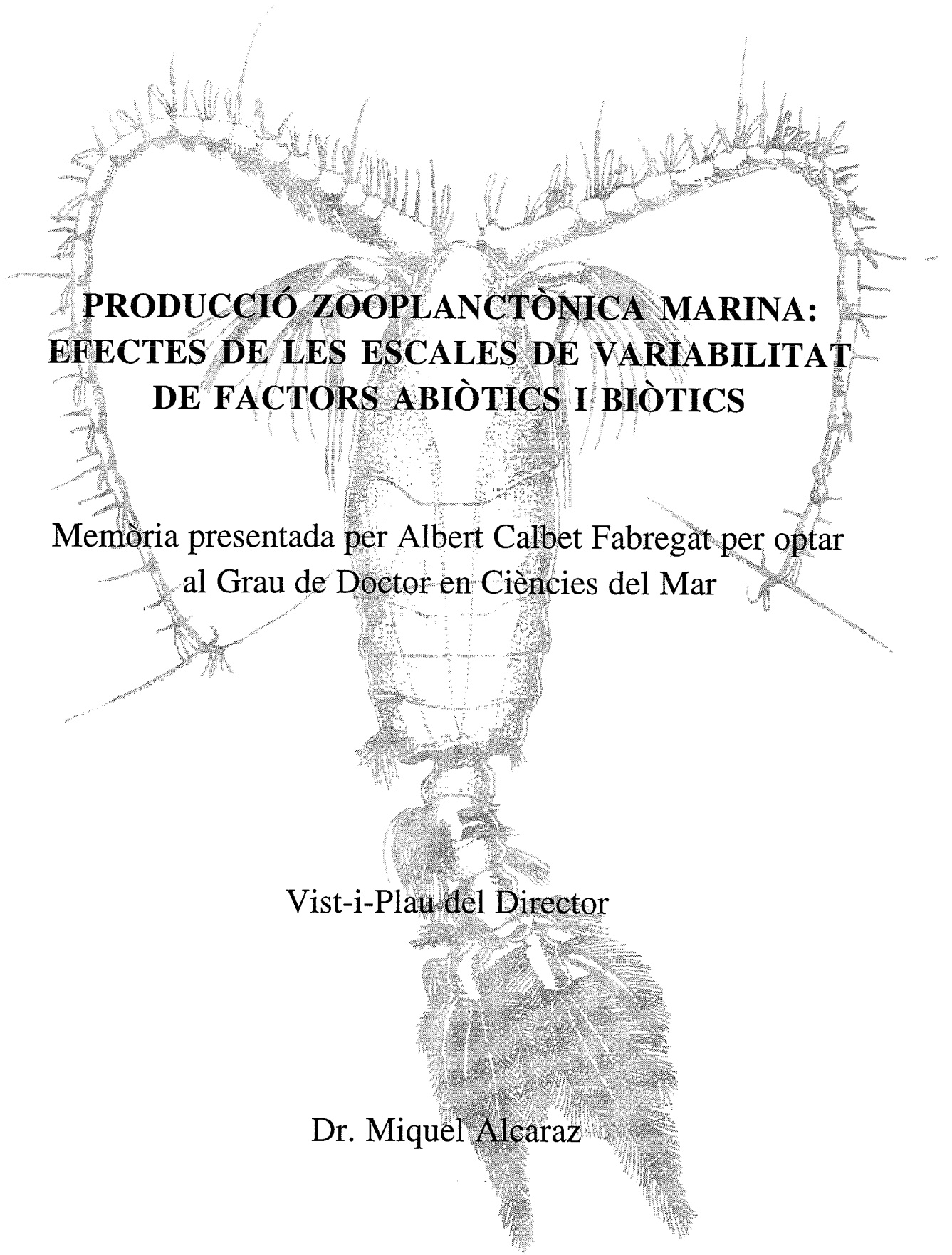

# PRODUCCIÓ ZOOPLANCTÒNICA MARINA: EFECTES DE LES ESCALES DE VARIABILITAT DE FACTORS ABIÒTICS I BIÒTICS

Memòria presentada per Albert Calbet Fabregat per optar al Grau de Doctor en Ciències del Mar

Vist-i-Plau del Director

Dr. Miquel Alcaraz

Barcelona 1997

A la meva família

## PRÒLEG

Quan un es planteja el fet, o tan sols la possibilitat, d'embarcar-se en una tesi doctoral sempre es deixa portar per l'emoció i la ignorància i no té mai en compte les tones i tones de temps i esforç (la majoria de vegades infructuós) que aconseguir un títol de doctor (si més no per als biòlegs) comporten.

Jo, com molts d'altres, em vaig deixar portar, o més aviat arrossegar, per tot un seguit de circumstàncies que em van dur on sóc ara, davant d'un ordinador tractant de donar forma a quatre anys de feina.

Han passat moltes coses des que vaig entrar per primer cop en aquesta casa amb cara d'espantat, i vaig creuar les primeres paraules amb en Miquel. He après molt, he conegut molta gent, he visitat molts països, he fet molts "creuers de plaer", i el que és més important he fet molts amics.

He de reconèixer, però, que no tot han estat flors i violes, hi ha hagut moments en els què he arribat a creure en malediccions i mal d'ull, però mica a mica me n'he anat adonant que el treball de laboratori, especialment amb bèsties vives, comporta tot una mena de riscos que "a priori" no es tenen mai en compte. Perquè, qui es podia pensar que una càmara isotèrmica fallés tres vegades seguides engegant a rodar dos experiments, o que quan la càmara funcionava s'espatllesin els escalfadors, o que el simple fet de canviar el lloc de procedència de l'aigua de mar fos suficient per acabar de cop amb la vida de les pobres bèsties experimentals o ....?. Són molts els exemples en els què la fatalitat pren forma, però són precisament aquests inconvenients els que fan tan valuosos els resultats obtinguts. Si no tinguéssim que suar una mica per obtenir bones dades, el fruit del nostre treball

perdria interès, i les tesis estarien enllestides en quatre dies. I a més als becaris sempre ens ha agradat patir una mica.
No obstant tot això, no me'n penedeixo d'haver entrat en aquesta casa ara fa quatre anys, ni de la feina feta, ni de les estones viscudes, només espero que el que presentaré a continuació no sigui del tot soporífer, i que pugui servir a algú més que a mi mateix.

L'estructura peculiar dels capítols, resum en català i la resta en anglès és deguda a la prèvia o futura publicació dels resultats en revistes científiques d'àmbit internacional.

## Agraïments

Aquesta potser és una de les parts més difícils d'una tesi, i no pel fet en sí d'agrair quelcom a algú, sinó pel temor de deixar-te alguna persona en la inacabable llista de gent que directa o indirectament han col.laborat en la realització de la tasca que aquí es presenta.

Primer de tot vull agrair al Dr. Miquel Alcaraz la direcció d'aquesta tesi i la paciència que ha tingut envers mi, que tot just m'iniciava en el món de la biologia marina. Agraeixo també especialment l'ajut i suport que m'ha proporcionat en tot moment l'Enric Saiz, una persona clau en la tasca que he estat realitzant en el transcurs d'aquests quatre anys.

No oblidem però, que aquesta tesi no hagués pogut dur-se a terme sense el finançament econòmic del Ministeri d'Educació i Ciència i del Consell Superior d'Investigacions Científiques, ni sense les instal.lacions de l'Institut de Ciències del Mar. A aquestes institucions, i als seus responsables i directors agraeixo la confiança posada en mi durant tot aquest temps. També agraeixo als caps dels programes en els què he participat (Drs. M. Alcaraz, M. Estrada, C. Duarte, S. Agustí i R. Anadón) les facilitats atorgades i l'experiència que m'han permès d'obtenir en el transcurs de totes les campanyes oceanogràfiques en les que he participat.

En Xabier ha estat un bon company de campanyes i de tasca de laboratori, i també ho ha estat un cop acabada la feina. A l'Esther li agraeixo la paciència que ha tingut durant els darrers tres anys; reconec que no ha de ser gens fàcil conviure en el mateix despatx amb algú tan desordenat com jo.

Agraeixo també la col.laboració de tot un seguit de gent que també han tingut alguna mena de participació directa en aquesta tesi, o si més no, m'han aclarit dubtes importants o m'han donat bons consells que m'han facilitat, i força, la feina. A l'entranyable Antonia, que tant em va ajudar al començament, i que tant he trobat a faltar al final. A en Joan Armengol, que em va animar a demanar la beca que he estat gaudint aquests anys. A l'Àngela per realitzar tan ràpid les anàlisis d'àcids nuclèics. A la Isabel, per la seva participació en el cultiu de copèpodes i per renyar-me quan em deixava les coses pel mig. A l'Elisa per la correcció del català i els seus coneixements sobre cultius de fitoplàncton. Al Pep, Cesc, Víctor i la Dolors, pel seu ajut informàtic i estadístic. Al Sr. Elías Barrenechea, cap del Port Esportiu de Masnou, que sempre em va donar totes les facilitats necessàries per tal d'obtenir mostres vives per a la tasca experimental. Als capitans, tripulacions i personal tècnic dels B.I.O García del Cid i Hesperides. A en Justo, l'Urbano i en Carles, els quals, cadascun per la part que els corresponia, van fer possible que gaudís del material necessari per a treballar. A la Rita a la que tants maldecaps li he donat. I a tanta gent (companys de campanyes, la Marta i la Glòria de la Biblioteca, la Pili i la Maria a les que tants pots he fet rentar, la Luisa i la Dolors dels serveis de neteja, els nois i noies de l'INEM, a la resta de gent dels serveis generals de l'Institut, etc.), que des de l'ombra han posat el seu granet de sorra en la realització d'aquesta tesi.

Hi ha hagut molts companys i amics que si bé no han tingut un paper directe en la tasca dels darrers quatre anys, m'han ajudat ocasionalment, o m'han donat suport moral per a continuar endavant en els moments en que els ànims m'abandonaven. Entre ells m'agradaria destacar a la Cèlia, en Carles, en Josep Maria, en Xelu, la Patrícia, la Lluïsa, la Núria, en Juani, en Maxi, la Cova, la Catherine, la Laura, la Marta, en Rafel, la Magda, la Roser, en Jordi i darrerament en Mikel, que m'ha donant uns quants bons consells per facilitar-me la vida durant el meu Postdoc. A tots ells moltes gràcies.

Finalment, vull agrair a la meva família i als meus amics la paciència i suport que m'han demostrat al llarg de tota la tesi.

**ÍNDEX**

# INTRODUCCIÓ

# INTRODUCCIÓ

## Distribució espacial i variabilitat temporal del plàncton marí

Una propietat característica dels ecosistemes pelàgics és el caràcter no uniforme de la distribució dels organismes, els quals es troben formant taques en un ampli espectre jeràrquic d'escales espacials i de persistència temporal. A l'espectre de variabilitat de les escales d'espai i temps en l'abundància dels organismes correspon, al seu torn, una distribució espectral similar en les variables físiques que en són responsables.

Els mecanismes de formació i manteniment de la distribució en taques dels organismes planctònics poden ser molt diversos, i són conseqüència de la interacció entre factors físics i processos biològics (Mackas et al. 1985). L'heterogeneïtat local de les condicions ambientals favorables al desenvolupament de les comunitats són conseqüència de mecanismes purament físics d'acumulació, de l'existència de pautes de comportament (natació) i d'interaccions de tipus tròfic.

Una condició que determina el manteniment de la distribució en taques dels organismes planctònics, és l'existència d'un balanç positiu entre les taxes d'increment i pèrdua d'organismes. La pèrdua d'organismes s'esdevé principalment per difusió i té lloc majoritàriament a través de les fronteres de la taca de plàcton. Per contra, el creixement dels organismes té lloc en tot el volum de la taca. Per tant, la relació superfície/volum és de vital importància a l'hora d'acotar la mida mínima de taca que es pot mantenir estable, o inferir-ne el temps de persistència. En el cas del fitoplàncton, aquesta relació superfície/volum, a més, determina la probabilitat d'ésser aprofitada pel zooplàncton. Per exemple, taques de fitoplàncton petites amb elevades relacions superfície/volum no

podran contrarestar els efectes de la difusió amb el creixement de les algues, i seran disgregades ràpidament. Aplicant el model bidimensional de difusió de Kierstead-Slobodkin-Skellam (model KISS, Kierstand and Slobodkin, 1953),

$$R = 2.4048\ (D/k)^{1/2}$$

on "R" és el radi crític en dos dimensions, "D" és la difusivitat ($L^2T^{-1}$) i "k" és la taxa de creixement de les algues ($T^{-1}$), trobem que aquesta mida crítica de taca capaç d'amortir els efectes dispersadors de la difusió està al voltant de dos quilòmetres considerant una k corresponent a 1 divisió per dia (Harris, 1980). Aquest valor teòric es correspon amb alguns dels resultats obtinguts a la natura: d'un a tres Km en una badia a Nova Escòcia (Platt et al. 1970), al voltant de 20 Km en el Pacífic Subtropical (Perú) (Lorenzen 1971), de més de 100 Km a l'oest del canal d'Anglaterra (Holligan, 1977), d'aproximadament 10 Km en sistemes frontals (Strass, 1992). Tanmateix, també hi ha referències de mides de menys d'un quilòmetre associades a sistemes frontals (Steel, 1978), d'un a dotze metres en zones estuàriques (McAlice, 1970) o de menys de 1 metre en la dimensió vertical (Owen, 1989). Aquests valors indiquen la importància d'altres factors (a part del simple creixement de les algues) que poden ajudar al manteniment d'una taca: circulació de Langmuir, picnoclines, termoclines, excreció de substàncies adherents per part de les algues, la pròpia motilitat dels organismes, etc. D'altra banda, petites proliferacions fitoplanctòniques de l'ordre de centímetres a metres, tot i no ser gaire estables molt de temps, podrien ésser explotades per organismes convenientment adaptats.

<u>Alimentació fluctuant, producció secundària i conseqüències ecològiques</u>

Respecte dels consumidors, encara que els agrupaments de zooplàncton de l'ordre de desenes a centenars de metres són freqüentment descrits a mar obert (per ex. Wiebe, 1970), petits eixams d'animals planctònics monoespecífics, de

diàmetres de centímetres a pocs metres (copèpodes, Anraku, 1975; Ueda et al., 1983) són corrents en àrees costaneres. En aquests casos s'ha demostrat experimentalment la importància del comportament individual en relació a la distribució en taques de l'aliment, amb la disminució significativa de la motilitat quan entren en una zona amb elevada concentració de menjar (Tiselius, 1992). Això permet optimitzar el temps de disponibilitat de menjar. Altres estudis (Poulet i Ouellet, 1982), indiquen que l'agrupament en eixams pot ser degut a respostes en front a estímuls químics produïts per les secrecions de les pròpies algues. Així doncs, sembla ser que fins i tot petites taques de fitoplàncton podrien ser detectades i aprofitades pels copèpodes.

El caràcter heterogeni en la distribució espacial dels productors primaris (fitoplàncton) i els seus consumidors (zooplàncton herbívor) (Estrada, 1979; Hutchings, 1981; Mackas et al., 1985; Delgado et al., 1992; Strass, 1992; etc) juntament amb llurs variacions en el temps (Walsh, 1976; Haury et al., 1978; Andrew i Hutchings, 1980; Sreekumaran Nair, 1992; etc) determinen fluctuacions d'amplitud i freqüència variables en la disponibilitat d'aliment pel zooplàncton.

Les escales espacials d'heterogeneïtat horitzontal tenen ordres de magnitud més grans que les verticals. Per a un copèpode és molt difícil, si no impossible d'accedir a taques de menjar separades per quilòmetres. Per exemple, *Acartia tonsa*, quan està cercant menjar, neda a una velocitat aproximada de 3.7 mm $s^{-1}$ (Tiselius 1992). Això vol dir que necessitaria al voltant de 3 dies per a recórrer un quilòmetre. En canvi, patrons verticals d'heterogeneïtat amb escales de desenes de metres són compatibles amb la capacitat de desplaçament i pautes de migració dels consumidors (zooplàncton). Són moltes les referències sobre la relació entre el patró de distribució vertical del fitoplàncton (màxims superficials o profunds) i les pautes diàries de migració vertical del zooplàncton (Cushing, 1951; Dumont,

1972; Dagg, 1985; Ohman, 1990, Hays et al., 1994; etc). En l'escala temporal, això es tradueix en una oscil.lació en la disponibilitat d'aliment d'aproximadament 12 hores, normalment durant la nit (el fet que els copèpodes no romanguin en els màxims de clorofila tot el dia sembla lligat amb la depredació).

Al llarg de l'any, deixant de banda els fenòmens purament estacionals (de gran importància a nivell de comunitat, però massa llargs per a ésser integrats com a canvis en el medi en el transcurs de la vida d'un individu en concret), es donen tanmateix moltes variacions en la disponibilitat d'aliment pel zooplàncton. Pertorbacions en l'ecosistema en forma de tempestes, aportaments puntuals de nutrients, etc, es reflecteixen en fluctuacions en la concentració de fitoplàncton a escales d'hores a dies (Dagg 1973, Walsh 1976), que corresponen plenament a la durada dels cicles de vida de molts dels organismes del zooplàncton.

L'aprofitament final d'aquests recursos puntuals per part del zooplàncton herbívor dependrà, en bona part, del fet que aquells apareguin en una fase adequada (hipòtesi del "match-mismatch", Cushing, 1978). Fluctuacions en l'abundància de productors primaris es traduiran en l'establiment d'una població de consumidors només en el cas que tinguin lloc en una freqüència capaç d'ésser integrada per la comunitat. Un aportament de menjar capaç de generar una resposta reproductiva serà ineficaç si no es manté el temps suficient, o si no es repeteix prou freqüentment com per a permetre el creixement de la nova generació fins a arribar a completar el seu cicle de vida.

Els mecanismes d'acoblament entre les escales (espacials o temporals) de variabilitat de productors primaris i zooplàncton herbívor tenen una gran transcendència pel que fa al destí final del carboni biogènic marí. La transferència de la matèria i l'energia fixades pels productors primaris (fitoplàncton) es fa mitjançant dues modalitats principals de xarxes tròfiques: la xarxa tròfica clàssica

(basada en la utilització del fitoplàncton pel zooplàncton herbívor) i la xarxa tròfica microbiana (basada en la utilització del fitoplàncton i dels seus productes metabòlics per microheteròtrofs, principalment, bacteris, flagel.lats i ciliats). Del predomini d'una o altra modalitat de transferència depèn, en bona mida, la taxa de retorn a l'atmosfera (turnover) del carboni fixat pel fitoplàncton. En el cas de la xarxa tròfica microbiana el carboni tindria una vida "curta" (amb un turnover de l'ordre de $10^{-2}$ anys). La xarxa clàssica permetria el "segrest" del carboni en el sediments i resultaria en una taxa de retorn superior als $10^2$ anys (Legendre i Le Fèvre 1992). El paper de l'oceà com a font o desguàs de $CO_2$, i les conseqüències que se'n poden derivar en el context del canvi climàtic global, poden ser, doncs, funció de la importància relativa d'ambdues modalitats de transferència tròfica.

En realitat, ambdues xarxes tròfiques no són excloents. El més freqüent és observar, per a una zona concreta, certa alternança en la importància relativa de l'una o l'altra via de transferència de carboni, sobre tot quan els factors de forçament físic, responsables dels episodis de fertilització, són aperiòdics (Calbet et al. 1996). Quan aquests tenen una periodicitat concreta (marees), es pot donar una persistència notable en les característiques tròfiques (Le Fèvre i Frontier 1988).

Per altra banda, per la majoria de components del zooplàncton, clàssicament descrits com a herbívors, la qualitat tròfica i la mida de l'aliment, més que el seu origen vegetal o animal, semblen ser determinants a l'hora de la seva explotació (Berggreen et al., 1988; Cowles et al., 1988). Per tot això, el zooplàncton juga un paper de primer ordre com a element modulador de les xarxes tròfiques per la seva capacitat de controlar per predació tant els productors primaris (fitoplàncton) com els microheteròtrofs (ciliats i flagel.lats).

Dins el zooplàncton, els copèpodes, a part de ser els metazoos més abundants de la biosfera (Humes, 1994), acostumen a dominar tant en nombre com en termes de biomassa en la majoria d'ecosistemes marins (Runge, 1988; Verheye et al., 1992; Barangé, 1994; Calbet et al. 1996). Una característica que confereix als copèpodes un paper transcendental en les xarxes tròfiques marines és la seva mida. Degut al seu tamany, els copèpodes es situen en el llindar entre el món viscós i l'inercial (Naganuma 1996). Mengen en el primer (Alcaraz et al. 1980), i són menjats en el segon (Strickler 1977). Aquest fet els converteix en un important víncul d'unió entre productors primaris (i també microheteròtrofs), i consumidors secundaris (peixos), essent una mena de filtre esmorteidor de la variabilitat entre aquests dos nivells tròfics (Runge, 1988).

Objectius i contingut d'aquesta Memòria.

Malgrat el reconeixement de les importants conseqüències ecològiques del caràcter fluctuant de les condicions ambientals, tant biòtiques com abiòtiques, de les què en resulta l'heterogeneïtat espacial i temporal dels organismes planctònics, els seus efectes sobre la producció zooplanctònica rarament s'han tingut en compte.

En aquesta memòria s'ha tractat d'avaluar l'efecte que tenen sobre la producció zooplanctònica les escales de variabilitat de factors biòtics (variacions en la quantitat i disponibilitat temporal de menjar, presència de depredadors, etc) i abiòtics (llum i temperatura). Especialment s'ha escollit treballar amb copèpodes degut a la seva importància en nombre i funció en els ecosistemes marins, i s'ha intentat en tot moment que les escales de variabilitat fossin incloses dins el rang propi dels sistemes naturals o si més no, que fossin suficientment properes.

Com a punt de partida, en el primer capítol s'ha intentat acotar la sensibilitat dels mètodes d'estimació de la producció en copèpodes, principalment la resposta a les condicions d'incubació (temperatura i concentració de menjar). El mètode d'estimació de la producció en copèpodes més utilitzat està basat en la detenció del creixement somàtic en els adults (excepte canvis en la quantitat de substàncies de reserva). Aleshores, gran part de l'aliment ingerit és redireccionat cap a l'activitat reproductora. Així doncs, per estimar la producció d'individus adults, ens hem de basar en la producció d'ous en femelles, o d'espermatòfors en mascles. Normalment, degut a la dificultat que comporta treballar amb mascles i als problemes per quantificar la producció d'espermatòfors, es mesura la producció d'ous per les femelles adultes (Kiørboe et al. 1985).

Una de les assumpcions bàsiques del mètode és que la producció obtinguda és conseqüència de les condicions de menjar a què ha estat sotmès l'individu en les 24 h anteriors. Així doncs sembla que els resultats d'una incubació de 24 h serien independents de les condicions en les que aquesta incubació es realitzi, malgrat que hi ha dades que ho fan posar en dubte (Dagg 1977, Tester and Turner 1990, Durbin et al. 1992).

Pel que fa als estadis juvenils, la producció s'estima en funció del creixement somàtic dels individus en el transcurs d'un interval discret de temps. Aplicant l'equació:

$$W_t = W_o * e^{gt}$$

(on $W_t$ és el pes al temps "t", $W_o$ és el pes inicial), s'obté una taxa instantània de creixement (g).

És un fet àmpliament assumit en els càlculs de producció secundària (Kiørboe i Johansen 1986, Kiørboe et al. 1990), encara que no ben provat (Fryd et al. 1991, Berggreen et al. 1988) que ambdós mètodes (producció d'ous per femelles adultes i creixement de juvenils) són equivalents. Aquest fet, conjuntament amb la dificultat que presenta el treball amb estadis juvenils de copèpodes, fa que el mètode més freqüentment utilitzat sigui el de la producció d'ous.

En el mateix capítol, a més de comparar les respostes funcionals d'ambdós mètodes a canvis en l'abundància de l'aliment, s'explora la possibilitat d'utilitzar d'altres més innovadors i força esperançadors, no basats en incubacions, com ara els canvis en la concentració específica dels àcids nuclèics (RNA per copèpode).

Un cop esclarida la part metodològica, es van estudiar els efectes que té sobre la producció de copèpodes (ja sigui estimada per producció d'ous, com per creixement de nauplis) una alimentació fluctuant a diferents freqüències en front d'una de continua. A tal efecte, el capítol segon recull tot un seguit d'experiments en els què es sotmeten fases adultes i juvenils d'*Acartia grani* a tot un ventall de fluctuacions temporals en la disponibilitat d'aliment amb freqüències i intensitats comparables a les dels sistemes naturals (Walsh, 1976; Haury et al., 1978). També s'estudien els efectes de períodes llargs de dejuni seguits d'un aportament saturant d'aliment en la producció d'adults, i el paper de la temperatura com a modulador de la resposta obtinguda.

Les diferències observades en la producció, conseqüència de les condicions d'il.luminació quan l'aliment era disponible (i la seva relació amb pautes de comportament tan importants com les migracions nictemerals), van aconsellar dur a terme una sèrie d'experiments relacionats amb els ritmes d'alimentació i amb els factors que poden alterar aquests ritmes, tals com la concentració de menjar, la

presència de depredadors, o la intensitat de llum. Els resultats d'aquests estudis estan recollits en el capítol tercer.

Al capítol quart es discuteix la variabilitat temporal i l'efecte de singularitats hidrogràfiques sobre la producció de zooplàncton, i en particular de copèpodes, en sistemes naturals. Es presenten dades de camp corresponents a quatre campanyes oceanogràfiques realitzades en el Mediterrani i a una en aigües Antàrtiques. El fet de treballar en ecosistemes tan diferents com poden ser el Mediterrani i l'Antàrtic, encara que parcialment circumstancial, és degut a la intenció de comparar la resposta d'organismes adaptats a medis sotmesos a diferents escales de variabilitat tan de factors biòtics com abiòtics. Els copèpodes antàrtics han hagut d'enmmotllar el seu comportament, i fins i tot el seu cicle de vida a un ambient amb una marcada estacionalitat. Aquest fet els hauria de conferir una major capacitat d'esmorteir petits canvis temporals de les condicions tròfiques a les que es veuen sotmesos. En canvi, animals d'ecosistemes comparativament més estables, com pot ser el Mediterrani, possiblement dependran més estretament de lleugeres variacions en el medi que els envolta.

L'espècie escollida com a model per a la majoria del treball experimental al laboratori fou el copèpode calanoid *Acartia grani*, típic d'ecosistemes costaners o semi-confinats, caracteritzats per un alt grau d'inestabilitat dels factors tant físics (temperatura, salinitat) com biològics (aliment). D'altra banda, aquesta espècie mostra una forta estacionalitat i només és present en les costes Catalanes durant els mesos de tardor i hivern. Aquest fet, conjuntament amb la necessitat de treballar amb animals de la mateixa edat, i criats en condicions controlades, ens van portar a realitzar un cultiu en continu o en semi-continu al llarg dels darrers anys de tesi. Degut al fet novedós que un cultiu d'aquesta mena representa, s'ha considerat necessari afegir un annex al final de la tesi en el que es detalla la metodologia utilitzada per a portar a bon fi un cultiu de copèpodes.

La síntesi resumeix els resultats obtinguts al llarg de tota la tesi i intenta de relacionar i discutir globalment les conclusions finals.

# CAPÍTOL I

## AVALUACIÓ DELS MÈTODES D'ESTIMACIÓ DE LA PRODUCCIÓ EN COPÈPODES

**Avaluació dels mètodes d'estimació de la producció en copèpodes**

Els copèpodes planctònics juguen un paper molt important en els mecanismes de transferència de matèria i energia entre productors primaris i consumidors de segon ordre. D'altra banda, degut a la seva posició clau en les xarxes tròfiques clàssiques, exerceixen un control directe sobre el reclutament en peixos. Però malgrat la seva importància (tant pel que fa al possible rendiment dels recursos explotables, com pel destí final del carboni biogènic) els problemes inherents a l'estimació de la producció secundària en copèpodes no han estat del tot resolts.

Són diversos els mètodes que s'han utilitzat per a mesurar la producció en aquest grup. Actualment, els dos més utilitzats estan basats, respectivament, en la producció d'ous per adults, i en el creixement somàtic per a juvenils.

Els copèpodes passen per diversos estadis larvaris fins a completar el seu desenvolupament. Un cop són adults, l'única forma de creixement (producció) està restringida a la producció d'ous en femelles i la producció d'espermatòfors en mascles. Una assumpció bàsica del mètode de la producció d'ous és que aquesta reflecteix el creixement "in situ" dels animals, independentment de les condicions en les què es realitzen les incubacions.

Una altra assumpció bàsica (encara que no ben demostrada) a l'hora d'estimar la producció de copèpodes és que el creixement específic (producció) dels adults és equivalent al de les fases juvenils.

Hi ha força discrepàncies respecte a la validesa d'ambdues assumpcions, essent un assumpte encara no ben resolt. L'estudi d'aquest fet és important en una tesi sobre producció zooplanctònica, especialment si es volen donar dades de la producció "in situ" de la comunitat de copèpodes.

Els objectius principals d'aquest estudi han estat els següents:

1) Estimar el paper de les condicions d'incubació en les estimes de producció (mètode de la taxa de posta d'ous). En el capítol 1.1 es demostra la importància d'efectuar les incubacions en condicions el més semblant possible a les que es trobaven els animals en el medi original. En aquest capítol també es proposa un protocol a seguir per tal d'aconseguir que les estimacions de producció siguin suficientment acurades.

2) Comparar l'equivalència entre taxes de producció específiques d'adults (ous) i juvenils (creixement somàtic). En el capítol 1.2 s'estudien les respostes funcionals a la concentració d'aliment i la temperatura d'ambdós estimadors de la producció en copèpodes.

3) Intentar desenvolupar un mètode nou per tal de reduir els problemes de metodologia i de consum de temps que presenten els dos mètodes anteriorment esmentats, la qual cosa disminueix considerablement la resolució tant espacial com temporal a l'hora d'obtenir estimacions acurades de producció secundària. En el capítol 1.3 es discuteix la validesa d'un nou mètode basat en la relació entre creixement i contingut específic d'ARN.

# 1.1. FOOD AVAILABILITY AS A POTENTIAL SOURCE OF BIAS ON THE EGG PRODUCTION METHOD FOR COPEPODS

(Basat en l'article del mateix títol fet amb la col.laboració d'altres autors i acceptat a J. Plankton Res.)

## INTRODUCTION

One of the ultimate goals of oceanography since its beginning has been to determine and understand the different physical and biological factors involved with the yield of fisheries, with the aim to predict its variability. Within this scope, the study of secondary production, in special regarding copepods, has become an important issue (Poulet et al., 1995). Copepods are the main food item for many first-feeding fish larvae and juveniles, and consequently play an important role in the control of fish recruitment.

The study of secondary production by copepods, however, has not resulted an easy task. Basically there have been two main approaches: one is based on methods regarding population dynamics of copepods; a second group of methods is based on the estimation of growth rates (see discussion in Berggreen et al., 1988; Poulet et al., 1995). In practice, the application of the first kind of methods to populations of zooplankton has not succeeded, in part due to the characteristics of copepod population dynamics (difficulty to establish a well defined cohort when many species have a continuous reproduction, etc.) and also to the difficulty to follow it in time and space.

The second group of methods pursues the estimation of growth rates by different ways. Some are based on physiological budgets; as Huntley and López (1992) pointed out, they bear the inconvenience of an accumulated error due to the sometimes huge variability obtained in the determination of the different components of the budget. Other approaches derive growth rates from development times determined in the laboratory; the estimates rendered this way tend to overestimate "in situ" rates (Berggreen et al., 1988).

**Table 1.** Review of some egg production literature indicating the procedence of the water employed for the incubations and the range of temperatures (°C) at which the determinations were done. When any of these features were not clearly specified or absent in the paper, this is distinguished with a question mark.

| Source | Water | Temp. |
|---|---|---|
| Ambler, 1985 | integrated | 14-28 |
| Ambler, 1986 | integrated | 14-28 |
| Ayukai, 1988 | surface (5 m) | 7.8-19.5 |
| Bellantoni and Peterson, 1987 | integrated | 19 |
| Dagg, 1978 | FSW, enriched | 2-22 |
| Durbin et al., 1992 | integrated | 4-16 |
| Hassett et al., 1993 | ?, enriched | 6 |
| Hay et al., 1991 | surface? | 5-12.5? |
| Hay, 1995 | ? | 7-12? |
| Hutchings et al., 1995 | fluorescence max. | 16-21 |
| Ianora and Buttino, 1990 | FSW, surface? | 14-18 |
| Jónasdóttir et al., 1995 | surface (5 m) | 4 |
| Kimmerer, 1984 | surface | ? |
| Kiørboe and Johansen, 1986 | chl max. | ? |
| Kiørboe and Nielsen, 1994 | surface | 4-20? |
| Kiørboe et al., 1985 | collection depth | 1-5 |
| Kiørboe et al., 1988 | collection depth | 8-11? |
| Kiørboe et al., 1990 | upper mixed | 4-9? |
| Kleppel, 1992 | integrated | 14.6-21.5 |
| Kleppel et al., 1991 | upper 30 m | ? |
| López et al., 1993 | surface | 0.5 |

**Table 1.** (Continuation)

| Source | Water | Temp. |
|---|---|---|
| McKinnon and Thorrold, 1993 | surface | 21-29 |
| Mullin, 1991 | 10-20 m; enriched | 11-20 |
| Nakata et al., 1994 | FSW | 18 |
| Nielsen and Hansen, 1995 | surface? | -0.5 - 5? |
| Nielsen et al., 1993 | surface | 7-11? |
| Park and Landry, 1993 | FSW, surface | 22.5-26.5 |
| Peterson and Kimmerer, 1994 | surface (5 m) | 1-21 |
| Peterson et al., 1990 | 10-15 m | 15? |
| Peterson et al., 1991 | ? | 16-17 |
| Plourde and Runge, 1993 | FSW, enriched | 1-5.5 |
| Rosenberg et al., 1990 | surface | 7-10? |
| Runge, 1985 | 12-15 m | 8-14 |
| Runge, 1985 | surface (5 m) | 6-8 |
| Smith, 1990 | surface | -2.3 |
| Tiselius et al., 1991 | surface | 9-18.7 |
| Uye and Sano, 1995 | surface | 10-30 |
| Uye and Shibuno, 1992 | surface (5 m) | 7.8-28 |
| Ward and Shreeve, 1995 | surface? | 3-5 |
| White and Roman, 1992 | surface | 15-29 |

The method that nowadays holds major acceptance because of its feasibility in the field and the robustness of its output is the egg production method (Kiørboe and Johansen, 1986; Berggreen et al., 1988). Although fecundity in the field had been measured before as an indicator of feeding conditions, it is

not until one decade ago that it was applied to the study of secondary production (Poulet et al., 1995). This method, widely used in both laboratory and field studies, is based on the fact that adult copepods do not molt and that under steady conditions adult females express all their production not in somatic growth but in egg mass. One of the advantages of this method is that it provides estimates of growth rates with a high temporal and spatial resolution (it is both time- and site-specific), impossible to obtain with other methods. It is very suitable, thus, for the study of mesoscale processes affecting copepod production.

Another advantage of the egg production method lays on the fact that for several copepod species the adult growth estimated by this method has been shown to be comparable to the growth experienced by juveniles (Berggreen et al., 1988; Fryd et al., 1991). Consequently, measuring egg production one should be able to estimate both adult and juvenile growth rates. However, there is still controversy whether or not this laboratory results can be applied in the field (Peterson et al., 1991), and if it can be extended to other copepods (see discussion in Poulet et al., 1995).

The egg production method overall is relatively easy, although laborious. Adult females are individually picked out from the plankton tow and incubated for about 24 hours in bottles usually filled with surface water (Table 1). Incubations are performed on deck at a temperature similar to "in situ" conditions. Incubations can be performed in individual animals or in groups, depending on the individual size. Other aspects vary according to the researcher's preferences. The bottles can be kept standing or on a wheel; in darkness or with a determined light regime; in very small containers or in larger ones. All this variance in methodological details is in part a consequence of the basic (and "a priori" robust) assumption of the method: that the observed egg production reflects the food environment experienced by the copepod

while was at sea (present-food independence of egg production). This precludes any exigent requirements for the incubations to be performed. In a way, it allows the researcher to obtain trusty estimates of "in situ" growth (production) independently of the conditions in which the incubation is performed.

In this paper we demonstrate that the basic assumption of present-food independence of egg production is not always true. Some indications of this fact can be found scattered in the literature, mainly for species of the genus *Acartia* (Dagg, 1977; Durbin et al., 1992; but see Ianora and Buttino, 1990; Hassett et al., 1993). Here we generalize these previous records and formalize the topic. We present both field data from the Northwestern Mediterranean and data from laboratory experiments that indicate that food conditions during the incubation might affect significantly the egg production obtained (i.e. that egg production does not only reflect past food conditions). This fact is shown for a variety of copepod species very common in neritic and open sea waters all over the world. We also show that this effect is temperature-dependent and finally discuss (and suggest) what precautions should be taken to obtain reliable data on egg production rates.

The interest on this study arose from our personal difficulties to establish a protocol for egg production incubations in cruises in the Western Mediterranean. The Western Mediterranean, as many other oligotrophic areas, is characterized by a deep chlorophyll maximum (DCM) that persists for most of the year. A mesozooplankton maximum seems to be associated to the DCM during daytime (Alcaraz, 1985), while at night zooplankton ascends to surface waters. Saiz and Alcaraz (1990) found that feeding rates of copepods were always higher for copepods in the DCM than at surface, instead of the "typical" situation where zooplankton ascend at night to feed at surface. This previous knowledge guided us to use water from the DCM for the egg production incubations in cruises. However, data from a recent cruise (Irigoien

et al. in prep.) indicated that this feeding pattern might not be always valid and poses the question whether or not the origin of the water used in the incubation is important regarding the estimation of "in situ" egg production rates. The scenario explicated here can be extended to any systems where food concentration presents a relevant degree of vertical heterogeneity.

## OBSERVATION AND DISCUSSION

### Field data

Table 2 shows a compilation of egg production rates for different copepod species determined in the Mediterranean Sea. They correspond to a sampling period during summer 1995 in several nearshore stations, and also to the VARIMED95 cruise onboard the research vessel "BIO HESPERIDES" that took place in June 1995 in a transect between Barcelona and Mallorca. The methodology in both cases was similar. Vertical WP2 (for the cruise) or horizontal Juday-Bogorov 200-μm nets (nearshore stations) fitted with a 5-10 l plastic bag were towed at low speed to collect the copepods. Adult females were picked out under the stereomicroscope and incubated for 24 h in groups of 4-5 individuals (10 for *Oithona*) in 650-ml screw-cap bottles filled with either surface water (nearshore stations, ca. 14 m deep) or water coming from the deep chlorophyll maximum (VARIMED95 cruise). Experimental water was previously screened through a 53-μm mesh by inverse filtration to exclude any zooplankters (except for the nearshore stations, where the presence of a high abundance of diatom chains precluded this procedure). In general, the bottles were incubated at surface temperature and with a light regime similar to the one in the field. The bottles were left standing and gently turned upside down one or more times through the incubation. At the end of the incubation the contents of the bottles were poured on a 20-μm submerged sieve, the females checked and the eggs counted under a

**Table 2.** Egg production rates (EPR, eggs per female and day; ± SE) of different copepod species from the North Western Mediterranean determined in different cruises and stations. Incubations lasted 24 hours and were performed simultaneously with the standard procedure ('in situ' water either from surface for nearshore stations or from the deep chlorophyll maximum in other cases), and with either filtered seawater (FSW) or an enrichment of the flagellate *Rhodomonas baltica* (or both). Temperature (°C) during the incubation is also shown. For *Oithona* spp., which carry egg sacs, egg production rates were determined only on individuals without sacs at the start of the incubation. Consequently, for this species, our estimates are biased and have only a relative value.

| Species | Temp. | EPR Standard | EPR Enriched | EPR FSW |
|---|---|---|---|---|
| *Acartia clausi* | 22 | 9.15±0.86 | 20±5.69 | 2.29±0.65 |
| *Acartia clausi* | 18 | 27±1.08 | 34.37±3.78 | 11.5±1.1 |
| *Centropages typicus* | 20 | 7.4±3.69 | 31.9±8.96 | |
| *Centropages typicus* | 20 | 31.5±6.03 | 67.4±6.4 | |
| *Centropages typicus* | 20 | 27.9±6.36 | 38.3±8.26 | |
| *Centropages typicus* | 20 | 22.2±9.64 | 27.5±4.32 | 16±4.67 |
| *Centropages typicus* | 20 | 8.4±2.24 | 13.8±3.76 | |
| *Centropages typicus* | 17 | 21.6±5.17 | 31.2±10.71 | |
| *Centropages typicus* | 13 | 6.6±1.66 | 6.6±5.01 | |
| *Centropages typicus* | 20 | 2.7±0.88 | 15.8±0.86 | |
| *Clausocalanus* sp. | 20 | 17.7±6 | 41±10.01 | |
| *Clausocalanus* sp. | 20 | 7.6±4.84 | 0±0 | |
| *Clausocalanus* sp. | 20 | 4.2±2.39 | 2.7±2.63 | |
| *Clausocalanus* sp. | 13 | 0.3±0.09 | 2.5±2.25 | |
| *Clausocalanus* sp. | 20 | 3.2±1.51 | 0±0 | |

Table 2. (Continuation)

| Species | Temp. | EPR Standard | EPR Enriched | EPR FSW |
|---|---|---|---|---|
| *Oithona* sp. | 23.5 | 15.03±1.44 | | 5.42±0.83 |
| *Oithona* sp. | 22 | 7.02±0.99 | | 2.33±0.59 |
| *Oithona* sp. | 22 | 7.52±1.57 | | 0.79±0.56 |
| *Oithona* sp. | 22 | 4.7±0.44 | 5.28±0.58 | 1.02±0.24 |
| *Oithona* sp. | 17 | 1±0.36 | 0.4±0.13 | |
| *Paracalanus parvus* | 20 | 14.1±2.48 | 17.6±3.56 | |
| *Paracalanus parvus* | 20 | 15.8±6.2 | 21.2±1.12 | |
| *Temora stylifera* | 20 | 17.2±1.65 | 80.2±15.58 | |
| *Temora stylifera* | 20 | 8.5±3.17 | 49.3±11.82 | |

stereomicroscope. In general no egg cannibalism was observed; empty shells, however, were considered if present. Blank bottles with experimental water were set up to take into account any eggs already present in the incubation water (most often no eggs were found). Egg production rates (EPR) are expressed as eggs laid per female and day.

Together with the above-explained procedure, that we will call hereafter 'standard' as the standard procedure for egg production in our laboratory, we performed parallel incubations in which the copepods were placed in either 0.2 filtered seawater (hereafter called FSW) or in enriched water. This "enriched water" consisted of the same water as used for the standard procedure to which we added a very high concentration (ca. 7 ppm) of the flagellate *Rhodomonas baltica*. This algae has proved to be a good food item

for many copepods, in special for the species studied here (Støttrup and Jensen, 1990).

Two main results appear from the study of Table 2. First, enrichment overall affected quite significantly the outcome of the egg production experiment in the 24-h incubations [average increases of 73% for *Acartia clausi*, 137 % for *Centropages typicus*, 126% for *Clausocalanus* sp., -24% (decrease) for *Oithona* sp., 29 % for *Paracalanus parvus*, and 423% for *Temora stylifera*]. Second, egg production rates of copepods incubated in filtered sea water were sensibly lower that the ones of the copepods incubated with our standard procedure (average decrease of 66% for *Acartia clausi*, 28% for *Centropages typicus* and 75% for *Oithona* sp.). Both results indicate that the basic assumption of the egg production method is not true, at least for our experiments. The food availability during the incubation affects considerably the output of the egg production method. This result also suggests that other factors affecting feeding during the incubation (like light regime) might require further attention.

It is also noticeable the variability observed within and between species in the response to food enrichment. The variability within species is very likely due to differences in the physiological state and previous history of the wild females collected at different places and times. That maybe the case for *Clausocalanus* spp., which showed a very variable response. In one case the response to enrichment consisted of doubling egg production rates, while in the other occasions no effect (or negative) was observed. [However, the latter incubations were followed by significant increases in egg production when these incubations in enriched water were followed for more than one day (data not shown)].

The variability between species can be due to real differences in their behaviour and also to the suitability of the food items employed for the enrichment. For instance, notice the large enhancement of egg production due to enrichment for *Temora stylifera*, compared with the negative response by *Oithona* spp. In this case it does not seem to be necessarily a real difference in the behaviour of the species ("a priori" one could argue that *Oithona* responds relatively slowly to food changes - see Paffenhöfer 1993), because a clear and relevant decrease in the egg production rate of *Oithona* was observed when incubated in filtered seawater after 24 h. Very likely *Rhodomonas baltica* was not a suitable food for *Oithona* to make apparent the effects of enrichment.

