of the total ingested live carbon.

18.- The contribution of carbon ingested from particles < 100 μm was about half (46 ± 39 %) of that of the zooplankton reported in a previously study.

19.- Because the abundance of *P. clavata* in some Mediterranean sublittoral communities, it has been estimated than the species could daily remove between 1 - 22 % of diatoms, 1 - 9 % of nanoeucaryotes, 1 - 26 % of the dinoflagellates, and 2 - 99 % of ciliates per m^3 of water adjacent to the bottom.

Respiration and energy surplus of three species of benthic suspension feeders

20.- Respiration rates of the three species over the year showed a marked seasonal pattern. Metabolic rates of both active suspension feeders (sponge and ascidian) appeared to be markedly affected by temperature changes. In contrast, respiration rates of the passive suspension feeder (gorgonian) did not exhibited any significant response to the temperature changes in the field.

21.- The seasonal pattern in respiration rates of the gorgonian and the ascidian species followed the seasonal reproduction patterns. This results point out that although a positive relationship exist between respiration rate and temperature, the production can also explain an important fraction of the observed seasonal patter in the respiration.

22.- The G/C index evolution over the year showed that winter and spring were the most favorable periods for investment in secondary production for the sponge. Late spring and fall for the ascidian and winter for the gorgonian. However, preliminary data on the lipid contents of the gorgonian specie showed an important decrease in lipids during summer and fall. Thus during winter the specie could store lipids which could be used like fuel energy demands when food would be scarce.

23.- The relationship between the G/C index and the abundance of resources exhibited a clear pattern, although it differed among the species. The G/C index of the ascidian did not exhibited any significant relationship with resource abundance. In contrast, the G/C index of the gorgonian and sponge species showed that it was depended on resource abundance.

24.- The results of both the sponge and the gorgonian species are consistent with the hypothesis that a trophic-energetic phenomenon may be underlying the seasonal dynamic of benthic suspension feeders in the Mediterranean. The ascidian species appears to be an

exception for which other factors rather than food concentration appears to be determining the seasonal dynamic of the species.

Heterotrophic feeding in tropical gorgonian corals

25.- Mean zooplankton capture rates by *Plexaura flexuosa* and *Pseudoplexaura porosa* was 0.09 and 0.23 prey polyp^{-1} d^{-1}. Moreover, *P. flexuosa* captured 7.2 ± 1.9 microorganisms polyp^{-1} d^{-1} including ciliates dinoflagellates and diatoms, but they did not appear to graze significantly on organisms smaller than 5 mm.

26.- Zooplankton and microbial prey accounted for only 0.4 % of respiratory requirements in *P. flexuosa*, but they contributed 17 % of nitrogen required annually for new production (growth and reproduction).

27.- Although the contribution of microbial prey to gorgonian energetics was low, dense gorgonian populations found on many Caribbean reefs may implies a daily removal of 9 % of the diatoms, 10 % of the dinoflagellates and 11 % of the ciliates of the water mass within 1 m of the reef. This suggest that gorgonian populations may be important grazers of plankton communities in this zones.

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RESUM DE LA TESI

DIETA I TAXES D'ALIMENTACIÓ DE SUSPENSÍVORS BENTÒNICS MARINS EN RELACIÓ ALS SEUS REQUERIMENTS METABÒLICS I A LA COMPOSICIÓ DEL SESTON

I. INTRODUCCIÓ

Els mars temperats càlids com la Mediterrània ofereixen als organismes que l'habiten un ambient estacionalment variable en factors tant físics com biologics. Seguint una aproximació energètica (quantificació de les entrades i sortides d'energia), s'ha plantejat estudiar quin era l'efecte de l'estacionalitat observada en factors com la disponibilitat d'aliment i la temperatura de l'aigua en el balanç metabòlic d'algunes espècies marines. Els organismes objecte d'estudi tenen com a característica comuna el fet de ser sèssils i per tant tròficament dependents de l'aliment que arriba a les seves estructures capturadores. A més també tenen en comú l'estratègia per capturar l'aliment així tots son suspensívors. L'estratègia suspensívora es àmpliament difosa en els organismes bentónics aquàtics els quals han d'obtenir els seus recursos alimentaris en un ambient on les partícules es troben en suspensió i normalment molt diluïdes. A més les preses potencials tenen un ampli ventall de talles des de menys d'una micra com els bacteris fins a centenars de micres com el zooplancton. En aquest estudi es centrarà l'atenció en els suspensívors bentònics que viuen en la zona de l'infralitoral rocós i on es troben representats grups tan diversos com els mol.luscs, celenteris, tunicats, esponges i briozous.

L'hipòtesi de partida va sorgir a partir d'una sèrie d'estudis previs on es feia palès que la dinàmica en la producció secundaria (Creixement i reproducció) i també l'activitat d'alguns suspensívors bentònics mediterranis era clarament estacional (Llobet et al 1991, Coma et al 1998, Becerro & Turon 1992, Garrabou 1997, Boero & Fresi 1986). La majoria d'aquest treballs varen hipotetitzar que la dinàmica observada en aquelles espècies podria estar directament relacionada amb la disponibilitat d'aliment. De fet les primeres evidències directes de restriccions tròfiques en la dinàmica d'alguns suspensívors bentònics varen ser aportades seguint una aproximació energètica, o sigui, avaluant les entrades (aliment) i les sortides (creixement, reproducció, respiració i excreció) (Coma et al 1998). En aquest estudi es va proposar l'estiu com una estació energèticament desfavorable en la Mediterrània per les dues espècies de cnidaris estudiades. Esperonats per l'idea d'aportar més evidencies que consolidessin o no l'hipotesi d'una crisi trófica estival pels organismes bentònics a la Mediterrània, ens intentàrem complementar l'estudi de Coma et al (1998) ampliant el rang de preses estudiades així com incrementant el nombre d'espècies.

El plantejament de partida era continuar amb una aproximació energètica tot i que d'una manera mes simplificada degut a la laboriositat de l'estudi de tots els components de l'equació del balanç energètic. D'aquesta manera ens vàrem proposar l'estudi de les entrades energètiques, o sigui avaluació de la dieta i taxes d'alimentació i també de la respiració com a representant de les sortides o pèrdues. Amb l'avaluació d'aquest dos paràmetres podríem calcular l'excedent energètic disponible per nova producció ja fos somàtica, reproductora. Segons aquest plantejament i si es complia l'hipòtesi de crisis tròfica a l'estiu, s'hauria d'observar uns mínims en els excedents energètics en aquesta època degut tant a una

disminució en l'ingesta com a un suposat increment en la respiració per les altes temperatures en aquesta època de l'any. Òbviament, degut al plantejament més ecològic que fisiològic d'aquest estudi, un dels objectius principal fou assegurar amb la màxima fidelitat possible les condicions ambientals a les que estaven sotmesos els organismes, i per tant es feia obligat un estudi in situ. Les espècies escollides per dur a terme aquest estudi pertanyen a grups filètics diferents de cara a avaluar quin era l'abast en l'efecte de la hipotètica crisi tròfica estival. Els organismes objecte d'estudi varen ser l'esponja *Dysidea avara*, l'ascidi *Halocynthia papillosa* i com s'ha assenyalat abans, de cara a completar els coneixements previs, la gorgonia *Paramuricea clavata*.