Laboratory data

Field data has evidenced that for a diversity of copepod species the egg production method based on on-board incubations is sensitive to food availability during the incubation. This sensitivity of the method will depend on the species and probably it is related to the species-specific differences in metabolic rates. Following this reasoning, we would predict that at higher metabolic rates (for instance, induced by a higher temperature) this potential bias of the method would be more stressed. It seems also reasonable that the magnitude of this bias will be affected by the previous food history experienced by the copepods.

In order to test both this temperature and previous food history dependence, an egg production experiment was conducted in the lab. The aim of the experiment was to determine the effect on egg production rates of "present food" during the incubation in relation to the "past-food" conditions, and how the intensity of this effect was dependent on temperature. Our hypothesis was

that the effect of "present-food" availability would be exaggerated when the copepods were inhabiting relatively warm waters due to the physiological increase in metabolic rates at high temperatures.

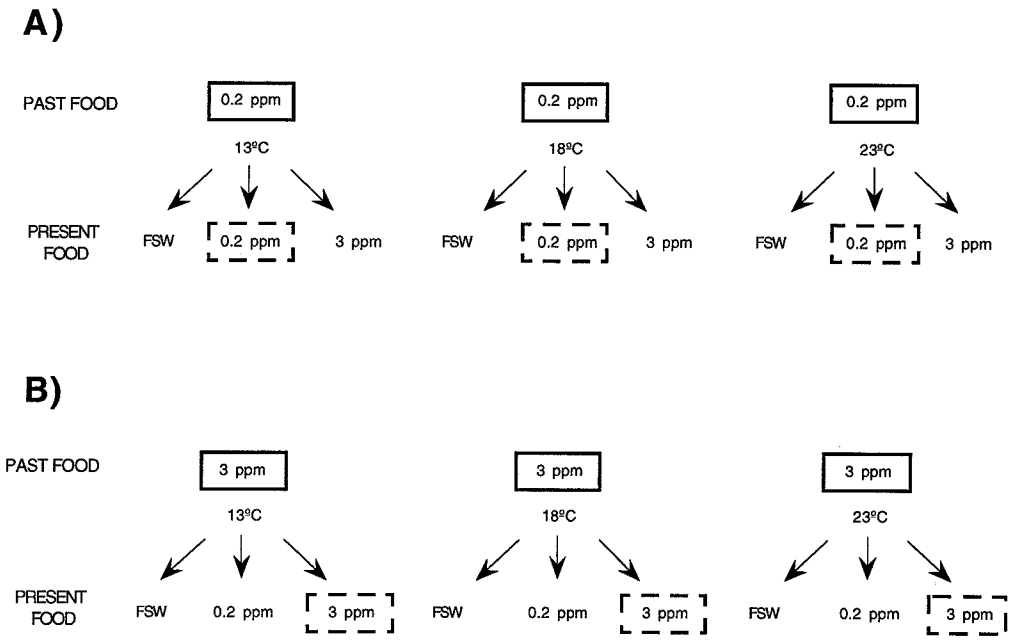


**Figure 1**. Outline of the experimental design. The boxes in dashed line correspond to the respective control treatment for each "past-food" condition.

The experimental design (Fig. 1) consisted of two "past-food" concentrations (nominally 0.2 and 3 ppm of the diatom *Thalassiosira weissflogii*), three "present-food" concentrations (nominally 0.2-μm filtered seawater, 0.2 and 3 ppm of *T. weissflogii*) and three temperatures within the range of surface temperatures in the Mediterranean Sea (13, 18 and 23 °C).

The procedure was the following. Adult females of the copepod *Acartia grani* were collected from a culture in our laboratory. The females had molted during the three days prior to the start of the experiment. Groups of 15-16 females and 3-4 males of *Acartia grani* were conditioned for three days in 1.25-l

screw-cap bottles at each combination of temperatures (13, 18 and 23 °C) and "past-food" conditions (0.2 and 3 ppm of *T. weissflogii*) (Fig. 1). Five conditioning bottles were set for each temperature-"past-food" combination. The bottles were kept standing and in one occasion during the incubation they were gently mixed by repeatedly turning them upside down. During the conditioning period the copepods were daily transferred to a new suspension of algae previously adjusted to the corresponding temperature. The batch cultures of *T. weissflogii* were kept in exponential growth through the experiment. A small amount of nutrients (25 ml of f/10 medium per litre) was added to the suspension of algae for the incubations to ensure nutrient availability for the growth of algae.

After the conditioning period the contents of the conditioning bottles were gently poured on a submerged sieve and the retained copepods (females) transferred by pipette to 625-ml Pyrex bottles filled with the corresponding suspensions of algae at the corresponding temperatures. The treatments were the following: for each temperature independently, copepods from each "past-food" condition were transferred in groups of 4-5 females to bottles with either of the following "present-food" treatments: filtered seawater (FSW), a 0.2 ppm or a 3 ppm suspension of *T. weissflogii* (see Figure 1). For the "past-food" treatment of 0.2 ppm, the egg production at "present-food" of 0.2 ppm was employed as control; for the 3-ppm "past-food" treatment, we used as a control the 3-ppm "present-food" treatment. This procedure mimics two field scenarios, tested at three temperatures: first, copepods living in a high food environment ("past food" 3 ppm) and egg production experiments conducted at either the right environmental food concentration ("present food" 3 ppm), in filtered seawater, or at a wrong environmental food concentration ("present food" 0.2 ppm); this could be the case for instance if the copepods feed at a deep chlorophyll maximum and on deck incubations are performed with

surface water or FSW. The second scenario would be one where the copepods feed in poor waters ("past food" 0.2 ppm), for instance at poor surface waters at night, but the egg production incubations are conducted in water from a deep chlorophyll maximum ("present food" 3 ppm) where the copepods stay during the day (but where they might not feed). Both scenarios are likely to happen in the Western Mediterranean Sea and in other areas where a deep phytoplankton maximum develops.

Figure 2 shows the egg production rates as a function of past food and present food for the three temperature tested. Statistical analyses of the data were conducted by two-factor ANOVA tests. The tests were performed for each temperature separately, using square-root transformation when required to assure the homoscedasticity of variances.

The results show that even at relatively low temperatures (13 °C), egg production rates obtained from 24-h incubations are affected by food availability during the incubation and previous food history. At 13 and 18 °C, both "past-food" and "present-food" treatments had a significant effect ($P<0.001$), with no interaction ($P>0.5$). At 23 °C, separated one-factor analyses were conducted due to significant interaction between "past-food" and "present-food" treatments ($P<0.001$). While for the 0.2-ppm "past-food" treatment the three "present-food" conditions yielded significantly different EPR ($P<0.001$), for the 3-ppm "past-food" treatment there was only a weakly significant difference between the 0.2-ppm and the FSW "present-food" conditions ($0.05<P<0.051$).

Figure 3 shows the ratio Egg production rate in present food versus Egg production rate in past food as a function of temperature, "past-food" and "present-food" conditions. This ratio indicates the changes in egg production relative to the control ("past food") conditions.

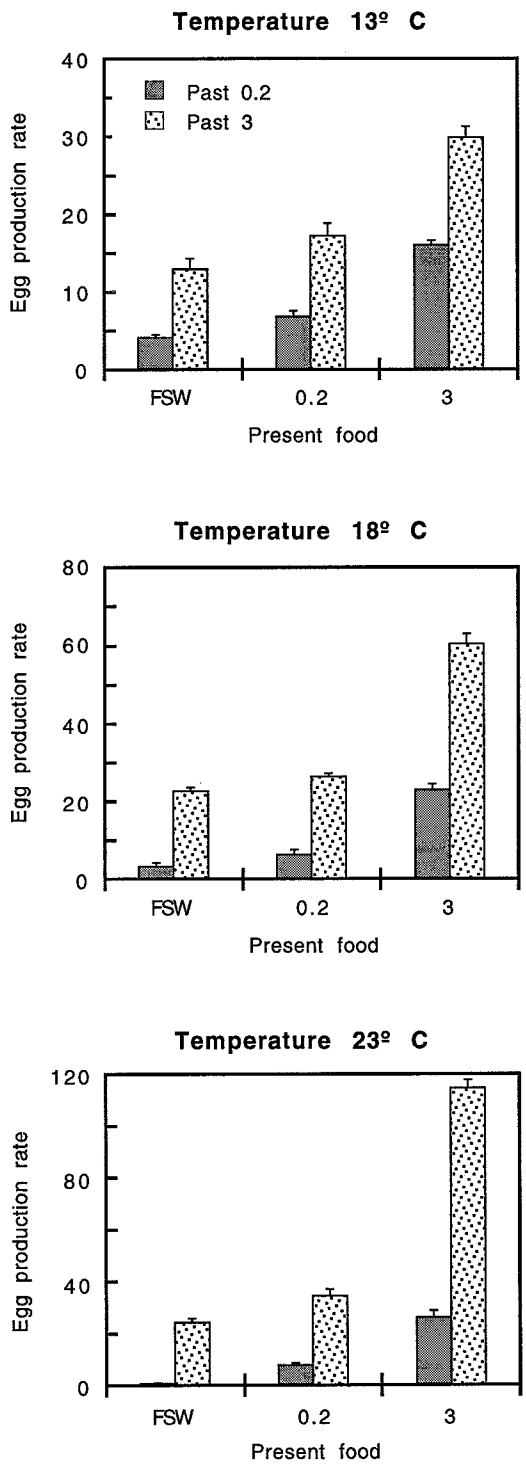


**Figure 2.** Egg production rates (EPR, eggs laid per female and day) of *Acartia grani* fed *Thalassiosira weissflogii* as a function of past-food, present food and temperature conditions. Error bars are ± 1 SE.

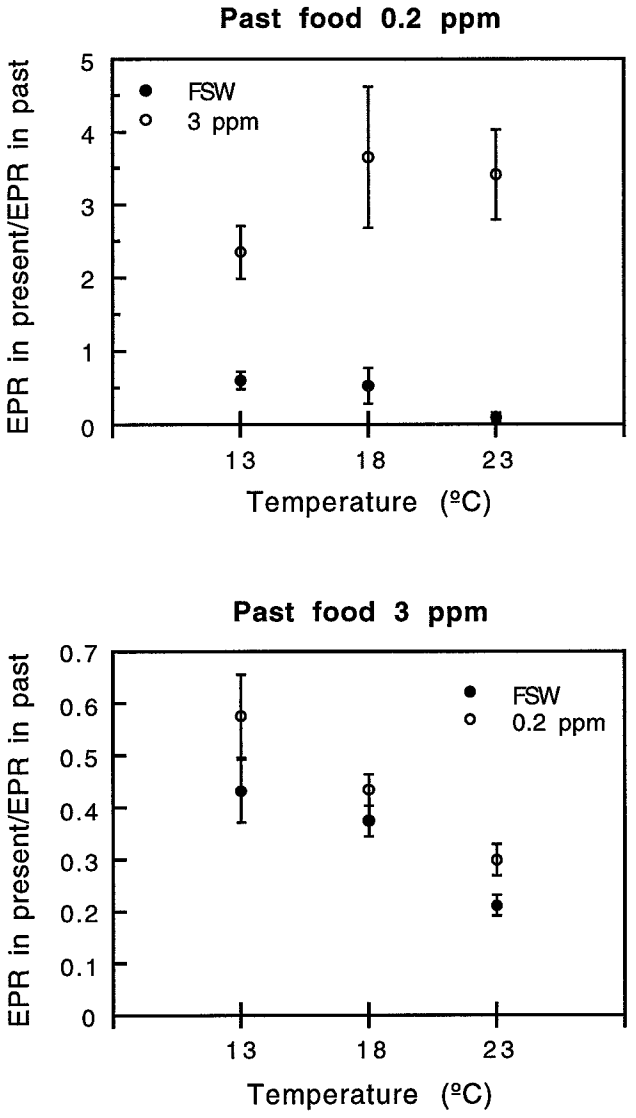


**Figure 3**. Ratio EPR in present food/EPR in past food (control) as a function of "past-food", temperature and "present-food" conditions. Error bars are ±1 SE computed for the ratios.

Regarding the first scenario ("past food" 0.2 ppm; incubations at 3 ppm and in FSW), there is a conspicuous increase of EPR at 3 ppm (ratios between 2.35 and 3.66) and a clear decrease in FSW (ratios from 0.60 to 0.10). In both cases

the ratios of EPR present food/EPR past food were temperature dependent, although the pattern differed for the incubations performed in FSW and at 3 ppm. There was a decline of the ratio with temperature for the animals incubated in FSW, very likely reflecting the increase in metabolism induced by higher temperature, which would lead to more severe starvation conditions. On the contrary, incubations performed with 3 ppm resulted in higher ratios at higher temperatures. This might be explained if the rate of building body and egg mass (biosynthesis) is more affected by temperature than the basal metabolism (i.e. the benefits of a higher feeding rate override the expenses due to respiration and excretion).

There appears to be some saturation effect at very high temperatures for incubations performed with 3 ppm, as it would be expected given that the copepods were laying eggs at very high rates (110 eggs laid per female and day), probably close to their physiological maximum, and consequently the rate of increase with increasing temperature was physiologically limited.

It is also remarkable the fact that the rate of change of the ratio with temperature was much lower in the FSW treatment than in the 3-ppm treatment (Fig. 3). The response of the copepods to starvation (FSW) was much less drastic than the response to a high food concentration. This must be a physiological adaptation to reduce the effects of starvation on one hand, and to enhance the ability of the individual to exploit ephemeral patches of food on the other hand. It can also be a consequence of the past food concentration.

The lower graph of Figure 3 shows the response to present food and incubation temperature of copepods coming from 3-ppm "past-food" conditions. Both "present-food" treatments (0.2 and FSW) resulted in EPR ratios lower than 1, being the effect more relevant at higher temperatures. Still

the ratios for the 0.2-ppm "present-food" treatment were higher than for animals incubated in FSW, reflecting a comparatively higher egg production (see Fig. 2). The response here is similar to that observed for animals conditioned at 0.2 ppm and incubated with FSW.

We must conclude from our experiment that it has confirmed for *Acartia grani* the initial hypotheses: 1) that the bias in the method due to present-food availability depends on the "past-food" conditions, and 2) that the effect of food availability during incubation relative to "past-food" conditions is temperature dependent, and 3) that egg production experiments do not always entirely reflect food intake in the previous day.

Recommendations

The conclusions from this experiment do not only concern *Acartia grani*, but very likely can be extended to other species (Table 1). Copepods like *Temora*, *Oithona* and *Centropages* are common in most coastal waters and their production has been widely estimated by the egg production method. It is well known that small neritic copepods have low lipid reserves and "a priori" their growth rate should respond quicker to changes in food availability. Thus, it seems reasonable that the conditions in which the incubations are performed can severely bias the estimation of egg production rates.

Obviously the intensity of this effect will depend on the species. In our study large copepods like *Calanus* or *Eucalanus* were absent. They store large amount of lipid reserves, which could help to dampen changes in food conditions and prevent them to be so tightly linked to the immediate food environment (but see Armstrong et al., 1991 for *Calanoides carinatus*). For these species it seems that the basic assumption of the egg production method

(i.e. that the measured EPR reflects "in situ" egg production) might be warranted (Plourde and Runge, 1993; Laabir et al., 1995) and the researcher does not have to worry about food availability during the incubation [but notice that Mullin (1991) described significant changes in egg production rates for *Calanus pacificus* of the California Current System according to food availability in the experimental containers in 24-h incubations].

How to do the incubations when working with small, neritic copepods, then? For studies where the goal is the comparison between areas/seasons of different characteristics, the choice of FSW or surface water as a standard concentration for the incubation can be acceptable, specially if working at low temperatures. The EPR estimated this way allows the comparison between areas of interest. It is noticeable, however, that if the areas/seasons surveyed differ considerably, differences in EPR will be stressed according to the water used for the incubations (see Fig. 3; the ratio of decrease of EPR for animals in FSW changes from 0.6 to 0.4 according to "past-food" concentration).

For studies where accurate estimates of "in situ" egg production rates are required, i.e. with the purpose to obtain estimates of secondary production, the use of FSW or surface water is not suitable. The optimum conditions would be to incubate the copepods in water coming from the depth stratum where they feed, and in a volume of water large enough to reduce as much as possible a significant decrease in food availability throughout the incubation.

Although alternative approaches have been proposed (Runge, 1987; Nakata, 1994; Roff et al., 1994), the egg production method by on-board incubations is still the most useful and feasible method to determine "in situ" growth rates nowadays. However, precautions must be taken in order to obtain reliable estimates in the field. Few studies have tried to determine the requirements for the method, and still there is not an agreement for a standardization (Poulet et

al., 1995). This contribution has attempted to stablish this standardization, showing some of the weaknesses of the method and optimizing the experimental protocol. These (and others) improvements will contribute to enhance our knowledge of the secondary production in the oceans.

## RESUM

El mètode de la producció d'ous és àmpliament utilitzat com a estimador de creixement (producció) de femelles adultes de copèpodes tant en el camp com al laboratori. En aquest treball es demostra que el fet d'assumir que la producció estimada durant la incubació reflecteix les condicions "in situ" no és sempre del tot cert, i a més, és depenent de la temperatura. L'increment de les taxes metabòliques induït per altes temperatures accentua el biaix del mètode. En aquest capítol es donen també uns consells per portar a terme les incubacions a fi d'obtenir resultats més propers als reals.

# 1.2. NAUPLIAR GROWTH VERSUS EGG PRODUCTION IN THE CALANOID COPEPOD *ACARTIA GRANI*

## INTRODUCTION

Once a copepod reaches the adult stage it stops the somatic growth and directs the excess of energy towards the reproductive effort. Therefore, eggs are the only form of production in adult females.

Specific growth rates of juvenile copepods and specific female egg production rates have been determined in several copepod species (Berggreen et al. 1988 and Fryd et al. 1991). In all cases they found that both rates were comparable. This assumption is of great importance when we calculate the secondary production for the whole community from egg production rates.

The objective of this work was to determine the growth rates of early naupliar stages under different food concentrations, and to compare naupliar production with simultaneous estimations of adult production (female egg production rates) in the calanoid copepod *Acartia grani.*

## METHODS

Naupliar stages of the planktonic marine copepod *Acartia grani* were obtained from a laboratory culture reared at 18 $\pm$ 1 ° C in a temperature-controlled room, in a 12 h light-dark cycle, and fed "ad libitum" a suspension of *Rhodomonas baltica* (6.9-7.3 μm diameter, 4-5 ppm by volume). The algae were maintained in exponential growth in order to avoid changes in nutritional quality by the daily addition of f/2 medium (Guillard, 1975). The experimental nauplii were collected from eggs hatched during 12-24 h intervals in order to obtain a maximum degree of homogeneity in initial naupliar stage and size. The experiments were conducted under the same light and temperature regime as described for the culture conditions.

Algal concentrations (volume) were measured by means of a Multisizer Coulter Counter, and the conversion to carbon contents was done according the volume-carbon relation given by Berggreen et al. (1988).

Stage-specific biomass in *Acartia grani* was estimated from the length-dry weight equations given by Durbin and Durbin (1978) for *Acartia clausi* . Eggs dry weight (DW) came from values of Kiørboe et al. (1985) for *Acartia tonsa.* Organismal size was measured with an Image Analysis System (dissecting microscope with camera) and NIH Image software on organisms fixed with acidic lugol. Instantaneous specific growth rates of nauplii (g) were calculated from the exponential change in biomass (DW):

$$g = \frac{1}{t} \ln \frac{DW_{t1}}{DW_{t0}}$$

About 500 nauplii II - III (average size 172.98 ± 2.1, equivalent to 0.13 ± 0.004 µg DW) were placed into 1.25 liter Pyrex bottles filled with a suspension of *R. baltica* at different concentrations (0, 0.5, 1, 2, 4 and 6 ppm, equivalent to 0, 135, 270, 540, 1081 and 1620 µgC $l^{-1}$ respectively). Experiments were run by triplicate. A slow-rotating Ferris wheel (0.2 r.p.m.) was used in order to avoid algal sedimentation. The experiment lasted three days, and the animals were transferred to a new algal suspension every day. An aliquot of the bottle contents, calculated to obtain about 30-40 nauplii per replicate, was daily filtered through a 20 µm-mesh submerged sieve. Subsequently the nauplii were fixed with acidic lugol and measured under a dissecting microscope by means of the described image analysis system.

In order to compare the naupliar growth (production) with adult female egg production rates, groups of 7 adult females were incubated in 625 ml Pyrex bottles filled with concentrations of *R. baltica*. similar to those as for naupliar growth rates. The experiments were run by triplicate. After three days of acclimation to food concentrations the following 24 h eggs were collected in a 20 µm submerged sieve and counted under a dissecting microscope.

## RESULTS

The temporal evolution of naupliar dry weight (DW) in relation to food concentration is represented in Fig. 1, and the corresponding values of instantaneous specific growth rates (g, $d^{-1}$) are shown in Table 1. The stage-composition of the cohorts for each food concentration at the end of the experiment is also indicated. Naupliar growth saturated at around 430 µgC $l^{-1}$, 1.6 ppm (Fig. 2).

The comparison of specific adult production rates (as egg production rates) and naupliar growth rates is represented in Fig. 2. Data have been fitted to the Holling type II equation (Holling 1959):

$$g = \frac{aC}{1 + abC}$$

Where "g" is the instantaneous growth rate, "C" is the food concentration and "a" and "b" are constants.

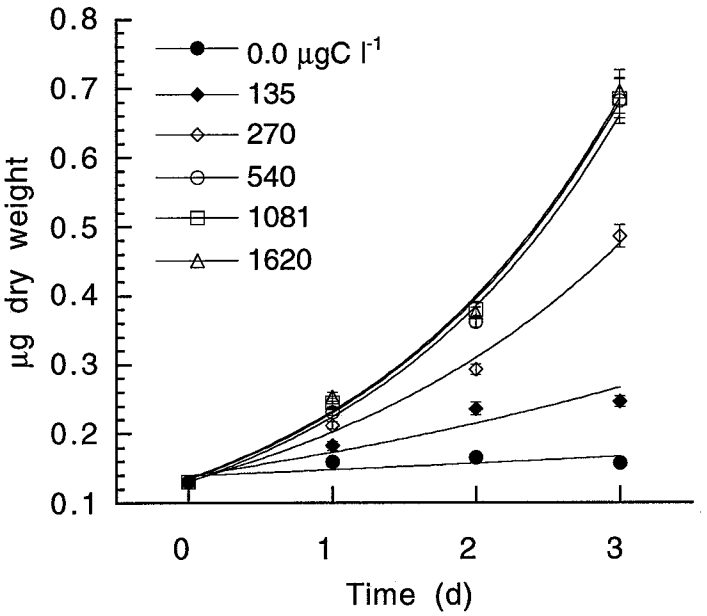


**Fig. 1**. Temporal evolution of naupliar dry weight (DW) in relation to food concentration of *Rhodomonas baltica*. (in µgC $l^{-1}$). Error bars represent SE.

**Table 1.** Functional growth responses of early naupliar stages of *Acartia grani* to food concentration. F: Food concentration, in ppm and µgC $l^{-1}$. g: Instantaneous specific growth rates ($d^{-1}$ ± SE); $r^2$: coefficients of determination; (%): demographic composition at the end of the experiment (3 days), as percentage of developmental stages (N: nauplius, C: copepodite). Experiments started at nauplius II-III.

| ppm | F µgC $l^{-1}$ | g | $r^2$ | Stage (%) |
|---|---|---|---|---|
| 0.0 | 0.00 | 0.060 ± 0.038 | 0.49 | NIV (100) |
| 0.5 | 135 | 0.218 ± 0.049 | 0.89 | NV (45) - NVI (55) |
| 1 | 270 | 0.427 ± 0.029 | 0.99 | NVI (89) - CI (11) |
| 2 | 540 | 0.470 ± 0.033 | 0.99 | NVI (60) - CI (40) |
| 4 | 1081 | 0.466 ± 0.026 | 0.98 | NVI (58) - CI (42) |
| 6 | 1620 | 0.458 ± 0.038 | 0.98 | NVI (54) - CI (46) |

To determine saturating concentrations, we have arbitrarily chosen a value of 2 ‰ for the rate of change of the curve. This value of 2 ‰ was close to the asymptotic stabilization of the rate of change of the curve.

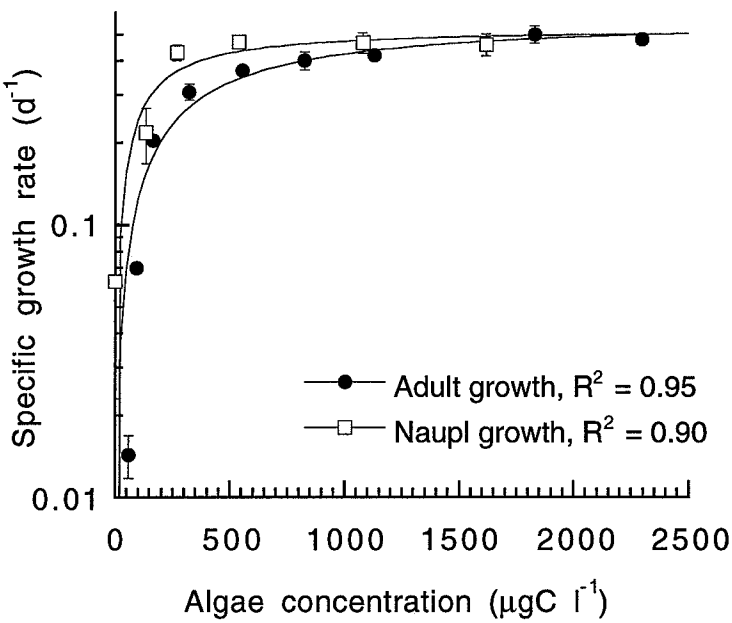


**Fig. 2.** Specific growth rate (± SE) of nauplii and adult (egg production rate) *Acartia grani* as function of food concentration (µgC $l^{-1}$ of *Rhodomonas baltica*). The lines correspond to the Holling type II equation (see text). The fitted parameters were: for adults a = 1.57 x $10^{-3}$ and b = 1.73; for nauplii a = 4.43 x $10^{-3}$ and b = 1.89.

Food saturation for adult egg production occurs at around 650 µgC $l^{-1}$, 2.4 ppm.. The instantaneous rate of growth for naupliar stages coincided with the values obtained for adult production at saturating food concentration (NII-CI g: 0.45 ± 0.01; adult g: 0.45 ± 0.024).

## DISCUSSION

The effects of food concentration on specific growth rates for early naupliar stages of *Acartia grani* were comparable to those observed for other congeneric species (*Acartia tonsa*, Berggreen et al. 1988) at similar temperature and feeding the same algal species. Maximum specific growth and growth-saturating food concentration coincided for both species.

The similar response of early nauplii and adult *A. grani* to food concentration appear to confirm the feasibility for translating production estimates based on definite age-classes to the whole community (Berggreen et al 1988, Fryd et al. 1991). This assumption is reinforced by the similarity in the values of instantaneous specific growth rates for naupliar stages and adult production. However, the differences in the food concentration at which adult copepod production (as egg production rates) and naupliar growth are saturated could induce minor errors in estimating copepod production for the whole population in the range at which growth is not saturated.

**RESUM**

Un cop els copèpodes assoleixen l'estadi adult aturen el seu creixement somàtic i redireccionen l'excés d'energia cap a l'esforç reproductiu.

El mètode de la producció d'ous ha estat sovint utilitzat com a estimador del creixement-producció de tota la comunitat, encara que no està del tot clar que, mitjançant aquesta metodologia, es puguin extrapolar els valors obtinguts per adults als estadis juvenils.

L'objectiu d'aquest treball era determinar les taxes de creixement d'estadis naupliars del copèpode marí *Acartia grani*, i comparar-les amb estimacions simultànies de producció en adults (producció d'ous).

Els resultats van mostrar que si bé ambdues estimacions són comparables a condicions saturants de menjar, per sota d'aquest llindar els resultats s'han de prendre amb precaució.

## 1.3 RNA CONTENTS OF COPEPODS AS A TOOL FOR DETERMINING ADULT GROWTH RATES IN THE FIELD

(Basat en l'article del mateix títol fet amb la col.laboració d'altres autors i enviat a Limnology)

## INTRODUCTION

The estimation of growth rates of copepods in the field is still a not completely solved problem. Very often zooplanktologists search for methods that allow to obtain reliable estimates of zooplankton growth rates at temporal and spatial scales short enough to cope with micro and mesoscale hydrographic variability. For this purpose the approaches based on the following of the development of well defined cohorts, if ever possible in the field, are precluded. The method nowadays more accepted is the egg production method, which is species- and site-specific (Kiørboe and Johansen 1986; Berggreen et al. 1988). This method is based on the determination of egg production rates of adult female copepods and on the assumption (validated in the laboratory for several species) that juvenile growth rates of copepods are similar to the rates of egg production in adults (Berggreen et al. 1988; Fryd et al. 1991).

However, the egg production method bears some trade offs that make a plea for the look for alternative/complementary methods for the determination of in situ growth rates of copepods. The main drawbacks are: i) that egg production is usually estimated from onboard incubations, which are not always free of flaw by changes in food availability during the incubation or by not reflecting properly the in situ food (patchiness) and temperature conditions (Saiz et al. in press), ii) it is tedious and time consuming because adult females (in good condition) of dominant species must be searched, while natural populations are usually composed mainly by juvenile stages, iii) it has been showed that this assumed correspondance between growth rates of juveniles and adult copepods might not be always valid (Peterson et al. 1991), and iv) in practice the method does not allow to accomplish a very intensive sampling with very short time and spatial scales when working with more than one or two species. Here we propose and

test an alternative to the egg production method that could cope with some of these drawbacks.

The nucleic acid RNA is the main responsible molecule for the synthesis of proteins which play both structural and catalytic roles in the cell. Therefore, the amount of RNA per cell or individual must be directly related to the intensity of growth of the cell/individual. This feature has encouraged in the past the use of the RNA content (or RNA/DNA ratios) as a method to estimate growth rates of organisms on phyla so diverse as bacteria, microalgae and fish. In general a good correlation between RNA contents and growth rate has been observed (see Sutcliffe 1970; Båmstedt and Skjoldal 1980; Clemmensen 1994 and references therein).

In the case of copepods (or other zooplankters) the search for a method to determine growth rates based on their nucleic acid content has been attempted several times in the past two decades with variable success. Båmstedt and Skjoldal (1976) reported dependency of nucleic acid contents of *Euchaeta norvegica* on season and body mass, and indirectly determined a good correlation between growth rates of late copepodites and RNA contents. Also, Båmstedt (1983) found a good agreement between the seasonal peaks of RNA contents and the occurrence of the breeding season of high latitude zooplankton.

However, data against the suitability of methods based on the nucleic acid contents also appeared. Dagg and Littlepage (1972) found fairly good relationships between growth rate and RNA content for *Artemia salina* and *Euchaeta elongata*, but these relationships appeared to be species-specific and the data showed high variability. These facts made them conclude that any general relationship between RNA contents and growth rate would lack sufficient accuracy to be used as a predictive tool. Ota and Landry (1984) also tried to establish any correlation between growth rates and nucleic acid contents of the

copepod *Calanus pacificus*. They concluded that the dependence of nucleic acid contents on temperature and stage (size) would act as confounding effects and make difficult to use methods based on nucleic acids as a predictive tool for growth rates of zooplankton.

In most of these previous attempts the aim of the research was to obtain a general predictive relationship for the whole zooplankton community. However, some of the poor correlations observed could have actually been a consequence of working with mixed species, each with specific nucleic acid contents, or due to the heterogeneous demographic composition of samples. It has been argued that for juvenile crustacea the process of ecdisis (molting) can result in peaks of high cell activity (high RNA content) but low growth (increase in biomass) (Baudouin and Scoppa 1975; Ota and Landry 1984).

An additional problem for the development of methods based on nucleic acid contents was that the quantification of RNA and DNA required a considerable amount of biomass for the analysis. New spectrofluorometric methods have been developed in the last few years allowing a much higher sensitivity (Clemmensen 1993; Fara et al. 1996).

Nakata (1990), following a species- and age-specific approach, found a good association between RNA/DNA ratio of *Paracalanus* sp. and phytoplankton abundance in the water column. More recently, Nakata et al. (1994) observed a good correlation between egg production rates and RNA contents of *Paracalanus* sp. and other species (Nakata, pers. comm.). Stemming from this work, here we present a laboratory study, under controlled conditions, of the suitability of the nucleic acid content as a tool to determine adult copepod growth rates. We have used a very sensitive spectrofluorometric method for the quantification of nucleic acids, recently developed for microplankton (Fara et al. 1996). Also, as Ota and Landry (1984) suggested, we have tested the potential

interaction of temperature with the correlation between nucleic acid contents and growth rates. Due to the enhancement effect of temperature on chemical reactions, we would predict that the amount of RNA per individual characteristic of a determined growth rate will be higher at lower temperatures.

Our final aim is to develop a method for the estimation of copepod growth rate, alternative to the egg production method, with the same reliability but lacking the inconveniences of sorting individuals onboard and of incubations. These features would allow higher time and space resolution in intensive sampling schedules.

## MATERIAL AND METHODS

Description of the experiments. - The experiments were designed in order to test the effects of temperature and the concentration of food on the nucleic acid contents of the marine copepod *Acartia grani*. Three different experiments were conducted:

a) Exp#1: Test of the relationship between egg production rate and RNA content of adult copepods, and interaction with temperature.

The experimental design consisted of two crossed factors: food concentration and temperature. Adult females from our *Acartia grani* culture were picked out and preconditioned in groups of 21 females to the experimental conditions in 1.1-l screw-cap bottles for ca. 48 h. A few males (4 per bottle) were also introduced to ensure fertilization of females. Six different nominal food concentrations were tested (0.2, 0.4, 0.7, 1.0, 2.0 and 3.0 ppm of the diatom *Thalassiosira weissflogii;* 1 ppm of *T. weissflogii* is ca. 1131 cells $ml^{-1}$) at respectively 17.8 and 23.3° C. The bottles were left standing during the incubation, being periodically turned upside down (twice a day) to prevent settling of the algae. Light:dark cycle was 12h:12h.

After the first day of preconditioning, the animals were transferred to new suspensions of algae at the corresponding concentrations. At the end of the second day of preconditioning, copepods were checked and any dead animals or in bad shape were taken out. The remaining copepods (males included) were introduced into new suspensions of algae at the corresponding food concentrations and temperatures. The experimental bottles (two replicates for each food concentration) were incubated for 24 h and then, their contents filtered through 180- and 20-μm sieves. The number of copepod eggs retained on the 20-μm sieve were counted under a stereomicroscope. The adult females were checked and one group of 18 females for each bottle was transferred to precombusted GF/F filters and frozen in liquid nitrogen until analysis of their nucleic acid contents. This procedure allowed to determine the egg production and nucleic acid content of the same individuals.

We tested by covariance analysis the linear relationship between RNA content and egg production rate, and also the effect of temperature on this linear relationship. We further applied a multiple regression model to predict egg production rate as a function of RNA content and temperature.

b) Exp#2: Test of the coupling between the time response of changes in RNA content and of changes in egg production rate.

The aim of the experiment was to expose the copepods to extreme changes in food availability and to determine to what extent the fast response of egg production to food availability (Calbet and Alcaraz 1996, Saiz, et al. in press) is matched by similar changes in the RNA content of the copepods.

The overall procedure was similar to that of Exp#1. Groups of 20 adult females and 4 males of *Acartia grani* were introduced in ten 1.1-l bottles filled with a suspension of 3.5 ppm of *Thalassiosira weissflogii.* The bottles were left

standing, at 17.8° C and 12h:12h light:dark cycle. After one day and a half of acclimation, the animals were transferred to new suspensions and the time of the transfer logged. After 24 h (Day 1), two bottles were taken and the contents filtered through 180- and 20-µm sieves. The eggs laid were counted, and the females were processed for RNA analysis as explained above. For the remaining bottles, the animals were put in starving conditions by transferring to 0.2-µm filtered sea water, which was changed daily. During the next two days (Day 2 and Day 3), two bottles were processed every day for the determination of egg production rates and nucleic acid contents of the female copepods. After Day 3, the animals were transferred again to 3.5 ppm suspensions of *Thalassiosira weissflogii* (renewed every day), and for the following two days (Day 4 and Day 5) two bottles were taken daily for egg production and nucleic acid contents determinations. The daily change of water (either filtered or with algae) allowed us to obtain daily determinations of egg production.

c) Exp#3: Effect of formaline preservation on the determination of nucleic acid contents of copepods.

Adult females of *Acartia grani* were picked out from the laboratory culture and 6 batches of 20 females prepared. Three batches were frozen in liquid nitrogen and the remaining three were preserved in 4% formaline and kept for ca. 3.5 days before nucleic acid content analysis. The purpose of this experiment was to determine whether nucleic acid contents could be conducted in formalin preserved samples.

RNA and DNA quantification. - Analyses were conducted following the fluorometric method of Fara et al. (1996). Copepods were placed onto precombusted (450° C, 6h) GF/F glass fiber filters and frozen in liquid nitrogen. At the moment of the analysis the filters were taken from the liquid nitrogen and immediately ground on 5 ml Tris buffer (0.9 mM $CaCl_2.2H_20$, 0.9 mM

$MgCl_2.6H_20$, 100 mM NaCl, 100 mM Tris(hydroxymethil)-aminomethane, pH 7.5) in a Potter-Elvehjem tissue-homogenizing tube for 2 min at 0°C. The homogenates were then centrifuged at 3500 rpm for 10 min at 0 °C, and the supernatant fluid was divided into three subsamples. One aliquot was used to quantify blank fluorescence. For the nuclease digestion, one aliquot was incubated 20 min at 37 °C with DNase-free, RNase (0.5 μg $ml^{-1}$, final concentration in the assay) and the other aliquot was incubated for 20 min at 25° C with RNase-free, DNase (10 units $ml^{-1}$, final concentration in the assay). After the enzymatic digestion the subsamples were stained with Thiazole Orange. Fluorescence values of the aliquotes treated with either RNase or DNase (previously corrected for the blank fluorescence) were used to determine DNA and RNA concentrations, respectively. For calculations, DNA and rRNA from *Escherichia coli* were used as standards. Preliminary studies indicated that the number of *Acartia grani* individuals required for analysis ranged between 15 and 20 (Fig. 1). Much higher numbers (data not shown) produced quenching effect.

RNA and DNA contents were expressed as μg RNA or μg DNA $copepod^{-1}$. The DNA content is an estimator of biomass (analogous to dry weight); thus, the RNA/DNA ratio normalizes the RNA content to biomass.

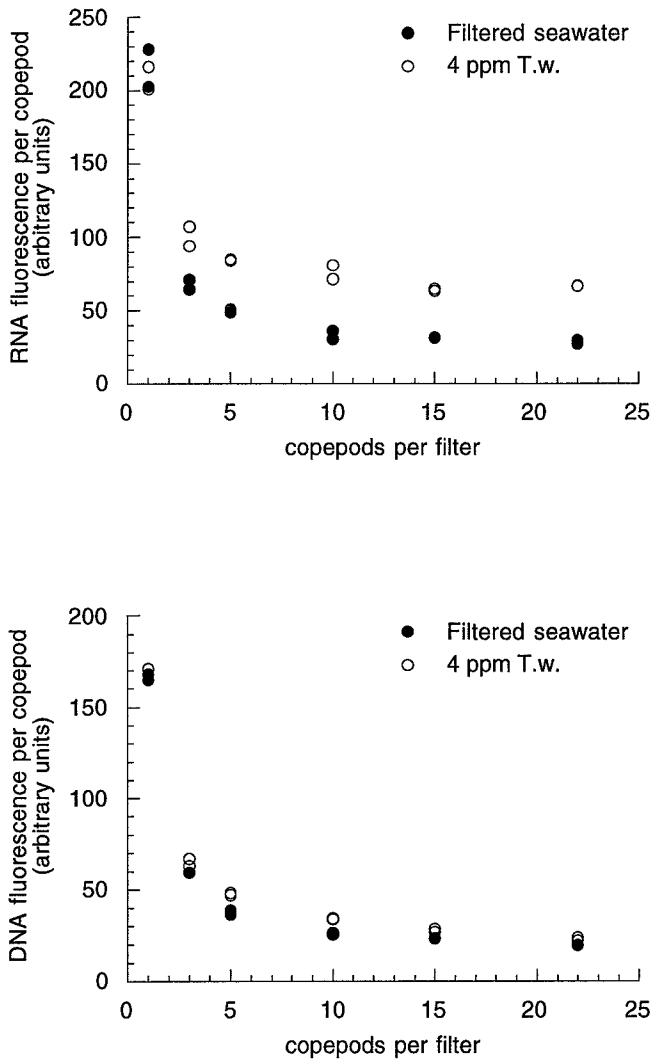


**Figure 1**. Plot of RNA and DNA fluorescence per copepod as a function of the number of copepods on the analyzed filter. Data correspond to a preliminary experiment conducted to determine the minimum number of copepods required for the nucleic acid content analysis. Adult *Acartia grani* females were incubated either in filtered seawater (filled circles) or in a 4-ppm suspension of *Thalassiosira weissflogii* (open circles), and afterwards groups of individuals were transferred to precombusted filters and frozen until analysis. The fluorescence values for both RNA and DNA become linear (i.e. independent of the number of copepods) after a minimum of 5-10 females per filter.

## RESULTS

Figure 2 shows the relationship between egg production rate and food concentration at the two experimental temperatures. A difference in temperature of ca. 5° C resulted in maximum egg production rates more than two times higher at the highest temperature (respectively 28 and 64 eggs $ind^{-1}$ $d^{-1}$ at 17.8 and 23.3° C). The $Q_{10}$ value for the maximum egg production rate in that range of temperatures was 4.4.

Figure 3 shows the linear relationship between egg production rate and both RNA content per copepod and RNA/DNA ratio. The relationship was highly significant for the experiments at both temperatures using either RNA per copepod or RNA/DNA as regressors (see Table 1). A slightly better fit was obtained using the RNA/DNA ratio as a regressor (which takes into account the biomass of the individuals analyzed). In the following we will use only the RNA/DNA ratio as regressor.

The effect of temperature on the linear relationship between egg production rate and the RNA/DNA ratio resulted in a significant change in the slope of the linear relationship (ANCOVA, $F_{1,20} = 32.6$, $P<0.001$), but not for the intercept ($F_{1,20} = 2.66$, $P<0.12$). For instance, a RNA/DNA ratio of 20 would result in an egg production of 16 eggs $ind^{-1}$ $d^{-1}$ at 17.8° C, and 39 eggs $ind^{-1}$ $d^{-1}$ at 23.3° C.

We have also built a predictive model of egg production rate by multiple regression. The best model (Table 2) included only two regressors: the RNA/DNA ratio and the product temperature x RNA/DNA (interaction factor in the ANCOVA).

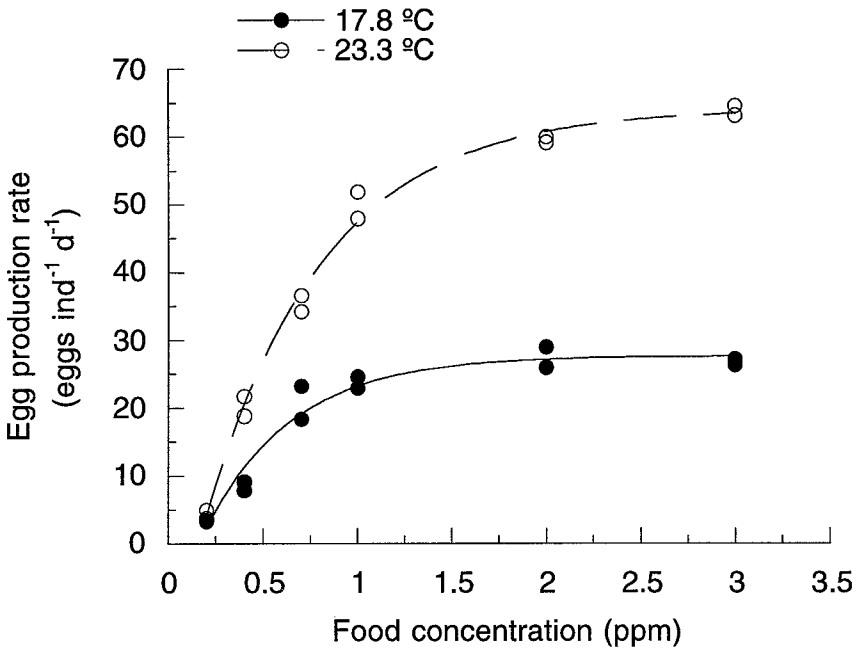


**Figure 2.** Functional response of egg production rate (EPR) of *Acartia grani* to food concentration (in abcissae) and temperature. Ivlev's fits to the data were EPR = 27.6 $(1\text{-}e^{-2.18(food-0.16)})$ at 17.8° C (continuous line) and EPR = 64.1 $(1\text{-}e^{-1.61(food-0.16)})$ at 23.3° C (broken line).

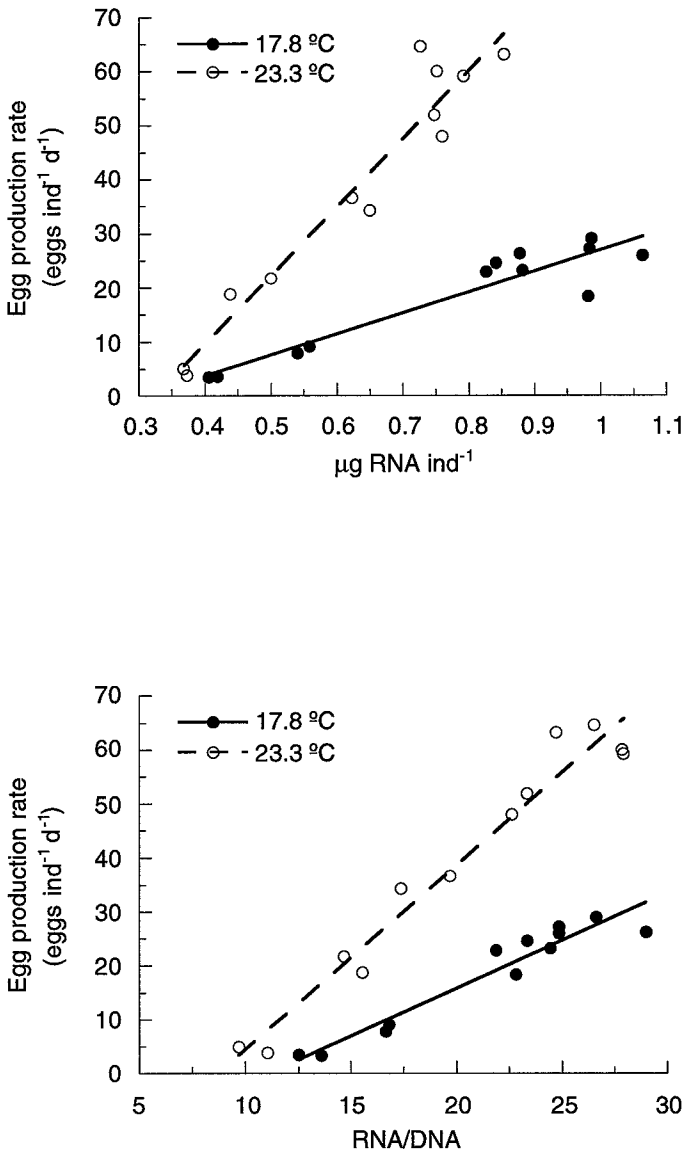


**Figure 3.** Linear relationship between egg production rate and RNA content per copepod (above; µg RNA $ind^{-1}$) and RNA/DNA ratio (below) at both experimental temperatures.

**Table 1.** Egg production rate of *Acartia grani* (EPR, eggs $ind^{-1}$ $d^{-1}$) as a function of either RNA content (µg RNA $ind^{-1}$) or RNA/DNA ratio at each experimental temperature. Values for the parameters (± SE) of simple linear regression fits (EPR = a + b X) are showed. * P<0.001

a) RNA content as regressor

| | 17.8° C | 23.3° C |
|---|---|---|
| a | -11.8±3.66 | -40.8±6.95 |
| b | 38.7±4.50 | 126.0±10.65 |
| $r^2$ | 0.88* | 0.93* |

a) RNA/DNA as regressor

| | 17.8° C | 23.3° C |
|---|---|---|
| a | -19.4±3.52 | -29.6±4.69 |
| b | 1.76±0.159 | 3.41±0.223 |
| $r^2$ | 0.92* | 0.96* |

**Table 2.** Multiple regression model to predict egg production rates (eggs $ind^{-1}$ $d^{-1}$) of *Acartia grani* adult females as a function of the variables temperature and RNA/DNA ratio. Parameters (± SE) are showed.

Model

$$EPR = a + b\,RNA/DNA + c\,(Temperature \times RNA/DNA)$$

$r^2$=0.96, n=24

$F_{2,21}$=264.6, P<0.001

Parameters

a= -25.7 ± 3.14, P<0.001

b= -1.67 ± 0.305, P<0.001

c= 0.21 ± 0.013, P<0.001

Figure 4 shows the temporal evolution of egg production rates and RNA/DNA ratio in Exp#2. It takes two days for well fed *Acartia grani* females to reduce their egg production to almost zero values when transferred into filtered seawater. The recovery of egg production, once the copepods were put back in plenty of food, is slower, as already showed for the same species by Calbet and Alcaraz (1996). The overall trend followed by the RNA/DNA ratio is very similar to the one showed by egg production. However, while for egg production the recovery due to food presence (Days 4 and 5) was 30% slower than its decline due to food privation (Days 2 and 3), the RNA/DNA ratio responded much slowlier to food presence (60%).

The use of formaldehyde as a fixative resulted in the lost of fluorescence of the samples, i.e. in negative or very low values of RNA and DNA per copepod. The RNA content estimated in the formalin preserved samples was -0.02±0.011 (SE) μg RNA $ind^{-1}$, compared to the 0.49±0.017 (SE) μg RNA $ind^{-1}$ measured in the frozen samples. In the case of DNA, the two treatments gave 40±0.8 and -4±3 ng DNA $ind^{-1}$ respectively for liquid nitrogen and formalin preservation. This result precludes the use of formaldehyde as a fixative for samples to be procesed for nucleic acid contents. Probably formaldehyde does not allow Thiazole Orange to stain the cells.

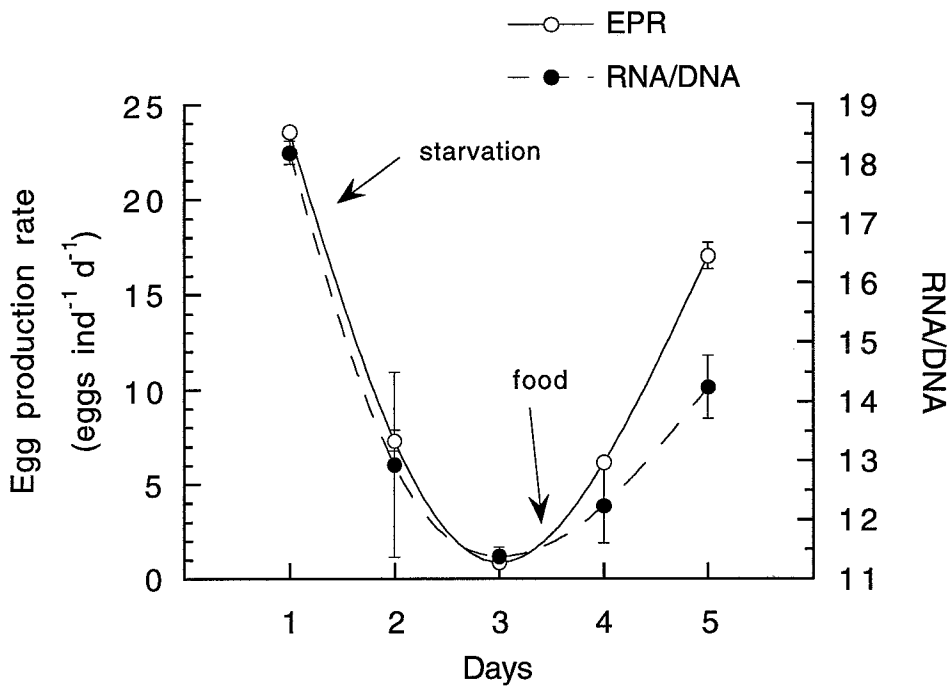


**Figure 4.** Time response of egg production rate (EPR) and RNA/DNA ratio of *Acartia grani* to changes in food availability (Exp. #2). The arrows indicate when the copepods were first transferred from saturating food conditions to filtered seawater ("starvation"), and finally put back into plenty of food ("food").

## DISCUSSION

We have shown that there is a significant relationship between the RNA content of adult copepods and their growth rate (estimated as egg production rate). This finding confirms the study by Nakata et al. (1994) and sets the base for further application of this method to determine growth rates of copepods in the field. Although in our experiments we only used one type of food item (*Thalassiosira weissflogii*), this relationship must be independent of food type and quality, what facilitates its application to field studies. Further, this relationship should be unique for a particular copepod species. Laboratory experiments would easily allow to

establish powerful regression models relating RNA content with egg production of targeted species that could be further applied to field preserved (frozen) samples.

Our data also showed that the linear relationship between RNA content and egg production rate was highly dependent on temperature. Metabolic processes are dependent on temperature and this holds also for the amount of RNA per copepod. This result emphasizes the fact that temperature must be taken into account when applying some biochemical methods to determine in situ growth rates of any kind of organisms. Our investigation showed that the changes induced by temperature on the linear relationship between nucleic acid content and growth can be quantified and are predictable. Although our data are restricted to two temperatures, we have been able to built a significant predictive multiple regression model that explains 96% of the variability in our data. Likely, considering three or four temperatures would improve the obtained model. It is important to note that the specific RNA content should be related to any biomass unit (e.g. dry weight or DNA content) rather than per individual.

Two main limitations were noted by Ota and Landry (1984) for the use of nucleic acids content as estimator of growth rates. First, they argued that this kind of methods might not be appropriate in the case of juvenile copepods. Developmental and physiological changes during the period to adulthood could induce variations in the RNA content not strictly related to growth (change in biomass). This seems to be the case for fish larvae during the first days after hatching (Clemmesen 1994) and probably the molting process in crustaceans would have a similar effect. Because our study was conducted on adult females this question should be addressed in further studies.

The second objection pointed out by Ota and Landry (1984) was that differences in growth rates due to food limitation might be poorly represented by changes in

the RNA contents (see also Dagg and Littlepage 1972). In contrast, our experimental data showed that the RNA content of adult Acartia grani females reflected changes in egg production rates even under extrem food conditions (Fig. 3). This fact warrants the validity of the method.

Our experiments have evidenced that under steady food conditions (after two days of preconditioning) RNA/DNA ratio was well correlated with growth rate (estimated as egg production). In addition, we investigated the time response of RNA content changes related to variations of growth induced by food availability (Fig. 4). Although RNA should respond quickly to slow down or building up changes in growth rates, some authors (Dagg and Littlepage, 1972; Sapienza and Mague, 1979) have argued that due to differences in stability of the different pools of RNA, total RNA content might not respond quickly to sudden reductions in growth rates. In contrast, our experiments showed that under slow down conditions the RNA contents paralleled the decline in egg production (growth) rates. Unexpectedly, when food was supplied, the egg production response was faster than the RNA building up. This result may have arisen from the experimental design. For this test, the animals were maintained in abundant food for a few days in order to achieve saturated and stable conditions. Then, the transfer to filtered seawater for two days induced the observed reduction in RNA content and egg production rate. After these two days of starvation, food was resupplied. We feel that the recovery of egg production was faster than the building-up of RNA during this last period because this egg production very much reflected the maturation of oocytes produced during the previous feeding history. Very likely, had the starvation period been longer, a better coupling in the time responses of RNA and egg production would have appeared.

Our study offers encouraging perspectives for an alternative method for the estimation of in situ growth rates of adult copepods. For the application of this

method, a calibration in the laboratory under temperature and food concentration controlled conditions is required. The quantification of RNA content or RNA/DNA ratios allows good time- and space-specific resolution. Although it can only be applied onto selected targeted species, not to the whole community, this problem is also valid for other methods like the egg production one. Furthermore, it can also get rid of some drawbacks with the egg production method in the field, like food availability during the incubation (Saiz et al. in press). It may also be used on copepods which carry their eggs in sacs (e.g. *Oithona*). For these organisms, the determination of their in situ growth rates is usually based on the quantification of the time interval between clutches from temperature-dependent relationships (Uye and Sano 1995), although they can depend also on food availability during incubation (Saiz et al. in press).

Our aim in the near future is to apply this methodology to field populations of selected species and to compare our predictions of in situ growth rates with the standard procedures. However, first further studies should extend the observed relationship in *Acartia grani* to other copepod genera. Also, the capacity of the method to detect fast increases in egg production rate due to sudden increases in food availability should be further studied.

**RESUM**

Es va estudiar sota condicions controlades de laboratori un mètode alternatiu d'estima de producció específica de copèpodes basat en el contingut de RNA. El mètode en qüestió permet l'obtenció d'estimes de producció a partir de mostres prèviament congelades (no a partir de mostres fixades amb formol), la qual cosa facilita el mostreig sobre espècies difícils de tractar durant les campanyes oceanogràfiques, i augmenta la resolució tan espacial com temporal de les determinacions de producció secundària.

Els resultats obtinguts sota diferentes concentracions de menjar van mostrar molt bones regressions entre la producció d'ous i el quocient RNA/DNA. La temperatura tenia un efecte modulador del pendent de les esmentades regressions. Tanmateix, és possible elaborar un model predictiu de producció que tingui en compte tant la temperatura com el quocient RNA/DNA. El contingut de RNA responia a la mateixa velocitat que la producció d'ous al dejuni. Per contra, ho feia més lentament quan es tornaba a subministrar menjar. Aquest resultat ens porta a pendre amb cura les estimes de producció a partir d'aquest mètode en condicions d'alimentació extremes, en canvi, en els sistemes naturals on la variabilitat de les condicions tròfiques és més baixa el mètode és perfectament aplicable.

Encara que els resultats presentats són força encoratjadors, és necessari seguir investigant sota diferents condicions i amb més espècies per tal de poder assegurar el rigor i la validesa del mètode per a poblacions naturals.

# CAPÍTOL II

# EFECTE DE LES FLUCTUACIONS EN LA DISPONIBILITAT D'ALIMENT SOBRE LES TAXES DE PRODUCCIÓ EN COPÈPODES

**Efecte de les fluctuacions en la disponibilitat d'aliment sobre les taxes de producció en copèpodes.**

La dinàmica de les poblacions de zooplàncton és fortament controlada per les condicions de disponibilitat d'aliment. D'altra banda, l'heterogeneïtat en la distribució espacial del fitoplàcton i el zooplàncton en forma de taques, i llurs canvis en el temps, determinen que la disponibilitat d'aliment pel zooplàncton segueixi fluctuacions d'amplitud i freqüències variables, de les que depèn en gran part el tipus preferencial de xarxa tròfica que es desenvolupa, que com s'ha dit determina en gran part el destí final del carboni fixat pels productors primaris.

Però malgrat l'evident caràcter variable de la disponibilitat d'aliment, els efectes d'una alimentació intermitent o fluctuant sobre la producció en copèpodes són pràcticament desconeguts. És important doncs, conèixer la resposta del zooplàncton a fluctuacions en el subministrament de menjar sota condicions controlades de laboratori. D'altra banda és lògic pensar que diferents freqüències d'oscil.lació de l'aliment no tindran el mateix efecte sobre els adults que pels diferents estadis de desenvolupament.

Amb motiu d'esbrinar l'efecte d'una dieta fluctuant sobre la producció i supervivència de copèpodes es van efectuar una sèrie d'experiments tant amb adults com amb fases juvenils del copèpode marí *Acartia grani*.

En aquest capítol es compara l'efecte que té sobre la producció d'ous un subministrament continu d'aliment en front d'un altre de fluctuant a vàries freqüències (cada 12, 24 o 48 h). També s'avalua l'efecte de períodes de dejuni prolongats en la caiguda i posterior recuperació de la posta d'ous.

En el capítol 2.2. s'intenta determinar la tolerància dels estadis de desenvolupament d'*A. grani* a condicions de dejuni i s'avaluen els efectes de diferents freqüències de fluctuació en el subministrament d'aliment sobre el creixement i mortalitat de nauplis comparades amb un aportament continu.

## 2.1 EFFECTS OF CONSTANT AND FLUCTUATING FOOD SUPPLY ON EGG PRODUCTION RATES OF *ACARTIA GRANI* (COPEPODA: CALANOIDA)

(Basat en un article del mateix nom fet amb la col.laboració d'altres autors i publicat a Mar. Ecol. Prog. Ser. Vol. 140:33-39, 1996)

## INTRODUCTION

The discontinuous nature of marine phyto- and zooplankton distributions and their temporal variability cover a wide spectrum of scales, which are closely related to similar scales of physical variability (Stommel 1963, Haury et al. 1978, Mackas. et al. 1985, Strass 1992). Structure and dynamics of planktonic ecosystems are, in turn, strongly dependent on the coupling between the different trophic components of the system with regard to these spatial and temporal scales of variability (Cushing 1974, Le Fèvre and Frontier 1988).

For herbivorous zooplankton, food availability depends mainly on the relationships between spatial dimensions of the patchiness field of phyto- and zooplankton, and the organism's mobility. At fine scale (meters to hundreds of meters, minutes to days, Haury et al. 1978), is where the trophodynamic interactions for plankton take place. The fate of zooplankton populations is thus strongly dependent on the modifications induced on growth and production parameters by the time scales (frequency) of alternate periods of feeding and starvation conditions.

Studies on survival during starvation conditions of calanoid copepods indicate specific differences in tolerance of starvation which are inversely related to egg production rates (Dagg 1977), and similar studies dealing with the functional response to fluctuating food conditions (Borcher and Hutchings 1986, Nival et al. 1990, Davis and Alatalo 1992) suggest, for some copepod species, the existence of a certain capacity to buffer food variability.

In this work we have tried to evaluate the effects of fluctuating food conditions on calanoid copepods' production at time scales comparable to the "fine scale" variability in natural systems (Haury et al. 1978).

The species chosen, *Acartia grani*, is a copepod typical of coastal, semi-confined ecosystems, conditioned by a high degree of instability of physical (temperature and salinity) and biological (food) conditions.

The 2 goals of the study were: I) To test the effect on egg production rates and on egg production periodicity of equivalent average food concentrations, either continuously supplied, or fluctuating at different frequencies (12, 24, and 48 hours); and II) To evaluate the effect of the duration of longer starvation periods on the decline and further recovery of egg production rates, and to estimate how these variables are affected by temperature.

## MATERIAL AND METHODS

### Experimental organisms

Male and female (926 ± 24 and 1003 ± 28 μm length, respectively) *Acartia grani* were reared at the laboratory in a temperature-controlled room at 18 ± 1°C, and fed "ad libitum" a suspension (5 ppm by volume) of the diatom *Thalassiosira weissflogii* (13-14 μm diameter, 1420.3 ± 3.46 $\mu m^3$ $cell^{-1}$). This diatom was cultured in f/10 medium (Guillard 1975) at the same temperature as the copepods. The algae were maintained in exponential growth rate in batch cultures in order to avoid changes in their nutritional quality which can affect the production and hatching success of the eggs (Kiørboe 1989, Jónasdóttir 1994). All experiments

were conducted in a 12:12 hours Dark/Light natural cycle (intensity of light = 50 to 60 μE $m^{-2}.sec.^{-1}$).

**Effects of fluctuating food availability**

The experiments on the effects of different frequencies of fluctuating food concentration were designed in order to provide the copepods with equivalent average amounts of food under two conditions: continuous supply at constant concentrations, which were considered the controls, and fluctuating, high food-low food concentrations, simulating the food variability experienced in natural fluctuating systems. The concentrations were chosen in the range in which egg production rates are proportional to the food concentration (Saiz et al. 1992a). The maximum food concentration was always maintained below ingestion rate saturating food concentration for the species (Saiz et al. 1992a).

Recently molted adult *A. grani* were acclimatized to a suspension of 0.9 ppm (by volume) *T. weissflogii* at 18°C and 12:12 hour Dark/Light, natural cycle for two days. Six females and three males were gently sorted with a wide-mouth pipette and placed in egg-laying chambers (Fig. 1). These chambers consisted of two concentric perspex cylinders: the inner (15 cm long, 9 cm diameter) was covered on the bottom with 200 μm nitex mesh, so that copepods were retained and eggs could sink and pass through the bottom mesh to avoid predation. Eggs laid were retained by the outer cylinder (15 cm long, 10 cm diameter) which was covered on the bottom with 40 μm nitex mesh.

Egg-laying chambers were suspended in 15 l plastic containers (6 chambers per container) with filtered sea water and algae at the chosen concentration. In order to avoid algal sedimentation and to secure water flow through the egg-laying chambers, water was circulated with a peristaltic pump at a rate of 0.2 l $h^{-1}$ in each

chamber, equivalent to an exchange rate of more than 6 times per day. Food concentration was monitored by means of a Coulter Multisizer every 6 h, and corrected if necessary. Each day water was changed by a new suspension of *T. weissflogii* at exponential growth rate.

Fluctuations in food concentration were simulated by gently changing the egg-laying chambers from the high food to low food containers and vice versa at the required frequencies, and the eggs laid were collected twice a day (at the end of the 12 h light and dark periods) and counted under a dissecting microscope. All the experiments were run in triplicate.

For high frequency (12 h fluctuation), two controls, with food supplied continuously at two constant concentrations and at 12:12 h Dark/Light natural period, simulated the average (0.9 ppm, averaged food) and maximum (1.6 ppm, maximum food) experimental food concentrations. Fluctuating food conditions consisted on 12 h high food (1.6 ppm) and 12 h low food(0.2 ppm) supply, combining two light situations: High food supplied at night (high food-dark) and high food supplied in daylight (high food-light).

For lower frequency fluctuations, the effects of two frequencies of food pulses: 24F (1 day high food - 1 day low food), and 48F (2 days high food - 2 days low food) were tested . The experimental design was the same as for 12 h fluctuation, with the same food concentrations for high food and low food conditions, and a control of 0.9 ppm, (averaged food), equivalent to the average high food-low food concentrations. The light conditions were 12:12 h Dark/Light natural period. Eggs laid were collected daily at the end of the dark period and counted under a dissecting microscope.

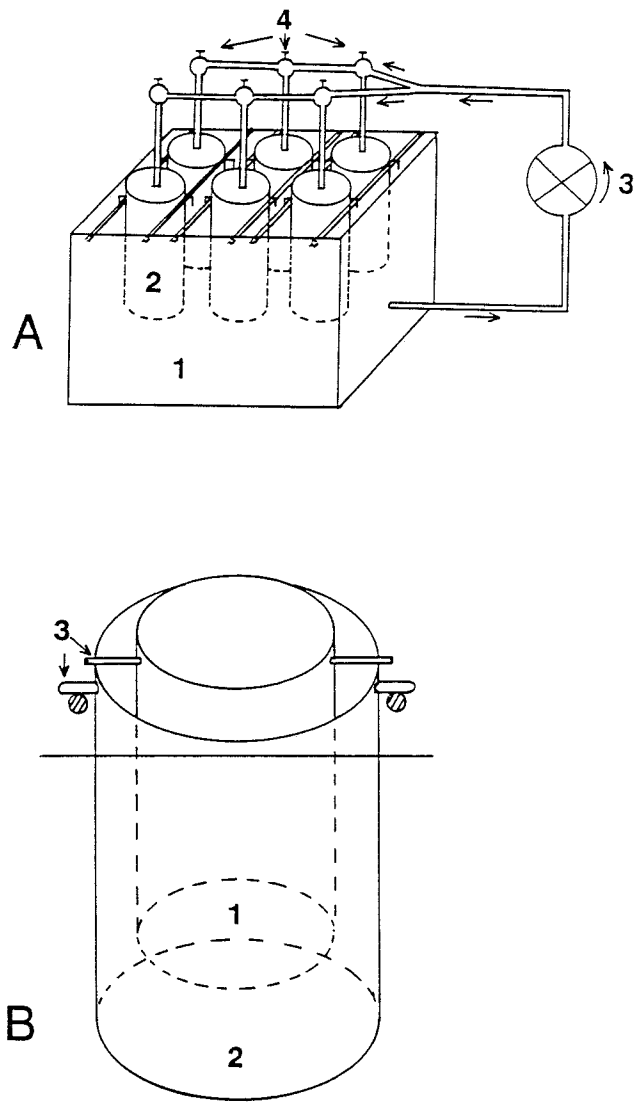


Fig 1. A) Schematic diagram of the incubation system used in the food fluctuation experiments. (1) 15 l plastic container, (2) egg-laying chambers, (3) peristaltic pump, (4) flow regulator. B) Digram of an egg-laying chamber. (1) 200µm nitex-mesh, (2) 40 µm nitex-mesh, (3) hanging system.

**Effects of starvation on the decline and recovery of egg production rates**

Adult females were acclimatized for 3 days at 18°C, 12:12 h Dark/Light natural cycle and saturating (5 ppm) food concentration. Five females were placed in 620 ml Pyrex bottles filled with filtered sea water and maintained under starvation conditions for 3, 4 and 5 days respectively (S3, S4 and S5). After the starvation periods, females were fed again with a suspension of 3.5 ppm *T. weissflogii*. Non-starved females, fed continuously 3.5 ppm of the same algae, were used as control. Every day the contents of the control and experimental bottles were filtered through a 20 μm mesh submerged sieve to collect eggs. Healthy copepods were placed again in the same bottles with either filtered sea water or food, depending on the treatment. Crumpled empty eggs were also included in the counting.

**Effects of temperature on the recovery of egg production**

In order to study the relationships between temperature and the recovery of egg production rates after a period of starvation, adult males and females were maintained for two days at low food (0.3 ppm *T. weissflogii*) and 12:12 h Dark/Light natural cycle at three temperatures (13, 18 and 23°C, four replicates per temperature). Five females and two males were then picked out and introduced into 650 ml Pyrex bottles and fed again with saturating (3.5 ppm) food, and the egg production was monitored daily for four days.

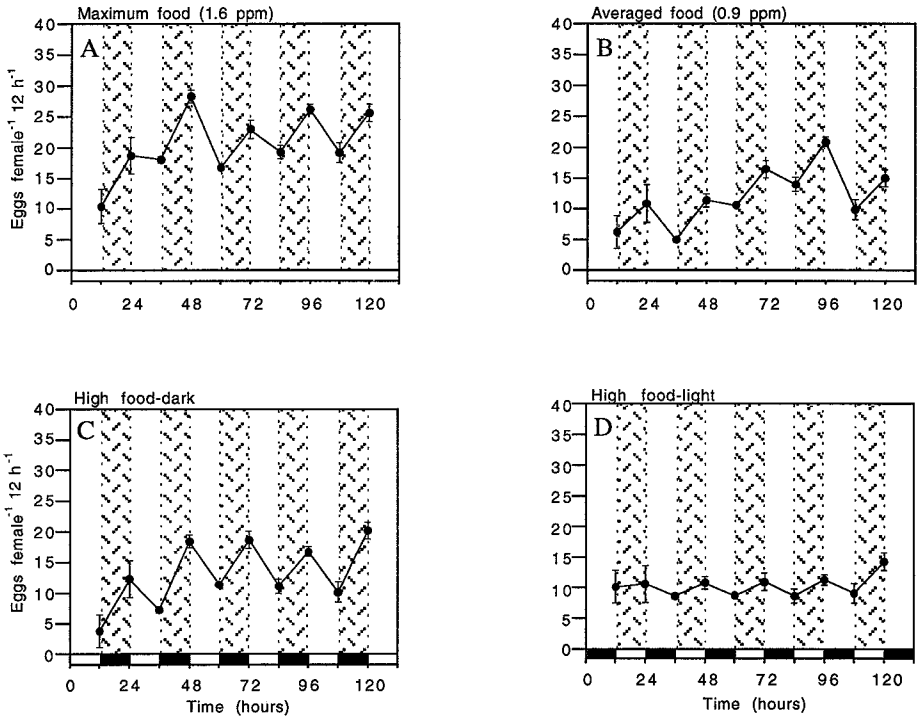


**Fig. 2.** Egg production corresponding to the short period (12 h) of food fluctuating treatments. A) Continuous food supply at maximum food concentration. B) Continuous food supply at averaged food concentration. C) 12 h oscillation treatment, High-food supplied at night. D) 12 h oscillation treatment, High-food supplied at day. Dark zones in abscissae represent the time spent in the presence of high food concentration. Vertical shaded areas the indicate dark periods. Vertical bars correspond to SE.

## RESULTS

### Effects of fluctuating food concentration on egg production rates

In the 12 h-fluctuation experiments, egg production resulted in a clearly rhythmical pattern for both continuous (controls) or fluctuating (12 h period) food conditions (Fig. 2), and was significantly higher during the dark hours ($p < 0.001$, one-way ANOVA). This rhythmical pattern in egg production (EP) was maintained even when high food concentration was supplied during daylight hours (high food-light, Fig. 2D). The light conditions at which high food conditions occurred also affected egg production (table 1). The higher values were observed in high food-dark individuals ($113 \pm 4$ eggs female$^{-1}$, compared to $84 \pm 1$ eggs female$^{-1}$ high food-light, four day cumulative EP, $p < 0.06$, Tukey's test, Fig. 2 C).

In controls, cumulative egg production was also dependent on food concentration (Fig. 2A, B), with $100 \pm 9$ eggs female$^{-1}$ at average food conditions, and $171 \pm 9$ eggs female$^{-1}$ at maximum food conditions (1.6 ppm). Significant differences were found ($p < 0.01$, one-way ANOVA) between maximum food and the 12 h fluctuating treatments. All calculations were done excluding the first day due to a possible effect of previous conditions.

Fluctuations of 1 and 2 days in food concentration (24F and 48F) significantly affected egg production (Fig. 3A, B), especially at 2 days frequency (48F). The number of eggs laid fluctuated according to the alternating high food and low food concentration.

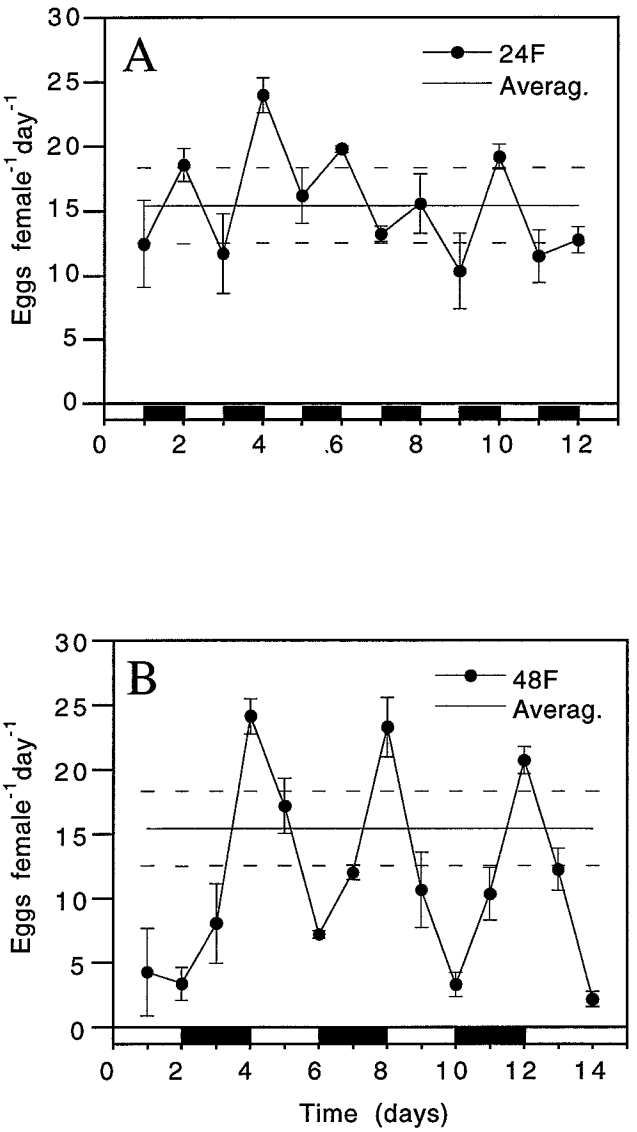


**Fig. 3**. Temporal pattern of egg production rates as a function of food supply fluctuations. A) 1 day (24F) fluctuation, B) 2 days (48F) fluctuation. Horizontal line represents the average egg production for a continuous, average food supply. Horizontal dashed lines are the limits of SE. Dark zones in abscissae represent the time spent in high food concentration. Vertical bars correspond to SE.

No significant differences were observed in the cumulative egg production in 24F (131 ± 4 eggs, eight day cumulative egg production) as compared with the control (averaged food, 130 ± 3 eggs, table 1). However, egg production in 48F was significantly lower (105 ± 1 eggs, $p < 0.006$, Tukey's test) than in 24F or average food (table 1).

**Table 1.**- Cumulative egg production (per female during "n" days) ± SE for the different food treatments (see text).

| Treatment | Eggs Produced (cumulative) | days |
|---|---|---|
| 12 h oscillation | | |
| Averaged food | 100 ± 9 | 4 |
| Maximum food | 171 ± 9 | 4 |
| High food-dark | 113 ± 4 | 4 |
| High food-light | 84 ± 1 | 4 |
| 24-48 h oscillation | | |
| Averaged food | 130 ± 3 | 8 |
| 24F | 131 ± 4 | 8 |
| 48F | 105 ± 1 | 8 |
| Starvation treatment | | |
| Saturated | 291 ± 25 | 5 |
| S3 | 167 ± 20 | 5 |
| S4 | 134 ± 15 | 5 |
| S5 | 119 ± 14 | 5 |
| Temperature treatment | | |
| 13°C | 39 ± 5 | 5 |
| 18°C | 108 ± 5 | 5 |
| 23°C | 198 ± 17 | 5 |

The oscillations of daily egg production closely followed the fluctuations of food abundance. The average ratio of increase and diminution of daily egg production for the different frequencies of high and low food, respectively, are indicated in Table 2.

**Table 2.**- Relative variation of egg production rates (after high food, 1.6 ppm and low food, 0.2 ppm conditions) as compared with constant supply of average food concentration, according to the time scales of food fluctuation (12, 24, 48 and 72 h).

* individuals in filtered sea water

| Time scale (hours) | High food | Low food |
|---|---|---|
| 12 | +0.077 | -0.098 |
| 24 | +0.125 | -0.226 |
| 48 | +0.397 | -0.754 |
| 72 | +0.72 | -1.0 * |

**Effects of starvation on egg laying decline and recovery**

The egg production rate decreased considerably after 24 h starvation and was close to zero after 48 h. Although females resumed spawning after 24 h from the re-start of feeding, the time needed to recover their normal egg production was directly related to the duration of the starvation period (Fig. 4). The duration of the starvation period had a negative effect in cumulative egg production, the

number of eggs laid during five days in relation to the days under starvation following a negative exponential function in the range of starvation time considered (Fig. 5).

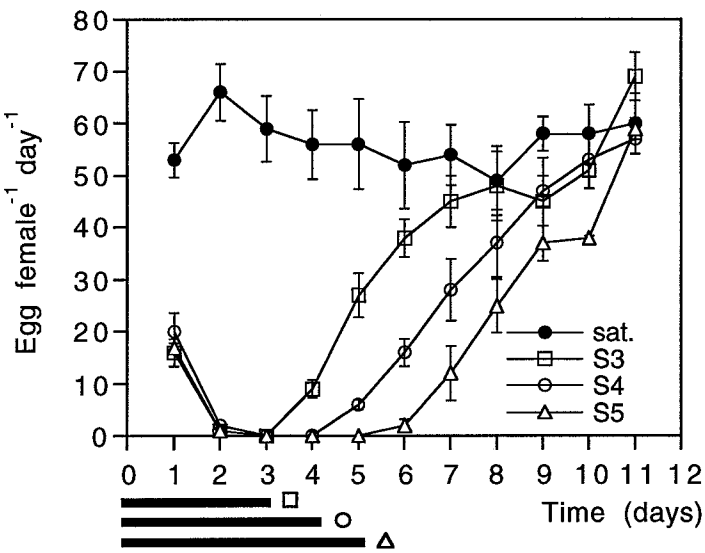


**Fig. 4**. Time evolution of daily egg production rates under saturating food concentrations after starvation periods of 3 days (S3), 4 days (S4) and 5 days (S5). Control corresponds to the egg production rates for a saturating, continuous food supply (sat.). Horizontal dark bars represent the starvation time for each treatment. Vertical bars correspond to SE.

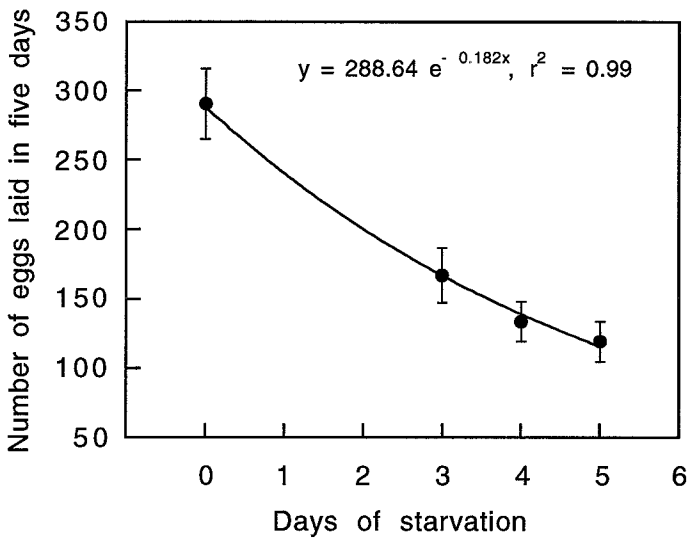


**Fig. 5**. Exponential relationship between starvation time and cumulative egg production per female after re-starting saturating feeding conditions. Vertical bars correspond to SE.

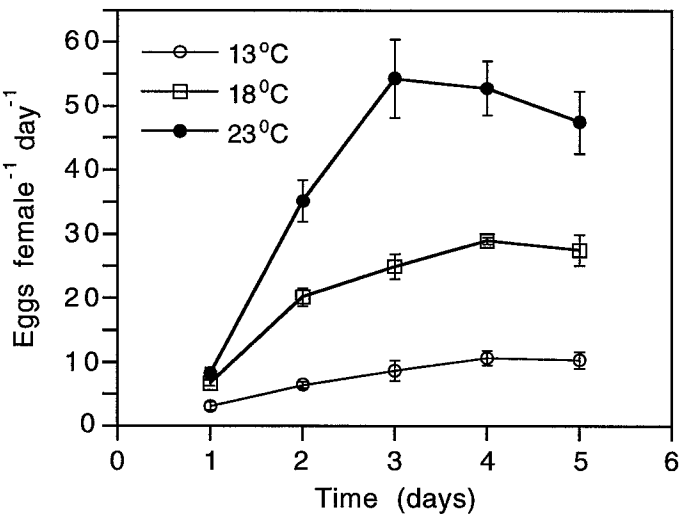


**Fig. 6.** Time evolution of egg production rates under saturating food conditions at 13, 18 and 23°C after 2 days starvation. Abscissae: Days after re-starting feeding. Vertical bars correspond to SE.

Temperature had a significant effect on both the maximum egg production rate, and the recovery time to achieve maximum egg production rate (fig. 6). Maximum egg production rates (EPR, eggs female$^{-1}$ d$^{-1)}$ were 51 ± 5 at 23°C, 28 ± 1 at 18°C and 10 ± 1 at 13°C, which corresponded to a $Q_{10}$ value of 3.2.

Recovery time (time to achieve maximum EPR after the onset of food saturating conditions) ranged from 2 days at 23°C to 3 days at 18°C (Fig. 6). At 13°C, the time needed to reach maximum egg production is more than 4 days (maximum egg production rates at this temperature come from authors' unpubl. data). The estimated $Q_{10}$ corresponding to the recovery of egg production is about 2.3.

## DISCUSSION

For the food concentrations at which experiments were run, egg production rates were linearly related to food concentrations (Saiz et al. 1992a). As a consequence, food fluctuations should have effects on egg production similar to a constant supply of food at average concentration, at least at time scales comparable to the diel activity rhythms of copepods. The diel spawning rhythm, also observed for other copepod species (Marcus 1988), seems to be independent of the feeding conditions. However, when comparing our results with the continuous, non-cycling spawning pattern in *Acartia hudsonica* under continuous light (Parrish and Wilson 1978), the light regime could be functionally

related to egg-laying cycles. The light conditions in the period at which the algae are available also affect the rates of egg production. Copepods feeding on high food during the night produce more eggs than copepods fed on high food during the day. The differences in egg production in relation to food and light conditions are a consequence of the control exerted by feeding rates on copepod's egg production (Kiørboe et al. 1985, Saiz et al. 1992a), and confirm not only the higher nocturnal feeding activity of *A. grani* (Bautista et al. 1988), but the short time (hours) at which female *Acartia* sp. transform the food ingested into eggs (Tester and Turner 1990).

The different consequences for egg production derived from the light conditions at which rich-food patches are found by copepods reinforce the importance of match-mismatch mechanisms (Cushing 1974) even at short time scales (hours). Thus, the coupling between copepod diel activity rhythms (feeding, vertical migration), light regime (night-day), and the appropriate environmental trophic conditions (surface or deep phytoplankton rich layers) is of paramount importance from the point of view of plankton dynamics (Saiz and Alcaraz 1990, Saiz et al. 1992b).

The lack of differences in average egg production between alternating, 24 h high food - low food supply (24F), and the control (constant supply of average food concentration), were in agreement with the results obtained in the 12 h fluctuation experiments. However, daily egg production rates fluctuated around the average values according to the alternation of high food-low food conditions. At alternating, two days high food-low food concentration (48F), *A. grani* produced less eggs than in the control. This is in agreement with the results obtained by Dagg (1977) with *A. tonsa*, suggesting a low capacity for tolerance of relatively long starvation periods.

Apart from the night-day rhythms in egg production, food fluctuations determine oscillations of egg production rates which are a function of the frequencies at which high food and low food concentrations are supplied. The relative decrease of egg production after low food conditions, as compared to egg production at average, constant food supply, is higher than the corresponding increase after high food conditions, the difference being also a function of the time scale of food fluctuations. The consequence is the lower total egg production rates determined by increasing time scale fluctuations of food supply.

The sequence of changes derived from long starvation periods in copepods include the mobilization of the energy reserves (Conover and Corner 1968, Ikeda 1974) and a decrease in the metabolic rates (Conover and Corner 1968, Mayzaud 1973, 1976, Tsuda 1994), swimming activity (Tiselius 1992), and egg production (Parrish and Wilson 1978, Checkley 1980, Kiørboe et al. 1985, Hirche 1989). The time required to recover the reserves used during the starvation period, and to resume the normal rate of egg production, seems to be species-specific (Attwood and Peterson, 1989, for *Calanus australis*, Borchers and Hutchings, 1986 for *Calanoides carinatus*). In our experiments, the time required to reach the normal (control) egg production in *Acartia grani* is proportional to the length of the starvation period. However, egg production always starts after 24 h in the presence of food, although the recovery rate during the first day after the restart of feeding is inversely proportional to the starvation time. This would explain the negative exponential relationship between starvation length and the number of eggs laid in five days.

Maximum production rates are strongly dependent on temperature, the corresponding $Q_{10}$ (3.2) coinciding with that observed by Deacon (1980) for ingestion rates by *Acartia clausi*. However, the time needed to recover maximum

egg production rates after starvation appears to be weakly controlled by environmental temperature.

The egg production response of *A. grani* to varying frequencies of food availability, and its capacity to quickly recover from moderate starvation periods, are indicative of a weak capacity to buffer, in terms of fecundity, the changes in food abundance. In the coastal, semi-enclosed habitats usually occupied by the species, high temporal variability and patchiness of phytoplankton, at scales comparable with the experimentally induced fluctuations, are the most characteristic feature (Walsh 1976). These are probably environmental conditions which explain the niche partitioning of estuarine congeneric associates (Alcaraz 1983, Rodriguez and Jimenez, 1990). The consequences are their segregation according the spatial gradient of environmental variability, or their temporal succession when the gradient in variability is no spatially but temporally forced (Jeffries 1962). Nevertheless, a better understanding of the control exerted by food variability on the dynamics of copepod populations would require further studies about the significance of food fluctuation for the different developmental stages.

**RESUM**

Es va determinar experimentalment l'efecte que tenen les fluctuacions de la concentració de menjar i els períodes de dejuni sobre la producció d'ous del copèpode marí *Acartia grani*. La taxa de posta oscil.lava d'acord a la freqüència a la què el menjar era subministrat. Per les freqüències més altes (12 hores), la presència o no de llum quan el menjar estava disponible tenia un efecte significatiu sobre la taxa de producció d'ous, essent més elevada quan l'aliment era present durant la nit.