Si be l'objectiu general d'aquesta tesi era el d'aportar noves evidencies o excepcions a l'hipòtesi tròfica estival pels organismes suspensívors mediterranis, hi van haver tota una sèrie d'objectius concrets previs i necessaris abans de la generalització. Un primer objectiu va ser la posta a punt d'una metodologia que permetés l'estudi in situ tant de l'alimentació com de la respiració de suspensívors bentònics. Aquest objectiu metodològic va incloure el disseny i posta a punt de nous aparells així com la utilització de tècniques de laboratori ja conegudes per la quantificació dels grups del seston que es pretenien estudiar. Un segon objectiu va incloure la descripció i quantificació estacional de les possibles presses disponibles pels suspensívors objecte d'estudi en la massa d'aigua propera al fons i en contacte amb ells. Un tercer objectiu va ser l'estudi de la dieta natural (tipus de preses capturades) i la seva quantificació en termes de carboni així com les taxes d'ingesta al llarg d'un cicle anual. Un quart objectiu incloïa un seguiment de les taxes respiratòries al llarg de l'any.

Fora dels objectius concrets i general de la tesi i de cara a mostrar la versatilitat de la metodologia utilitzada, es va dur a terme un estudi puntual en una espècie de suspensívor tropical, la gorgonia *Plexaura flexuosa* molt abundant en el carib de Panamà. A més l'interès en l'estudi d'aquesta espècie venia donat per una estratègia tròfica alternativa a la gorgonia mediterrània estudiada, l'autotrofia. La gran quantitat d'algues simbionts que presentava l'espècie tropical així com la controvèrsia de l'autotrofia-heterotrofia com a mecanismes d'obtenció d'energia en suspensívors tropicals, ens va animar a dur a terme aquest estudi.

II. MATERIAL I MÈTODES

Lloc d'estudi

La major part d'aquest treball s'ha dut a terme en una estació costanera en la Mediterrània Nord-Occidental (42°, 3'N, 3° 13'E) dins la reserva marina de les Illes Medes (Fig 1). L'estudi de *Plexaura flexuosa* i *Pseudoplexaura porosa* va tenir lloc majoritàriament a l'arxipèlag de San Blas (Panamà) prop de l'estació de camp de l'Institut

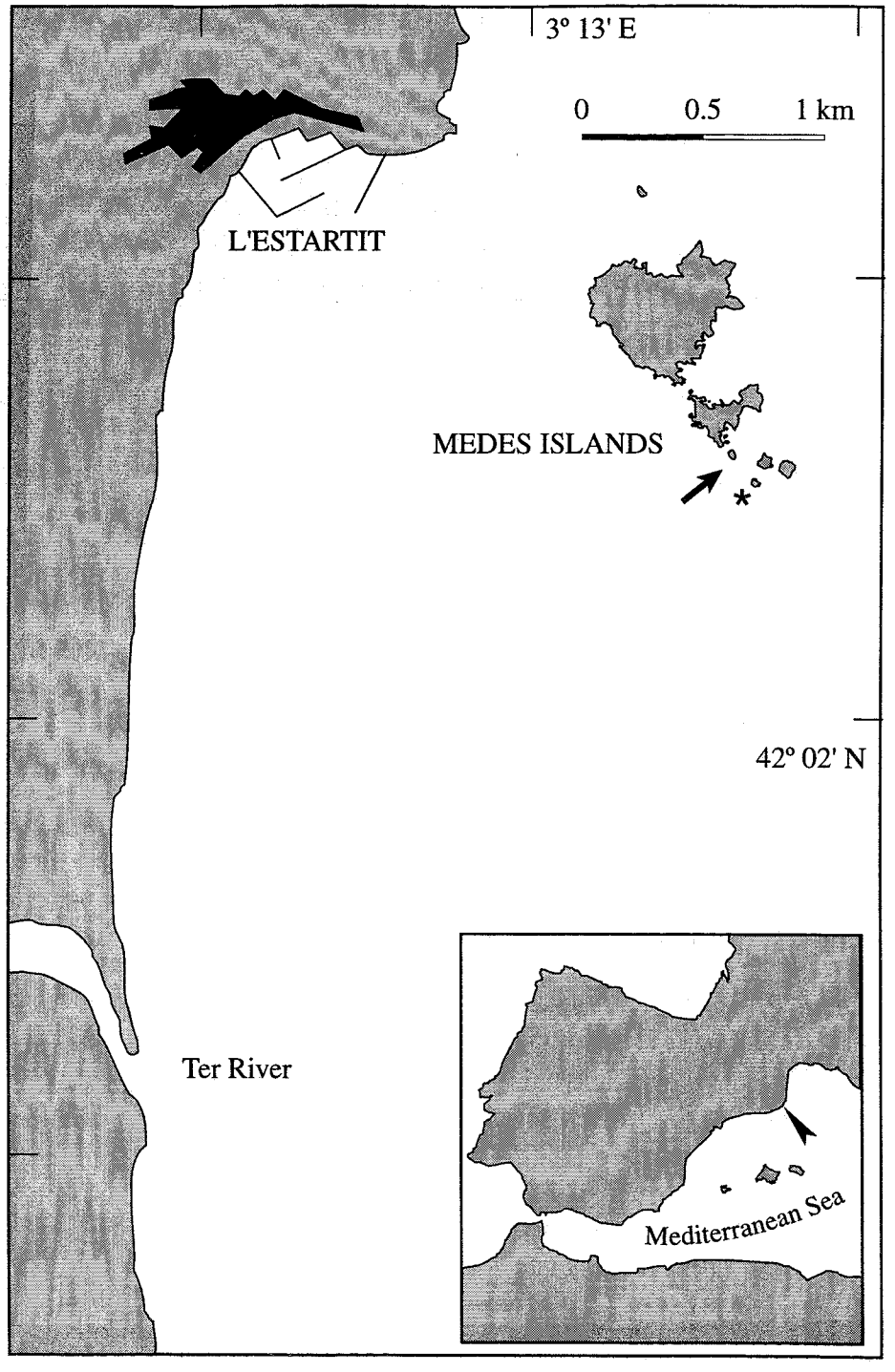

Fig. 1 Mapa de la zona d'estudi. (Illes Medes, Mediterrània nord-occidental). Fletxa i asterisc mostren la zona de mostreig i la zona de seguiment de la temperatura respectivament.

Smithsonian d'estudis tropicals (Brazeau and Lasker 1992). A més algunes mostres complementaries de *P. porosa* foren agafades a Florida (Escull de Pickles: 24 ° 59'N, 80° 24'W, Escull de Conch: 24 ° 57' N, 80° 27'W).

Estudi de l'alimentació

L'estudi de la captura de preses del pico- nano- i microplancton així com del carboni particulat detritic i el carboni dissolt, per part de les espècies de suspensívors estudiades, es va dur a terme amb l'utilització de campanes incubadores de circuit tancat i amb un flux constant. Les campanes incubadores (una experimental amb organisme i una altra control) estaven fetes de metacrilat i tenien un volum aproximat de 3 litres. Mitjançant unes bombes submergibles alimentades per bateries tancades dins d'una caixa estanca, es va poder mantenir un flux constant dins les campanes al llarg dels experiments. Prèviament als experiments d'alimentació (entre 3 i 4 setmanes abans), els organismes eren arrancats del seu substrat natural i col·locats en un suport artificial que permetia tornar-los a fixar al seu habitat natural de manera remobible. A l'inici de cada experiment, un individu prèviament preparat sobre el suport artificial era col.locat sobre la base de la campana experimental. Tot seguit s'obtenia una mostra d'aigua de l'interior de les dues campanes (mantenint el flux obert per evitar el buidament parcial de la campana) (mostra inicial) i després d'un temps d'incubació variable segons l'espècie en la que el circuit es mantenia tancat, es tornava a agafar una altra mostra d'aigua (mostra final). La taxa d'aclariment i l'ingesta es varen calcular a partir de la caiguda en la concentració de preses en la campana experimental relativa a la campana control. Els grups de preses considerades com a font potencial d'aliment pels organismes estudiats incloïen procariotes (bacteris heterotròfics, cianobacteris i proclorofits), pico- i nano- eucarionts, ciliats, fitoplancton, carboni particulat detritic i carboni dissolt.

Per a la quantificació del nombre de preses ingerides i la seva contribució en termes de carboni, es van seguir una sèrie de protocols de laboratori que incloen la citometria de flux per a la numeració de procariotes pico i nano- eucarionts, microscopia d'epifluorescencia i anàlisi d'imatges per a la mesura dels seus tamanys, microscopi invertit per la numeració de fitoplancton i ciliats i anàlisi d'imatges per a la mesura dels seus tamanys, autoanalitzador C:H:N per a la quantificació del carboni particulat detrític i un autoanalitzador TOC per a la quantificació del carboni dissolt. La microscopia electrònica d'escandillatge s'ha utilitzat en alguns casos per buscar evidències directes en la captura d'algun tipus concret de presa.

Estudi de la respiració

Per l'estudi de la respiració es va dissenyar i posar a punt un prototipus de respiròmetre que inclou, apart del sistema de campanes i bombes utilitzat pels experiments

d'alimentació, tot un dispositiu localitzat dins d'una caixa estanca, i per tant submergible, que conté, un parell d'oxímetres amb sensor de temperatura, una tarja electrònica que funciona com a magatzem de dades i com a suport del "software" i unes bateries que alimenten tot el sistema. A través d'un programa informàtic dissenyat per aquest treball es va poder dur a terme un seguit de mesures continuades de la concentració d'oxígen i temperatura en les dues campanes (experimental i control). Cada experiment de respiració incloïa un cicle de 24 hores on la tarja anava recollint les dades de concentració d'oxígen i temperatura aproximadament cada dos minuts, per separat per les dues campanes. L'activació (a través de la tarja) d'una tercera bomba connectada a les dues campanes quan la concentració d'oxigen disminuïa per sota d'un determinat valor respecte el valor inicial en la campana experimental, va evitar el problemes d'anoxia i acúmul de productes d'excreció inherents a les incubacions en circuits tancats.

Amb les dades de ingesta i respiració en termes de carboni, al llarg d'un cicle estacional i per cadascuna de les espècies mediterrànies es va calcular l'excedent d'energia disponible pel creixement somàtic i/o reproductor. Això ens va permetre comparar si els màxims en els excedents d'energia coincidien amb estudis previs fets sobre la producció secundaria d'algunes espècies. Els resultats obtinguts varen ser discutits en funció de l'evolució del contingut tròfic de l'aigua en la zona estudiada al llarg d'un cicle anual.

III. RESULTATS

Variació estacional del carboni dissolt i particulat (viu i detrític)

El promig anual de DOC i POC en la massa d'aigua propera al fons i en contacte amb els suspensívors bentònics estudiats va ser de 2560 ± 180 (SE) μg C l^{-1} i 387 ± 35 (SE) μg C l^{-1} respectivament (Fig 2). Durant tot el cicle anual, el carboni orgànic d'origen detrític (detritus = POC total - Carboni viu), va ser el principal component del total de Carboni orgànic. El Carboni viu va presentar un promig anual de 24 ± 9 μg C l^{-1} (Fig. 2). L'hivern i la primavera varen ser les estacions amb els màxims valors de POC i la primavera i l'estiu els màxims valors de DOC. Els bacteris heterotròfics, amb una abundància promig de 5.16 ± 0.08 10^4 cel.lules ml^{-1}, foren el grup que majoritàriament va contribuir a la fracció del Carboni viu (26 ± 7 %). Durant l'hivern, els bacteris heterotròfics varen disminuir la seva biomassa en un 40 % degut al decrement en el seu biovolum promig per cèl.lula (Fig. 2). Les abundàncies de *Synechococcus* sp i *Prochlorococcus* sp foren de 2.24 ± 0.09 10^4 cèl.lules ml^{-1} i 1.05 ± 0.07 10^4 cèl.lules ml^{-1} respectivament. Però, mentre que els *Synechococcus* sp foren presents durant tot l'any a la columna d'aigua, els *Prochlorococcus* sp no varen ser detectats des d'Abril a Juliol (Fig. 3). L'abundància promig de fitoplancton (diatomees i dinoflagelades) fou de 2.06 ± 0.40 10^4 cèl.lules l^{-1} i la seva biomassa va arribar