L'alternança d'alta i baixa concentració de menjar cada 24 hores no tenia efectes significatius en la posta mitjana. D'altra banda, freqüències més baixes (més de 48 hores) reduïen la posta respecte al control (subministrament d'aliment en continu). La duració del període de dejuni i la temperatura determinaven el temps requerit per a restablir una producció d'ous normal (control), a més la temperatura exercia un control sobre les taxes de producció màximes.

L'estret acoblament entre la disponibilitat de menjar, i la producció d'ous d'*Acartia grani* es tradueix en una feble capacitat d'esmorteir oscil.lacions en l'abundància d'aliment, la qual cosa podria explicar la presència, gairebé exclusiva, d'aquest copèpode en zones semi-confinades o costaneres on les escales de variabilitat espacial i temporal són les adequades per a la supervivència i millor competència d'aquesta espècie.

## 2.2. GROWTH AND SURVIVAL RATES OF EARLY DEVELOPMENTAL STAGES OF ACARTIA GRANI IN RELATION TO FOOD CONCENTRATION AND FLUCTUATIONS IN FOOD SUPPY

(Basat en un article del mateix títol fet amb la col.laboració d'altres autors acceptat a Mar. Ecol. Prog. Ser.)

## INTRODUCTION

The dynamics of zooplankton populations is strongly dependent on the conditions of food availability (Holling 1959, Dagg 1977, Kleppel et al. 1996), which are mediated by the rate of temporal variability and patchy nature typical of planktonic communities (Holligan 1984, Mackas et al. 1985, Le Fèvre 1986, Cushing 1989). In this variable food environment, herbivorous zooplankton would perceive the succession of phytoplankton pulses or biomass patches as fluctuations in the amount of food available (Dagg 1977, Le Fèvre and Frontier 1988, Calbet and Alcaraz 1996).

Food fluctuations are known to play a significant role in the control of copepod populations, mainly through changes in mortality and female fecundity rates (Dagg 1977, Nival et al. 1990, Davis and Alatalo 1992, Calbet and Alcaraz, 1996). Nevertheless, most of the available data have been concentrated on adult copepods. Studies on the life-history parameters for early developmental stages refer in general to the control exerted by predation (Landry 1978), or food concentration (Paffenhöfer 1970, Berggreen et al. 1988, Klein Breteler et al. 1982, Tsuda 1994), but the effects of fluctuations in food availability for early developmental stages of copepods are less known.

In this work, we have evaluated the functional effects of starvation and food fluctuations on growth and mortality rates for early developmental stages of the marine pelagic copepod *Acartia grani*. The objectives were:1) To estimate the tolerance of the different developmental stages to starving conditions; and 2) To evaluate the effects of different frequencies of food fluctuations on naupliar

growth rate and mortality as compared with an equivalent concentration of food continuously supplied.

## METHODS

Experimental organisms and general procedures

Naupliar stages of the planktonic marine copepod *Acartia grani* were obtained from a laboratory culture reared at the described conditions in the chapter 2.2. The experimental nauplii were collected from eggs hatched during 12-24 h intervals in order to obtain a maximum degree of homogeneity in initial naupliar stage and size. All the experiments were conducted at 18 $\pm$ 1 ° C, in a 12 h light-dark cycle. Algal concentrations (volume) were measured by means of a Multisizer Coulter Counter, and the conversion to carbon contents was done according the volume-carbon relation given by Berggreen et al. (1988).

Stage-specific biomass in *Acartia grani* was estimated from the length-dry weight equations given by Durbin and Durbin (1978) for *Acartia clausi* . Organismal size was measured with an Image Analysis System and NIH Image software on organisms fixed with acidic lugol. Instantaneous specific growth rates of nauplii (g) were calculated from the exponential change in biomass (dry weight) as is shown in the chapter 2.2:

Survival to starving conditions

Eggs obtained from the laboratory culture of *A. grani* were hatched and grown under excess food (5-6 ppm *R. baltica*, equivalent to 1350-1620 $\mu gC\ l^{-1}$), and the evolution (length and stage) of the cohort was monitored from nauplius I to adult. Along the development of the cohort, about 50 individual of the successive

naupliar or copepodite stages were placed individually in 2.5 ml chambers filled with filtered sea water and checked once a day for mortality. The control consisted in a parallel group of individuals of the same developmental stage incubated in similar receptacles but filled with a suspension of *R. baltica* at excess food concentration. 2/3 of the culture suspension in the controls and of the filtered sea water in the experimental chambers were daily changed. Survival time to starvation for each developmental stage was estimated as the days elapsed in filtered sea water until mortality reached 50 % of the initial population.

Effects of fluctuating food availability in naupliar growth and survival

The experimental design to test the effects of different frequencies of fluctuating food concentration on naupliar growth rates consisted in providing the copepods with equivalent average amounts of food in two conditions: Fluctuating, high food-low food concentrations, simulating the food variability of natural, variable systems, and continuous supply of food (controls) at a concentration equivalent to the average food experienced by the organisms in fluctuating conditions (Calbet and Alcaraz 1996). The chosen concentrations were maintained in the range at which growth rates were proportional to the food concentration. The maximum food concentration was always maintained below saturating conditions for growth.

About 500 recently molted nauplii II were placed in each experimental chamber. These chambers consisted of a perspex cylinder (15 cm long, 10 cm diameter) with the bottom covered with 40 μm nitex-mesh. Experimental chambers were suspended in 15 l plastic containers (6 chambers per container) with filtered sea water and algae at the chosen concentration. In order to avoid algal sedimentation and to provide water flow through the egg-laying chambers, water was circulated with a peristaltic pump at a rate of 0.4 l $h^{-1}$ in each chamber. Food

concentration was monitored by means of a Coulter Multisizer four times a day and corrected if necessary. Every day the culture medium was exchanged by a new suspension of *R. baltica* at exponential growth rate.

Fluctuations in food concentration were simulated by gently changing the experimental chambers from the high food to low food containers and vice-versa at the required frequencies, and an aliquot of the nauplii were collected once a day. All the experiments were run by triplicate.
Three frequencies of food fluctuations were tested:

- 12F (12 h low food, 0.1 ppm equivalent to 27 $\mu gC\ l^{-1}$, and 12 h high food, 1.2 ppm equivalent to 324 $\mu gC\ l^{-1}$), combining two light situations: High food supplied at night (high food-dark, 12FN) and high food supplied during the day (high food-light, 12FD).
- 24F (1 day low food - 1 day high food)
- 48F (2 days low food - 2 days high food).

The control consisted of a food concentration equivalent to the average low food-high food concentration (0.65 ppm, 175.5 $\mu gC\ l^{-1}$) continuously supplied.

Parallel experiments were run in order to estimate the effects of 24F and 48F food fluctuations on the naupliar survival rates. The experimental setup was the same as for the study of growth rates under fluctuating food conditions. After four days of running the experiment, the number of surviving individuals was counted.

## RESULTS

The cohort development under saturating food conditions is shown in fig. 1. The time needed to reach adult stage under saturating food conditions and 18° C is 15 days, about one stage per day.

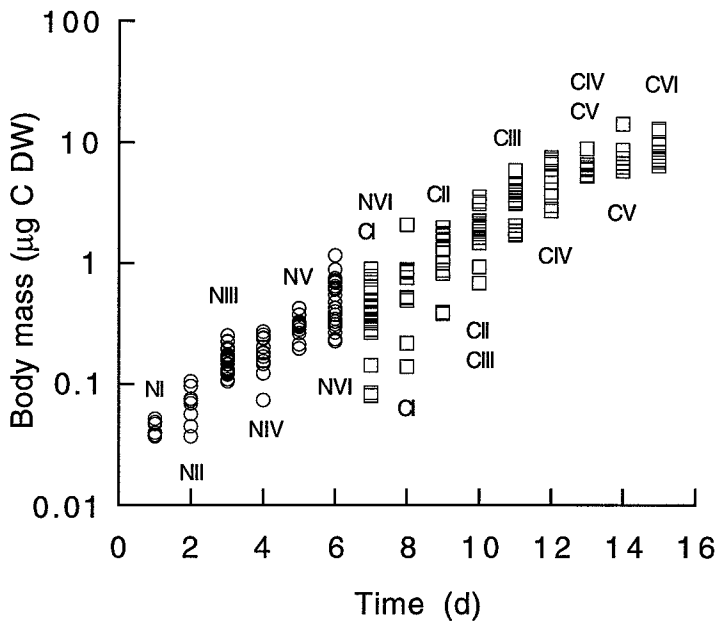


**Fig. 1**. Cohort development of *Acartia grani* expressed in dry weight. The most abundant stages present for each day is given also in the figure.

The survival time of the different developmental stages to starvation is represented in fig. 2. In general, there was a progressive increase in the tolerance to starving conditions according the cohort development, except for nauplius III. Survival rate in control animals was 100 %.

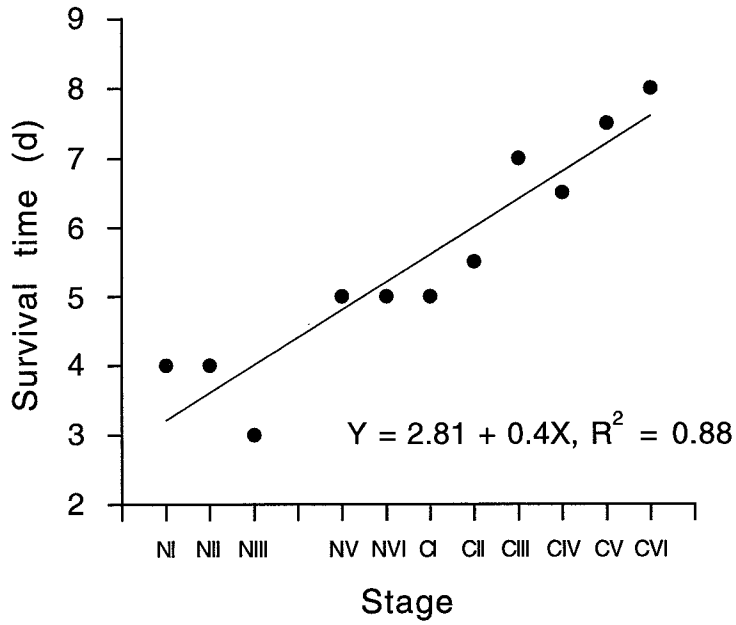


**Fig 2.** Starvation tolerance of the different stages of *A. grani*. In abcissae: development stage. In ordinates: time elapsed (in days) under starving conditions to reach 50 % mortality.

The temporal evolution of naupliar DW in relation to the different frequencies of food fluctuations is represented in Fig. 3 A and B. The effect of short-term fluctuations (12 h) on growth rate depended on the light conditions at which the food was available (Fig. 3 A). When high food concentrations were supplied at night (12FN), naupliar growth rates were slightly higher than for those feeding continuously on average food concentration (controls), although differences were not statistically significant. However, high food concentrations supplied during daylight hours (12FD) resulted in significantly lower naupliar growth rates (Tukey test, $p<0.05$).

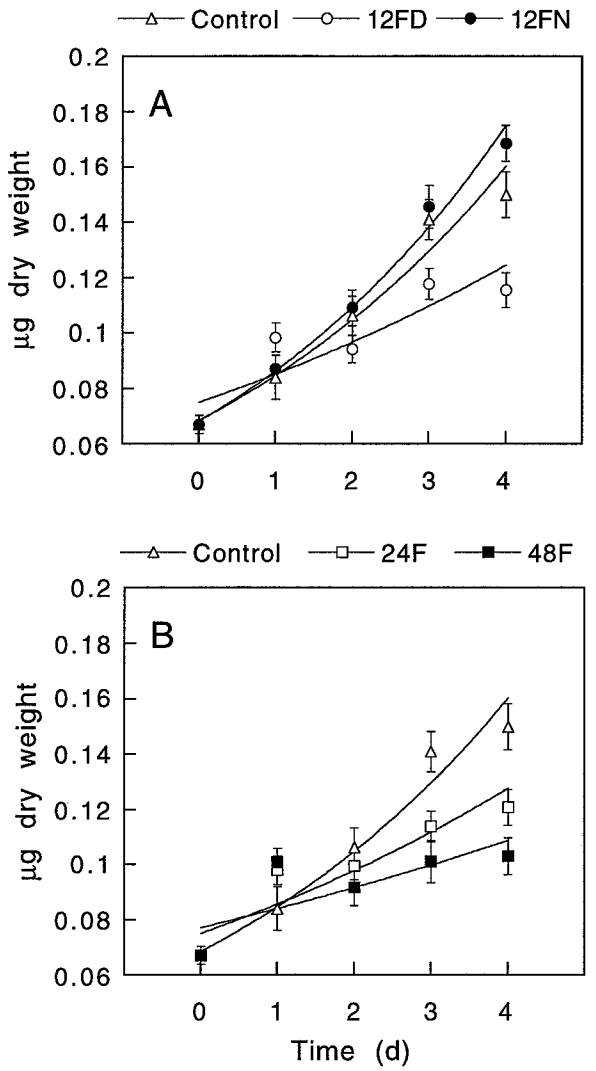


**Fig. 3.** Temporal evolution of naupliar dry weight (± SE) in relation to the frequency of food fluctuations. A) 12 h food fluctuations. Control: continuous average supply; 12FD: high food supplied during the day; 12FN: high food supplied at night. B) 24-48 h food fluctuations. Control: continuous average supply; 24F:1 day low food - 1 day high food; 48F: 2 days low food - 2 days high food.

Under longer term food fluctuations (24F and 48F, Fig. 3 B), naupliar growth rates were significantly lower ($p<0.05$) than for controls or 12FN, and similar to 12FD fluctuations (Fig. 4). Although 48F food fluctuations gave the lower growth rates, differences respect 24F or 12FD were not statistically significant.

Mortality rates (% of nauplii alive at the end of the experiment) induced by 24 or 48 h food fluctuations (34 % and 29 % respectively) were significantly higher than for the control (11 %, $p < 0.005$), but were not different between them.

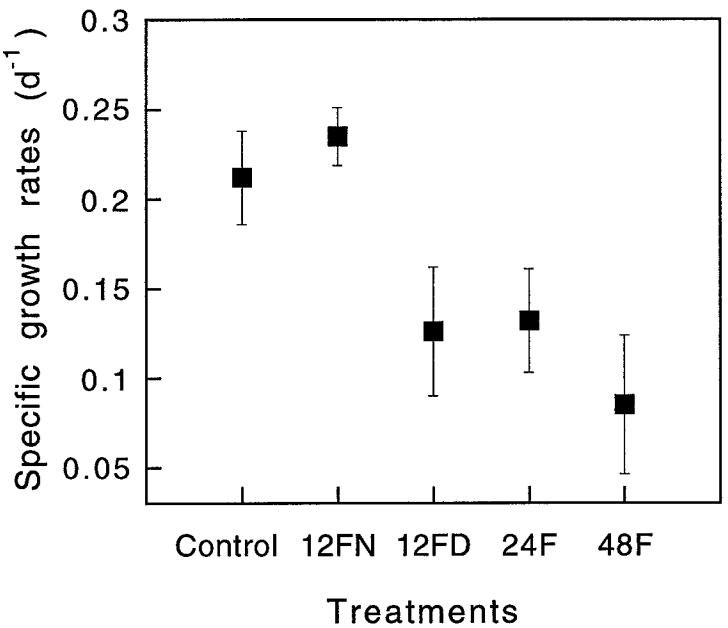


**Fig. 4**. Effects of the different frequencies of food fluctuations on specific growth rates (± SE) in the fluctuating treatments. Control: continuous average supply, 12FD: high food supplied during the day, 12FN: high food supplied at night, 24F:1 day low food - 1 day high food, 48F: 2 days low food - 2 days high food.

## DISCUSSION

The food concentration threshold at which growth rates are saturated (chapter 2.2), and the capacity to survive, should be carefully taken into account in order to scale both the intensity and frequency of food oscillations to the response-range of the organisms (Calbet and Alcaraz 1996).

The increase in survival time to starving conditions along the copepod development coincided with a similar tendency observed by Burns (1985) for three *Boeckella* species, Borchers and Hutchings (1986) for *Calanoides carinatus*, and Tsuda (1994) for *Pseudocalanus newmani*, although in all the cases the tolerance to starvation was significantly higher than for *A. grani.* In our experiments, survival time to starvation increased linearly with age (instar). The lower survival rate to starvation for NIII in our experiments suggests a critical change in the naupliar trophic condition, probably the start of active feeding, as observed by Marshall and Orr (1956) for *Calanus finmarchicus.*

The significant differences in naupliar growth rates observed in short-term food fluctuations (12 h), in relation to the light conditions at which high food concentrations are available, confirm previous results obtained by Calbet and Alcaraz (1996). As for egg production rates, naupliar growth is significantly higher when high-food concentrations occur during the night. This can only be explained by higher nocturnal feeding rates in nauplii, similar to those described for adults (Bautista 1988).

The negative effects of food fluctuations of longer period (24 and 48 h) on life-history parameters of early naupliar stages (i.e., lower growth and higher mortality rates) contrast with the almost negligible role attributed by Huntley (1996) to food availability for copepod growth. Their hypothesis is based in the inappropriate time and space scales at which food is experimentally supplied to copepods in most of laboratory experiments (i.e., homogeneous distribution and constant supply). Nevertheless, the observed age-specific response of growth and mortality rates of *A. grani* to even high-frequency food fluctuations (12 h), confirm the modulation imposed by fluctuations in food availability to copepod production.

*A. grani* is a clear example of copepod with low capacity for dampening food variability and high egg production rates (Dagg 1977). The negative effects of

relatively short (few days) food fluctuation periods on naupliar growth are compensated by its high reproduction rates in favorable environments. In marine areas where phytoplankton variability occurs mainly at micro and fine scales (Walsh 1976, Owen 1989), *A. grani* can favourably outcompete other copepod species (Calbet and Alcaraz 1996), thus explaining its dominance in the innermost parts of harbours and estuaries (Alcaraz 1983).

The control exerted by food availability conditions (abundance and frequency of fluctuations) on life-history parameters of copepods confirm the importance of match-mismatch mechanisms between extensive properties (i.e., phyto and zooplankton biomass and distribution) and rate-processes in plankton systems (Cushing 1989, Calbet et al. 1996).

A better understanding of the ecological implications for zooplankton dynamics of the scales of variability of the different trophic groups would require a global trophodynamic approach, and its study in relation to the scales of physical variability in natural systems.

**RESUM**

Les escales de variabilitat temporal i espacial dels recursos alimentaris en els sistemes marins són factors determinants en el control de les poblacions de zooplàncton. Per copèpodes adults, les taxes de producció d'ous depenen de la freqüència de fluctuació de la disponibilitat de menjar. Tanmateix, es coneixen molt poc els efectes de les fluctuacions d'aliment en estadis juvenils de desenvolupament de copèpodes.

Es van estudiar les taxes de creixement i supervivència d'estadis naupliars de desenvolupament del copèpode marí *Acartia grani* en relació a la concentració i seqüència de les fluctuacions en la disponibilitat de menjar.

Les taxes de creixement dels nauplis foren altament dependents de l'abundància de menjar, i la tolerància a la manca de menjar (temps de supervivència) va anar augmentant al llarg del desenvolupament de la cohort.

Per fluctuacions de menjar de curta durada (alternança en l'abundància de menjar de 12 h), les taxes de creixement dels nauplis depenien de les condicions de llum en les què el menjar estava disponible. Baixes freqüències de fluctuacions (alternança en l'abundància de menjar de 24 o 48 h) reduïen significativament les taxes de creixement i la supervivència dels nauplis.

L'elevada sensibilitat de les taxes de creixement a les fluctuacions de menjar que mostren els estadis naupliars, i la seva baixa tolerància a condicions de manca de menjar, constitueixen evidències adicionals que expliquen el confinament d'*A. grani* a ambients costaners.

# CAPÍTOL III

# LLUM, DISPONIBILITAT D'ALIMENT I PRESÈNCIA DE DEPREDADORS COM A FACTORS MODULADORS DELS RITMES D'ALIMENTACIÓ EN COPÈPODES

**Llum, disponibilitat d'aliment i presència de depredadors com a factors moduladors dels ritmes d'alimentació en copèpodes.**

Una característica del comportament alimentari del zooplàncton, i dels copèpodes en particular, és l'existència de ritmes d'alimentació. Potser els més coneguts corresponen a les taxes altes d'ingestió nocturna que s'observen generalment relacionades amb ritmes nictemerals de migració (ascens nocturn del zooplàncton a capes superficials, amb concentracions generalment altes de fitoplàncton). Però no sempre els ritmes nictemerals de migració estan acoblats amb la presència d'aliment en capes superficials. Quan es desenvolupa un màxim profund de fitoplàncton, un comportament migratori dóna lloc a esmerçar la nit en capes superficials on l'aliment és generalment escàs. De qualsevol manera, existeix gairebé unanimitat en l'acceptació que la llum és un inhibidor de l'activitat alimentària en copèpodes.

Els depredadors no visuals detecten la presència de les preses mitjançant les "pistes" o pertorbacions de microescala que aquestes produeixen en l'aigua en desplaçar-se. És, doncs, possible que els copèpodes hagin seleccionat pautes de comportament que tendeixin a minimitzar la seva detectabilitat, mitjançant la reducció de la seva activitat en presència de depredadors d'aquest tipus, però no relacionades amb les condicions d'il.luminació, si la detecció es fa únicament per mitjà de mecanosensors.

Per contra, la presència de depredadors visuals, que necessiten llum per tal de detectar l'aliment, només modificaria el comportament alimentari durant el dia (o en condicions d'il.luminació equivalent).

En aquest capítol s'estudien els efectes dels factors esmentats (concentració d'aliment, llum, presència real o simulada d'un depredador, etc.) com a possibles responsables del control dels ritmes d'alimentació en un copèpode planctònic (*Acartia grani*). La simulació dels depredadors s'ha fet mitjançant imitacions animades, la presència visual del depredador o dels seus exudats.

## 3.1. EFFECTS OF LIGHT, FOOD AVAILABILITY AND PREDATOR PRESENCE ON *ACARTIA GRANI* DIEL FEEDING BEHAVIOR

(Basat en un article del mateix títol fet amb la col.laboració d'altres autors i enviat a J. Plankton Res.)

## INTRODUCTION

Diel feeding rhythms displayed by marine zooplankters, and specially by copepods, is a characteristic feature and has been widely described since the first studies on the ecophysiology of zooplankton (see review by Haney 1988). The reasons for such behaviour have been thoroughly discussed, and although the existence of an endogenous component has been proposed (Duval and Geen 1976), more recent studies indicate a major role of exogenous components like the presence of light (Stearns 1986).

Part of the discussion comes from the fact that, besides the so-called normal pattern (i.e. higher ingestion rates at night, Fuller 1937, Petipa 1958, Dagg and Grill 1980, Saito and Taguchi 1996), there are abundant observations in which no rhythms are evident, or even the pattern is a reversed one (Daro 1985, Dagg 1985, Kiørboe et al. 1985, Roman et al. 1988).

Two variables that have been addressed only very recently as potential triggering factors of zooplankton diel feeding rhythms are food availability and risk of predation. Feeding implies conspicuouity to predators, either by increased motility (increased encounter rate) or activity per se (hydrodynamic signals to be detected for potential predators). One would predict, therefore, that under the presence of potential predators, zooplankters will have to balance and optimize their behaviour between the risk of predation and hunger. Due to the fact that visual predators (only active during daytime) are probably the most important predators of marine copepods, it would not be surprising the appearance of diel feeding rhythms, possibly selected through evolution.

Diel feeding rhythms are commonly associated to some sort of vertical migration and it has been shown that the presence of predators can affect the extent of

vertical migration (Bollens and Frost 1989, Bollens and Stearns 1992, Bollens et al. 1994).

It is well known that low food availability usually induces the zooplankters to spend more time searching for food (higher clearance rates). The increase in the time spent searching for food (Piontkovskii and Petipa 1976, Tiselius 1992, Saiz 1994) is associated with an increase in conspicuouty. Following the reasoning exposed above, one would expect that under low food availability, the conflict between the need to feed and the need to hide from predators will be more extreme, and at some point it would pay off the lack of feeding rhythms and to feed as much as possible.

The few attempts made in the laboratory to determine the effect of food concentration in diel feeding behaviour of copepods have failed to show any effect (Durbin et al. 1990, Hasset and Blades-Eckelbarger 1995), and diel rhythms were present in spite the experiments were conducted in the absence of predators. However, in nature predation risk is a common fact.

Here we present a study on the effects of light intensity, food concentration and predation risk in the diel feeding behaviour of the coastal copepod *Acartia grani*, both in wild animals and in organisms cultured for more than ten generations in laboratory conditions and in the absence of predators. Predation risk was simulated using either live predators (small fish and carnivorous copepods) or mimics, and in different ways we attempted to determine what kind of signal released by the predator would be the cue that might affect *Acartia* feeding behaviour.

## METHODS

Effects of food concentration

A series of four experiments were conducted in order to clarify the effects of food concentration in the diel pattern of food ingestion and egg production. The experiments consisted of two series of incubations, one during the night and the other during day time with the same animals. The first three experiments were performed with cultured female *Acartia grani* (1020±6.68 (SE) μm length) reared in laboratory for more than ten generations. Experimental conditions were similar, except for light intensity. In Expt 1 and 2 the light intensity was 10-30 μE $m^{-2}$ $s^{-1}$ and in Expt 3 the light intensity was higher (35-100 μE $m^{-2}$ $s^{-1}$). This range of values is equivalent to the light intensity commonly found between 30 and 50 m in near coastal waters. The experiments were conducted in a 12:12 hours Dark/Light cycle. *A grani* males and females were acclimatized during two days to the experimental temperature (19±1.5°C) and food concentrations: 0.3, 0.6, and 2 ppm by volume of the diatom *Thalassiosira weissflogii* (TW, 13-14 μm diameter, 1420.3±3.46 (SE) $\mu m^3$ $cell^{-1}$) at exponential growth rate. These food levels will be hereafter called low, medium and high respectively. Up to 25 and not less than 10 females (depending on the experiment) were placed in 1200 ml screw-cap bottles filled with the corresponding algal suspension plus nutrients (5 ml f/2 medium per litre) and incubated on a slow rotating Ferris-Wheel. Each treatment consisted of 4-5 bottles with copepods and 4-5 controls in order to quantify the growth of the respective algae suspension. After 10-12 h (dark period) the contents of the bottles were gently filtered through two submerged sieves. One of 180 μm to keep the females and an other of 40 μm in order to collect the eggs. The females were checked, and animals suspected to be in bad conditions were removed from the incubation. The remaining animals were transferred to a new algal suspension for the second 12 h (light period)

incubation. Eggs were counted under a dissecting microscope. Algae concentrations were determined with a Multisizer Coulter Counter.

The last experiment was conducted with wild *Acartia grani* (872.15±6.28 (SE) μm length). Copepods were caught in Masnou harbor (a town 20 Km N of Barcelona) by means of slow-speed horizontal tows, with a Juday-Bogorov plankton net provided with a 5-10 l plastic bag as cod-end in order to prevent damage in the animals. Samples were quickly transported to the laboratory and females of *Acartia grani* picked out.

The set-up for this experiment was the same as for Expt 3. The food concentrations tested were 0.3 and 2 ppm TW for *A grani* (high and low food levels respectively). Light intensity was 35-100 $\mu E\ m^{-2}\ s^{-1}$.

Effects of predator presence

Several experiments were performed in order to study the effects of visual, mechanical or chemical presence of a predator on diel feeding rhythms. All these experiments were run at the same light and temperature conditions as described for Expt 3, and were performed with cultured copepods.

1) Effects of the presence of a non-visual predator:

The treatments consisted of incubations in suspensions of 0.4 ppm TW (8 adult *A grani* per bottle), conducted either in the absence or presence of a non-visual predator, adult females of *Candacia armata* (1.95-2.7 mm length). There were 3 replicate bottles per treatment plus additional controls for algal growth. Incubations were performed in 1200 ml screw-cap bottles on a slow rotating (0.2 rpm) Ferris Wheel. After 24 h copepod and algae were collected and counted in order to detect any change in the daily ingestion rates.

2) Effect of mechanical and visual presence of potential predators:

Adult male and female *A grani* were introduced in three 10-l perspex cylinders filled with a suspension of 0.7 ppm TW and exposed to three treatments: 1) Presence of a mimic plastic fish (a lure, 8 cm long. fig 1A), that was manually moved every 15 minutes for periods of 1 minute. With this treatment we intended to simulate the effect of the mechanical and visual presence of a potential predator over the ingestion rates. 2) Emulation of the visual perception of a real predator (fig 1B). Consisted of a glass bottle (1200 ml) filled with two free-swimming fishes (*Liza* sp, 4 cm long.) placed inside the experimental cylinder; and 3) Control without any kind of external disturbance (fig 1C). This experiment was run during the day time. Every two hours, 3 samples of 15 copepods each were taken from each cylinder and immediately frozen in liquid nitrogen for posterior chlorophyll gut contents analysis.

3) Effect of chemicals released from a potential predator:

Our aim was to determine the effects of fish exudates (as a chemical signal of the presence of a predator) on diel ingestion rhythms. We tested two different treatments. In both we had 10 *A grani* females in 625 ml bottles (4 replicates) filled with a suspension of 0.5 ppm TW. To prepare these suspensions, in the treatment called "exudate" we used 50% filtered sea water and 50% water coming from a 10-l bucket in which we had kept five fishes (*Liza* sp, 4 cm length) for 3 days. To avoid any enhancement of algal growth due to the presence of exudates, we added 5 ml F/2 per litre of suspension. The control of algal growth was made in bottles without copepods (4 replicates) with and without exudates, depending on the treatment. After the first 10-12 h (dark period) the contents of the bottles were gently filtered through two submerged sieves and healthy animals were returned to the bottles that were filled with new experimental suspension for the next incubation period (light period). Algal concentrations

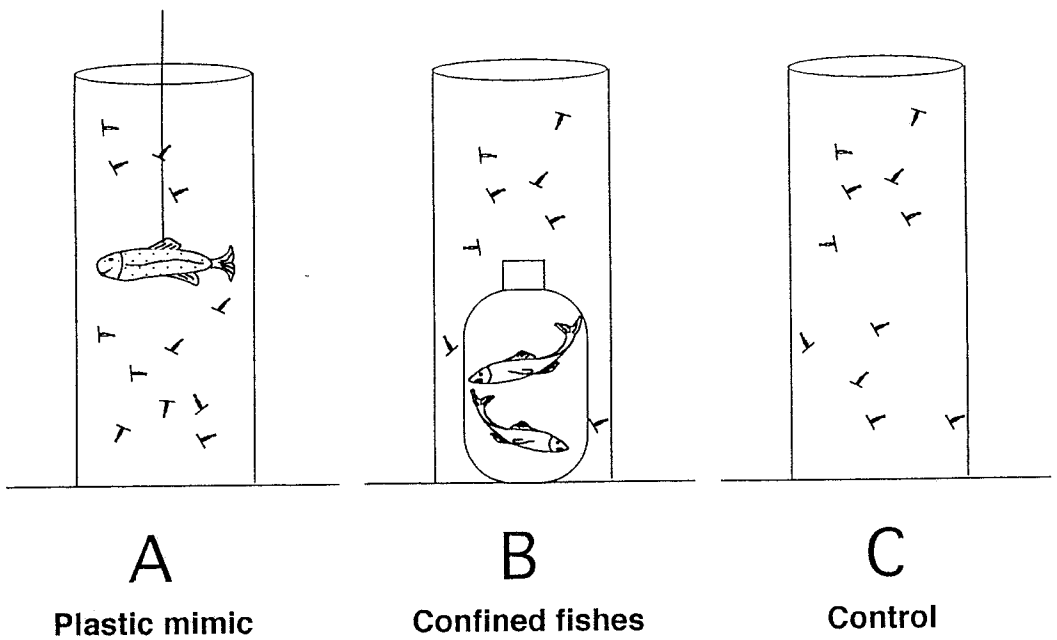


**Fig. 1**. Experimental set-up for testing the effects of potential predators on Acartia grani feeding rhythms. A) Plastic fish mimic, B) Alive fishes inside a glass bottle and C) Control without predators.

were measured by means of a Coulter Counter. Mortality was always negligible (less than 2%).

Effects of light on ingestion rates

In order to know if light has a direct control in diel feeding patterns of *A grani*, we carried out an experiment in which adult females that had been growing in a 12:12h Dark-Light cycle were exposed durng night-time to normal light cycle (i.e. no light at nigh) or to the presence of light during night. The incubations were performed in 620 ml screw-cap bottles (15 copepods per bottle; 4 replicates per treatment and per algae controls) filled with a suspension of 3 ppm TW. The experiment had a duration of 12 h, and after this period of time copepods and algae were counted. Light intensity was 40-60 $\mu E\ m^{-2}\ s^{-1}$.

**RESULTS**

Effects of food concentration

The ratios between night/day values of ingestion, clearance and egg production rates in relation to food concentration are presented in fig 2. The ratios have been calculated for each individual bottle (i.e. the same individuals). The statistical analysis (two-way ANOVA, table 1) showed differences in clearance and ingestion rates between day and night in all food concentrations. However, this feeding rhythm consisted of only a 30% increase in ingestion rates during night-time. It is important to notice that although for 0.6 and 2 ppm the highest ingestion rates corresponded to the dark period, for the low food concentration (0.3 ppm treatment) the ingestion rates were higher during the daylight hours.

**Table 1**. Ratios (± SE) of ingestion, clearance and production as calculated by night/day quotiens. Asterisks indicate the level of significance (* $p<0.05$, ** $p<0.01$)

| Treatment | Ratio Ingestion | Ratio Clearance | Ratio Egg product. |
|---|---|---|---|
| Cultured *A grani* | | | |
| Low food | 0.8 ± 0.35** | 0.9 ± 0.43* | 1.4 ± 0.10** |
| Medium food | 1.3 ± 0.10* | 1.4 ± 0.14* | 1.3 ± 0.11* |
| High food | 1.2 ± 0.07* | 1.3 ± 0.11* | 0.9 ± 0.04 |
| Wild *A grani* | | | |
| Low food | 0.8 ± 0.05* | 0.8 ± 0.07** | 1.5 ± 0.20* |
| High food | 1.4 ± 0.08* | 1.6 ± 0.09** | 0.8 ± 0.06* |

There were no differences between the three experiments performed with cultured copepods, except for Expt 2 at high food concentration ($p<0.05$). This result cannot be attributed to changes in light intensity due that Expt 1 and 3 were not statistically different at any food level. Feeding and clearance rhythms in wild and laboratory cultured *Acartia grani* (table 1) were not different although the ratios (night/day) were always more apparent in wild copepods. Regarding egg production, *A grani* seemed to have higher nocturnal spawning rates at low food and medium food concentrations. At high food concentrations egg production followed an opposite trend.

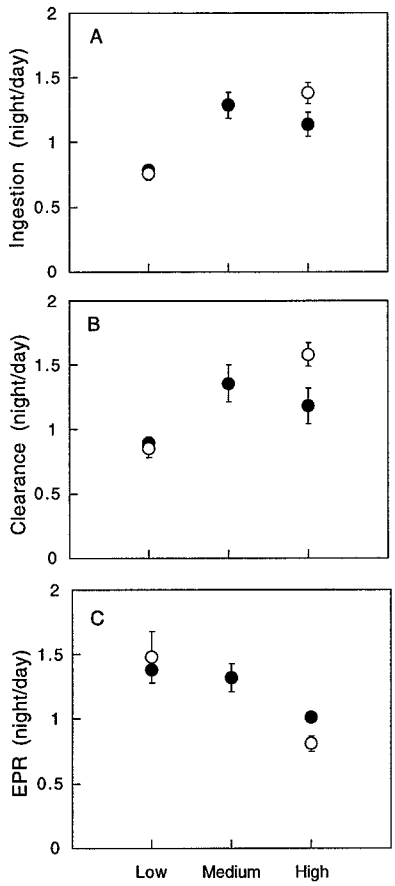


**Fig 2.** Ratios of ingestion (A), clearance (B) and egg production rates (C) calculated as the quotient between night and day rates. Filled and open symbols correspond to cultured and wild *Acartia grani* respectively. The food treatments "Low", "Medium" and "High" correspond to 0.3, 0.6 and 2 ppm TW. Error bars represent ±1 SE.

Effects of predator presence

1) Effect of the presence of a non-visual predator

The presence of a non-visual predator like *Candacia armata* appeared to have no significant effects in ingestion rates (0.97±0.10 (SE) ppm TW copepod$^{-1}$ d$^{-1}$ in controls and 0.91±0.09 (SE) ppm TW copepod$^{-1}$ d$^{-1}$ in incubations with *Candacia*). Although the results integrate a period of 24 h incubation, the lack of reduction of feeding rates by the presence of a non-visual predator suggests that

feeding rhythms were not modified either. All experimental animals were intact when recovered, indicating that although *A grani* is probably chased by *C armata*, it cannot succeed feeding on them.

2) Effect of mechanical and visual presence of potential predators

The differences between the three treatments regarding the daylight ingestion rates of *A grani* were not statistically significant. However, it seemed that a mechanical disturbance with a fish mimic and the visual presence of a predator tended to reduce feeding rates (as chlorophyll gut contents) in comparison to the control (fig 3).

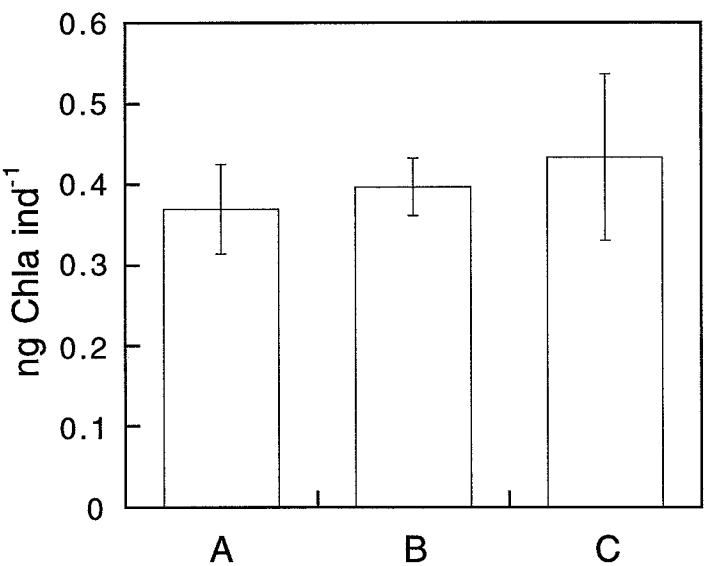


**Fig 3.** Average (± SE) Chlorophyll*a* gut-contents along daylight period of copepods incubated in the presence of a fish mimic (A), with alive fishes in a glass bottle (B) and without any kind of external disturbance (C). Error bars represent ±1 SE.

3) Effect of chemicals released from a potential predator

Although the presence of fish exudate clearly affected (inhibited) ingestion ($p<0.05$), there were no differences between day and night (fig 4). However, the exudates effect was stronger during day than during night (27% reduction in ingestion during day versus 14% during night). This result suggests that the copepod response to chemical signals is modulated by the presence of light. However the concentration of exudate was not determined.

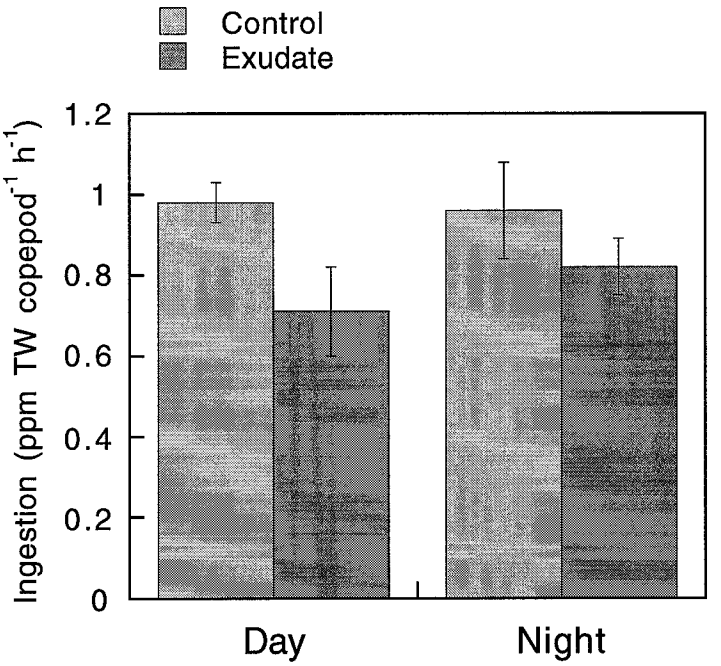


**Fig 4.** Ingestion rates during day and during night of *A grani* females incubated with fish exudate respect to the controls incubated without fish exudate. Error bars represent ±1 SE.

Effects of light on ingestion rates

There was a weak although significant effect of light during night-time on ingestion and clearance rates ($p<0.05$, fig 5). Copepods that were exposed to light during night showed lower feeding rates (~ 20%) than the control. It seems thus, that light plays an important role in the control of diel rhythms. The effect of light could be enough to explain diel changes in ingestion and clearance rates.

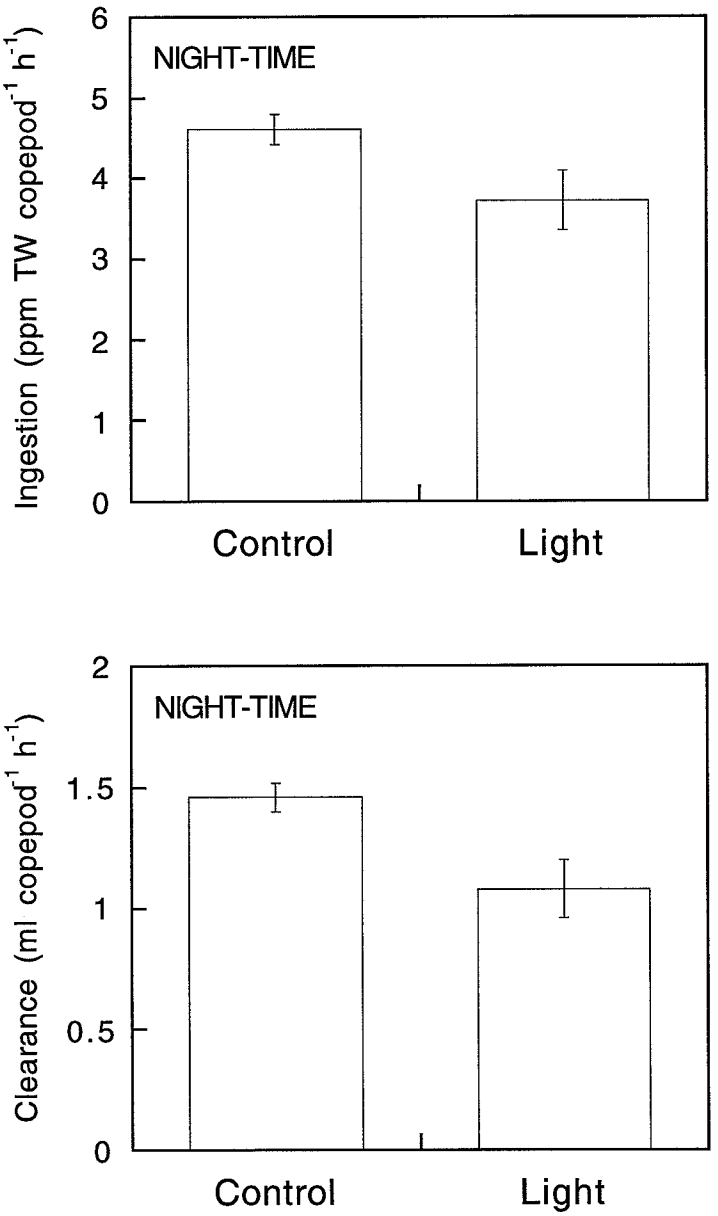


**Fig 5.** Ingestion and clearance during night-time of *A grani* females incubated under darkness or in presence of light.

## DISCUSSION

The persistence of feeding rhythms at low food levels (Durbin et al. 1990, Hasset and Blades-Eckelbarger 1995) is indicative of a strong adaptive response to predation. Our results evidence a higher response to hunger than to predation for both cultured and wild *A grani*. Although the ratios of clearance and ingestion (night/day) found at medium and high food concentrations (around 1.3 for ingestion and 1.4 for clearance on average, see table 1) seems to be less marked than found for *Acartia tonsa* in literature (e.g. 3.5x, Stearn 1986; 2.1x, Stearns et al. 1989; 3x, Durbin et al. 1990) it is important to take into account that we are presenting data about integrated overall day or night time ingestion, not only the maximum rates. It is expectable that maximum rates would have been much higher in our experiments than overall day or night ingestion presented here. At low food concentrations the ratios obtained (0.8 for ingestion and 0.9 for clearance on average) denoted a higher feeding activity during day. This fact, previously described for other species (Daro 1985, Kiørboe et al. 1985), suggest a strong hungry response that overcomes the risk of predation. Probably, the defense for this species to predation was more addressed towards higher reproduction and growth rates than behavioral changes directed to avoid predation. This assumption could contribute to explain the almost mono-specific and strongly seasonal presence of *A grani* in the innermost parts of harbors and estuaries (Alcaraz 1983).