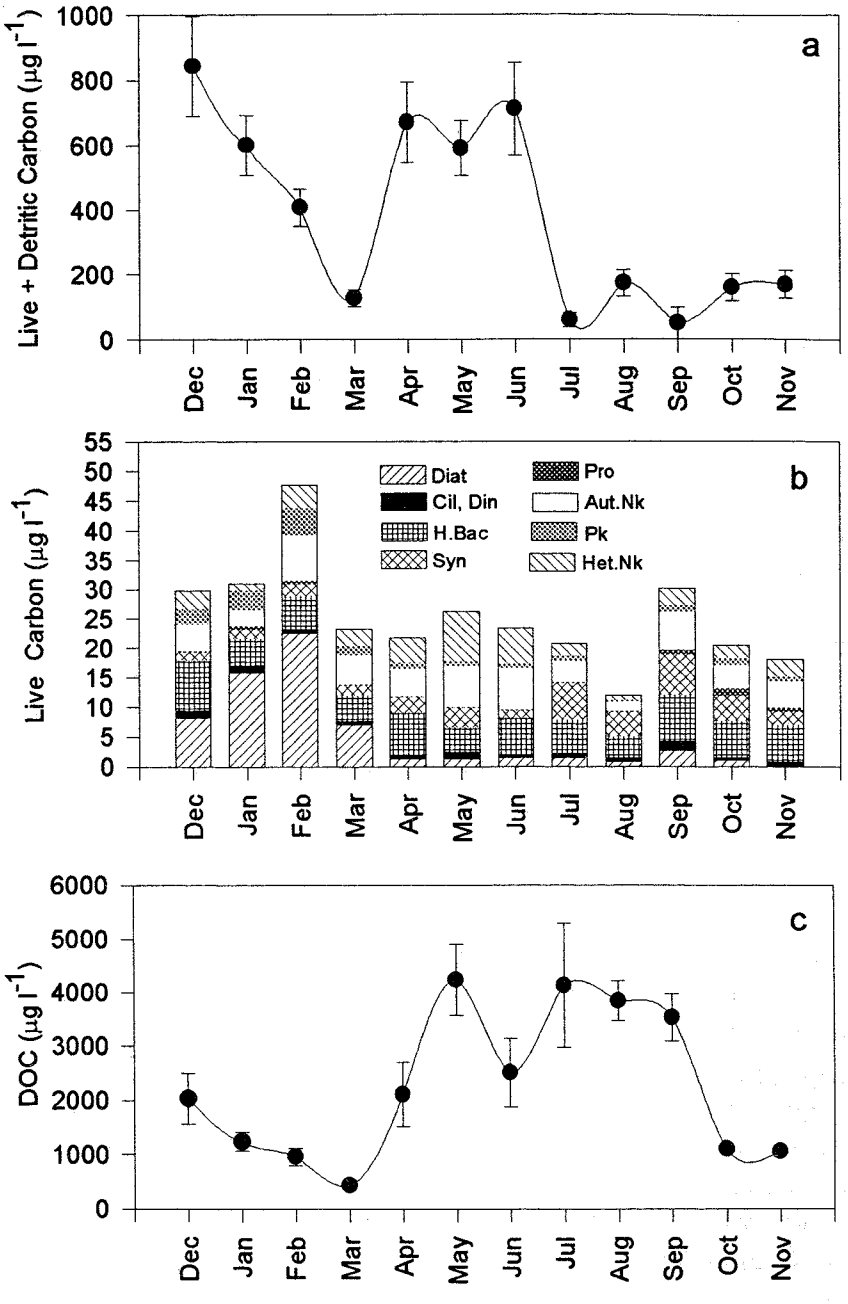

Fig. 2 Dades mensuals de µg C l^{-1} de a) Carboni viu i detritic a partir de l'anàlisi C:H:N, b) Carboni viu a partir del comptatge de cèl.lules i la composició en: Het. Nk: nanoeucarionts heterotròfics, Pk: picoeucarionts, Aut Nk: nanoeucarionts autotròfics, Pro: *Prochlorococcus*, Syn: *Synechococcus*, H.Bac: Bacteris heterotròfics, Din: dinoflagelades, Cil: ciliats, Diat: diatomees, c) Carboni orgànic disolt.

als màxims valors durant els mesos d'hivern coincidint amb mínimes temperatures i màxima concentració de nutrients (Fig. 4). La composició de talles del Carboni viu va mostrar dos períodes clarament diferents durant l'any. Des de Desembre fins a Març, el Carboni viu va ser dominat en biomassa pel microplancton, mentre que d'Abril a Novembre varen dominar el pico- i nanoplancton.

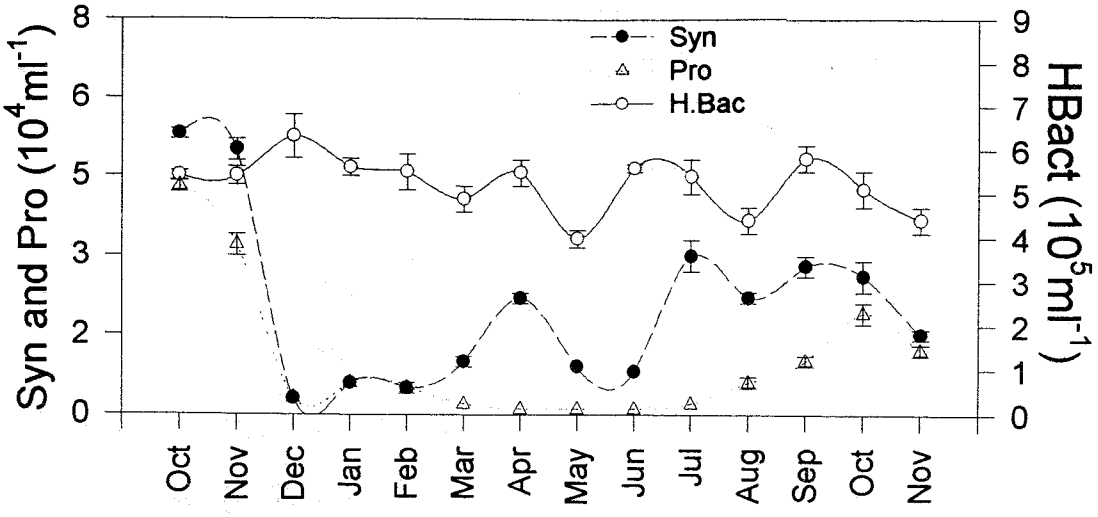

Fig. 3 Dades mensuals de la concentració (cèl.lules ml^{-1}) de bacteris heterotrofics (H Bact), *Synechococcus* (Syn) i *Prochlorococcus* (Pro).

Dieta i taxes d'ingestió de l'esponja *Dysidea avara*

L'estudi estacional in situ de la dieta i taxes d'aclariment de l'esponja *D. avara* mostren una considerable heterogeneïtat en el tipus de presa capturada que inclou: bacteris heterotròfics, *Prochlorococcus* sp, *Synechococcus* sp, pico- i nanoeucarionts, fitoplancton i ciliats. Aquestes preses es troben dins d'un rang de talles des de 0.5 ± 0.3 μm (bacteris heterotròfics) fins a 70 ± 0.3 μm (diatomees pennades). La taxa d'aclariment pel grup dels procariotes va ser superior a la dels altres grups, suggerint una major eficiència en la captura per aquest grup. La taxa d'aclariment per unitat de biomassa va decréixer en funció de la talla de l'esponja altrament no va variar en funció de la concentració de preses ni amb la temperatura. El grup dels procarionts va contribuir en un 74 ± 14 % del total del Carboni ingerit per l'esponja, els pico- i nanoeucariotes en un 11 ± 3 % i el fitoplancton en un 11 ± 10 %. Segons això *D. avara* va obtenir un 85 % del Carboni ingerit de la fracció del plancton més petita de 5 μm mentre que la fracció més gran de 5 μm va contribuir en un 15 % (Taula 1). Aquesta contribució parcial dels diferents groups al Carboni ingerit per l'esponja va variar al llarg de l'any en funció de la composició planctónica a la columna

d'aigua. Durant l'hivern el fitoplancton va aportar una important quantitat de Carboni (26 %) mentre que la resta de l'any tant sols ho va fer en un 7 % (Taula 1).

Dieta i taxes d'ingestió de l'ascidi *Halocynthia papillosa*

Els resultats de l'estudi estacional de la dieta i taxes d'aclariment de l'ascidi *H.*

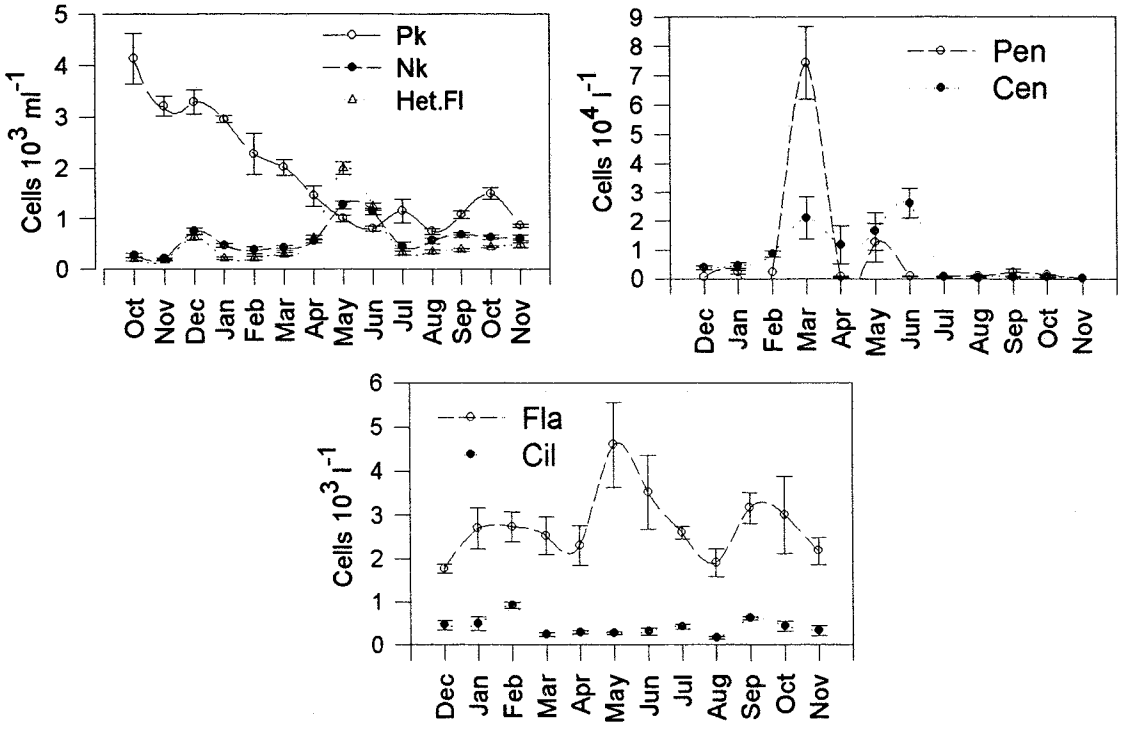

Fig. 4 *Halocynthia papillosa*. Dades d'abundancia de picoeucarionts (Pk), nanoeucarionts autotròfics (Nk), nanoeucarionts heterotròfics (Het. Fl), diatomees cèntriques i pennades (Cen, Pen), dinoflagelades (Fla), Ciliats (Cil).

papillosa mostra que aquesta espècie captura grans quantitats de detritus, bacteris heterotròfics, *Prochlorococcus*, *Synechococcus*, pico- i nanoeucarionts fitoplancton i ciliats. Aquesta varietat de preses inclou un rang de talles des de 0.6 ± 0.3 mm (bacteris heterotròfics) fins 70 ± 22 μm (diatomees penades). Les taxes d'aclariment per unitat de biomassa varen variar estacionalment i mostren un patró d'increment juntament amb afectar la taxa d'aclariment de l'ascidi. El carboni ingerit per l'espècie va presentar marcades diferencies estacionals, amb una màxima ingesta de detritus durant la primavera mentre que el carboni viu va presentar els seus màxims de captura durant l'estiu i la tardor (Fig. 5). En promig, els espècimens de *H. papillosa* utilitzats en els

Taula 1. Estima de la taxa d'ingestió (μg C g AFDW^{-1} h^{-1}) de *Dysidea avara*. Els valors han estat estimats a partir dels extrems de talles trobades durant els experiments (0.2 - 1.6 g AFDW). % diet es la composició del carboni ingerit i % plankton es la proporció del carboni viu en la columna.

	SUMMER			FALL			WINTER			SPRING		
	Ingestion	% diet	% plankton	Ingestion	% diet	% plankton	Ingestion	% diet	% plankton	Ingestion	% diet	% plankton
g AFDW	0.2 - 1.6			0.2 - 1.6			0.2 - 1.6			0.2 - 1.6		
Het B	76 - 4.30	43	32	60 - 3.96	33	33	59 - 3.90	35	19	63 - 3.50	35	24
Pro	2 – 0.10	1	1	9 - 0.60	5	4	1 - 0.04	0,4	1	1 - 0.02	0,2	1
Syn	74 - 4.20	42	25	86 - 5.64	47	20	37 - 2.42	22	4	57 - 3.20	32	14
Pic	2 - 0.10	1	4	2 - 0.12	1	5	3 - 0.22	2	9	2 - 0.10	1	5
Nan	11 - 0.60	6	28	15 - 0.96	8	25	20 - 1.32	12	14	22 - 1.20	12	33
Diatoms	12 - 0.70	7	7	7 - 0.48	4	7	44 - 2.86	26	49	11 - 0.60	6	18
Din	0.2 - 0.01	0,1	1	1 - 0.04	0,3	1	1 - 0.04	0,4	1	2 - 0.10	1	1
Cil	2 - 0.10	1	2	2 - 0.12	1	4	2 - 0.11	1	3	23 - 1.30	13	4
TOTAL	176 - 11			183- 12			169 - 11			180 - 10		