The lack of differences between cultured (more than ten generations in laboratory) and wild animals implicate that the ingestion rhythms are genetically determined, and are independent on the presence of predators, although this factor could modify the amplitude of rhythms.

The nocturnal spawn that showed *A grani* at low and medium food concentrations agree with the results found repeatedly in the literature (i. e. Stearns et al. 1989, Checkley et al. 1992, Cervetto et al. 1993, Calbet and Alcaraz 1996). However, there was no clear pattern at high food concentrations, and even in wild animals the maximum spawn was during day. The changes in diel egg production pattern could be explained in two different ways. The ingestion rhythms could be absolutely independent of the rhythm in egg production or on the contrary, the eggs produced could be the result of the conversion of food in a species specific dependent period of time (Tester and Turner, 1990). It might be that the time required to transform ingested food into eggs was also food dependent. Our results suggest that the food used to produce eggs could have been ingested in a longer period at low food concentrations than at high food concentrations (9.5 h for *A tonsa* at saturating food conditions, Tester and Turner, 1990). This hypothesis would explain partially our results; probably in the low food treatment the lag between ingestion and egg production was around 12 h and in the other treatments it was less than this period, being difficult to find any clear trend. The advantages of a nocturnal spawn are several. During night, eggs are not so conspicuous to visual predators than during day. Furthermore, if this nocturnal spawn is coincident with vertical ascension, the previous mentioned advantage is reinforced by an increasing in sinking time that is important in order to get hatching in nearer surface layers with higher food resources. This could be of major importance for nauplii with low swimming capabilities.

There are evidences that copepods are able to respond to visual (Buskey et al. 1986, 1987), mechanical (Bollens et al. 1994) and chemical (Folt and Goldman 1981) signals. This abilities can be used to detect and avoid predators. It might be expected that copepods in presence of potential predators would reduce feeding activities and increase their escape responses, and consequently this behavior would translate into a decrease in the amount of food ingested. Therefore, the

presence of predators in incubations might have effect on ingestion rates, and this effect could be more apparent during day time. In our experiments neither the presence of a non-visual predator, nor the fish mimics, nor the visual presence of a predator had any significant effect in feeding rates. However, fish exudates reduced ingestion, specially during day.

*A grani* seemed not to respond negatively to the presence of other copepods (*Candacia armata* ) able to chase and attack them, or at least they did not change its feeding patterns enough to be detected as changes in ingestion.
Regarding visual detection of potential predators, there are evidences than *Acartia tonsa* and other estuarine copepods are able to respond by jumping to a shadow that simulates a predator (Buskey et al. 1986, 1987). However, in our experiments we did not detect any changes in ingestion (as chlorophyll gut contents). Possibly, due to the experimental set-up, only a few number of animals near the fish could react to it, and even if they react, this behavior did not affect significantly ingestion rates. However we have to take into account that fishes of this size (~ 4 cm length) are habitual predators in harbors and semi-confined areas where coexist with *A grani*, and that these animals are extraordinarily efficient in capturing copepods (author's personal observation). On the other side, the visual perception in copepods is quite rudimentary (they are able to detect only shadows). Probably the visual reaction distance for copepods is less than the attack distance for fish, and if they are able to detect the fish it is by means of mechanical or chemical signals.

We did not find any response either in the presence of a fish mimics that could be visual and mechanical detected. However, Bollens et al. (1994) generated vertical migrations in *Acartia hudsonica* with fish mimics, but they did not present data about ingestion. It seems thus, that *A grani* have not well developed neither the visual nor the mechanical capacity of predators detection or if it have it well

developed, they do not change its feeding activity. Again, *A grani* seems to direct more effort towards feeding (that can be translated in reproduction) than towards escaping of predators.

On the other hand, fish exudates had an effect on clearance and ingestion, and this effect was more apparent during day. Similar results were found in fresh water with the copepod *Diaptomus tyrelli* exposed to a chemical released of its predator *Epischura nevadensis* (Folt and Goldman 1981), but Bollens et al. (1994) did not find any effect of exudates on vertical migrations. The work carried out with fish exudates have to be taken carefully due to the difficulty to quantify the amount of substance released by fishes and the triggering component that can be detected by copepods. In our experiment we used a mixture of filtered sea water and 50% of "fish water" and we obtained a response, but in a preliminar experiment with only a 10% of "fish water" we did not find differences between treatments. This fact indicates that there is a threshold concentration at which fish is chemically detected by copepods and that the response could be concentration dependent.

Light had a negative effect on ingestion and clearance rates (~20%) and this effect was enough to explain diel changes in feeding activity observed at medium and high food concentrations, although not at low food concentrations. Similar results were observed by *Acartia tonsa* (Stearns 1986). It seems thus, that light is the direct responsible of the induction of diel feeding rhythms in *A grani* by means of an inhibition effect. Probably, copepods feed at high rates during dark periods and when they detect the presence of light they reduce its feeding. This idea contrasts with the results found by Duval and Geen (1976) who proposed the existence of an endogenous control of diel feeding rhythms, what suggest the need of further research regarding the control exerted by light in copepods diel feeding patterns. We did not found any effects of light intensity on diel ingestion

rates in the experiments, indicating that the effect of light over *A grani* ingestion rates was not intensity dependent, at least in the range tested. Previous experimental work in this area showed a weak although significant negative correlation between light intensity and copepod gut fullness (Stearns 1986), however the range of values at which animals were exposed (from 0 to 1000 μE $m^{-2}$ $s^{-1}$) was too extreme, and the scatter observed were too big to be compared with our results.

In summary, light could induce the diel feeding rhythms in *Acartia grani*, but these depend strongly on the food concentrations and on the presence of chemical signals released by potential predators. Other factors, like mechanical and small visual disturbances, are not able to produce any significant change in *A grani* feeding patterns.

**RESUM**

Es van estudiar els efectes de diversos factors possiblement responsables del control dels ritmes d'alimentació en *Acartia grani* (concentració de menjar, presència real o simulada d'un depredador, llum). Ritmes diaris d'alimentació (major ingestió de nit) van ser observats a concentracions de menjar intermèdies i elevades. Per contra, a concentracions de menjar baixes, les ingestions més altes van tenir lloc durant el dia. Aquest resultat va ser observat tant en copèpodes de camp, com en copèpodes cultivats al laboratori, la qual cosa indica una feble resposta selectiva a la depredació.

La presència de depredadors no visuals (*Candacia armata*) no produïa cap efecte sobre les taxes d'ingestió. *A grani* tampoc responia (amb canvis en els seus hàbits alimentaris) ni a imitacions animades, ni a la percepció visual d'un peix. Per contra, la presència d'exudats de peix a l'aigua reduïa la intensitat de l'alimentació, especialment durant el dia. La llum juga un paper molt important en els ritmes diaris d'alimentació mitjançant una activitat depressora de les taxes d'ingestió. Els resultats obtinguts suggereixen que la llum té un efecte directe sobre els patrons diaris d'alimentació, però que aquests es poden veure modificats per altres factors com ara la concentració de menjar o la depredació.

# CAPÍTOL IV

# VARIABILITAT TEMPORAL I HETEROGENEÏTAT ESPACIAL DE LA PRODUCCIÓ ZOOPLANCTÒNICA EN ECOSISTEMES MARINS.

**Variabilitat temporal i heterogeneïtat espacial de la producció zooplanctònica en ecosistemes marins.**

Els ecosistemes planctònics marins presenten un ampli ventall d'escales de variabilitat, tant espacials com temporals, en bona part relacionades amb les fluctuacions del sistema atmosfera-oceà. En general, la intensitat d'aquestes està inversament relacionat amb la seva freqüència. Els organismes planctònics, i els copèpodes en concret, han hagut d'emmotllar els seus límits de tolerància, cicles de vida i comportament a les condicions de variabilitat dels seus hàbitats. Així, en latituds altes, els copèpodes han de subsistir en un medi amb una marcada estacionalitat, i han d'ajustar els seus cicles vitals a períodes productius anuals o plurianuals. En aquestes condicions, els petits canvis de curta durada són poc rellevants en comparació amb l'elevada intensitat de les fluctuacions lligades als fenòmens purament estacionals. Aquest fet hauria de conferir als copèpodes típics d'aquests ecosistemes una major capacitat per a esmorteir les petites fluctuacions d'alta freqüència que es poguessin donar en el medi.

Per contra, les latituds més baixes estan caracteritzades per una variabilitat de més alta freqüència, però de menor amplitud. A les poblacions de copèpodes es succeeixen diverses generacions al cap de l'any, per la qual cosa la producció depèn més estretament de lleugeres variacions en el medi.

D'altra banda, dins de cada ecosistema existeixen gradients espacials tant en la freqüència com en la intensitat de les fluctuacions, normalment en relació amb estructures hidrogràfiques característiques. Aquest és el cas dels sistemes frontals o les zones costaneres, ocupades per espècies seleccionades per tal d'explotar un ambient fluctuant i inestable.

En aquest capítol, s'ha estudiat l'eficàcia d'alguns indicadors d'acoblament entre productors primaris i zooplàncton, així com la variabilitat a escala fina i mesoscala

en la producció dels copèpodes en el medi natural. Aquest estudi s'ha dut a terme en dos sistemes contrastats pel que fa a la importància relativa de les oscil.lacions estacionals i de més alta freqüència. La velocitat de resposta a variacions de curta durada en els factors biòtics (aliment) i abiòtics (temperatura) dels indicadors de producció en copèpodes també ha estat analitzada.

Com exemples d'ambients naturals diferenciats, s'han triat quatre zones marines:

i) Zones costaneres (Mediterrani). Caracteritzades per una elevada variabilitat temporal no predictible a curta escala. Les comunitats de copèpodes es componen d'espècies típiques adaptades a aquest tipus d'ambient.

ii) Fronts de densitat (Mediterrani). Elevada variabilitat temporal no predictible a curta escala.

iii) Mar obert (Mediterrani). Variabilitat temporal més baixa, tampoc predictible, a curta escala.

iv) Mars Antàrtics. Relativament baixa variabilitat temporal a curta escala comparada amb l'amplitud dels fenòmens estacionals. Hi ha espècies típiques fortament adaptades a aquest ambient.

L'estudi s'ha dut a terme en quatre campanyes oceanogràfiques en el Mar Mediterrani (capítols 4.1 i 4.2) i en una en aigües Antàrtiques (capítol 4.3). Les característiques particulars que presenta el Mediterrani i més concretament el Mar Català (oscil.lacions estacionals relativament poc importants en comparació amb les fluctuacions d'alta freqüència, presència de singularitats físiques com ara el front Català de densitat, etc.) i la seva accessibilitat, el fan el marc d'estudi ideal com a model d'ecosistema d'elevada freqüència i baixa amplitud de variabilitat en les condicions ambientals. Per contra, tal com ja s'ha esmentat, els mars Antàrtics són exemple d'un ecosistema de característiques totalment oposades.

# 4.1 PLANKTONIC HERBIVOROUS FOOD WEBS IN THE CATALAN SEA (NW MEDITERRANEAN): TEMPORAL VARIABILITY AND COMPARISON OF INDICES OF PHYTO-ZOOPLANKTON COUPLING BASED IN STATE VARIABLES AND RATE PROCESSES.

(Basat en un article del mateix títol fet amb la col.laboració d'altres autors i publicat a J. Plankton Res. Vol. 18(12): 2329-2347)

## INTRODUCTION

The knowledge of the turnover rate and ultimate fate of biogenic carbon in planktonic marine systems are of paramount ecological importance, and are directly related to the modality of matter and energy transfer from primary producers to the different groups of heterotrophs. While carbon circulating through microheterotrophic food webs (microbial loop) is part of the "short-lived" carbon pool, and returns to the atmosphere in relatively short time periods (i.e., $<10^{-2}$ years), the classical, herbivorous pathway (primary producers exploited by herbivorous zooplankton) leads to "long-lived" or "sequestered" carbon pools, whose permanence in the marine domain can be much longer (from $10^{-2}$ to $>10^{2}$ years, Legendre and Le Fèvre 1992).

The predominance of either of both trophic pathways is highly dependent on the hydrodynamic conditions, the level of mechanical energy being apparently an important factor controlling the trophic characteristics of pelagic systems (Legendre et al. 1993). But apart from the intensity of auxiliary energy (Margalef 1978), the frequency and duration of energy pulses leading to phytoplankton outbursts, and their coupling with the time response of heterotrophs, are also important modulators of the characteristics of planktonic food webs (Le Fèvre 1986).

When mechanical energy inputs are periodical (i.e., tidally induced), the coupling between phytoplankton pulses and the time response of the different groups of heterotrophs are usually spatially determined and temporally persistent (Le Fèvre and Frontier 1988, Holligan et al. 1984 a, b). In those conditions, the trophic characteristics of the system are reflected by its extensive properties, and the intensity of the transfer of matter and energy through herbivorous food webs can

be estimated from state variables like size spectrum, planktonic community structure, relative distribution and biomass of phytoplankton and herbivorous zooplankton, etc. In contrast, aperiodical fertilization events (i.e., weather induced) preclude the stablishment of persistent food webs, the alternance of match-mismatch mechanisms (Cushing 1989) leading to shifts from one to another trophic modes. In such conditions, the dominant modality of energy transfer should be less clearly defined by the structural properties of the system. Is in unstable hydrographic structures, like fronts and ergoclines in general, where the temporal changes in the trophic structure are more important, mainly due to the amplification of mechanical energy inputs and the co-variant effect of small-scale turbulence (Le Fèvre 1986, Kiørboe 1993). In the Catalan Sea (NW Mediterranean), the most characteristic hydrographic singularity is a permanent density front of variable intensity, at the external border of the Liguro-Provençal-Catalan current (Castellón et al 1991). Between the front and the Balearic Islands, the stratification is more clear, and the physical variables present a dome-like structure. These hydrographic structures have been shown to determine the phytoplankton distribution (Margalef and Estrada 1987), the relatively high primary production in the area (Estrada and Margalef 1988), and the spatial features of zooplankton distribution, metabolism and feeding rates (Alcaraz 1988, Alcaraz and Packard 1989, Saiz et al. 1992 a).

Recently, Alcaraz et al. (1994b) have discussed the spatial trends in temporal variability of zooplankton excretion in this area. The contribution of regenerated nitrogen to phytoplankton requirements showed higher temporal variability at the vicinity of the front, where occasionally ammonium was in apparent excess, than in the stations located offshore. The variability appeared to be due to the imbalance between standing stocks and rate processes of producers and consumers, due to the unstable frontal dynamics.

In the present paper we discuss the importance of the matter and energy transfer between phytoplankton and herbivorous zooplankton in the Catalan Sea during two hydrographic situations: the autumn weakening of the thermocline (October-November 1992) and the summer stratification period (June 1993). The goals of the study are twofold: 1) to compare the usefulness of phyto-zooplankton coupling indices based either on structural properties or on rate processes, and 2) to estimate the variability of energy transfer through classical herbivorous-based food webs at the front, as compared to offshore stations, during two different hydrographic situations.

## METHODS

### Area surveyed

The two cruises took place during October-November 1992 (FR92) and June 1993 (ME93), on board the R/V "Garcia del Cid" and the R/V "Hesperides", respectively. On both occasions, the area sampled included a transect running from Barcelona to the channel between the islands of Mallorca and Menorca, crossing the Liguro-Provençal-Catalan current, the Catalan front at the outer margin of the current (Castellón et al. 1991), and the dome in the central part of the Catalano-Balearic sea (Fig. 1 and 2).

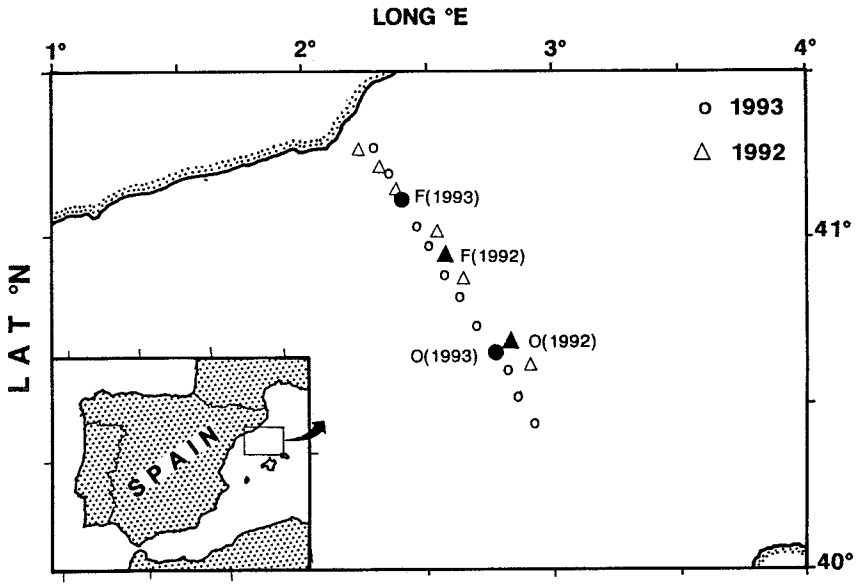


**Fig. 1.** Map of the study area with the position of the transect stations during FR92 (October-November 1992, triangles) and ME93 (June 1993, circles) cruises. The intensively studied front (F) and offshore (O) stations corresponding to both cruises are indicated.

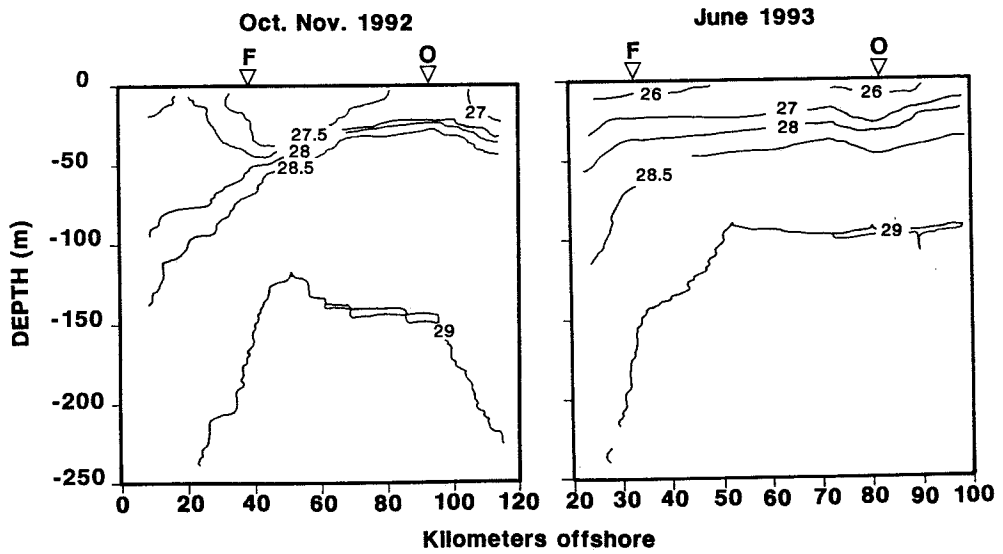


**Fig. 2.** Density distribution along the transects on the FR92 and ME93 cruises. The position of front and offshore stations are indicated.

Sampling strategy

The sampling strategy was similar for both cruises: quick series of transects of "hydrographic stations" perpendicular to the frontal axis provided basic information on the physical structure; according by, the position of two long (12-24 h) "biological stations" was determined, one at the vicinity of the Catalan Front, and the other offshore, in the stratified zone and above the central dome.

At hydrographic stations, temperature, salinity and "in situ" fluorescence were recorded with a Seabird-25 CTD (FR92 cruise), and a Neil-Brown MARK-V CTD (ME93 cruise), both equipped with a Sea Tech fluorometer. Water samples at 10 m intervals down to 200 m, for nutrients and chlorophyll determinations, were taken by means of 5- l Niskin bottle arrays after the CTD profiles (FR92), or during the ascending CTD casts with a rossette (ME93). After each CTD cast, a vertical, 200 m-surface zooplankton sample at a speed of 30 m per min was taken by a 200 μm WP-2 net.

At biological stations, after an initial CTD cast, and according to the vertical physical structure and fluorescence profiles, six depths were chosen for sampling between surface and 80-100 m depth. The variables considered for the estimation of the herbivorous transfer included, apart from those mentioned for hydrographic stations, phytoplankton pigments and primary production, mesozooplankton biomass and community structure, and mesozooplankton oxygen consumption. Other complementary measurements included biomass-specific chlorophyll gut contents of mesozooplankton (FR92), and copepod egg production rates (ME93). The sampling at both biological stations was repeated every 2 to 8 days for a period of 16 days (FR92) or 18 days (ME93) in order to study the temporal variability.

Phyto and zooplankton biomass and activity

Phytoplankton biomass (chlorophyll *a*) was measured by fluorimetry on acetone extracts (Yentsch and Menzel 1963) without acidification. The details concerning phytoplankton pigment extraction, fluorometer calibration and fluorescence measurements can be found in Estrada (1985 b) and Latasa et al. (1992). Chlorophyll concentration was transformed into phytoplankton carbon ($C_{phyto}$) using a carbon-to-chlorophyll ratio of 50 (Antia et al. 1963).

Primary production was measured by $^{14}C$ uptake. On the FR92 cruise, simulated incubations on deck and two in situ incubations (one at the front and one offshore, 7 light levels) were conducted; on the ME93 cruise, the $^{14}C$ uptake was measured in situ at 7 levels between the surface and the depth of 1% surface irradiance. (For details of sampling and methodological procedures, see Estrada, 1985 a, b, and Estrada and Margalef, 1988).

Vertical distribution of mesozooplankton biomass was estimated on discrete samples taken at noon at the six depths chosen for the general biological sampling. 40-l water samples were taken by Van Dorn-type bottles and filtered through 200 µm nylon netting (Alcaraz 1982). The organisms retained were transferred to GF/C glass-fibre filters, dried and stored for organic carbon ($C_{zoo}$) analysis (Carlo-Erba HCN analyzer). The coefficient of variation between repeated casts at the same depth of the mesozooplankton C or N estimated by this method was 18.9 % (Alcaraz 1985).

The ratio consumers/producers (here considered as an estimator of trophic efficiency of the system, Holligan et al., 1984 a, b ,and Table 1) was calculated as the quotient $C_{zoo}/ C_{phyto}$. The oxygen consumption rates of mixed mesozooplankton were estimated from organisms obtained by 100-0 m vertical tows at low speed (ca 10 m per min) with a 200 µm WP-2 net equipped with a non-filtering cod end. The contents of the cod end was split into 1-l jars and

diluted with air-saturated, GF/F-filtered sea water. After 1 h acclimation at the experimental temperature (17 °C) under dim light, several aliquots of the diluted samples were introduced in three 500-ml respiration chambers. A fourth respiration chamber without organisms was the control. Oxygen consumption was measured by an ENDECO-pulsed $O_2$ electrode meter. The system was set to measure oxygen concentration each 30 min in incubations lasting from 12 to 24 h. After incubations, the contents of respiration chambers were filtered through GF/F glass fibre filters and the carbon contents of the organisms analyzed as for the vertical distribution of zooplankton biomass. Biomass-specific respiration rates in $\mu l\ O_2.\mu g\ C_{zoo}^{-1}.day^{-1}$ were transformed into zooplankton carbon losses (equivalent to the minimum C requirements for routine metabolism) considering an RQ = 0.97 (Omori and Ikeda 1984). 1 ml $O_2$ consumed would be thus equivalent to 0.52 mg C.

The proportion of primary production directed towards mesozooplankton (table 1) was estimated as the quotient between the carbon required for mesozooplankton metabolism and the carbon assimilated by phytoplankton.

The zooplankton herbivory index (biomass-specific $C_{phyto}$-gut contents, see table 1) was measured during FR92 on mixed mesozooplankton. Samples were obtained at noon, by 100-0 m vertical hauls at a speed of 30 m per min with a 200 μm WP-2 net. Organisms were immediately transferred into graduated cylinders which were filled with filtered sea water to a volume of 500 ml. Chlorophyll gut contents was measured by quadruplicate on 5-ml aliquots of the thoroughly mixed zooplankton sample. Aliquots were transferred to GF/C glass fibre filters, introduced in borosilicate test tubes with 6 ml of 90 % acetone and left overnight at 4 ° C to extract phytoplankton

**Table 1.-** The indices used as quantitative descriptors of phyto-zooplankton coupling and the variables considered in their calculation.

| Index | Variables |
|---|---|
| Structural | |
| Trophic efficiency (the ratio consumers biomass/ producers biomass, $C_{zoo}/C_{phyto}$) | $C_{zoo}$ (mesozooplankton carbon) and $C_{phyto}$ (chlorophyll-derived phytoplankton carbon) |
| Herbivory index (biomass-specific phytoplankton gut contents of zooplankton) | $C_{phyto}$-gut contents of zooplankton (derived from biomass-specific chlorophyll gut contents of zooplankton) |
| Functional | |
| Proportion of primary production required for zooplankton metabolism (ratio carbon required/ carbon produced) | C produced by phytoplankton (derived from $^{14}C$ fixation rates) and zooplankton C requirements (derived from zooplankton respiration rates and |
| Copepod production | Specific C production by female copepods (derived from egg production rates and C contents of eggs) and female C (derived from female abundance and specific female C contents) |
| C ingested by copepods | Derived from copepod production assuming a gross-growth efficiency of 30 % |

pigments from the gut contents. Four other 5-ml aliquots of the sample were transferred immediately onto GF/C glass fibre filters, dried and stored for analysis of organic C in order to estimate the biomass-specific chlorophyll gut contents of zooplankton. Herbivory indices (μg Chl*a*-gut.μg $C_{zoo}^{-1)}$ were expressed as mg $C_{phyto}$-gut. mg $C_{zoo}^{-1}$ considering the C/Chl*a* mentioned ratio of 50. The average coefficient of variation between aliquots was 20.4 % for pigment gut contents and 14.3 % for mesozooplankton biomass.

Copepod production (as egg production rates) was estimated during ME93 for five species: *Calanus tenuicornis*, *Paracalanus parvus, Clausocalanus* sp., *Acartia clausi* and *Centropages typicus*. Copepod s were collected by a WP-2 net (200 μm-mesh) with a non-filtering cod end, towed vertically from 70-60 m to surface at low speed (ca. 10 m per min.). This vertical range includes both deep phyto- and mesozooplankton maxima (Alcaraz 1985). Once on deck, the contents of the cod end was diluted in a 10-l isothermic container with water from the deep phytoplankton maximum (DPM) and experimental organisms were immediately sorted by species or genus (for *Clausocalanus*) and stage. Adult females were placed in 620-ml screw-cap Pyrex bottles filled with DPM water filtered through 100-μm mesh, and incubated for ca. 24 h in a room at 17° C. The number of individuals per bottle ranged from 1 for the biggest species (*Calanus*) to 6-9 for the smallest ones (*Paracalanus*). Some bottles without copepods were used as controls of egg abundance in the DPM water. During the incubation, the bottles were occasionally turned upside down to reduce settling of algae. At the end of the incubation, the whole contents of the bottles was filtered through a 20-μm mesh submerged sieve, the copepods checked for activity, and eggs and copepods transferred to glass vials and preserved with acidic Lugol's solution. In the shore laboratory, eggs and copepods were counted and sized under an inverted microscope or stereomicroscope. Egg production rates were transformed into biomass-specific carbon production rates according to the female size-carbon

contents relationship for each species (for *Clausocalanus*, Chisholm and Roff 1990; for *Paracalanus*, Uye 1991; for *C. typicus*, Davis and Alatalo 1992; for *Calanus*, Williams and Robins 1982, and for *A. clausi*, Uye 1982). The carbon contents of eggs were obtained from the equation of Huntley and Lopez (1992). The minimum carbon ingestion needed for the obtained egg production rates was calculated assuming a gross-growth efficiency of 30 % (Kiørboe et al. 1985). Differences between cruises and stations in the indicators of zooplankton herbivorism were statistically analysed by means of one-way ANOVA tests.

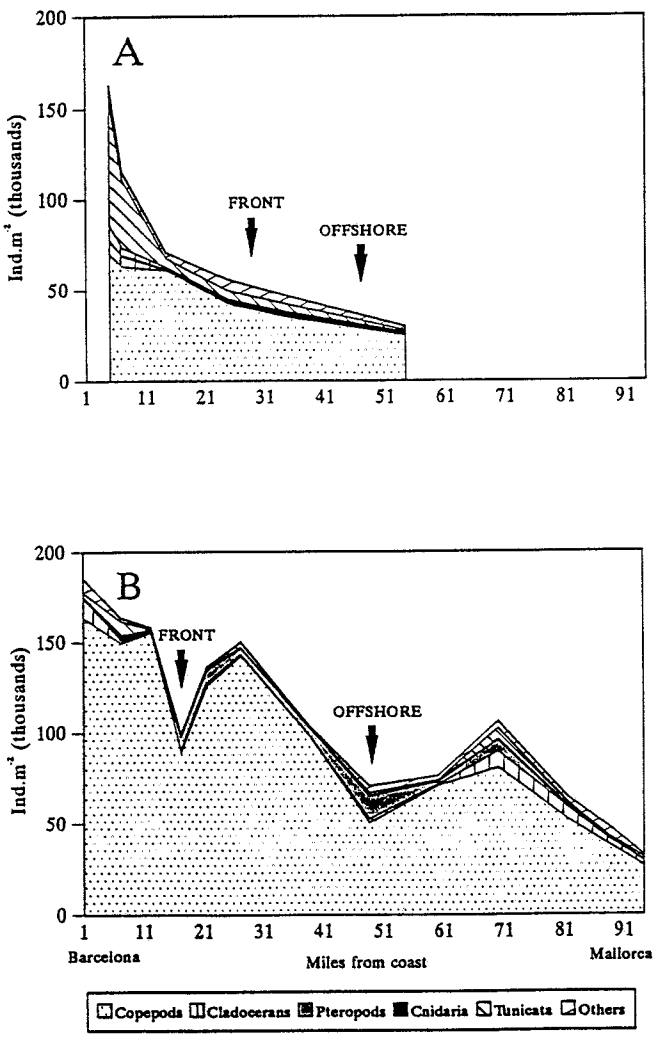


**Fig. 3.** Spatial trends in the abundance and taxonomic composition of mesozooplankton during FR92 (A) and ME93 (B) cruises. Integrated values between surface and 200 m depth. Arrows indicate the position of front and offshore stations.

## RESULTS

Hydrographic structure and zooplankton communities along the transect

The density structure along the transect corresponding to both cruises is represented in Fig. 2. The front, which was less clearly marked than in previous cruises (Saiz et al. 1992 a), was only conspicuous for FR92, and only in depth, below the thermocline. For ME93, the density gradients were even lower, and the position of the front less clear. During this cruise, the position of the station in the vicinity of the front was determined according to the higher horizontal gradient of density below the termocline.

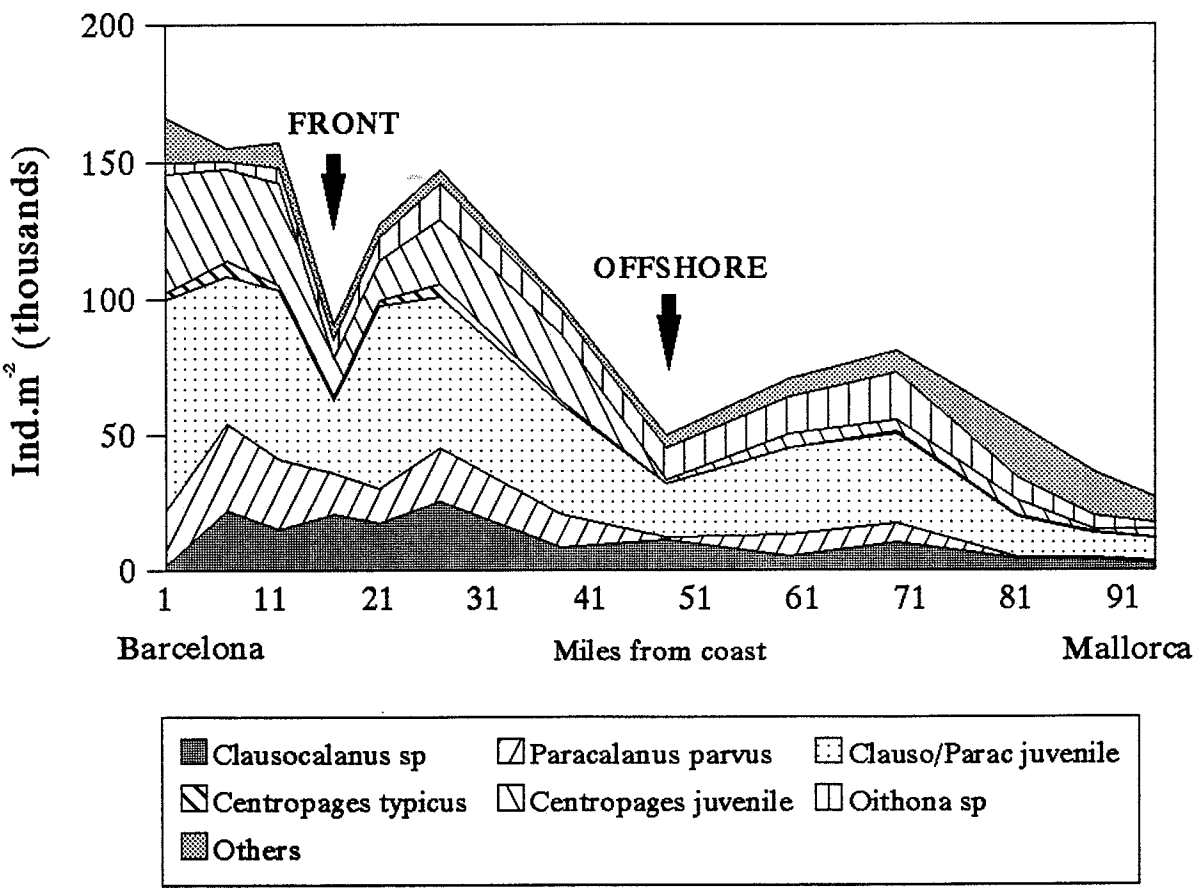


**Fig. 4.** Taxonomic composition of the copepods community during ME93 on the transect. Integrated values between surface and 200 m depth. Arrows indicate the position of frontal and offshore stations.

**Table 2.-** Average depth-integrated values (linear interpolation) of phyto and zooplankton biomass and rate processes measured along the successive samplings (Julian days) at front and offshore stations corresponding to October-November 1992 (FR92) and June 1993 (ME93) cruises. J.Day: Julian day. Avg.: Average values. Std.: Standard deviation. $C_{phyto}$: Chlorophyll-derived phytoplankton biomass (mg $C.m^{-3}$). P.P.: Primary production ($mgC.m^{-3}.d^{-1}$). $C_{zoo}$: Mesozooplankton biomass ($mgC.m^{-3}$): Res C: Respiration-derived specific carbon requirements of mesozooplankton ($mgC.mgC_{zoo}^{-1}.d^{-1}$). Res/Pro: Proportion of primary production daily required for mesozooplankton routine metabolism.

| | J. Day | Cphyto | P.P. | Czoo | Czoo/Cphyto | Res C | Res/Pro. |
|---|---|---|---|---|---|---|---|
| *FRONT* | | | | | | | |
| FR92 | 292 | 12.3 | 0.7 | 3.2 | 0.26 | 0.10 | 0.45 |
| FR92 | 295 | 12.3 | 0.9 | 4.5 | 0.36 | 0.13 | 0.65 |
| FR92 | 300 | 10.5 | 1.1 | 2.6 | 0.24 | 0.34 | 0.80 |
| FR92 | 303 | 13.8 | - | 2.7 | 0.19 | - | - |
| Avg. FR92 | | 12.2 | 0.9 | 3.2 | 0.26 | 0.19 | 0.63 |
| Std. FR92 | | 1.2 | 0.2 | 0.8 | 0.06 | 0.10 | 0.14 |
| ME93 | 161 | 17.3 | 4.5 | 7.2 | 0.42 | 0.16 | 0.26 |
| ME93 | 165 | 16.6 | 6.3 | 5.3 | 0.32 | 0.10 | 0.08 |
| ME93 | 174 | 15.9 | 6.5 | 5.6 | 0.35 | 0.28 | 0.24 |
| ME93 | 177 | 18.7 | 5.6 | 3.8 | 0.20 | 0.06 | 0.04 |
| ME93 | 179 | 17.3 | 5.4 | 4.9 | 0.28 | 0.05 | 0.05 |
| Avg. ME93 | | 17.2 | 5.6 | 5.4 | 0.31 | 0.13 | 0.13 |
| Std. ME93 | | 0.9 | 0.7 | 1.1 | 0.07 | 0.08 | 0.09 |
| *OFFSHORE* | | | | | | | |
| FR92 | 291 | 13.7 | 2.3 | 3.1 | 0.23 | 0.09 | 0.13 |
| FR92 | 299 | 14.2 | 0.8 | 2.2 | 0.15 | 0.13 | 0.35 |
| FR92 | 302 | 14.5 | 1.1 | 3.4 | 0.23 | 0.14 | 0.41 |
| Avg. FR92 | | 14.1 | 1.4 | 2.9 | 0.20 | 0.12 | 0.30 |
| Std. FR92 | | 0.3 | 0.6 | 0.5 | 0.03 | 0.02 | 0.12 |
| ME93 | 162 | 24.3 | 5.5 | 8.0 | 0.34 | 0.18 | 0.27 |
| ME93 | 166 | 16.7 | 5.3 | 5.1 | 0.30 | 0.28 | 0.28 |
| ME93 | 175 | 22.7 | 6.8 | 4.8 | 0.22 | 0.37 | 0.29 |
| Avg. ME93 | | 21.7 | 6.0 | 5.8 | 0.20 | 0.27 | 0.28 |
| Std. ME93 | | 2.9 | 0.6 | 1.3 | 0.05 | 0.07 | 0.008 |

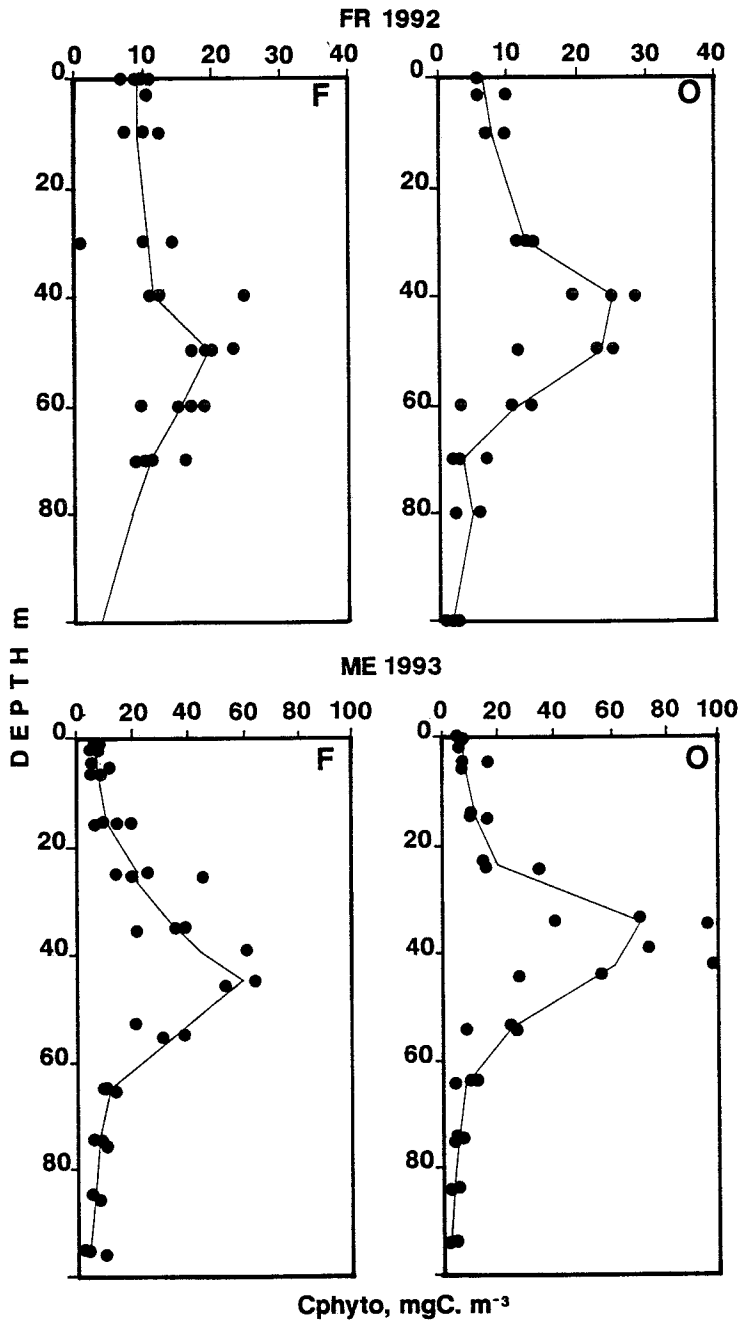


**Fig. 5.** Vertical distribution of phytoplankton carbon (as derived from chlorophyll *a* concentration) at front (F) and offshore (O) stations during FR92 and ME93 cruises. The continuous lines represent the LOWESS regression.

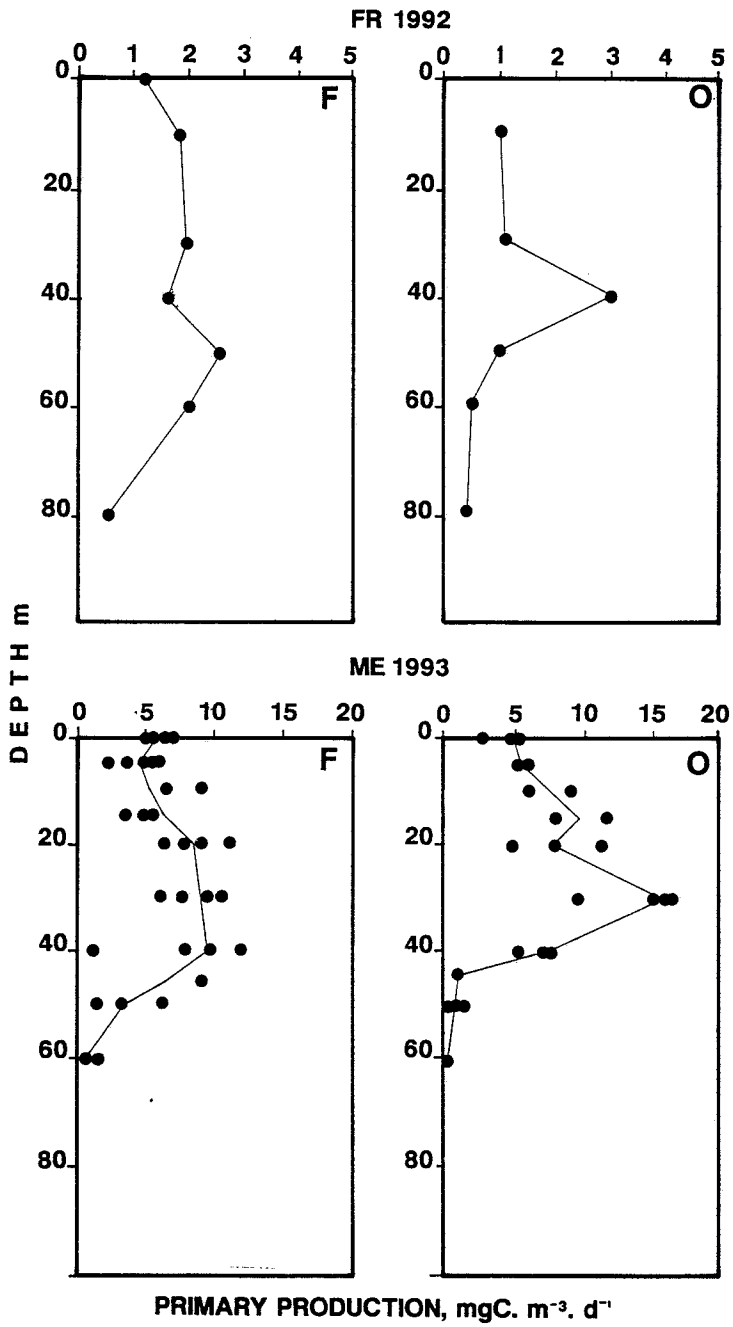


**Fig. 6.** Vertical distribution of in situ primary production values at front (F) and offshore (O) stations during FR92 and ME93 cruises. The continuous line for ME93 represents the LOWESS regression.