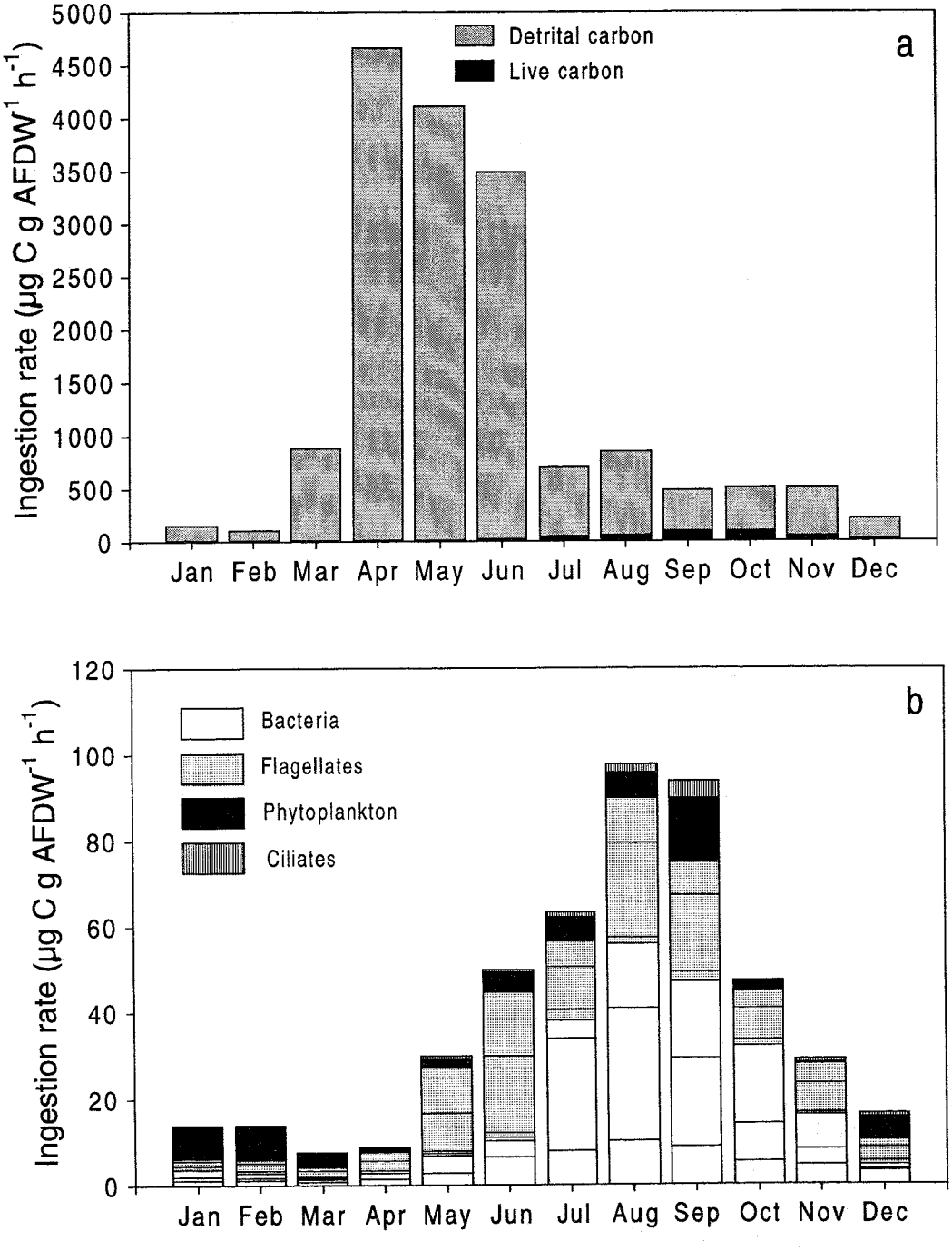

Fig. 5. Carboni orgànic ingerit (µg C g AFDW^{-1} h^{-1}) per *Halocynthia papillosa* al llarg d'un cicle anual. a)- Carboni ingerit en forma de detritus i viu. b)- Composició del carboni viu ingerit: Bacteria inclou bacteris heterotròfics, *Synechococcus* sp and *Prochlorococcus* sp; flagellates inclou picoeucarionts i nanoeucarionts i phytoplankton inclou diatomees i dinoflagelades.

experiments varen presentar una taxa d'ingestió de 1305 ± 496 μg C g AFDW^{-1} h^{-1} i uns 84 ± 16 μg N g AFDW^{-1} h^{-1}. El Carboni d'origen detrític va representar un 92 ± 2 % del total ingerit mentre que el viu va representar un 8 ± 2 % amb els màxims valors durant els mesos d'estiu i tardor. Per altra banda, la variació estacional del nitrogen ingerit a patir de cèl.lules, va explicar un 91 % de la variança al llarg de l'any en el desenvolupament gonadal. La comparació en les taxes d'alimentació amb el contingut tròfic de la columna d'aigua no varen mostrar cap mena de correlació.

Dieta i taxes d'ingestió de la gorgonia *Paramuricea clavata*

L'estudi de la dieta natural de la gorgonia mediterrània *P. clavata* mostra que aquesta espècie pot capturar preses d'un tamany superior a les 4 μm, això inclou