Zooplankton abundance showed a tendency to decrease in the offshore direction for both cruises, without significant changes in the relative proportion of taxa along the transect (Fig. 3). When comparing both cruises, the main differences consisted in a relatively high abundance of tunicates during FR92. The copepod community was the dominant, and accounted for more than 80 % (as abundance) of total zooplankton, although juvenile stages were very abundant (mainly *Clausocalanus* and *Paracalanus* copepodites). During ME93, the relative proportion of adult *Clausocalanus* sp., *Paracalanus parvus* and *Centropages typicus* decreased slightly offshore, while *Oithona* sp. and other copepods showed an opposite trend (Fig. 4).

Phytoplankton biomass and production.

The vertical trends of phytoplankton biomass ($C_{phyto}$) were similar during both cruises, with a deep maxima around 50 m at the frontal stations, and slightly shallower offshore (Fig. 5). $C_{phyto}$ maxima corresponded to both increased phytoplankton cell numbers and increased pigment content per cell (Estrada, pers. communication). Overall phytoplankton concentration was significantly higher for ME93, and the spatial trends of vertically integrated phytoplankton biomass were similar for the two cruises, with significant higher values offshore (15 % and 27 % higher for FR92 and ME93 respectively, $p<0.05$, Table 2). Although no definite trends were observable at short-time scale, the variation coefficient for successive samplings ranging from 2.1 % (FR92, offshore) to 13 % (ME93, offshore).

The vertical profiles of primary production indicated the existence of a deep maximum during both cruises. During FR92, primary production peaked at 50 and 40 m depth (at the front and offshore stations respectively), coinciding with the deep phytoplankton maximum, whereas for ME93, primary production maxima occurred above the deep phytoplankton maximum; the depth of

maximum production was around 20-40 m at the frontal station and between 20-30 m offshore (Fig. 6). Depth-integrated average values were slightly higher offshore for FR92, but very similar at both stations for ME93. On average, primary production during ME93 was higher than for FR92 cruise (Table 2).

Zooplankton biomass and activity.

The vertical distribution of mesozooplankton biomass ($C_{ZOO}$) differed for both cruises. While during FR92 zooplankton biomass was uniformly distributed, ME93 profiles peaked coinciding with the deep phytoplankton maximum at the station near the front, and at slightly deeper levels offshore (Fig. 7). Average $C_{ZOO}$ values were significantly lower during FR92 (Table 2), both at frontal and offshore stations (ANOVA, $p<0.03$ and $p<0.04$, front and offhsore respectively). Differences between frontal and offshore stations, although not significant, showed opposite trends for both cruises (slightly higher values near the front during FR92, and offshore for ME93, Table 2).

The trophic efficiency of the system, as described in Table 1 (average consumers/producers biomass ratio, $C_{ZOO}/ C_{phyto}$) was similar at offshore station for both cruises. Differences amongst stations or cruises, or between station for each cruise, were not statistically significant (Table 3).

Biomass-specific rates of mesozooplankton carbon losses, as derived from oxygen consumption rates, represented from 5 to 37 % of zooplankton carbon (table 2). At short-time scale, the variability was higher at the stations near the front (variability coefficient 52.6 against 16.6 % during FR92, front and offshore respectively, and 61.5 against 25 % during ME93, front and offshore respectively). The average metabolic requirements of mesozooplankton (considering the integrated community comprised between surface and 100 m depth) during FR92 represented from 63 % (frontal) to 30 % (offshore) of the

carbon fixed by phytoplankton. In contrast, during ME93, the mesozooplankton required from 13 % (frontal) to 27 % (offshore) of primary production (Table 2). The differences amongst cruises were statistically significant, but not amongst stations (Table 3.

Herbivory index (biomass-specific $C_{phyto}$ gut contents of mixed zooplankton, see Table I) during FR92 was slightly higher and showed higher temporal variability at the sation near the front, although differences between stations were not statistically significant (Tables 3 and 4).

**Table 3.**- Statistical probability of significant differences in the structural and functional indicators of herbivorous transfer (ANOVA). NS: not significant. * $p<0.05$. ** $p<0.001$.

| | Amongst cruises | Amongst stations | |
|---|---|---|---|
| | | FR92 | ME93 |
| $C_{zoo}/C_{phyto}$ | <0.095 (NS) | 0.270 (NS) | 0.637 (NS) |
| $C_{gut}$ | --- | 0.310 (NS) | ---- |
| Res/Pro | <0.003 ** | 0.063 (NS) | 0.0620 (NS) |
| Spec. C prod % | --- | --- | 0.017 * |

The egg production rates and the abundance of adult females of the five copepod species considered at the frontal and offshore stations during ME93 are indicated in Table 5. The corresponding female biomass ($mgC.m^{-3}$), their metabolic carbon requirements calculated from biomass-specific carbon losses of mixed zooplankton indicated inTable 2 ($mgC.m^{-3}.day^{-1}$), egg production rates ($mgC.m^{-3}.day^{-1}$), and other metabolic parameters are given in Table 6. At the frontal station, the carbon ingested (as estimated through the egg production rates) was enough to provide for the carbon requirements for only one of the five copepod species (*Paracalanus*). In contrast, at the offshore station three of the five species

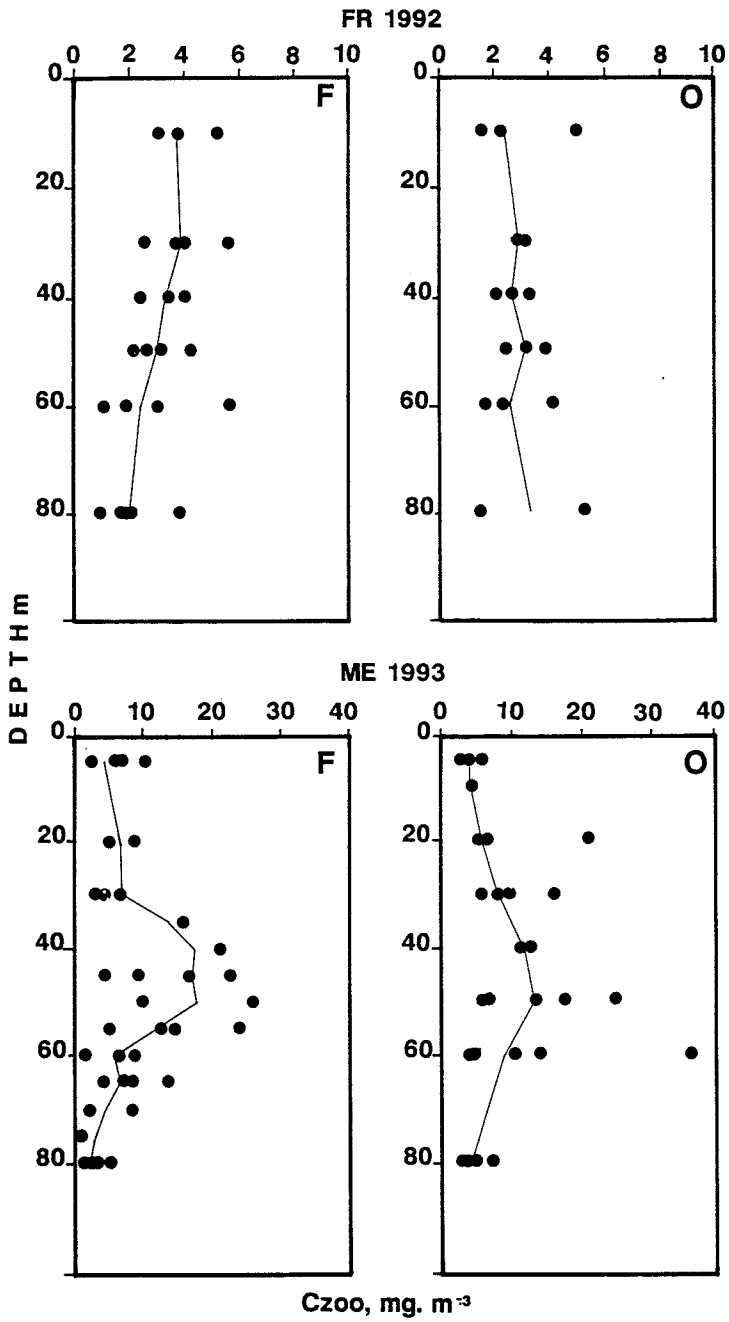


**Fig. 7**. Vertical distribution of zooplankton biomass, as organic carbon, at front (F) and offshore (O) stations during FR92 and FR93 cruises. The continuous lines represent the LOWESS regression.

ingested enough carbon to provide for their metabolic requirements. The specific carbon production was higher offshore for the five species. Integrated average values of carbon production at frontal and offshore stations were, as average, about three times higher at offshore station than near the front (Table 7), and were statistically significant (Table 3).

**Table 4.-** FR92: Zooplankton herbivory index (biomass-specific phytoplankton gut contents of zooplankton) at front and offshore station for 1992 cruise. J. day: Julian day. C-gut: μg $C_{phyto}$-gut.mg $C_{zoo}^{-1}$.In parentheses, the standard error (p>95 %).

| | C-gut | |
|---|---|---|
| J. Day | Front | Offshore |
| 291 | - | 5.2 (1.5) |
| 292 | 8.0 (3.1) | - |
| 295 | 4.3 (0.5) | - |
| 298 | - | 5.4 (0.7) |
| 298 | 6.3 (2.6) | - |
| 299 | - | 6.7 (2.1) |
| 300 | 9.4 (3.3) | - |
| 302 | - | 6.7 (2.8) |
| 303 | 5.2 (1.9) | - |
| 307 | 8.3 (3.2) | - |
| Average: | 6.9 (2.4) | 6.0 (1.7) |

## DISCUSSION

According the structural indices, the overall coupling between zoo- and phytoplankton seemed weaker during FR92. The relatively high abundance of tunicates during this cruise (Fig. 3) suggests a relatively higher proportion of matter and energy transfer through microhetrotrophic (microbial based) food webs than for ME93, thus reducing the fraction of phytoplankton carbon allocable towards other herbivorous zooplankton (Legendre et al. 1993). The same tendency is reflected by other structural indicators, such are the lack of coincidence between the profiles of zoo- and phytoplankton biomass or production, or the lower values of the trophic efficiency, coincident with significantly lower values of phyto- and zooplankton biomass. On the contrary, functional indices suggest that the overall transfer through herbivorous food webs was more intense during FR92 than for ME93. The fraction of primary production required to compensate for the zooplankton respiratory losses during the summer stratification (ME93) coincided with previous estimates for the same period in the study area (Alcaraz 1988), and were less than half those observed during the autumn weakening of the thermocline (FR92).

This contradictory trend in herbivorous food webs as estimated by the two categories of indices when comparing cruises could be explained by the conservative nature of the extensive properties of the system, like biomass, whose response time to environmental changes is longer than in the case of rate processes.

**Table 5.-** FR93: Female abundance (ind. $m^{-3}$) and egg production rates (eggs. $fem^{-1}.d^{-1}$, in parentheses) for the successive samplings (Julian day) at front and offshore stations. (-): Lack of data. Avg. F and Avg. O: Average values for front and offshore stations.

| | FRONT | | | OFFSHORE | | Avg. | Avg. |
|---|---|---|---|---|---|---|---|
| Julian day | 174 | 177 | 179 | 175 | 178 | F | O |
| *C. tenuicornis* | 0.0 | 0.0 | 0.0 | 0.55 | 0.55 | 0.0 | 0.6 |
| " | (-) | (-) | (-) | (3.2) | (3.6) | (-) | (3.4) |
| *P. parvus* | 183.3 | 53.3 | 16.6 | 22.7 | 11.7 | 84.4 | 17.2 |
| " | (2.1) | (5.2) | (-) | (-) | (12.4) | (3.4) | (12.4) |
| *Clausocalanus* sp. | 87.1 | 63.2 | 103.3 | 76.7 | 58.3 | 75.2 | 67.5 |
| " | (0.5) | (5.6) | (1.9) | (7.4) | (6.1) | (2.7) | (6.7) |
| *A. clausi* | 3.3 | 1.7 | 0.0 | 1.1 | 0.0 | 1.7 | 0.6 |
| " | (1.9) | (1.6) | (-) | (3.4) | (0.4) | (1.8) | (1.9) |
| *C. typicus* | 18.9 | 1.7 | 2.8 | 1.7 | 0.0 | 7.8 | 0.8 |
| " | (-) | (10.9) | (-) | (23.4) | (0.0) | (10.9) | (11.7) |

When comparing stations within each cruise, only slight differences could be ascertained by means of structural indices. Phytoplankton abundance was always higher at the offshore station, in agreement with previous observations in the same area (Saiz et al. 1992 a) and in the Liguro-Provençal frontal system (Boucher 1984; Boucher et al. 1987), while mesozooplankton biomass showed an opposite trend for both cruises. Average $C_{ZOO}$ was slightly higher at the front during FR92, and slightly higher offshore during ME93. However, the trophic efficiency at offshore stations was similar for the two cruises, suggesting that the magnitude of the transfer through herbivorous food webs in this hydrographically stable area is more constant than in the vicinity of the front. Regarding the herbivory index during FR92, the frontal-offshore tendencies coincide with the pattern shown by the trophic efficiency, althoug in both cases the differences were not statistically significant.

**Table 6.-** Biomass ($\mu gC.m^{-3}$) of females of the copepod species used in egg production experiments, their metabolic carbon requirements ($\mu gC.m^{-3}.day^{-1}$, calculated from the biomass-specific respiration rates of mixed zooplankton, Table II), the carbon produced daily as eggs ($\mu gC.m^{-3}.day^{-1}$), the carbon ingested assuming a gross-growth efficiency (from egg production) of 30 % of the food ingested ($\mu gC.m^{3}.day^{-1}$), and the percent of female biomass daily produced as eggs ($100*\mu gC_{eggs}.day^{-1}.\mu gC_{females}^{-1}$). Asterisks indicate the species and station in which the estimated carbon ingestion is enough to provide their metabolic carbon requirements. F= front, O= offshore.

| | | Fem. biom. | C req. | C prod. (eggs) | C ing. | Spec. C prod. % |
|---|---|---|---|---|---|---|
| *C. tenuicornis* | F | -- | -- | -- | -- | -- |
| " | O * | 20.3 | 5.4 | 1.7 | 5.7 | 8.4 |
| *P.parvus* | F * | 156.0 | 20.6 | 6.6 | 22.0 | 4.2 |
| " | O * | 32.0 | 8.4 | 2.9 | 9.6 | 9.0 |
| *Clausocalanus* sp. | F | 507.0 | 66.8 | 5.6 | 18.5 | 1.1 |
| " | O | 405.0 | 107.7 | 12.8 | 42.9 | 3.2 |
| *A. clausi* | F | 5.2 | 0.7 | 0.1 | 0.4 | 2.3 |
| " | O | 1.7 | 0.4 | 0.05 | 0.2 | 2.9 |
| *C. typicus* | F | 44.6 | 5.8 | 0.4 | 1.5 | 1.0 |
| " | O * | 4.8 | 1.3 | 0.5 | 1.6 | 9.8 |

Regarding functional indices, the tendencies were similar: the proportion of primary production required by zooplankton at offshore stations was equivalent for both cruises, while at stations near the front interannual variability was higher, and indicated a less intense herbivorous transfer for ME93.

The higher egg production rates of herbivorous copepods during ME93 at the offshore station confirms the higher herbivorous transfer as shown by zooplankton carbon requirements. The potential use by copepods of non-phytoplankton food sources, (i.e., ciliates), would not modify this conclusion as their abundance was similar at both stations (Saiz, unpublished data). However, the low egg production rates observed during ME93 at both stations suggest severe food limitation for copepods. Furthermore, their carbon requirements (estimated according female biomass and the biomass-specific carbon requirements for mixed zooplankton) and their carbon ingested (estimated from egg production rates) are unbalanced. Only in the case of *Paracalanus* at the front and offshore, and *Centropages* and *Calanus* at the offshore station, the ingestion rates derived from their egg production rates were enough to provide for their carbon requirements. It should be noted, however, that the calculated ingestion rates must be taken with caution, because the trophic efficiency considered here (30 %) corresponds to copepods of the genus *Acartia* (Kiørboe et al. 1985, Saiz et al. 1992 b).

**Table 7.**- Cummulative values at frontal (FRONT) and offshore (OFF) stations of biomass of females of copepod species used in egg production rates (Fem. biom., $mgC_{female}.m^{-3}$), daily carbon production as eggs (Egg C prod., $mg\ C.m^{-3}.day^{-1}$), carbon ingested (C ingest., $mgC.m^{-3}.day^{-1}$), and average percentage of female biomass daily produced as eggs (Spec. C prod %., $100*mg\ C_{eggs}.mgC_{female}^{-1}.day^{-1}$). In parentheses, standard deviation.

| | Fem. biom | Egg C prod. | C ingest. | Spec. C prod %. |
|---|---|---|---|---|
| FRONT | 0.713 | 0.010 | 0.042 | 2.15 (1.28) |
| OFFSHORE | 0.464 | 0.012 | 0.060 | 6.60 (2.98) |

The weak differences amongst cruises, or between stations within cruises, regarding the structural indices could be due in part to the less intense signature of the Catalan front during FR92 and ME93 as compared with previous years (Saiz et al. 1992 a). But apart from the reduction in the physical forcing due to small gradients even during the autumn weakening of the thermocline (FR92), the low variability and contradictory results provided by structural and functional indices should be attributed to their different time-scale response. The functional response of zooplankton to physical or biological environmental changes like temperature, water turbulence, or abundance, size, and quality of food, is much faster than any quantifiable modification in the structural properties of the system (Alcaraz et al. 1988, Saiz and Alcaraz 1992, Alcaraz et al. 1994 a, Calbet and Alcaraz in press). As a consequence, structural indices are appropriate estimators of the trophic characteristics of pelagic systems mainly when they are functioning at a near steady-state regime, but in areas where short-term fertilization events are generally aperiodic, the temporal variability preclude the stablishment of permanent plankton structures (Le Fèvre et al 1983).

Apart from the quick response of zooplankton rate processes to environmental forcing, either physical or biological, indices like the proportion of primary production required to compensate for the zooplankton metabolic losses, or egg production rates, appear directly related with feeding, respiration and excretion rates in planktonic copepods (Kiørboe et al. 1985, Saiz et al. in press, Calbet and Alcaraz in press). Although respiration rates and copepod egg production seem to respond to the previous food conditions experienced by the organisms (about 1 day, Kiørboe et al. 1985), the possible errors due to the comparison of respiration or egg production and simultaneous estimates of primary production are precluded by the duration of zooplankton respiration and copepod egg production experiments (about 24 h), and the low variance of food (phyto C, chlorophyll derived) concentration (Table 2).

In conclusion, the trophic characteristics of pelagic systems where energy inputs are aperiodic, and specially in the vicinity of hydrographically unstable physical structures like fronts, seem to be better reflected by the relationships between rate processes of the different trophic groups than by structural indices based on state variables of the system. This is in agreement with the conservative nature of extensive properties (biomass) of pelagic systems, whose changes are relatively slow and depend on cumulative, complex interaction between rate processes. As contrast, rate processes are rapidly changing, and respond almost immediately to biological (trophic) or physical (turbulence) estimulation.

When comparing multiyear data, the transfer through herbivorous food webs seems to be more intense at the Catalan front on average. However, its temporal variability as estimated by those and other estimators of phyto-zooplankton coupling (i.e., contribution of zooplankton excretion to phytoplankton nutrient requirements, Alcaraz et al. 1994) are also higher there, while at the more stable zone offshore and in the central dome, the low temporal variability suggests a quasi steady-state transfer through herbivorous food webs. However, it is unclear how the alternate periods of high and low intensity in the herbivorous-based food chains described here affect the general production, biogeochemical cycling and ultimate fate of biogenic carbon in the area. A better understanding of the problem would require the study of the frequency and intensity of hydrodynamic instabilities along extended time periods, and how they are transmitted through pelagic food webs.

**RESUM**

Diversos índexs d'acoblament basats respectivament en les propietats estructurals i relacions funcionals entre productors primaris i zooplàncton herbívor van ésser comparats en el transcurs de dos campanyes oceanogràfiques, FR92 (octubre-novembre de 1992) i ME93 (juny de 1993), en dos estacions fixes situades en un transecte a través del front de densitat present en el Mar Català. La primera estació estava situada en les proximitats del front, i la segona a mar obert. Ambdues estacions foren mostrejades a intervals variables en el transcurs de cada campanya per tal de determinar les possibles tendències en els canvis temporals d'acoblament entre el fitoplàcton i el zooplàncton. Aquest acoblament fou estimat d'acord amb dues categories d'indicadors quantitatius: 1) índexs estructurals, basats en les relacions entre variables d'estat de productors i consumidors (tals com distribució relativa de la biomassa de productors i consumidors, índexs d'herbivorisme i eficiència tròfica del zooplàncton) i 2) índexs funcionals, basats en taxes d'activitat corresponents a processos tals com requeriments metabòlics de carboni, la producció de copèpodes, etc.

Els índexs estructurals varen mostrar menys varabilitat temporal que els funcionals, ja sigui a curta escala (dins de cada campanya) o a llarga escala (entre campanyes). Els valors d'ambdós índexs a les estacions de mar obert varen coincidir per les dues campanyes, i foren similars a la mitjana dels valors obtinguts per la zona en campanyes anteriors. En les estacions properes al front, mentre que els índexs estructurals suggerien un major acoblament entre el fitoplàncton i el zooplàncton durant FR92 (encara que les diferències entre campanyes no van ser significatives), els funcionals indicaven un patró oposat, essent significativament més alts durant ME93.

Així en zones on la entrada d'energia responsable de polsos de producció no són

periòdiques, com el Mediterrani, els índexs funcionals semblen doncs, reflectir millor qualsevol canvi en la importància relativa de les diferents xarxes tròfiques que els estructurals. Això suggereix que la importància relativa de les xarxes tròfiques planctòniques pot ésser estimada d'una manera més acurada a través de la relació entre els processos que a través de relacions entre variables d'estat més conservatives, basades en la biomassa o l'estructura de la comunitat.

## 4.2 COPEPOD EGG PRODUCTION IN THE WESTERN MEDITERRANEAN IN JUNE 1995

## INTRODUCTION

The hydrographic instability associated with frontal systems may favour the occurrence of short fertilization events and brief phytoplankton outbursts (Le Fèvre 1986) consequence of mechanical energy inputs, whose timing may or may not be coped by the response in time of the zooplankton community (Le Fèvre and Frontier 1988). These match-mismatch processes determine the trophic characteristics of the system (energy transfer via the classical herbivorous food webs or via decomposers and the microbial loop), which are determinants for the export pathways of biogenic carbon (Legendre et al. 1993). The phyto-zooplankton coupling in areas where phytoplankton events are aperiodical have been better described by the indices based on the rate processes of the different trophic groups than by structural indices based on the state variables (Calbet et al. 1996).

Along the shelfbreak area of the Catalan Sea (North Western Mediterranean) there is a density front in the external margin of the Ligurian-Provençal-Catalan current, which flows south-westward. Several studies have shown that this hydrographic structure has a major influence on the composition and abundance of phyto- and zooplankton as well as on their metabolic rates, specially during their seasonal growth period (Margalef and Estrada 1987, Alcaraz 1988, Estrada and Margalef 1988, Alcaraz and Packard 1989, Saiz et al. 1992, Alcaraz et al. 1994, Calbet et al. 1996).

Data about zooplankton production in the area, and its relation with phytoplankton biomass are scarce (Calbet et al. 1996) and although these data suggest a high degree of temporal variability in the vicinity of the front, further estimates of zooplankton production through the different singularities that

characterize the Catalan Sea are needed in order to determine the spatial heterogeneity.

The aim of the study presented here was to determine the effect of the Catalan front on the abundance and growth rates of copepods during late spring-early summer. The period studied is characterized by a weakening of the front whose signature cannot be perceived in surface waters, the density gradient reaching only the bare of the thermocline.

## MATERIAL AND METHODS

### Area surveyed

Sampling took place in the Catalan Sea (Western Mediterranean) throughout June 1995 during two cruises (VARIMED-95, 2-14 June 1995 and FRONTS-95, 18-23 June 1995), on board the R/V "Hesperides" and the R/V "Garcia del Cid" respectively. The sampling stations were located in three transects perpendicular to the shoreline (fig. 1). During the VARIMED-95 cruise, the sampling was restricted to 3 stations in the central transect; In FRONTS-95, sampling was performed in a grid of nine stations. Each transect comprised three main stations, between Barcelona and the Balearic Islands, crossing the system defined by the Liguro-Provençal-Catalan current, the Catalan density front at the outer margin of the current, and the dome in the central part of the Catalano-Balearic sea. The three stations were located respectively at the shelf, the shelfbreak and the dome-like structure in the center of the Catalan Sea (fig. 1).

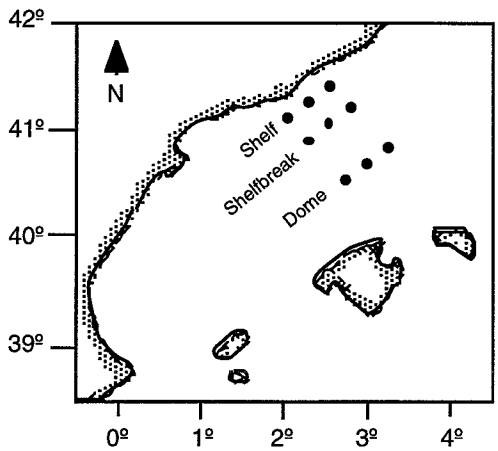


**Fig. 1**. Location of the study area with the position of the main stations (Shelf, Shelfbreak and Dome) in both cruises (VARIMED-95 and FRONTS-95).

Sampling strategy

CTD casts and chlorophyll concentration (total and >5μm) determination were routinely conducted at each station. Temperature, salinity and "in situ" fluorescence were recorded with a Neil-Brown MARK-V CTD (VARIMED-95), and Seabird-25 CTD (FRONTS-95), both equipped with a Sea Tech fluorometer. Chlorophyll determination were made on water samples taken at 10 m intervals down to 200 m, during the ascending casts with a rosette attached to the CTD. After an initial CTD cast, and according to the vertical physical structure and fluorescence profiles, six depths were chosen for sampling between surface and 80-100 m depth for the estimation of phytoplankton biomass (chlorophyll *a*). Chlorophyll was measured by fluorimetry on acetone extracts (Yentsch and Menzel 1963) without acidification.

For the determination of abundance and biomass of mesozooplankton, vertical tows were performed from 200 m depth to surface (or from near the bottom to surface at shallower stations) with a double WP-2 net fitted with 200-μm mesh, towed at 20-30 m $min^{-1}$. For collecting live zooplankton, the nets were towed

from 80 m depth (or near the bottom when shallower) at 10 m $min^{-1}$. The nets were fitted with 5-10 l plastic bags as a cod end to prevent damage of organisms. Once on deck, the contents of the bags was transferred to coolers and the organisms for experiments sorted.

Determination of the egg production rates of copepods

To determine egg production rates of the dominant copepod species during the cruise, live undamaged adult females were sorted under a stereomicroscope. The dominant species were overall *Centropages typicus* and *Clausocalanus* sp., and in some stations *Oithona* sp., *Paracalanus parvus* and *Temora stylifera*. Groups of four (ten for *Oithona*) adult females were picked out and incubated (independently for each species) in 625-ml bottles filled with water from the fluorescence maximum previously screened through 53 μm by reverse filtration. When possible, four replicate bottles for each species were prepared per station. The incubations were performed on deck in an incubator in which temperature was maintained by the circulation of surface water. Temperature oscillated between 18 and 20°C in the VARIMED-95 cruise and between 20 and 22°C in the FRONTS-95 cruise. The incubations were performed at the natural light cycle; however, the bottles were covered with coloured plastic to provide dim light. The bottles were frequently turned upside down during the incubations to prevent the settling of algae. After 24 hours the contents of the bottles were filtered onto 20-μm sieves, the condition of the animals checked, and eggs and copepods preserved for posterior counting and sizing in the laboratory.

In several occasions an extra batch of bottles was prepared in which the copepods were offered a very high (7 ppm by volume) suspension of the flagellate *Rhodomonas baltica*. The purpose of this procedure was to determine if the copepods were food limited and how long it takes for them to build up the

incubations lasted from one to three days (only for *C. typicus*), and each day egg production was determined. A single experiment was performed in order to test the effects of temperature in the egg production rates of *C. typicus*. Batches of four bottles with four individual each and a saturating suspension (7 ppm) of *R. baltica* were incubated for two days at four different temperatures: 13, 17, 20 and 25°C. The number of eggs laid and the status of the females were checked after 24 and 48 hours of incubating.

## RESULTS

The position of the frontal structure is represented by the salinity distribution along the central transect (fig. 2). Due to the thermal stratification, the presence of the front was only perceptible in depth, below the bare of the thermocline. Associated with this seasonal stratification (the thermocline), between 40-50 m depth, there was a deep chlorophyll maximum (DCM) that is a semi-permanent structure in the North Western Mediterranean, (Estrada et al. 1993). The front, although more evident than in previous cruises (Calbet et al 1996) seemed to have no significant effect over the chlorophyll *a* distribution (fig. 3), which reached the highest values in the central dome (fig. 4). However, while total chlorophyll tended to increase offshore, the >5 μm chlorophyll concentration was higher (1.3 times higher) at the shelf and shelfbreak stations than at the dome one. This differences are also apparent in the linear relationship between total and >5 μm chlorophyll *a* which differs for the shelf and shelfbreak waters respect to the dome station (fig. 5). At the shelf and shelfbreak stations the contribution of the >5 μm phytoplankton was about twice the one found at the dome station.

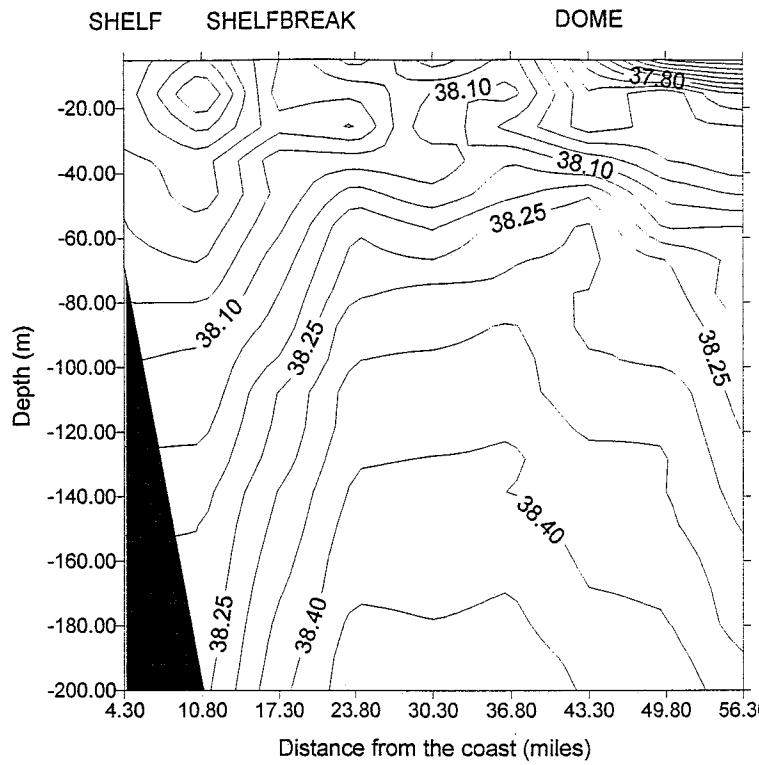


**Fig. 2.** Salinity distribution along the central transect in Varimed-95 cruise. The positions of the main stations surveyed are indicated.

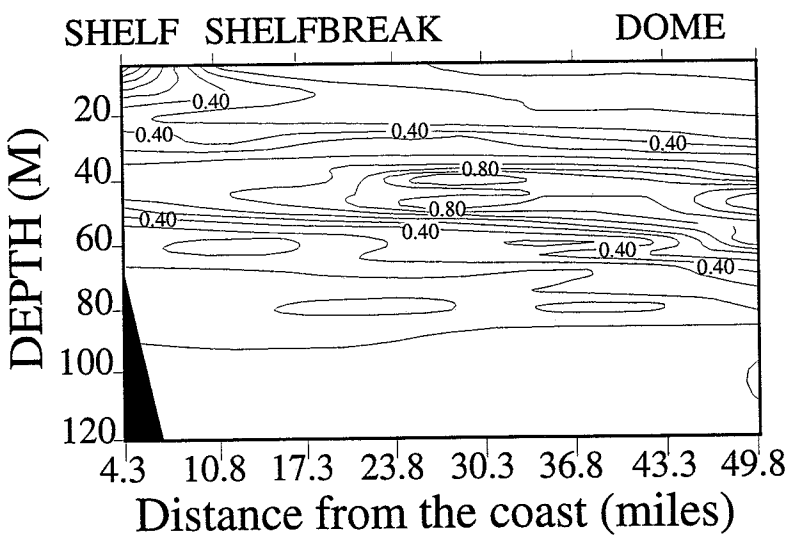


**Fig. 3.** Chlorophyll *a* distribution (mg $m^{-3}$) in the central transect sampled.

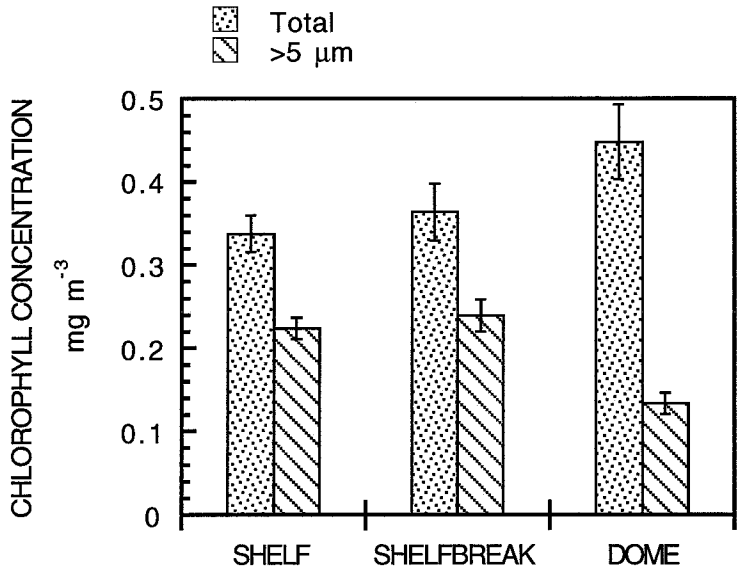


**Fig. 4.** Chlorophyll *a* and >5 µm chlorophyll concentration (mean ± SE; mg $m^{-3}$) at the shelf, shelfbreak and dome stations throughout the period studied. Average values have been computed using photic zone data only (60-70 m).

**Table 1.** Composition of the copepod community in the area studied. Average percentage (%) of abundance (respect to total copepod abundance) of *Centropages typicus*, *Clausocalanus* spp. and *Paracalanus parvus* together, *Temora stylifera* and *Oithona* spp.

| | SHELF | SHELFBREAK | DOME |
|---|---|---|---|
| *Centropages typicus* | 24 | 17 | 20 |
| *Clauso/Paracalanus* | 47 | 40 | 57 |
| *Temora stylifera* | 7 | 2 | 0 |
| *Oithona* spp. | 9 | 17 | 9 |
| Others | 13 | 23 | 14 |

The integrated (0-200 m) mesozooplankton abundance, and the relative taxonomic composition of the copepod community are indicated in fig. 6 and table 1 respectively. Total mesozooplankton was more abundant in the shelf waters, with an important contribution by gelatinous herbivorous zooplankton, cladocerans and meroplankton. Regarding copepods, no clear difference among stations was evident. No significant differences were observed in the relative abundance of the dominant copepod groups amongst the shelf, shelfbreak and dome stations except for *Temora stylifera* which was more abundant in shelf stations.

**Table 2.** Egg production rates (eggs female$^{-1}$ day$^{-1}$) in the three stations surveyed of the copepod species not presented in fig. 7. The values are average of replicates ± SE.

| Species | EPR |
|---|---|
| **SHELF** | |
| *Oithona* sp. | 3.3 ± 0.80 |
| *Paracalanus parvus* | 21.6 ± 3.60 |
| *Paracalanus parvus* | 14.1 ± 2.48 |
| **SHELFBREAK** | |
| *Acartia clausi* | 0.4 ± 0.28 |
| *Acartia clausi* | 1.0 ± 0.42 |
| *Oithona* sp. | 0.1 ± 0.11 |
| *Oithona* sp. | 1.0 ± 0.36 |
| *Oithona* sp. | 9.9 ± 5.26 |
| *Paracalanus parvus* | 15.7 ± 6.20 |
| **DOME** | |
| *Acartia clausi* | 0.8 ± 0.21 |
| *Oithona* sp. | 0.6 ± 0.36 |

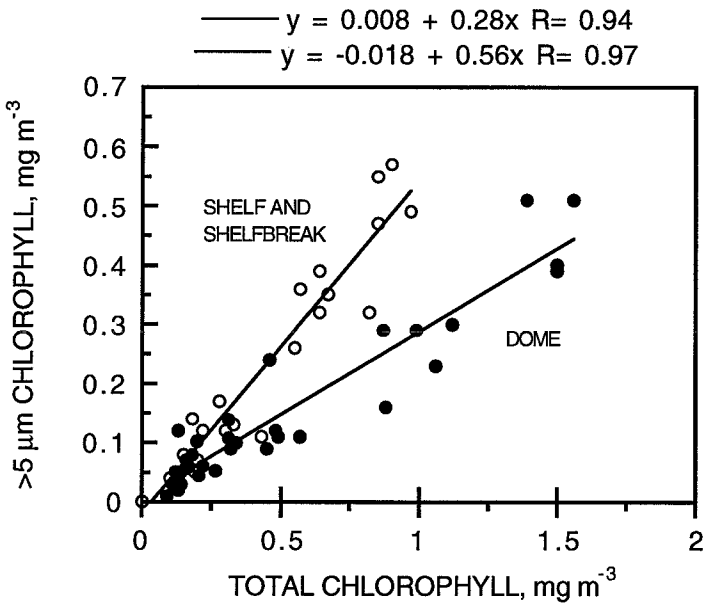


**Fig. 5.** Scatterplot of total chlorophyll versus >5 µm fraction. Open symbols: shelf and shelfbreak stations, filled symbols: dome stations.

*Centropages typicus* and the group *Clausocalanus* spp. + *Paracalanus parvus* comprised about 75% of copepods.

The egg production rates for the dominant species are represented in fig. 7. For *C. typicus* and *Clausocalanus* spp., egg production rates were 3-4 times higher at the shelf and shelfbreak than at the dome. The differences between shelf and shelfbreak were not statistically significant. Regarding *T. stylifera* not special trends in egg production were observed. For non-dominant copepods species like *Oithona* spp., *P. parvus* and *Acartia clausi* the spatial patterns in egg production were not clear (table 2). The sizes of the dominant copepod species in each station are presented in table 3.

The relationship between the >5 µm chlorophyll concentration and egg production rates (mean ± SE) for *C. typicus* and *Clausocalanus* spp. respectively

are represented in fig. 8. There was a tendency to higher egg production rates at higher concentrations of chlorophyll (which correspond to incubations conducted at the shelf and shelfbreak stations), but scatter precludes any good statistical relationship. The temporal evolution of the egg production rates (± SE) for *C. typicus* and *Clausocalanus* spp. at the shelf, shelfbreak and dome stations did not show any clear trend (fig. 9 and 10 respectively). However, there was a gradient on egg production rate from shelf waters to dome waters, similar to the one found for the >5 μm chlorophyll concentration. *C. typicus* showed some natural mortality in incubations at the end of the experimental period, coincident with the diminution in abundance previously described by Vives (1963) (these data have not been used in previous and further calculations). This was not an experimental artifact because concurrent incubations with *T. stylifera* and *Clausocalanus* spp. did not show any mortality. Furthermore, juvenile *C. typicus* incubated in parallel did not show any mortality either. The egg production rates after 24 h of "ad libitum" food supply (7 ppm *Rhodomonas baltica*) for the most common copepod species is shown in table 4.

**Table 3.** Copepod body size (length and width) in mm (average values ± SE) in the three stations surveyed.

| Species | Length | Width |
|---|---|---|
| **SHELF** | | |
| *Centropages typicus* | 0.93 ± 0.007 | 0.36 ± 0.003 |
| *Clausocalanus* sp. | 1.10 ± 0.012 | 0.41 ± 0.006 |
| *Temora stylifera* | 0.97 ± 0.008 | 0.43 ± 0.007 |
| *Paracalanus parvus* | 0.68 ± 0.016 | 0.26 ± 0.008 |
| *Oithona* sp. | 0.52 ± 0.008 | 0.17 ± 0.004 |
| **SHELFBREAK** | | |
| *Centropages typicus* | 1.00 ± 0.012 | 0.37 ± 0.004 |
| *Clausocalanus* sp. | 1.05 ± 0.011 | 0.41 ± 0.005 |
| *Temora stylifera* | 1.01 ± 0.010 | 0.44 ± 0.006 |
| *Paracalanus parvus* | 0.69 ± 0.008 | 0.25 ± 0.006 |
| *Oithona* sp. | 0.54 ± 0.012 | 0.17 ± 0.004 |
| *Acartia clausi* | 0.87 ± 0.009 | 0.26 ± 0.004 |
| **DOME** | | |
| *Centropages typicus* | 0.91 ± 0.005 | 0.34 ± 0.002 |
| *Clausocalanus* sp. | 1.07 ± 0.005 | 0.39 ± 0.003 |
| *Temora stylifera* | 0.98 ± 0.007 | 0.43 ± 0.005 |
| *Oithona* sp. | 0.51 ± 0.011 | 0.14 ± 0.014 |

The overall (average of all stations) maximum increase in egg production rate after a period of 24 h under high food concentration respect DCM water incubations corresponded to *T. stylifera* (an increase factor of 5.2), followed by *C. typicus* and *Clausocalanus* spp. (an increase factor of 2.4). The species less sensitive to the food increase was *P. parvus*, which only increased the egg production by a factor of 1.3. The temporal evolution of egg production along three days under the presence of saturating food conditions presented in fig. 11. The results demonstrated a clear food limitation in the dome stations (open sea). For the stations located in the shelf and shelfbreak, the pattern was not so clear. This result agree with the low >5μm chlorophyll concentrations found in the dome in comparison to the shelf and the shelfbreak stations.

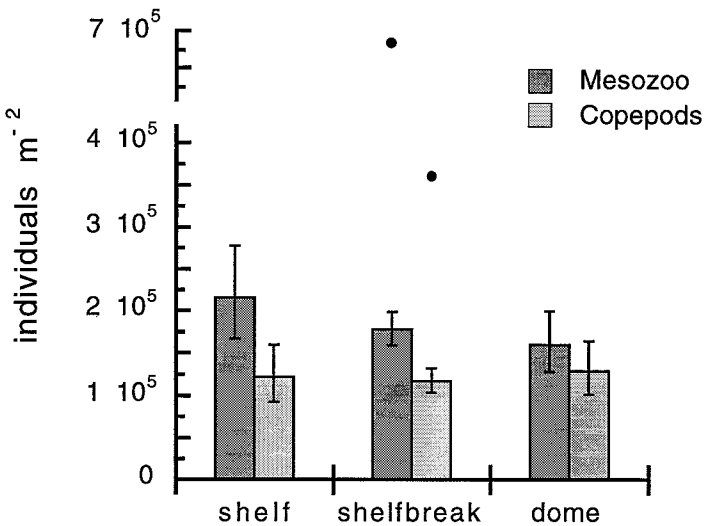


**Fig. 6.** Mesozooplankton abundance (integrated values: 0-200 m for shelfbreak and dome stations, 0-60 or 0-75 m for shelf station; geometric means ± SE) in the sampled area.

The functional response of egg production rate to temperature is represented in fig. 12. In the rang of temperatures tested, the highest egg production rates for *C. typicus* corresponded to 20°C. The effects of temperature become apparent after 48 h incubation (fig. 12). Temperatures equal or higher than 25°C had a negative effect in egg production.

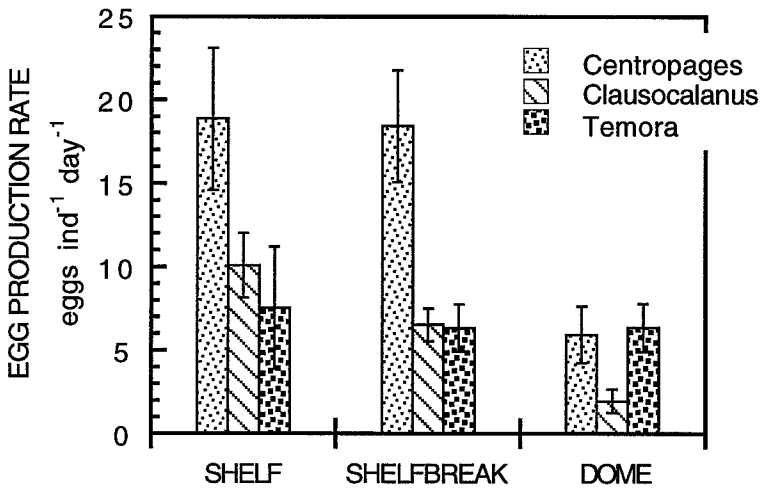


**Fig. 7**. Egg production rates (± SE) of *Centropages typicus*, *Clausocalanus* spp. and *Temora stylifera* at the shelf, shelfbreak and dome stations.

**Table 4.** Egg production rates (eggs female$^{-1}$ day$^{-1}$) of most abundant copepod species in the three stations surveyed. The values correspond to the average of replicates ± SE of incubations performed with deep chlorophyll maximum water (DCM) or enriched water (DCM + 7 ppm *R. baltica*).

| Species | DCM | Enriched |
|---|---|---|
| **SHELF** | | |
| *Centropages typicus* | 7.4 ± 3.69 | 31.9 ± 8.96 |
| *Centropages typicus* | 31.5 ± 6.03 | 67.4 ± 6.40 |
| *Centropages typicus* | 27.9 ± 6.36 | 38.3 ± 8.26 |
| *Clausocalanus* sp. | 17.7 ± 6.00 | 41.0 ± 10.01 |
| *Paracalanus parvus* | 14.1 ± 2.48 | 17.6 ± 3.56 |
| *Temora stylifera* | 17.2 ± 1.65 | 80.2 ± 15.58 |
| **SHELFBREAK** | | |
| *Centropages typicus* | 21.6 ± 5.17 | 31.2 ± 10.71 |
| *Centropages typicus* | 22.2 ± 9.64 | 27.5 ± 4.32 |
| *Clausocalanus* sp. | 7.6 ± 4.84 | 0.0 ± 0.00 |
| *Paracalanus parvus* | 15.8 ± 6.20 | 21.2 ± 1.12 |
| *Temora stylifera* | 8.5 ± 3.17 | 49.3 ± 11.82 |
| **DOME** | | |
| *Centropages typicus* | 6.6 ± 1.66 | 6.6 ± 5.01 |
| *Centropages typicus* | 8.4 ± 2.24 | 13.8 ± 3.76 |
| *Centropages typicus* | 2.7 ± 0.88 | 15.8 ± 0.86 |
| *Clausocalanus* sp. | 0.3 ± 0.09 | 2.5 ± 2.25 |
| *Clausocalanus* sp. | 4.2 ± 2.39 | 2.7 ± 2.63 |
| *Clausocalanus* sp. | 3.2 ± 1.51 | 0.0 ± 0.00 |

## DISCUSSION

Contrary to previous data for the same area (Saiz et al. 1992, Alcaraz et al. 1994) the presence of the density front seemed to have no effects mesozooplankton abundance, and the same occurred for the copepod community structure. In general, the copepod abundance found in June 1995 was higher than found in 1992 but similar than in 1993 (Calbet et al. 1996). However, in these two previous years the gradient between the shelf and offshore (dome) waters was more apparent than in the present study, and the slight differences in the community structure should be attributed to the abundance of T. stylifera, which was concentrated in the coastal zone.

Egg production rates of the dominant copepod species were lower at the stations located in the dome than either the coastal or frontal stations. Copepods from the front showed egg production rates similar to the ones at the shelf or intermediate between shelf and dome waters. Previous results in this area (Calbet et al. 1996) indicated higher egg production rates in the shelfbreak against dome station for herbivorous copepod species like *P. parvus* and *Clausocalanus* sp., although no differences were found in omnivorous species like *C. typicus*. On average, previous values of total chlorophyll and egg production rates (June 1993, Calbet et al. 1996) were lower than in this work (June 1995). The differences detected could be explained by the size of the phytoplankton or by the abundance of ciliates. Unfortunately, no chlorophyll fractions or estimates of the ciliates abundance were presented in 1993 data. It is well known that copepods feed preferentially over food size classes that keep a close relation to the size of the animal (Berggreen et al. 1988). In the present study, for the species found as most abundant in the areas surveyed it is difficult to ingest efficiently particles (i.e. phytoplankton cells) less than 5 μm diameter. This phenomenon could explain

the inshore-offshore difference in egg production rates, paralleled by the gradient on the relative abundance of >5 μm phytoplankters. Although statistical

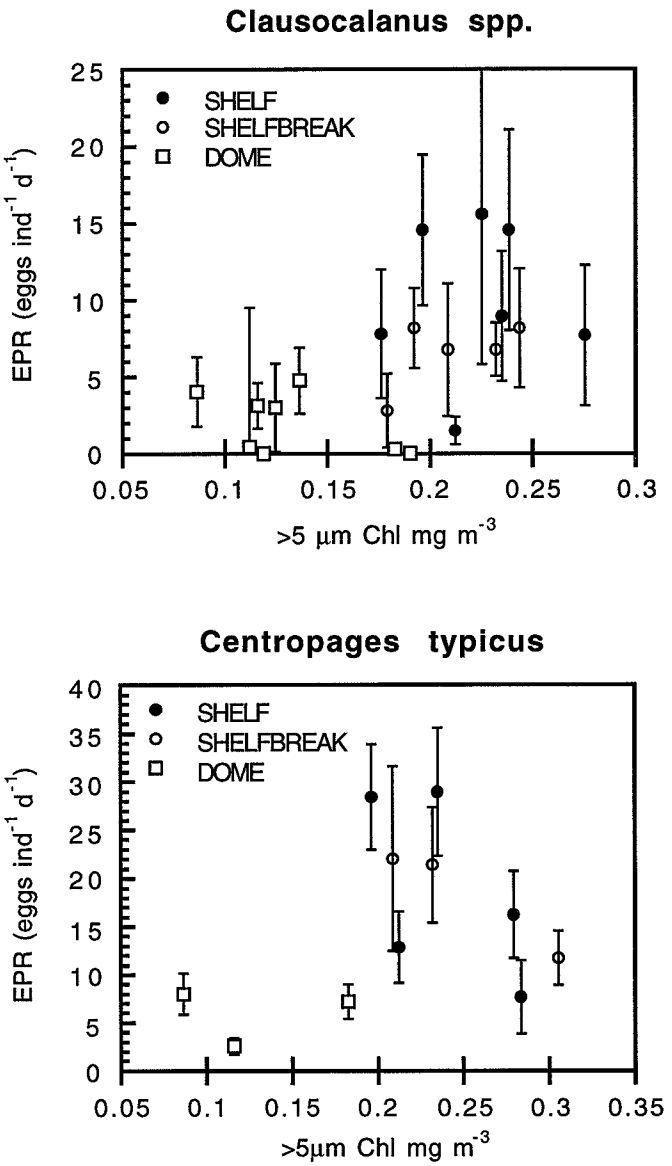


**Fig. 8.** Scatterplot of >5 μm chlorophyll concentration and egg production rate (mean ± SE) for *Centropages typicus* and *Clausocalanus* spp..

relationships are weak, there seems to be a correlation between the abundance of >5 μm chlorophyll and egg production rates. This assumption appears to be confirmed by the time evolution of egg production rates under saturating food conditions in open sea stations (dome), where despite the highest chlorophyll values found, the food limitation appears to be higher than in the other two stations, but agree with the > 5 μm chlorophyll concentration. This fact, and other potential sources of food like ciliates, carry to take with caution the lack of correlation between Chl *a* and egg production that sometimes is found in literature (i. e., Dagg 1978, Ward and Shreeve 1995), specially in areas where total and > 5 μm Chl *a* follow different spatial patterns.

For *Clausocalanus* spp., the time variability in egg production rates throughout the period studied is not significant. However, for *Centropages typicus* we observed that egg production rates were variable, specially at the shelf stations, according with the idea of a higher instability associated to the coastal waters (Walsh 1976). At the end of the period of study although egg productions were maintained a quite significant natural mortality of adult females was observed. This phenomenon might be related with the fact that the end of June is typically the end of the season for *C. typicus* to be significantly present in the North Western Mediterranean (Vives 1963). This mortality could be due to the ageing of the *C. typicus* population, produced for a diminution in the recruitment of new generations caused by a decrease in food resources (end of the spring phytoplankton bloom and beginning of the new summer community). The change in phytoplankton and ciliates community with the increase of temperature and stability could be thus the reasons for the substitution of *C. typicus* for other species more adapted to the new environmental and trophic conditions.

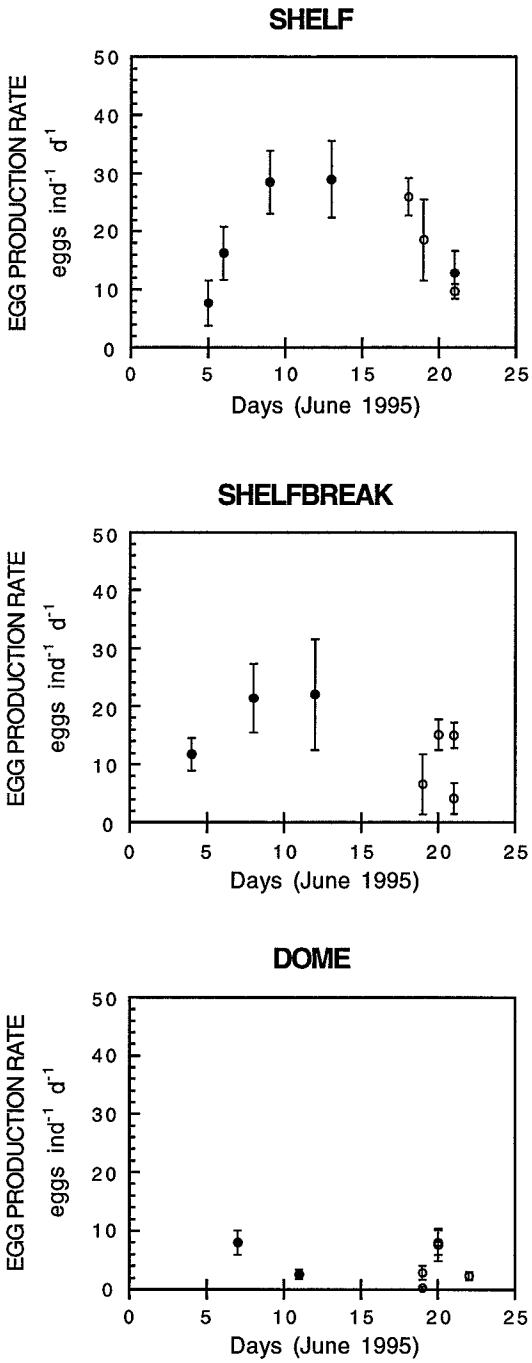


**Fig. 9**. Egg production rates (± SE) of *Centropages typicus* in the shelf, shelfbreak and dome stations throughout the period studied. Hollow circles correspond to incubations where quite a few of the incubated females died during the incubation.

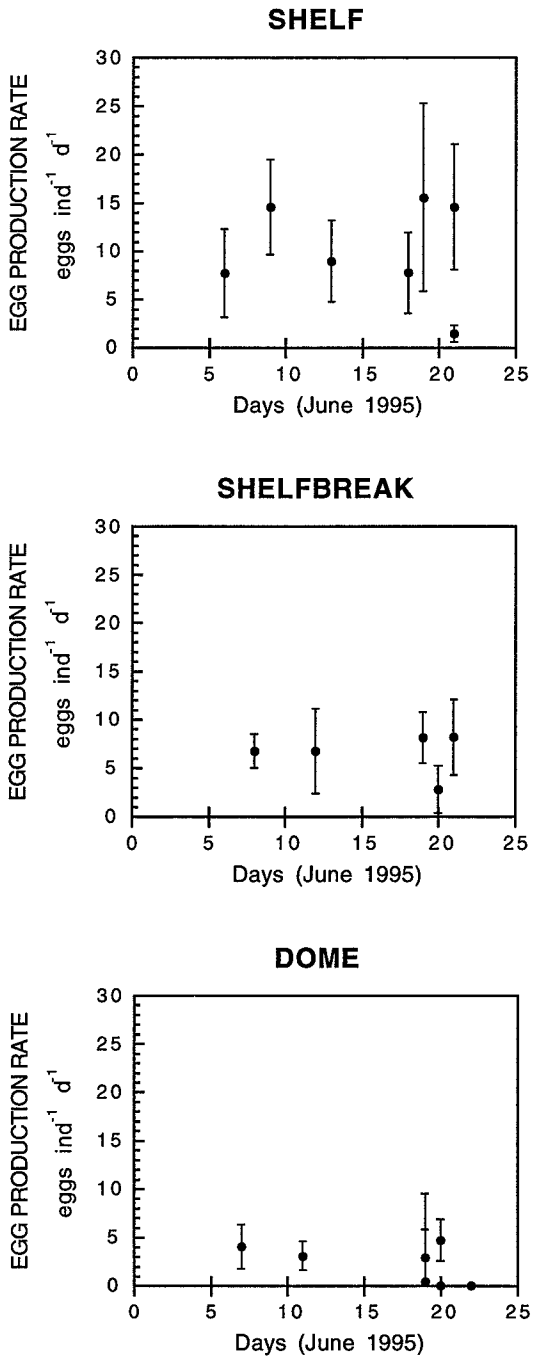


**Fig. 10**. Egg production rates (± SE) of *Clausocalanus* spp. in the shelf, shelfbreak and dome stations throughout the period studied.

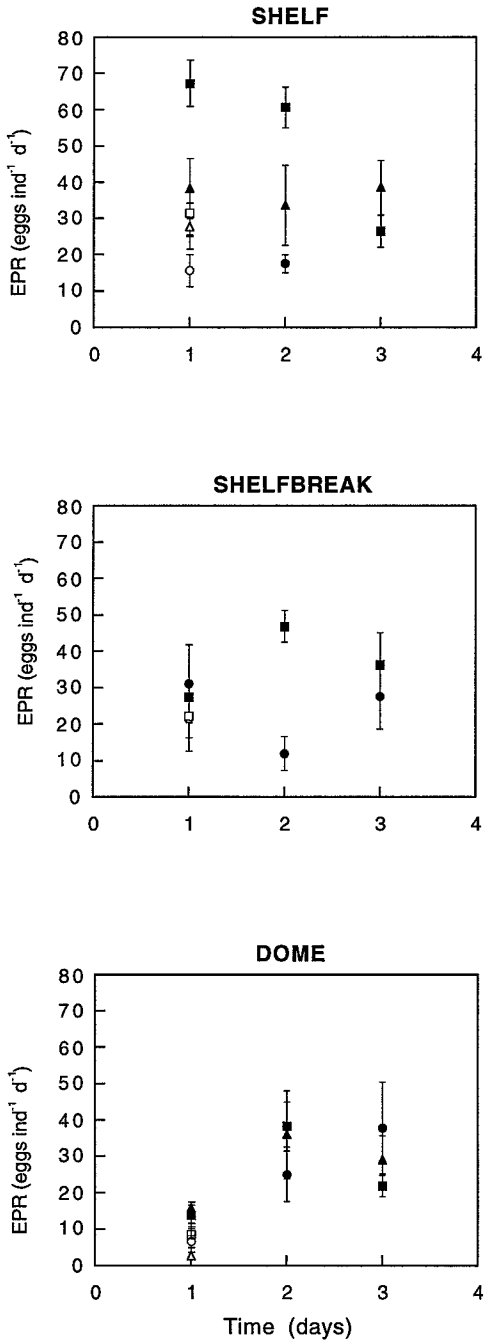


**Fig. 11**. Temporal evolution of egg production (means ± SE) along three days of presence of saturating food supply (7 ppm *R. baltica*). Open symbols: incubations performed with deep chlorophyll maximum water, filled symbols: incubations with enriched water (saturating conditions). The different shape of symbols correspond to different experiments.

Egg production in *Temora stylifera* responded faster to an extra supply of food rising in shelf stations maximum egg productions rates for this species at the working temperature (Ianora and Poulet 1993) in only 24 h. It means that this copepod (mostly located in coastal waters) is more adapted to benefit from a patchy and high-frequence fluctuating food environment than more oceanic species, where the temporal variability of processes follows a longer duration scale (Walsh 1976).

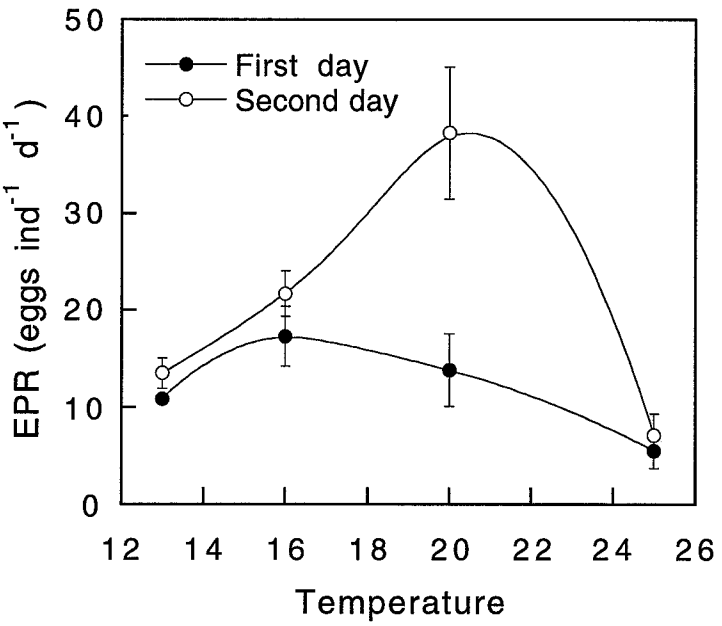


**Fig. 12.** Egg production rates of *Centropages typicus* as function the incubation temperature in saturating food conditions (DCM water + 7 ppm *R. baltica*). Filled symbols correspond to the first day incubation and open symbols to the second day incubation. Error bars represent SE.

*C. typicus* showed higher egg production rates at 20°C after 24 h acclimatization, being this difference not clear in the first 24 h incubation. It also seems that temperatures equal or higher than 25°C had a negative effect in egg production for this species. Such results contrast with the obtained by Carlotti et

al., in prep., who found that the same species presented higher egg production rates at 15°C than at 20°C. This fact could be explained regarding the temperature at which copepods were growing. It exists a temperature threshold after which the metabolic benefits of the increase of temperature are less important than losses due to the basal metabolism, and this threshold is around the temperature at which animals were growing. It means that animals preconditioned to low temperatures are more conspicuous to rise before the threshold at which temperature has negative effects over EPR than animals preconditioned to higher ones.

In conclusion, the shelfbreak conditions in summer does not seem to have any relevant biological effect regarding mesozooplankton abundance and production, what is consistent with the fact that the salinity front does not reach surface in that period due to thermal stratification.

**RESUM**

L'existència d'un front de densitat en les proximitats del talús continental del litoral català condiciona la composició i l'abundància de les espècies integrants del fitoplàncton i del zooplàncton, així com les seves taxes metabòliques. A més, els fronts són zones d'una elevada inestabilitat hidrogràfica que fa que l'entrada d'energia és tradueixi en polsos de producció, acoblats o no a les escales temporals dels processos en les comunitats de zooplàncton.

En l'estudi que es presenta, es va determinar la producció secundària planctònica mitjançant el mètode de producció d'ous de les espècies de copèpodes més abundants durant el juny de 1995 en tres estacions representatives de les condicions típiques de plataforma, front de talús i del dom central del Mar Català. Les taxes de producció d'ous al talús van ser similars a les de la plataforma, i en ambdós casos superiors a les que es van trobar al dom central. Aquest gradient de producció d'ous semblava estar relacionat amb un gradient en la concentració de clorofila en la fracció de mida superior a 5 μm. Així doncs, les condicions que es donen en el talús (presència del front de densitat) a l'estiu no semblen tenir efectes biològics rellevants sobre l'abundància i producció mesozooplanctòniques, la qual cosa és coincident amb el fet que en el període estudiat, degut a l'estratificació tèrmica, el front no assoleixi les capes superficials.

## 4.3 EGG AND FECAL PELLET PRODUCTION RATES OF THE MARINE COPEPOD *METRIDIA GERLACHEI* IN THE NW OF THE ANTARCTIC PENINSULA

(Basat en un article del mateix títol fet amb la col.laboració d'altres autors i enviat a Polar Biology)

## INTRODUCTION

The strong sesonality characteristic of southern ocean ecosystems induces specific adaptations in phyto- and zooplankton communities. The majority of Antarctic calanoid copepods have developed ecological adaptations to the periodical scarcity of food. The most common strategy is the concentration of the reproductive effort in a short time period, coinciding with appropriate trophic conditions, including a resting period for juvenile stages in deep water layers (diapause). However, some species like *Metridia gerlachei*, appear to have more than one spawning event (Kurbjeweit, 1993) or even be active most of the year (Schnack-Schiel and Hagen, 1995). *M. gerlachei* is a very common copepod in most areas of the Southern Ocean, and is specially abundant in the Gerlache and Bransfield Straits (Jazdzewski et al. 1982; Huntley and Escritor 1992). This species appears to feed in a large variety of preys from phytoplankton to protozoans and even on other metazoans (Hopkins, 1985; Lopez and Huntley, 1995; Metz and Schnack-Schield, 1995), although it is generally described as a small-particle grazer (Hopkins and Torres, 1989; Perissinotto, 1992). It is also know that *M. gerlachei* displays clear rhythms in feeding and a migratory behaviour (Huntley and Escritor, 1992; Perissinotto, 1992; Lopez and Huntley, 1995).

In general, the characteristics of the life cycle and population dynamics of *M. gerlachei* are recognized to be basically regulated, as in the majority of high latitude copepods, by the strong seasonality in primary production. However, the details of the control exerted by food availability on feeding and egg production are less known (Kurbjeweit, 1993).

The objectives of this study were: 1) To estimate the "in situ" egg and pellet production rates of *M. gerlachei* in the area around the Antarctic peninsula; 2) To

give insights about the control induced by temperature, food size and food concentration on the processes mentioned above; and 3) To estimate the relative proportion of body carbon addressed towards reproduction and its relationships with the specific metabolic carbon requirements of the species.

## MATERIAL AND METHODS

### Study area

The study took place during the Fruela-96 (January 1996) cruise in the Straits of Gerlache (G1, G2, G3, G4 and G5) and Bransfield (B1 and B2), and in the Drake Passage (D1, fig. 1).

At each station, temperature, salinity and "in situ" fluorescence were recorded with a Neil-Brown MARK-V CTD, equipped with a Sea Tech fluorometer. Water samples for chlorophyll determinations were taken by means of 10- 1 Niskin bottles fitted to a rosette during the ascending CTD casts. Phytoplankton biomass (as chlorophyll *a*) was measured by fluorimetry on acetone extracts according Yentsch and Menzel (1963).

### "In situ" egg and fecal pellet production rates

Copepods were collected by a WP-2 net (200 µm-mesh) with a 8-l plastic bag as a non-filtering cod end, towed vertically from 70-60 m to surface at low speed (ca. 10 m . min.$^{-1}$). Once on deck, the contents of the bag was gently poured into a 10-l isothermic container and experimental organisms immediately selected and translated into filtered sea water.

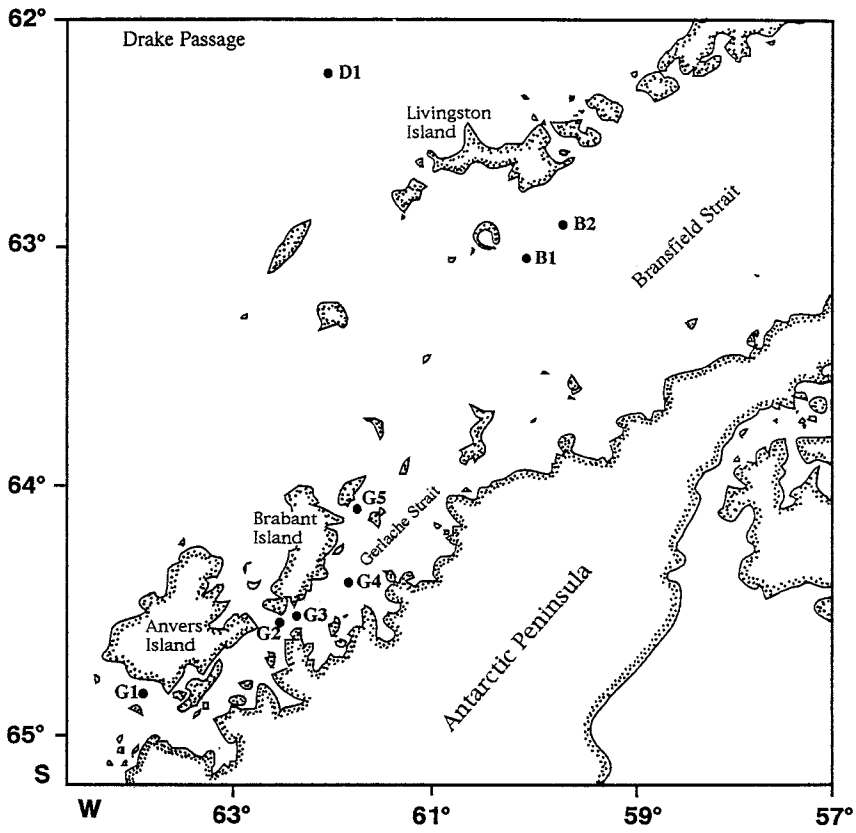


**Fig.** 1. Map of the area surveyed showing the position of the stations in Gerlache Strait (G1 to G5), Bransfield Strait (B1, 2) and Drake Passage (D1).

Four adult females were placed in 620 ml screw-cap Pyrex bottles (from 3 to 10 replicates for sample) filled with water from the phytoplankton maximum (hereafter FM) previously filtered through 330 μm mesh, and incubated at the temperature of the FM for approximately 24 h in a thermostatic bath. Some bottles without copepods were used as controls for eggs and fecal pellets abundance in the FM water. During the incubation, the bottles were occasionally turned upside-down to reduce algal settling. At the end of the incubation, the whole contents of the bottles was filtered through a 20 μm mesh submerged sieve, the copepods checked for activity, and fecal pellets and eggs counted under a dissecting microscope.

Food and temperature control of egg and fecal pellet production rates, and metabolic carbon requirements

The study of the functional response of egg and fecal pellet production to temperature, food size-spectrum and food concentration was held in the stations of the Gerlache strait. In general, the experiments followed the same methodology as for "in situ" estimates of egg and fecal pellet production.

The experiments about the influence of temperature on egg and fecal pellet production rates were made under saturating food concentrations. The experimental temperatures (-2, 0, 2.5 and 4 ± 0.1 ° C) were maintained by means of thermostatic baths. The temperature in the FM was 0 ± 0.5 ° C.

In order to test the effects of food size, copepods were incubated in FM water in which the original particle-size spectrum was modified by filtration through nylon meshes of appropriate pore size. The control consisted of unscreened FM water, and the experimental size-fractions were: > 200 μm, <200 >10 μm, <10 μm and <5 μm. This last size-fraction was not tested in station G5.

In the study of the functional response to food concentration, FM water was either diluted with 0.2 μm filtered sea water, or concentrated by reverse-flow filtration through 5 μm nylon-mesh until reaching 0, 25, 50, 75, 100 and 200 % of the "in situ" food concentration, which had been previously measured.

The individual variability and short-term spawning rhythm was estimated on 9 females which were incubated individually for three days in 330 μm filtered FM water. Every day the whole contents of the incubation bottles was filtered through a submerged double sieve: the upper, 330 μm sieve retained the adults, which were checked and returned to the incubation bottles with new FM water;

the lower, 20 μm sieve retained the eggs and fecal pellets, which were fixed and counted.

Specific oxygen consumption rates of female *M. gerlachei* were measured in the Gerlache area. Adult females were sorted from vertical zooplankton hauls made in the same conditions as for egg and fecal pellet production rates. About 15 individuals were introduced into 125 ml BOD bottles filled with aerated, GF/F filtered sea water, let acclimatize for 1 h, and incubated during 12 h at "in situ" (FM) temperature and dim light. Each experiment consisted in 3 replicates and 1 control. At the end of the incubations, samples for $O_2$ analysis were siphoned out through 100 μm mesh screen and the experimental organisms checked and fixed in 4% neutralized formaline. $O_2$ was analyzed by Winkler titration using a METROHM titroprocessor. Individual respiration rates were transformed into metabolic carbon losses (here considered as the minimum C requirements for routine metabolism) considering an RQ = 0.97 (Omori and Ikeda 1984). 1 ml O2 would be thus equivalent to 0.52 mg C.

Adult female and eggs were measured using an NIH image analysis system. Female size were translated into dry weight using the equations provided by Mizdalski (1988), and further converted to carbon assuming the C/dry weight ratio of 0.453 for *M. gerlachei* (Ikeda and Mitchell, 1982). Eggs were transformed into carbon using the equation from Huntley and Lopez (1992).

## RESULTS

"In situ" egg and fecal pellet production rates.

The average values of egg (EPR) and fecal pellets (PPR) daily produced per female *M. gerlachei* at the different stations are given in Table 1. The production rates measured at the stations located in the Gerlache Strait were significantly

higher than those at the Bransfield Strait or the Drake Passage ($p<0.01$), in accordance with the phytoplankton (chlorophyll) abundance in the different areas (table 1). Egg and fecal pellet production rates were significantly correlated with chlorophyll concentration ($p<0.01$, Fig 2). At turn, egg production rates were significantly correlated with the rates of fecal pellet production ($p<0.01$, Fig. 3).

**Table 1.** "In situ" average ± SE values of egg production rates (EPR, eggs $fem^{-1}$ $d^{-1}$) and fecal pellet production rates (PPR, pellets $fem^{-1}$ $d^{-1}$) for *Metridia gerlachei* in the stations surveyed. FM Chl*a*: Chlorophyll concentration in the fluorescence maximum (µg chl*a* $l^{-1}$). Integr Chl*a*: 0-20 m integrated chlorophyll in mg chl*a* $m^{-2}$. Stations as in Fig. 1.

| **Station** | **Average EPR** | **Average PPR** | **FM Chl*a*** | **Integr Chl*a*** |
|---|---|---|---|---|
| G1 | 2.8 ± 0.98 | 15.1 ± 2.04 | 6.6 | 48.5 |
| G2 | 3.5 ± 1.01 | 13.6 ± 1.20 | 3.9 | 50.7 |
| G3 | 6.2 ± 0.75 | 18.7 ± 1.52 | 7.6 | 58.9 |
| G4 | 4.3 ± 1.13 | 31.2 ± 2.90 | 5.5 | 48.0 |
| G5 | 3.9 ± 1.20 | 20.6 ± 1.76 | 2.6 | 47.9 |
| B1 | 0.0 ± 0.00 | 4.2 ± 0.61 | 0.8 | 11.9 |
| B2 | 0.3 ± 0.13 | 3.3 ± 0.42 | 0.8 | 10.4 |
| D1 | 0.0 ± 0.00 | 0.7 ± 0.07 | 0.2 | 3.4 |

Effects of food concentration

The response of egg and fecal pellet production rates to food concentration are represented in Fig 4. While egg production rates were not affected by food concentration, the rate of fecal pellet production increased according the food abundance and becomes saturated at around 2 µg $l^{-1}$. The FM in the Gerlache stations appeared to be at saturating food concentrations (table 1).

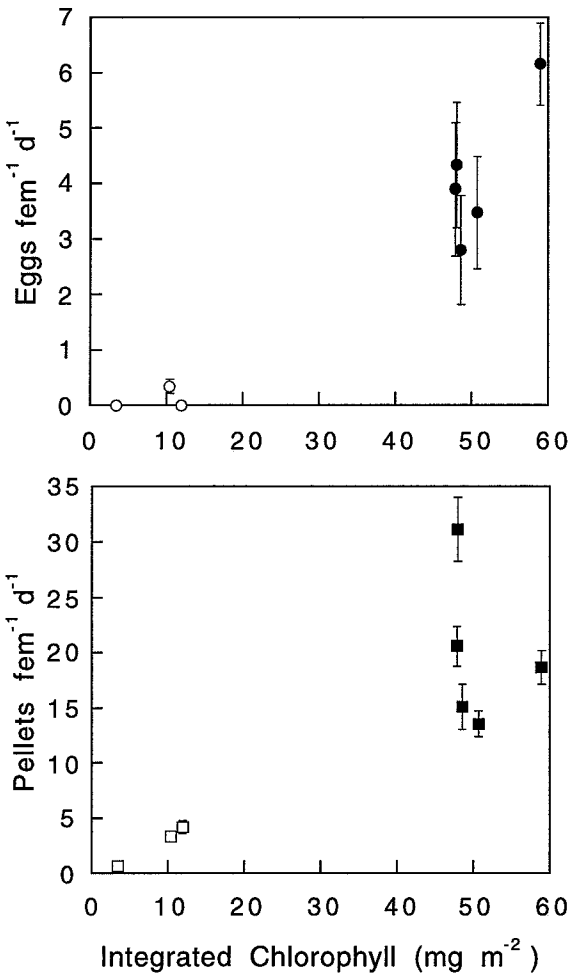


**Fig. 2.** Egg production rates (above) and pellet production rates (below) of *M. gerlachei* as function of the 0-20 m integrated chlorophyll *a* (mg $m^{-2}$). Open symbols correspond to the Bransfield and Drake stations, and filled symbols correspond to the Gerlache stations. Error bars represent SE.

Effects of food size availability

The rates of pellet production when *M. gerlachei* was fed the different size-fractions available at the FM indicated a clear preference for the <10 μm fraction (Fig. 5). In fact, the rates observed for the small, <10 μm size fraction were similar to those obtained for the controls (natural, unscreened water). Only at St. G1,

where the <10 μm fraction was very scarce (about 5 % of total food) the rates of pellet production were lower.

**Table 2.** Individual egg and pellet production rates (EPR and PPR respectively) of *Metridia gerlachei* along three days. Coefficients of variation (in %) are also indicated.

| | **Day 1** | **Day 2** | **Day 3** | **Cumulated** |
|---|---|---|---|---|
| **EPR** | | | | |
| Average ± SE | 6.4 ± 2.48 | 6.0 ± 2.79 | 6.0 ± 2.97 | 18.4 ± 4.69 |
| Coeff. of variation | 115.96 | 139.76 | 149.01 | 76.59 |
| **PPR** | | | | |
| Average ± SE | 18.4 ± 3.19 | 23.6 ± 2.80 | 9.0 ± 1.77 | 51.0 ± 6.64 |
| Coeff. of variation | 52.03 | 35.65 | 58.95 | 39.07 |

Functional response to temperature

The relationships between temperature and egg or fecal pellet production rates for *M. gerlachei* are represented in Fig. 6. Although egg production rates did not follow any clear pattern with temperature, maximum rates were observed at 0 ° C. Pellet production rates increased with temperature until 2.5 ° C, and further decreased at 4 ° C. The PPR at 2.5 ° C were significantly higher ($p<0.05$).

Individual variability.

Egg production rates were more variable, both in time and between individuals, than the rates of fecal pellet production. The only regularity observed is a tendency to the decay on fecal pellet production the last day of incubation. The average values and variability coefficients for both rate processes are indicated in table 2.

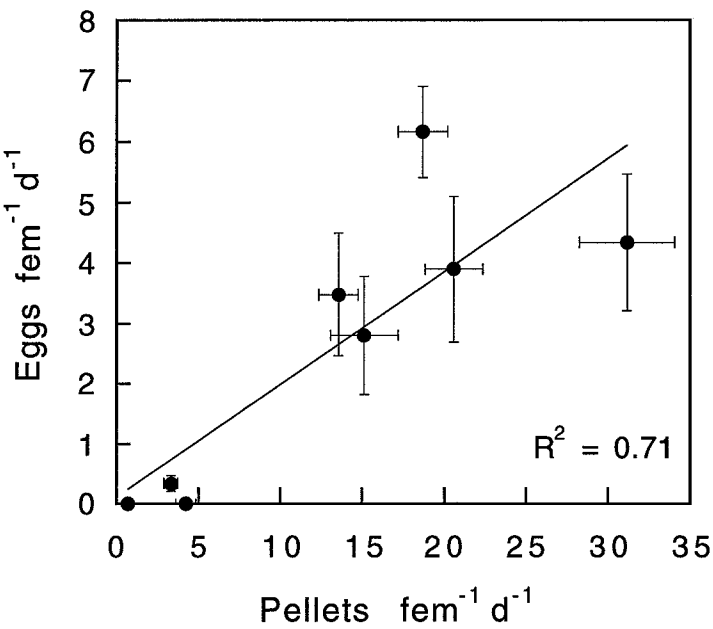


**Fig. 3.** Relationships between egg and pellet production rates in the stations sampled. Error bars represent SE.

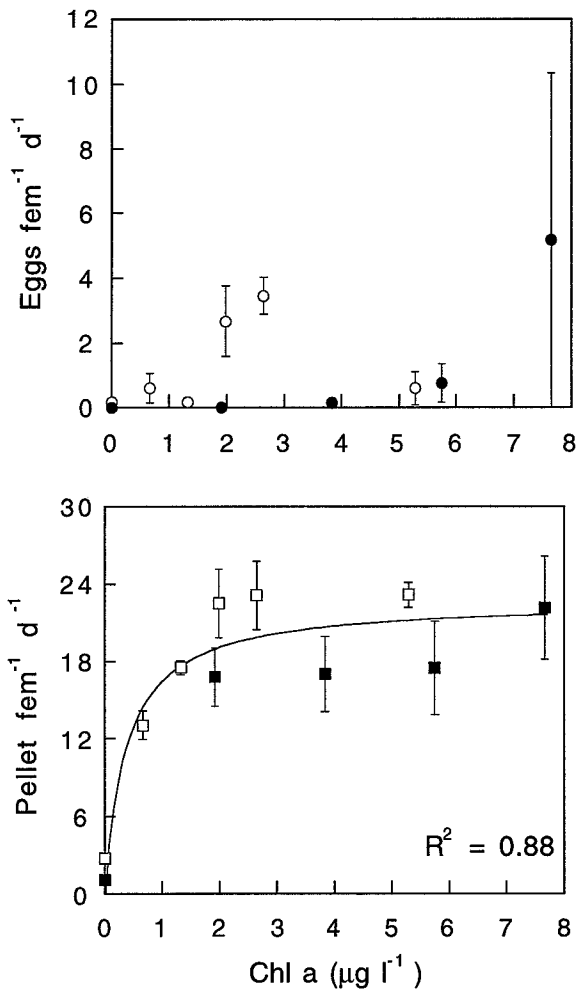


**Fig. 4**. Eggs (eggs fem$^{-1}$ d$^{-1}$) and fecal pellet aproduction rates (pellets fem$^{-1}$ d$^{-1}$) of *M. gerlachei* as function of chlorophyll *a* concentration. Filled symbols correspond to the station G3 and open symbols correspond to the station G5. Error bars represent SE.

Respiration rates and metabolic carbon requirements.

Respiration rates were around 0.01 ml $O_2$ $fem^{-1}$ $d^{-1}$, which in terms of carbon requirement represents about 5 % of the carbon body weight per day (table 3). There were no differences between the two stations surveyed (G1 and G3).

**Table 3.** Individual biomass (µgC) of *Metridia gerlachei* females used in egg production experiments, their respiration rates (ml $O_2$ consumed $fem^{-1}$ $day^{-1}$), the metabolic carbon requirements (µgC $fem^{-1}$ $day^{-1}$, calculated from the respiration rates), the carbon produced daily as eggs (µgC $fem^{-1}$ $day^{-1}$) and the carbon required for egg production assuming a gross-growth efficiency of 30 % (Kiørboe et al.1985), of the food ingested (µgC $fem^{-1}$ $day^{-1}$). Values are means ± SE.

| St | Ind biom | Resp rate | Metab C req | C prod | C req (eggs) |
|---|---|---|---|---|---|
| G1 | 93.0 ± 0.0044 | 0.009 ± 0.0012 | 4.9 ± 0.62 | 0.8 ± 0.28 | 2.4 ± 0.85 |
| G3 | 104.6 ± 0.0063 | 0.010 ± 0.0018 | 5.2 ± 0.96 | 1.8 ± 0.21 | 5.4 ± 0.65 |

## DISCUSSION

The "in situ" egg and fecal pellet production rates (EPR and PPR, respectively) observed in the study area suggest the existence of two well-defined trophic situations: In the Gerlache Strait, high chlorophyll (phytoplankton) concentrations allowed relatively high egg production and food ingestion (fecal pellet production). The Bransfield Strait and Drake Passage, on the contrary, with poor phytoplankton (chlorophyll) conditions had the lowest rates of egg and fecal pellet production.

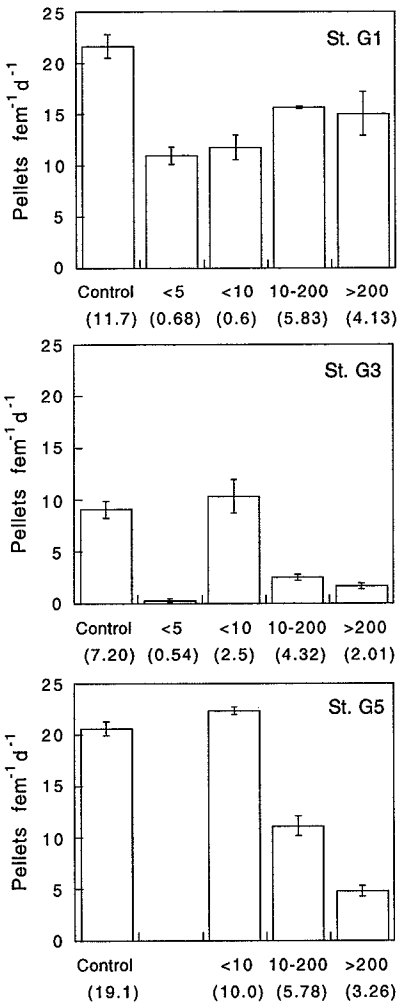


**Fig. 5**. Fecal pellet production rates of *M. gerlachei* when fed different size-fractions (in μm) of the food present in the fluorescence maximum (control) at the Gerlache Strait. Numbers in parentheses are the chlorophyll concentration for the corresponding size-classes. Error bars represent SE.

Although the EPR obtained at the Gerlache strait were on average lower than those given by Kurbjeweit (1993), these values seemed to be maximum rates for this specie at 0 ° C in the surveyed area. This fact is supported by the results

obtained in the experiments about the functional response to food concentration in stations G3 and G5. In these stations pellet production rates (PPR) at natural food concentrations were saturated or close to saturation. The low (or even null)

spawning rates of *M. gerlachei* in the food depleted waters outside the Gerlache strait confirms the hypothesis of Huntley and Escritor (1992), who considers the Gerlache strait as the recruitment area of this species for the surrounding zones (Bransfield strait and Drake passage).

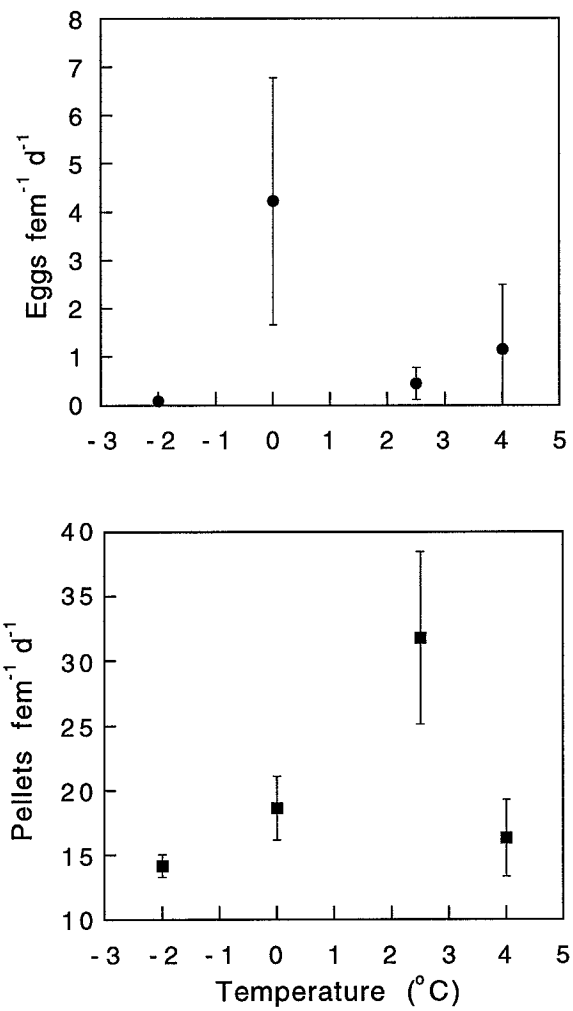


**Fig. 6.** Egg production rates (above) and pellet production rates (below) of *M. gerlachei* as function of the incubation temperature in the Gerlache Strait. Error bars represent SE.