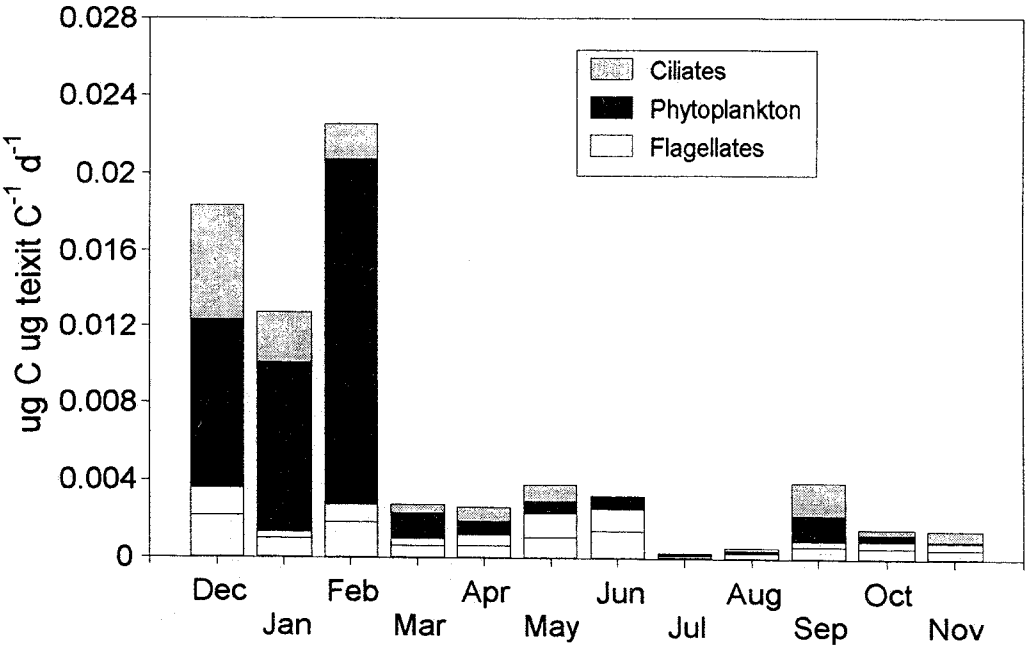

Fig. 6. Carboni orgànic ingerit (μg C g AFDW^{-1} h^{-1}) per *Paramuricea clavata* al llarg d'un cicle annual. Composició del carboni viu ingerit.

nanoeucarionts, diatomees, dinoflagelades i ciliats així com també partícules de detritus. Les taxes d'aclariment estimades per l'espècie varen mostrar una marcada estacionalitat, amb el màxims valors durant l'hivern coincidint amb la màxima concentració de detritus i fitoplancton. Altrament ni la temperatura ni la talla de les colònies varen explicar un percentatge significatiu de la variança observada en les taxes d'aclariment. Les taxes d'ingesta, calculades sempre amb els pòlips oberts, varen ser corregides pel ritme d'activitat

de l'espècie (obertura i tancament dels pòlips) el qual presenta una marcada pauta estacional. Així un pòlip de P. clavata pot capturar entre 31 - 794 cèl.lules per dia (promig ± DE, 276 ± 225) inclouen nanoeucarionts, dinoflagelades, diatomees i ciliats. En termes de Carboni ingerit, la gorgonia pot capturar un promig anual (mitjana ± DE) de 0.192 ± 0.175 μg C μg C^{-1} d^{-1} de la fracció sestònica inferior a les 100 μm (Fig 6). D'aquesta quantitat, les partícules de detritus varen aportar un 84 ± 14 % amb els màxims valors durant l'hivern i la primavera. Les cèl.lules de fitoplancton varen ser els principals contribuïdors en el carboni viu ingerit (48 ± 6 %) amb les màximes captures durant l'hivern (Fig. 6). La quantitat de Carboni ingerida a partir de partícules < 100 μm, va ser aproximadament la meitat (promig anual 48 ± 6 %) de la que l'espècie obté capturant zooplancton (dades de Coma et al 1994). Degut a l'estacionalitat en l'ingesta de detritus, durant l'hivern i la primavera la fracció estudiada en aquest treball va representar un 73 ± 28 % del carboni ingerit a traves del zooplancton. Una comunitat de P. clavata amb les densitats trobades en la Mediterrània, poden buidar diàriament entre 1-22 % de les diatomees, 1-9 % dels nanoeucarionts, 1-26 % de les dinoflagelades i un 2 - 99 % del ciliats en un m^3 d'aigua proper al fons.

Taxes de respiració i excedent d'energia

Les taxes de respiració de les tres espècies mediterrànies (*D. avara*, *H. papillosa* i *P. clavata*) estudiades a partir de cicles diaris, no varen presentar variacions al llarg de les 24 hores i per tant no es va detectar cap patró dia-nit en el consum d'oxigen. La taxa de respiració per a les tres espècies no va variar entre dies dins del mateix mes però si entre diferents mesos. La temperatura va explicar un percentatge significatiu de la variança trobada en l'esponja (r^2=0.91, n= 142, p<0.0001) i en l'ascidi (r^2 = 0.66, n = 140, p < 0.0001) però no en la gorgonia. L'esforç reproductor (només conegut per l'ascidi, dades de Becerro i Turon 1992, i per la gorgonia, dades de Coma i col·laboradors 1995) va explicar en ambdós casos un percentatge significatiu de la variança (r^2=0.61, n=12, p=0.027 per l'ascidi, r^2=0.96, n=12, p=0.0001). La taxa de respiració quan la gorgonia presentava els pòlips oberts fou d'un 26 % més que quan estaven tancats (ANOVA $F_{(1, 46)}$ = 4.03, p < 0.05). Els càlculs previs del carboni ingerit per les tres espècies juntament amb els valors de respiració aportats en aquest capítol varen permetre el càlcul del quocient Guany/Cost (G/C). L'evolució d'aquest quocient al llarg de l'any va mostrar els màxims valors durant l'hivern i la primavera per l'esponja, i durant l'hivern per la gorgonia. Altrament, l'anomenat quocient va aconseguir els seus valors més alts durant l'estiu per l'ascidi .El quocient G/C va mostrar una correlació positiva amb les variacions del contingut de Carboni a l'aigua al llarg de l'any en el cas de l'esponja i la gorgonia però no en el cas de l'ascidi. L'anàlisi del contingut de lípids totals en el teixit de *P.clavata* en les quatre estacions de l'any (10 branques de colònies amb menys de 10 cm d'alçada en cada estació), mostra una caiguda gradual en el contingut de lípids des dels màxims a l'hivern fins uns mínims a la tardor.

Dieta i taxes d'ingestió de les gorgònies tropicals *Plexaura flexuosa* i *Pseudoplexaura porosa*

La captura de zooplancton per part de les dues gorgonies tropicals, *P. flexuosa* i *P. porosa* estudiada a traves de l'anàlisi de continguts estomacals va mostrar que les dues espècies capturaven preses dins del rang de talles de 100 a 700 μm amb una taxa de captura de 0.09 i 0.23 preses $polip^{-1}$ d^{-1} respectivament. *P. porosa* va obtenir una major quantitat de carboni a partir d'aquestes preses (3.4×10^{-5} mg C $polip^{-1}$ d^{-1}) degut a un major nombre de preses capturades i a un major tamany d'aquestes en comparació a *P. flexuosa*. *P. flexuosa* va capturar un promig de 7.2 ± 1.9 microorganismes $polip^{-1}$ d^{-1} els quals incloïen només cèl.lules mes grans de 5 μm, bàsicament fitoplancton i ciliats. En *P. flexuosa*, el carboni ingerit en forma de zooplancton i microorganismes va cobrir tan sols un 0.4 % dels requeriments metabòlics de l'espècie calculats a partir del consum d'oxigen. D'altra banda, aquestes preses varen contribuir en un 17 % del nitrogen que l'espècie requereix per nova producció (reproducció i creixement somàtic). Degut a l'alta densitat de gorgonies que es troba en els esculls del carib, l'impacte de predació d'aquestes sobre les comunitat microbianes de la zona es va calcular que era al voltant d'un 10 % de cèl.lules capturades diàries en un m^2 d'escull.

IV. DISCUSSIÓ I CONCLUSIONS

Variació estacional del carboni dissolt i particulat (viu i detrític)

La major part del carboni orgànic en la massa d'aigua propera al fons estudiada es d'origen detrític fet que es relaciona tan amb el capdal del riu Ter proper a la zona com també amb la producció de les macroalgues. Tot i les grans quantitats de DOC i POC en la massa d'aigua propera al fons si es compara amb la resta de la columna d'aigua, la concentració de cèl.lules de les comunitats microbianes no varen mostrar un increment paral·lel. Aquest patró pot estar relacionat a les característiques fisiques i químiques de l'ambient així com al paper de controladors d'aquestes poblacions que podrien estar exercint les comunitats bentònics de la zona.

La variació estacional de la temperatura de l'aigua i de la concentració de nutrients foren els factors ambientals que millor van explicar els canvis observats en la contribució dels diferents grups planctònics al carboni viu de l'aigua. La menor biomassa de bacteris heterotròfics trobada en la massa d'aigua propera al fons respecte a la trobada en la columna d'aigua en una estació costanera propera va ser bàsicament deguda a un menor biovolum per cèl.lula en la primera. Les baixes temperatures durant els mesos d'hivern sembla ser el principal factor que regula els menor biovolum per cèl.lula trobat..

Dieta i taxes d'ingestió de l'esponja *Dysidea avara*

Tot i que l'habilitat de les esponges per capturar cèl.lules inferior a les 2 μm ha esta àmpliament demostrada, hi ha menys dades disponibles respecte al paper de les preses més grans en la dieta d'aquests organismes. *D. avara* va mostrar una amplia heterogeneïtat en el tipus de preses capturades incloguen cèl.lules mes grans de 2 μm tot i que les talles mes petites son mes eficientment capturades. La menor taxa d'aclariment en incrementar el tamany de la colònia ha estat interpretat com un decrement en el nombre de coanocits actius en esponges més grans.

L'heterogeneïtat en la dieta de l'esponja i la seva capacitat de capturar un ampli rang de preses l'hi va permetre mantenir una taxa d'ingesta de Carboni constant al llarg de l'any. Això es avantatjós per l'espècie ja que atenua els efectes dels canvis estacionals en la composició del plàncton. Aquesta plasticitat en la dieta podria contribuir a la amplia abundància i distribució del grup de les esponges arreu del mon.

Dieta i taxes d'ingestió de l'ascidi *Halocynthia papillosa*

L'estudi de la dieta de *H. papillosa* al llarg de l'any va mostrar que l'espècie no presenta un comportament selectiu en quan al tipus de presa capturada inclou des de les cèl.lules més petites com els bacteris fins a diatomees, a més a més d'una gran quantitat de detritus. La marcada estacionalitat en la taxa d'aclariment explicada majoritàriament per canvis en la temperatura de l'aigua és una característica comuna a altres espècies d'ascidis. La correlació significativa entre l'ingesta de Carboni i Nitrogen d'origen viu amb la producció gonadal, ha suggerit que tot i la gran quantitat de detritus capturat per l'espècie, les preses del pico- nano- i microplancton semblen tenir un paper més rellevant en la seva dieta en relació a les partícules detrítiques.

Dieta i taxes d'ingestió de la gorgonia *Paramuricea clavata*

La dieta de *P. clavata* mostra un rang de preses similar al descrit en altres espècies de gorgonies tropicals tot i que la taxa de captura és superior en l'espècie Mediterrània fet que es relaciona amb la seva exclusiva heterotròfia. La manca de captura de preses de talla inferior a les 3 μm sembla ser una restricció anatòmica del grup més que una capacitat selectiva ja que les preses mes grans son capturades amb més o menys quantitat en funció de la seva presència en la columna d'aigua. Els mecanismes de captura descrits en la literatura estan d'acord amb l'ampli rang de preses capturat per l'espècie. Les taxes d'ingestió de carboni calculades en aquest estudi representen un bon complement al carboni aportat per la captura de zooplancton sobretot durant els mesos d'hivern. L'alta densitat de colònies de *P. clavata* en algunes zones de la Mediterrània juntament amb les taxes de

captura de microorganismes mostren que aquestes població de suspensivors bentònics poden tenir un paper important en el control d'aquestes poblacions planctòniques en la massa d'aigua propera al fons.

Taxes de respiració i excedent d'energia

Les taxes de respiració estimades per les tres espècies mediterrànies es troben dins dels rangs aportats per altres espècies. Si bé els canvis en temperatura afecten el metabolisme d'organismes poiquiloterms, en la natura, altres factors com el seu estat fisiològic (creixement, reproducció, senescencia...) poden tenir un efecte mes marcat que la temperatura. Així ho mostra clarament les dades aportades en el cas de la gorgonia.

L'evolució de "l´exedent d'energia" al llarg de l'any ha mostrat que els seus màxims es corresponen amb els moments de màxima producció per l'ascidi i la gorgonia. En el cas de la gorgonia, la possibilitat d'emmagatzemar substàncies de reserva (en forma de lípids), pot permetre a l'espècie superar les èpoques amb una alta demanda energètica o amb una baixa disponibilitat d'aliment en la columna d'aigua. Els màxims valors en els excedents d'energia han coincidit amb el màxim contingut de carboni orgànic a l'aigua en el cas de l'esponja i de la gorgonia però no en el cas de l'ascidi. Això podria indicar que a diferencia dels dos primers, *H. papillosa* no estaria limitada tròficament i podria triar el millor moment per la reproducció en funció d'assegurar la supervivència de les larves.

Dieta, taxes d'ingesta i respiració de les espècies tropicals

Les taxes de captura de preses estimades per les gorgonies tropicals són un ordre de magnitud inferiors a les aportades per l'espècie Mediterrània. Tot i que la heterotrofia aporta una mínima quantitat de carboni per les espècies tropicals, aquesta pot ser una font significativa en l'aport de nitrogen.

La gran abundància de gorgonies en alguns sistemes tropicals com és el cas del carib, fan que les seves poblacions capturin, de manera global, grans quantitats de fitoplancton i ciliats. La localització a poc fons d'aquests cnidaris fan sospitar que poden tenir un important paper en el control d'aquestes poblacions microplanctóniques

Els capitols 2, 3, 4, 5 i anexe I han estat millorats gracies als comentaris dels revisors i als membres del tribunal. Els podreu trobar publicats al llistat que us dono a continuació. Sobre la resta de capitols.... hi estic treballant.

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