The Chl*a* - EPR and Chl*a* - PPR significant correlations suggest that, although other preys like eggs, nauplii or ciliates could be eaten by this copepod (Hopkins, 1985; Huntley and Escritor, 1992; Lopez and Huntley, 1995; Metz and Schnack-Schield, 1995), in the stations surveyed phytoplankton was the main food source. This is in agreement with the results of Huntley and Escritor (1992) who estimated the phytoplankton ingestion of *M. gerlachei* to be between 25 and 50 % of the body carbon per day, and suggested that this species can survive on a strict herbivorous regime. Furthermore, the carbon requirements in the Gerlache strait as estimated from respiration and from egg production rate (7.5-10 % of the body carbon per day, table 2), could be largely covered by the phytoplankton carbon ingestion rates given by Huntley and Escritor (1992) for the same area (25-30 %). This superfluous feeding could contribute to the lipid accumulation during the summer (Hagen and Schnack-Schiel, 1996). The significant correlations between "in situ" EPR and PPR contrast with the results obtained by Ward and Shreeve (1995), in which no correlation was found between Chl*a* and EPR for *Rhincalanus gigas*, *Calanoides acutus* and *Calanus simillimus*. This could be due to an almost steady-state environmental food conditions previous to our study, allowing the equilibrium between ingestion (PPR) and egg production, and to the different range of food concentration observed in both studies (0.6-16 μg Chl*a* $l^{-1}$, this work, against 0-3 μg Chl*a* $l^{-1}$, Ward and Shreeve, 1995).

The relatively low average egg production rates (EPR) found for *M. gerlachei* in this study (always less than 7 egg $female^{-1}$ $day^{-1}$ in all stations) confirm the assumption that this copepod spawns continuously at low rates during an extend reproductive period (from late winter to late summer), and produces two or three generations during the reproductive season (Huntley and Escritor, 1992; Schnack-Schiel and Hagen, 1995).

*M. gerlachei* has been considered as a small particle-grazer (Perissinotto, 1992; Hopkins and Torres, 1989). In agreement to this assumption, this specie does not follow the optimum particle size-prosome length relation (2-5% ) obtained by Berggreen et al. (1988) for other copepods. According to this relation, *Metridia gerlachei* (2.34 ± 0.014 mm prosome length, this study) should have its optimum ingestion in a size-fraction comprised between 47 and 117 μm diameter. Although *M. gerlachei* is able to eat in this range (see fig. 4) our experiments indicate a preference for small size particles (< 10 μm).

The lack of a clear response of EPR to temperature in the experimental range considered contrast with the general trend observed in other copepods (Saiz et al. 1996). The higher (although not significant) rates of egg production observed at 0 ° C could suggest a narrow temperature optimum for the species. The optimum would be very close to the "in situ" temperatures, and was not observed in our experiments given the relatively wide range of temperatures tested. The increase in PPR from -2 to 2.5 ° C indicate a different metabolic response for ingestion rates.

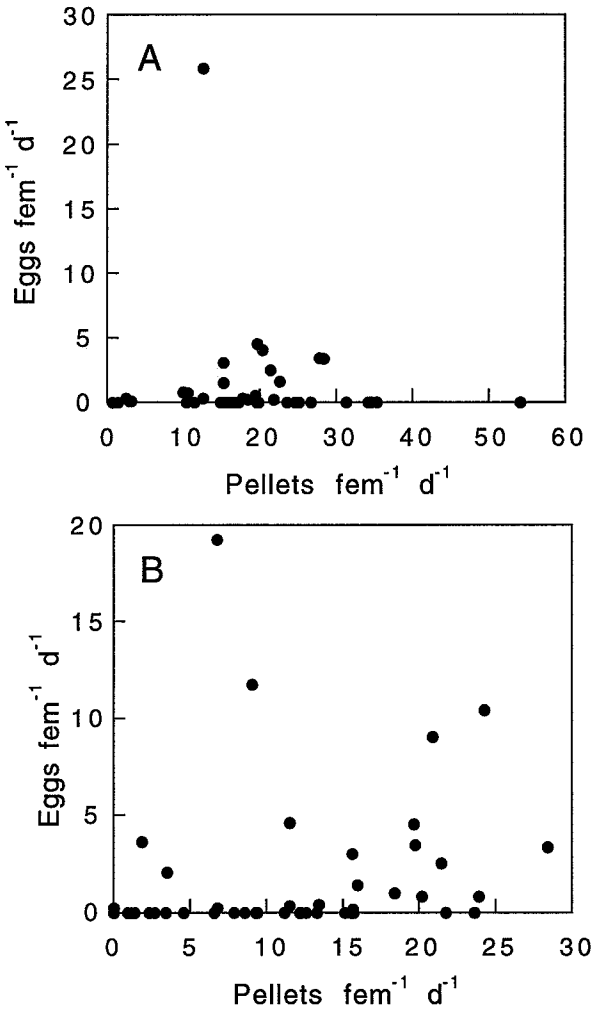


**Fig. 7.** Plot of the relationship between egg production rates and pellet production rates in experiments related to the functional response to food concentration (A) and food size-fraction effect (B).

Contrary to the field data (fig 2), no significant correlation were obtained in laboratory experiments between PPR and EPR (fig 7). This could be explained in part by the different time-response of both rate-processes to the food and temperature changes. In general ingestion (and its indirect estimator, PPR) shows an almost immediate response to food or temperature changes (Mackas and Burns, 1986; Runge, 1980), which would explain the direct relationship observed

in our experiments. In contrast, the lack of clear response in EPR could be related with the high variability observed in EPR for *M. gerlachei*, both temporal and between individuals (up to 149%, table 2). This high variability contrasts with the values obtained for other polar species like *Calanus finmarchicus* (40%, Runge 1985; 35% Hirche 1990). Our results could be explained by an interval between clutches higher than the incubation time.

The apparent delay between the changes in the ingestion (PPR) and EPR could be due to the capacity of the species to dampen changes in the food environment by using the lipids stored for egg production (Schnack-Schiel and Hagen, 1995). In the light of the results obtained, short-term experiments about the functional response of EPR in high-latitude copepods should be reconsidered.

**RESUM**

Es van mesurar les taxes de producció d'ous i de paquets fecals del copèpode antàrtic *Metridia gerlachei* durant gener de 1996. L'àrea d'estudi comprenia els estrets de Gerlache (G) i Bransfield (B), i el passatge de Drake (D).

Les taxes més elevades de producció d'ous i paquets fecals foren observades a les estacions de Gerlache, on també es van detectar concentracions de clorofila properes als nivells de saturació per aquesta espècie. A les estacions de Bransfield i Drake, on els nivells de clorofila eren menors, les taxes de producció d'ous van ser molt baixes o nul.les.

Les taxes de producció d'ous, encara que ben correlacionades amb els valors de clorofila, semblaven independents de la concentració de menjar a escales temporals curtes, mentre que la producció de paquets fecals va estar estretament relacionada amb l'abundància de menjar en els mateixos experiments. En general, les taxes de producció d'ous foren baixes, inclús a condicions de menjar saturants, i amb una elevada variabilitat individual.

Encara que en la majoria d'estacions de Gerlache, el 50% de la clorofila total (aproximadament) corresponia a la fracció més gran de 10 μm, *M. gerlachei* menjava preferentment en la fracció menor de 10 μm. La temperatura a la qual es van realitzar les incubacions no va tenir efectes clars sobre la producció d'ous. Tanmateix va tenir efectes significatius sobre la producció de paquets fecals, els quals van assolir valors màxims al voltant dels 2.5°C.

Tots aquests fets semblen estar en concordança amb l'estratègia reproductiva atribuïda a aquesta espècie, basada en relativament baixes taxes de producció d'ous esteses en llargs períodes reproductius.

## SÍNTESI

Al llarg d'aquesta memòria s'han presentat els estudis dels efectes que tenen sobre la producció zooplanctònica les escales de variabilitat de factors biòtics (aliment, depredadors) i la seva interacció amb variables físiques (llum, temperatura). En els primers capítols s'ha intentat determinar la resposta d'una espècie de copèpode escollida com a model (*Acartia grani*) sota diferents condicions experimentals que simulaven la variabilitat pròpia de l'ecosistema marí. En el darrer capítol (capítol 4), s'ha tractat de donar una visió global del tema mitjançant dades de camp, que ens serveixen per a situar els resultats obtinguts al laboratori en el marc físic del sistema marí, i ens donen una idea dels límits i ordres de magnitud dels processos en els què es mouen els organismes integrants del zooplàncton.

L'estudi dels principals mètodes de producció no podia faltar en un treball com el que s'ha dut a terme en aquesta Tesi. En el primer capítol s'ha demostrat la fiabilitat del mètode de la producció d'ous com a estimador de la producció "in situ" (sempre que es segueixin unes determinades recomanacions), i l'estret acoblament entre la producció d'ous i les variables físiques i tròfiques del medi. Tanmateix, s'ha observat que si bé sota condicions saturants de menjar es poden traslladar les estimes de producció d'adults a tota la comunitat de copèpodes, en condicions no saturants s'han de tenir en compte les petites diferències que mostren les taxes de producció específiques entre adults i juvenils.

Pel que fa a mètodes alternatius per a estimar la producció en copèpodes, els resultats obtinguts en aquest capítol referents a un nou mètode basat en la relació directa entre creixement i quantitat de RNA en individus de la mateixa espècie, són força encoratjadors. L'aplicació al camp d'aquesta nova

metodologia ens permetria augmentar considerablement la resolució tant espacial com temporal a l'hora d'obtenir estimes acurades de producció secundària. El mètode en qüestió, però, és dependent de la temperatura, la qual cosa obliga a la seva calibració tenint en compte les variacions d'aquest factor si es volen tenir bons regressors en els models de predictibilitat.

En el transcurs d'aquesta Memòria sovint s'ha fet esment de la naturalesa discontínua i fluctuant de les variables tròfiques que condicionen l'estructura i el funcionament d'un ecosistema. Era necessari doncs, conèixer com responien els copèpodes a oscil.lacions en la disponibilitat d'aliment, i a partir de quina freqüència els era impossible esmorteir aquestes oscil.lacions. També era important saber si la resposta en adults seguia el mateix patró que en juvenils o si per contra aquests responien més ràpid a canvis en les variables ambientals. Al llarg de les investigacions descrites en el capítol 2 amb el copèpode marí *Acartia grani*, hem comprovat que encara que una mateixa quantitat de menjar (algues) subministrada en continu o a freqüències iguals o inferiors a 24 h es traduïa en una producció acumulada similar, el període del dia en el que les algues estaven disponibles tenia un efecte directe sobre la producció d'ous. Copèpodes alimentats durant la nit mostraven majors produccions que els que havien estat alimentats durant el dia. A més, en ambdós casos les postes màximes d'ous sempre es donaven durant el període de foscor. Aquest resultat evidencia a més de la presència d'un ritme diari d'ingestió, el curt període de temps en el què *A. grani* converteix en ous l'aliment ingerit. Freqüències superiors a 24 h es traduïen en una reducció de la posta respecte del subministrament continu. A part dels ritmes diaris de posta, les fluctuacions en la disponibilitat de menjar determinen oscil.lacions en les taxes de producció d'ous, les quals són funció de les freqüències a les que l'aliment era subministrat. La taxa relativa de disminució en la producció d'ous després d'un període de

dejuni és superior a l'increment que s'obté després d'estar en presència de menjar, i aquesta diferència és funció del temps de dejuni.

Si bé la producció màxima d'ous era fortament dependent de la temperatura ($Q_{10}$ = 3.2), el temps necessari per recuperar els mateixos nivells de producció després de condicions de dejuni no semblava ésser controlat per la temperatura ambiental.

En els experiments amb juvenils de la mateixa espècie de copèpode, el temps de supervivència a l'absència de menjar anava augmentant al llarg del desenvolupament (seguia una relació lineal). Un aportament d'aliment nocturn produïa la mateixa resposta en creixement que la mateixa quantitat de menjar subministrada en continu. Per contra, quan el menjar estava disponible només en les hores de foscor el creixement fou significativament menor, i comparable a l'obtingut en períodes d'oscil.lació en la disponibilitat de menjar de 24 h. Aquest fet només pot ser explicat per una major ingestió d'aliment durant la nit com passava en els adults. D'altra banda, períodes de fluctuacions superiors o iguals a 24 h tenien un efecte clarament negatiu sobre les taxes de supervivència de nauplis. Aquest fet indica una baixa capacitat d'esmorteir oscil.lacions en l'abundància de menjar a freqüències que es poden donar fàcilment a la natura (Haury et al. 1978).

El control exercit per les condicions de disponibilitat de menjar (abundància i freqüències de fluctuacions) en les taxes de creixement (producció) i supervivència tant d'adults com de juvenils del copèpode *Acartia grani* expliquen, en part, la seva distribució quasi exclusiva en zones confinades com ports i estuaris, on la variabilitat en la disponibilitat de menjar és molt elevada.

La possible existència d'un ritme diari d'alimentació que s'inferia dels resultats obtinguts, va portar a realitzar uns experiments per tal de corroborar aquest fet i d'esclarir la importància relativa d'altres factors com ara la concentració d'aliment o el risc de depredació en aquest comportament.

S'ha especulat molt sobre les causes que indueixen una determinada espècie de copèpode a reduir les seves taxes d'alimentació durant les hores de llum. Sembla ser que la hipòtesi més acceptada relaciona aquest fet amb el risc de ser depredat per animals que utilitzen la vista per a localitzar les seves preses. Si això es cert, hom podria esperar que els ritmes es mantinguessin fins i tot a baixes concentracions de menjar. Els resultats obtinguts (capítol 3) van demostrar l'existència d'un ritme diari d'alimentació en *A. grani* i la seva dependència de la concentració d'aliment. Així doncs, a baixes concentracions de menjar, els copèpodes perdien el ritme i adreçaven la seva activitat vers l'alimentació contínua. Aquest resultat es va repetir tant en animals de laboratori (cultivats en condicions controlades i en absència de depredadors) com en animals de camp, fet que indica que aquest comportament no és induït, encara que els depredadors puguin modular-ne la intensitat del ritme. Els estímuls mecànics i visuals que simulaven la presència d'un depredador no provoquen cap tipus de resposta. Tampoc no va haver-hi resposta en canvis en el comportament alimentari en front a possibles depredadors no-visuals (*Candacia armata*). Per contra, els exudats provinents de peixos que usualment s'alimenten de copèpodes van afectar clarament les taxes d'ingestió, especialment durant el dia. Finalment, es va demostrar l'efecte depressor de la llum sobre les taxes d'ingestió nocturnes.

Tot això fa pensar que *A. grani* respon a la manca d'aliment amb una estratègia adreçada amb preferència a garantir l'èxit reproductiu (a major ingestió, major producció d'ous) encara que el comportament comporti un risc més alt de

depredació. La detecció d'alguns depredadors (peixos) sembla basat en la identificació d'estímuls químics.

Les investigacions realitzades en el transcurs de 5 campanyes oceanogràfiques presentades en el capítol 4 ens serveixen per situar els resultats obtinguts al llarg d'aquesta tesi en les escales de variabilitat que es troben en el sistema marí. A més, ens donen la possibilitat d'estudiar la resposta d'espècies d'ecosistemes tan diferents com puguin ser el Mediterrani i l'Antàrtic a variacions de curta freqüència en les condicions ambientals.

Abans d'intentar comprendre les relacions entre els organismes que integren les xarxes tròfiques d'un ecosistema és necessari conèixer quina mena d'aproximació es la més adequada per tal d'estudiar les variables que ens interessen. Els índexs estructurals (com per exemple la distribució i biomassa del plàncton) poden ser estimadors apropiats de les característiques tròfiques dels sistemes pelàgics principalment en els casos en què s'ha assolit un estadi proper a l'estacionari. En canvi, en àrees on les entrades d'energia exògena són aperiòdiques (per exemple, al Mediterrani), i es tradueixen en breus episodis de fertilització, la variabilitat impedeix la formació d'estructures planctòniques permanents. En aquests casos és imprescindible la utilització d'índexs funcionals (com ara la producció) per tal de caracteritzar les relacions existents dins i entre les diferents xarxes tròfiques.

Fou en aquestes zones d'elevada variabilitat temporal i espacial on es van donar les majors produccions i abundàncies de copèpodes, en concordança amb la distribució de clorofila present en la fracció menor de 5 μm (juny 1995). En canvi, en anys anteriors (juny 1993), les diferències en producció entre estacions van ser minses, encara que els valors de clorofila foren similars en els dos anys. Aquest fet reforça la necessitat de tenir en compte la variabilitat de

curta escala intrínseca al sistema a l'hora d'interpretar o d'entendre la dinàmica dels ecosistemes plantònics.

Un resultat força interessant que s'obté quan es revisen conjuntament les dades d'aquest capítol quart és la variació de resposta en la producció d'ous que mostraven espècies de diferents ambients en front d'un subministrament puntual de menjar. Copèpodes d'ecosistemes antàrtics, on els petits canvis de curta durada són poc rellevants en comparació amb l'elevada intensitat de les fluctuacions lligades als fenòmens purament estacionals, responien molt més lentament i amb menor intensitat que copèpodes de zones caracteritzades per una variabilitat de més alta freqüència, però d'amplitud baixa (Mediterrani). Dins aquests ambients, els organismes típics d'àrees costaneres (on la freqüència dels processos i la inestabilitat que comporten són generalment més elevades), com pot ser *Temora stylifera*, mostraven una resposta de major intensitat en front a un eventual augment en la quantitat de menjar que espècies considerades més cosmopolites.

Així doncs, els copèpodes responen a un aportament puntual d'aliment (que simula, per exemple, una taca de fitoplàncton) de diferent manera i d'acord amb les condicions de variabilitat de l'ecosistema en el què es troben.

La feina duta a terme en aquesta Tesi ha aclarit uns quants dubtes, però n'ha plantejat molts més. Cal treballar amb altres espècies amb capacitats d'esmorteïment de les variables ambientals més forta, cal introduir altres variables en l'estudi, com ara la qualitat de l'aliment (algues de diferents espècies, ciliats, etc.) o la turbulència de petita escala, i altres factors com el percentatge d'eclosió d'ous. És necessari també donar més importància a les fases juvenils de desenvolupament, les quals malgrat que juguen un paper clau en la dinàmica dels ecosistemes marins, són força desconegudes.

## CONCLUSIONS

1 Existeix un estret acoblament entre la producció d'ous i els canvis de curta durada (menors a 24 h) en les variables ambientals. Aquest fet obliga a realitzar les incubacions en condicions d'aliment i temperatura similars a les que es troben "in situ" si es volen obtenir bones estimacions de producció secundària mitjançant aquest mètode.

2 Per *Acartia grani*, les estimacions de producció en adults (producció d'ous) són equivalents a les de juvenils (creixement somàtic) en condicions saturants d'aliment. En canvi, per sota d'aquests nivells ambdues estimacions són lleugerament diferents.

3 S'han obtingut bones correlacions entre la producció d'ous i el contingut d'ARN en copèpodes. Encara que el mètode és força dependent de la temperatura, un cop realitzada la calibració inicial permet ampliar la resolució espacial i temporal de les estimacions de producció al camp.

4 La disminució relativa de la producció d'ous després d'un període de dejuni és superior a l'increment que s'observa després d'estar en presència d'aliment. La diferència obtinguda és funció del temps de dejuni.

5 La temperatura exerceix un control sobre les taxes de posta màximes de copèpodes, però no sembla afectar la velocitat de resposta a polsos d'aliment.

6 Oscil.lacions en el subministrament d'aliment de freqüències superiors a 24 h es tradueixen en la reducció de la producció d'ous d'*Acartia grani* respecte d'un aportament equivalent d'aliment en continu (control).

7 Les fases juvenils mostren una menor capacitat esmorteïdora de les fluctuacions d'aliment. Una freqüència en l'oscil.lació de l'abundància d'aliment de 12 h (menjar disponible de dia) o de 24 h és suficient per a produir una disminució significativa de les taxes de creixement respecte del control (alimentat en continu).

8 El temps de supervivència a condicions de dejuni permanent és funció (segueix una relació lineal) de l'edat de desenvolupament dels copèpodes.

9 Es confirma l'existència de ritmes d'alimentació en individus adults d'*Acartia grani*. Aquests ritmes han estat detectats també en les fases juvenils.

10 El ritme d'alimentació canvia a baixes concentracions de menjar, la qual cosa indica una tendència a mantenir l'activitat reproductora a expenses de la seva possible detecció per part dels depredadors. La detecció dels depredadors per part d'*Acartia grani* sembla estar mitjançada principalment per estímuls químics.

11 S'ha demostrat que la llum exerceix un efecte inhibidor de les taxes d'ingestió nocturnes dels copèpodes.

12 Els índexs funcionals semblen ser millors estimadors de les característiques tròfiques del sistema que els indicadors basats en propietats extensives del sistema quan la variabilitat és aperiòdica.

13 La producció de copèpodes en sistemes controlats per una elevada estacionalitat (Antàrtida, i en general latituds altes) no respon a curt termini a canvis sobtats en les condicions físiques i tròfiques. Contrariament, en zones on la importància relativa de l'estacionalitat és menor, els copèpodes tenen una capacitat més baixa de tamponar la variabilitat ambiental.

14 Al període estiuenc, a la Mediterrània Occidenta, s'observa un gradient de producció de costa cap a mar obert. Aquest gradient sembla estar determinat per un gradient similar en la disponibilitat de fitoplàncton de mida superior a 5 μm.

# ANNEX

# EL CULTIU DEL COPÈPODE MARÍ *ACARTIA GRANI* SOTA CONDICIONS CONTROLADES

## INTRODUCCIÓ

Les diferents cohorts que integren la població d'una determinada espècie de copèpode marí estan molt lluny de desenvolupar-se sincrònicament, i es troben força barrejades en el medi natural. Aquest fet comporta que quan tractem d'obtenir organismes del medi per tal de portar a terme un experiment, obtinguem un col.lectiu d'individus en el que es troben representats la majoria d'estadis de desenvolupament. Separar les diferents fases juvenils (sempre que siguin fàcilment identificables) no representa major problema que el d'haver-los de "pescar" un a un (la qual cosa no deixa de ser una tasca laboriosa). En canvi, la separació dels adults per classes d'edat no és factible ja que és del tot impossible esbrinar-ne l'edat. Treballar amb animals adults de diferents edats pot emmascarar els resultats experimentals obtinguts degut a una més gran variabilitat individual (per exemple, femelles joves tenen taxes de producció d'ous més elevades que femelles d'edat més avançada). Així doncs, el cultiu dels animals en laboratori neix de la necessitat de treballar amb organismes, desenvolupats sota condicions controlades, el que permet disposar d'animals de mida i edat homogènia, ja siguin estadis juvenils o adults. Si a aquests avantatges hi afegim la marcada presència estacional que mostren molts dels copèpodes del litoral català, és fa del tot comprensible la importància de mantenir un cultiu de copèpodes estable al laboratori.

És possible trobar referències en la literatura sobre cultius de copèpodes (vegis la revisió de Paffenhöfer, 1979), però la majoria eren a petita escala o es van portar a terme durant poques generacions. Per contra, Klein Breteler, 1980 i Støttrup et al., 1986 donen unes normes bàsiques que, amb certes modificacions, poden servir per a cultivar qualsevol copèpode de mida petita. A continuació es fa una descripció detallada de la tècnica i materials utilitzats durant més de 20

generacions per al cultiu del copèpode marí *Acartia grani* al Departament de Biologia Marina i Oceanografia de l'Institut de Ciències del Mar, CSIC.

**L'Espècie escollida**

*Acartia grani* és un copèpode calanoid típic de zones costaneres i àrees semi-confinades com ara estuaris i ports. La seva talla mitjana oscil.la entre les 900 i 1100 μm i es caracteritza per un clar dimorfisme sexual. Els mascles (fig. 1) són estilitzats i tenen la cinquena pota i una antena (la dreta del primer parell antenal) hipertrofiada i geniculada per tal d'aferrar-se a la femella en el moment de la còpula. Les femelles (fig. 2) són més robustes i es poden diferenciar clarament dels mascles per tenir les antenes iguals, el segment gonadal més aparent que la resta de segments abdominals, i per un parell d'expansions en forma d'aletes que es situen a cada costat del darrer segment toràcic.

El fet d'escollir aquesta espècie per a ésser cultivada respon a diversos motius:
1) Encara que de marcada presència estacional (només present a la tardor i hivern), aquest copèpode és extraordinariament abundant en zones portuaries, la qual cosa facilita la recol.lecció i posterior selecció dels animals.

2) Pel seu hàbitat (zones costaneres, ports i estuaris freqüentment sotmesos a elevats nivells de contaminació i a canvis sobtats de les variables fisico-químiques) és una espècie molt resistent a condicions extremes o a les alteracions que la tasca experimental pugui comportar.

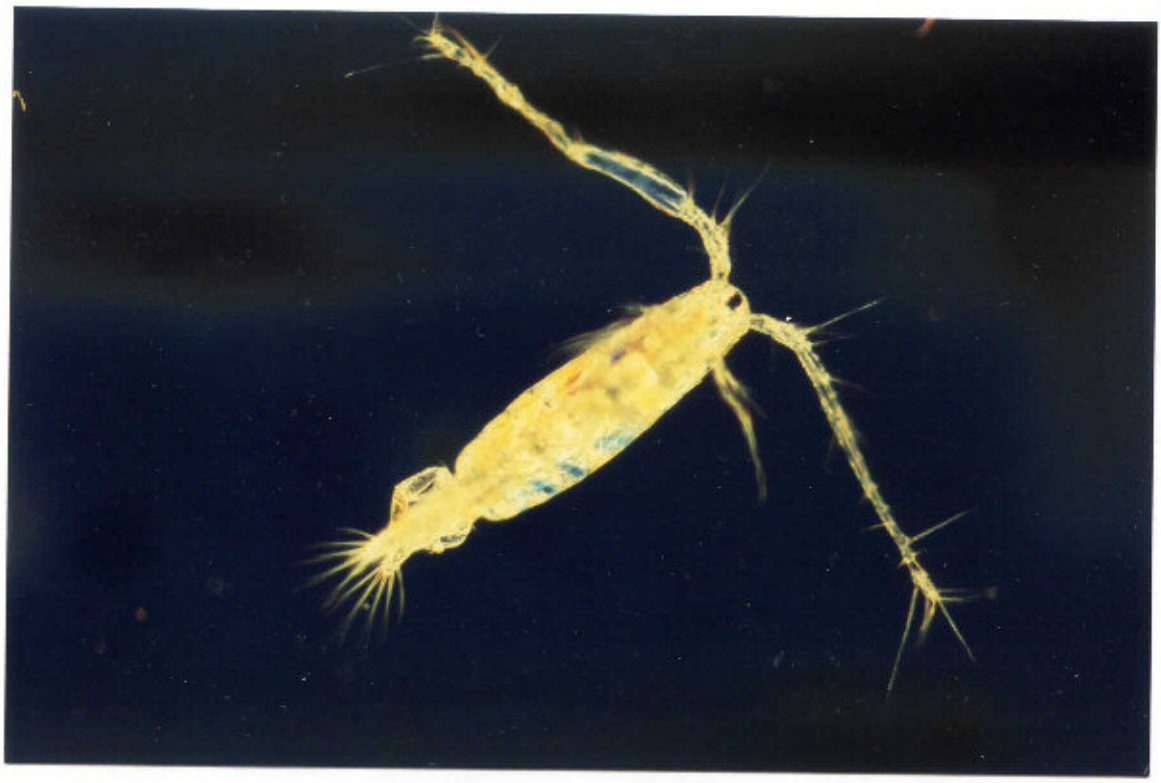

**Fig. 1**. Mascle d'*Acartia grani*.

**Fig. 2**. Femella d'*Acartia grani*.

3) Té elevades produccions d'ous (vegeu capítol 1.1) i creixement ràpid (capítol 1.2) la qual cosa facilita el poder disposar d'animals en qualsevol moment.

4) Els ous d'aquesta espècie sedimenten ràpidament, i per tant és molt fàcil separar-los dels adults sifonant el fons del flascó.

**Recol.lecció dels organismes**

La recol.lecció dels organismes es va efectuar en el port esportiu de Masnou, donada la seva proximitat a Barcelona i a les facilitats oferides en tot moment per part del capità del port i la resta de personal. Es van realitzar diverses pesques de curta durada (de 3 a 5 minuts) a baixa velocitat i el més properes al fons possible, amb una xarxa de plàncton Juday-Bogorov modificada. La modificació consistia en fer servir una bossa de plàstic de 10 litres en lloc del clàssic cubilet per tal d'evitar que els individus capturats resultessin malmesos en el procés de la pesca. El contingut de la bossa va ser transferit a una nevera portàtil i traslladat al laboratori. A continuació es va procedir a la selecció dels animals sota la lupa binocular. Les femelles adultes d'*A grani* van ser separades amb una pipeta Pasteur de boca ample i situades en pots de 2 litres amb abundant menjar. Per cada 5 o 10 femelles es posava també un mascle de la mateixa espècie ja que els copèpodes del gènere *Acartia* necessiten còpules freqüents per a la producció d'ous viables. Un cop separades unes 1000 femelles es van posar juntes en un tanc de 50 l amb aigua de mar filtrada per 0.2 μm i algues suficients (vegeu més endavant). Durant dos o tres dies es va recollir la posta d'aquestes femelles per sifonació i els ous es van posar a eclosionar en les condicions descrites en l'apartat de metodologia. Els animals procedents d'aquests ous van ser, doncs, la primera generació del cultiu.

**Producció de les algues**

Per tal d'alimentar els copèpodes es va escollir l'alga flagel.lada *Rhodomonas baltica* degut al seu tamany (7±0.5 μm) adequat per a ser ingerida tant per adults com per juvenils (Berggreen et al., 1988) i per les seves excel.lents qualitats nutritives (Støttrup, 1986; Jónasdóttir, 1994). El cultiu d'algues es porta a terme en bosses de plàstic transparents de 2 litres de capacitat amb aireació contínua. El règim de llum és de 12:12 h llum:foscor i la temperatura es manté a 19±1°C. Diàriament es procedeix a diluir les algues al 50% amb medi f/2 fresc, preparat segons el protocol descrit per Guillard (1975). Sota aquestes condicions la taxa de creixement de les algues és de 0.7 div.dia$^{-1}$, i el quocient C/N de 4.7±0.18. La concentració d'aliment en el cultiu de copèpodes s'intenta mantenir sempre per sobre de les 5 ppm en volum (1 ppm de *Rhodomonas baltica* és equivalent a 5772±157 cèl.lules per ml), encara que a les 24 h d'haver afegit menjar els copèpodes es poden trobar per sota d'aquest llindar degut a sedimentació i al propi consum. Aquesta concentració ha estat escollida atenent a la relació entre creixement i concentració de menjar, mantenint sempre una concentració saturant tant per a juvenils com per adults (capítol 1.2).

**El cultiu de copèpodes (metodologia)**

El cultiu es duu a terme en tancs blancs de PVC de 50 l de capacitat plens amb aigua de mar filtrada a través de 0.2 μm a la qual s'hi ha afegit les algues. Tant la temperatura com el cicle de llum és el mateix són els descrits pel cultiu de *Rhodomones*. Per tal de minimitzar la sedimentació de les algues i proporcionar oxigen suficient es fa bombollejar aire amb un airejador d'aquari. Experiments preliminars van mostrar que si bé la sedimentació de les algues en 24 h era només del 30% (fig. 3a) en un tanc sense agitació, al posar-hi aire, la concentració es

recuperava considerablement en molt poc temps (fig. 3b), encara que no s'arribava mai als valors inicials.

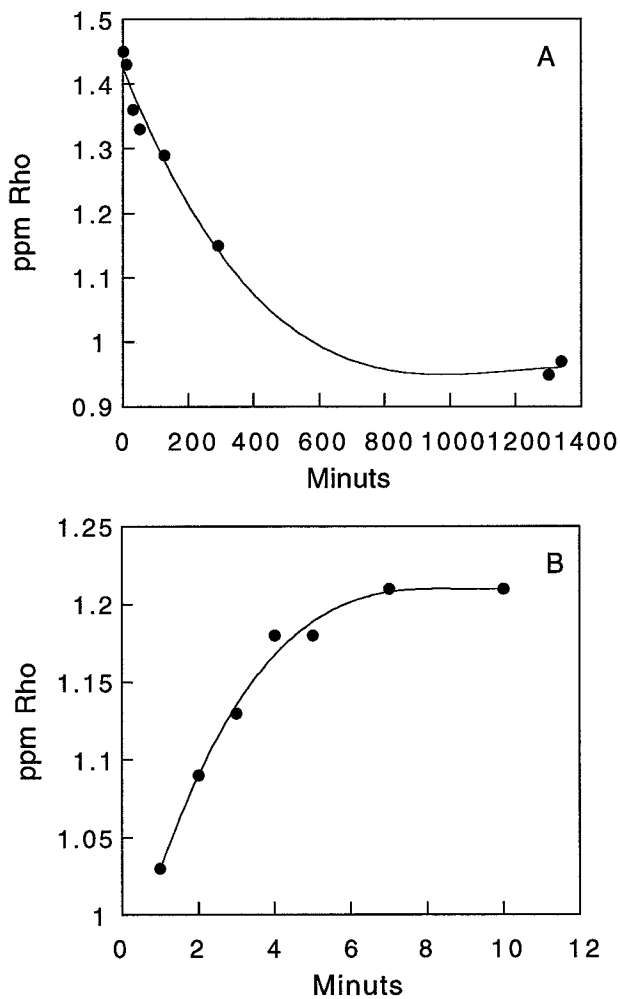


**Fig. 3**. A) Sedimentació de *Rhodomonas baltica* en funció del temps (minuts). B) Resuspenció per aireació de *R.baltica* al llarg del temps (minuts). Els experiments es van portar a terme en tancs de 50 litres (40 cm diàmetre per 50 cm alçada).

Per a començar un cultiu nou, es posen a eclosionar entre 50000 i 100000 ous en el tanc sota les condicions descrites. Transcorregudes 48 h es procedeix a sifonar el fons per a retirar els ous no eclosionats. Descartant aquests ous (que encara podrien eclosionar) es redueix bastant el percentatge d'eclosió (el qual

sota aquestes condicions només és del 25%) i el nombre d'individus finals, però això garanteix que la cohort obtinguda serà homogènia. El sifonat del fons es repeteix cada dos o tres dies pels estadis juvenils (fig. 4 i 5) i diàriament quan els copèpodes han arribat a adults. Amb aquesta operació s'extreuen del tanc les closques de les mudes, els "fecal pellets" (paquets fecals) i els individus morts, així i com uns 5 litres d'aigua, els quals són substituïts per aigua de mar filtrada nova. Quan es sifona el fons dels tancs que contenen estadis juvenils, els animals vius que hagin pogut ésser aspirats pel procés de sifonat del material no desitjable es separen ja sigui per sedimentació o per filtració.

Un cop els copèpodes han assolit l'estadi adult (15-16 dies a 18°C, vegeu capítol 4) s'ha de procedir a recol.lectar els ous diàriament. Els ous d'*A grani* tenen 75 µm de diàmetre, la qual cosa ens permet separar-los perfectament de la resta de material sifonat. A tal efecte es filtra suaument l'aigua amb la resta de material dipositat al fons del tanc per 200 µm per tal de separar els adults (que tornaran a ser introduïts en el cultiu) de la resta. Del material menor de 200 µm es recull la fracció entre 60 i 100 µm formada quasi exclusivament per ous. Amb aquesta posta recol.lectada es pot començar un nou cultiu o es poden conservar els ous per a més endevant.

**Fig. 5**. Naupli VI d'*A grani*.

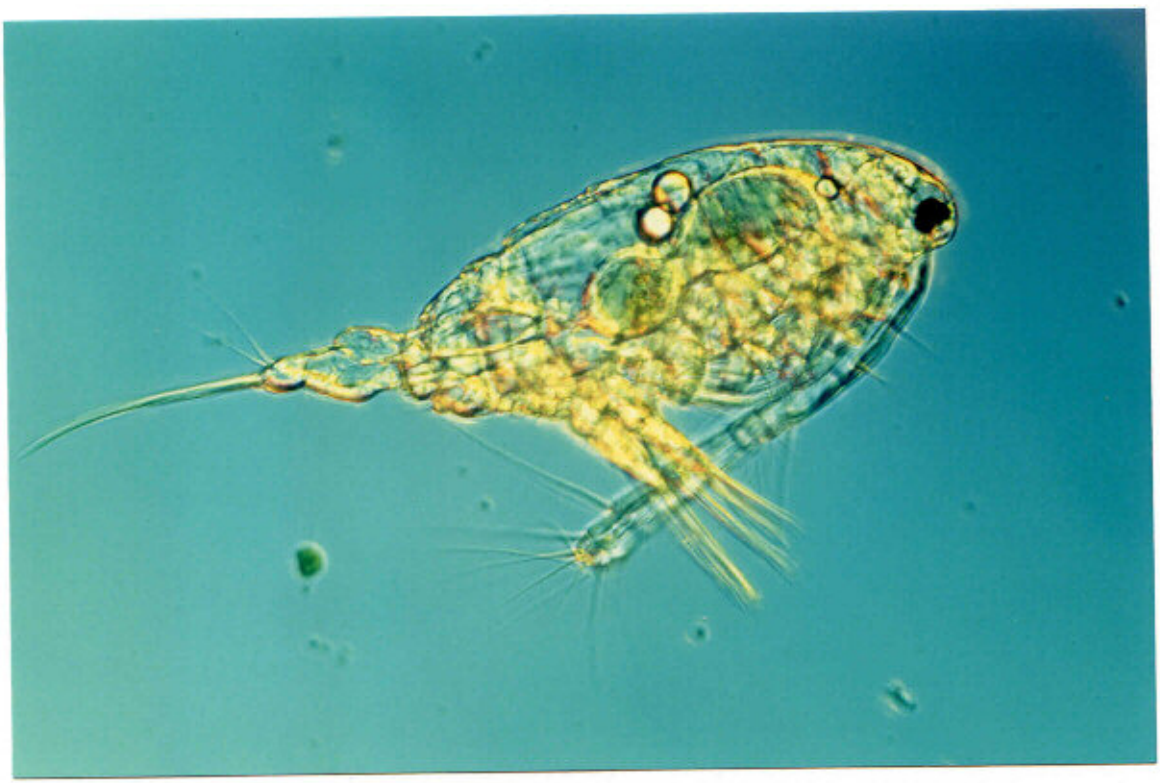

**Fig. 6.** Copepodit I d'*A grani*.

## Conservació dels ous

Per guardar ous de copèpode s'han d'eliminar o esmorteir tots aquells factors que en faciliten l'eclosió: llum (Landry, 1975a), l'oxigen (Marcus i Lutz, 1994; Madhupratap, 1996), temperatura (Tester, 1985). El mètode utilitzat consisteix en guardar els ous en tubs de plàstic de 10 ml a les fosques i a una temperatura de 6ºC. D'aquesta manera els ous es poden mantenir viables uns quants mesos (fins a un màxim de 5 ó 6 mesos). Cal tenir en compte però, que el tant per cent d'eclosió disminueix a mesura que passa el temps. Llavors, quan es vulgui començar un nou cultiu, només cal posar a eclosionar els ous que estaven enmagatzemats.

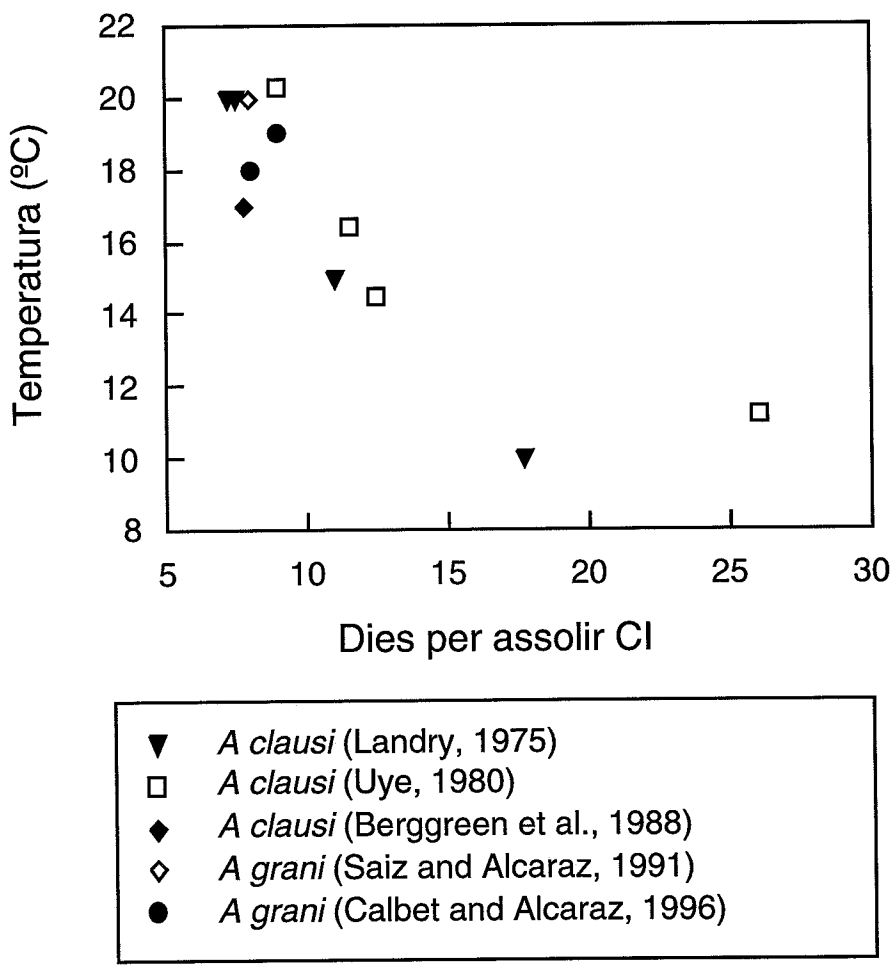


**Fig. 7.** Temps necessari per a assolir l'estadi de copepodit I per a diferents espècies d'*Acartia* en funció de la temperatura. Dades pròpies i de la literatura.

## DISCUSSIÓ

Amb lleugeres modificacions sobre la metodologia exposada es pot portar a terme un cultiu de qualsevol espècie de característiques similars a *A grani*. Per a copèpodes més grans, com ara *Calanus*, el sistema s'haurà d'escalar a la mida i requeriments tròfics dels animals. Sota les condicions descrites els copèpodes assoleixen l'estadi adult amb una mortalitat acumulada de naupli I a adult de ~ 30% a una taxa de creixement que és funció de la temperatura i la concentració de menjar (Miller et al., 1977; Landry, 1975b,c; Berggreen et al., 1988). A la figura 6 es pot apreciar l'efecte de la temperatura sobre el temps de desenvolupament de diferents espècies d'*Acartia*. Com es pot observar, l'increment en la temperatura està associat a la disminució del temps en assolir un determinat estadi (copepodit I).

D'acord amb aquesta mortalitat, d'un nombre inicial de 25000 nauplis, arriben a l'edat reproductiva al voltant de 17000 individus, dels quals el 50% són femelles (proporció sexual 1:1). Aquestes femelles sota condicions saturants de menjar i a una temperatura mitjana de 19°C haurien de produir uns 212000 ous per dia (25 ous femella$^{-1}$ d$^{-1}$, segons experiments previs). En canvi, la producció diària del cultiu acostuma a oscil.lar al voltant de 100000 ous per dia, la qual cosa representa una producció per femella i dia de 11-12 ous. Aquesta diferència entre la producció esperada i l'obtinguda pot ésser deguda a fenòmens de canibalisme, comuns entre copèpodes del gènere *Acartia*, i a una disminució en el transcurs del dia en la concentració d'aliment disponible per als copèpodes deguda a sedimentació i al propi consum de la cohort.

Aquestes taxes, encara que baixes, asseguren el manteniment del cultiu. En un dia de producció es generen prou ous per a un nou cultiu, i tenint en compte que les femelles d'*A. grani* són fèrtils durant un mínim dues setmanes, la posta

acumulada és suficient per a generar 14 noves cohorts. A manera de resum, en la figura 7 es mostra esquemàticament el procès habitual seguit per al manteniment del cultiu d'*A grani*.

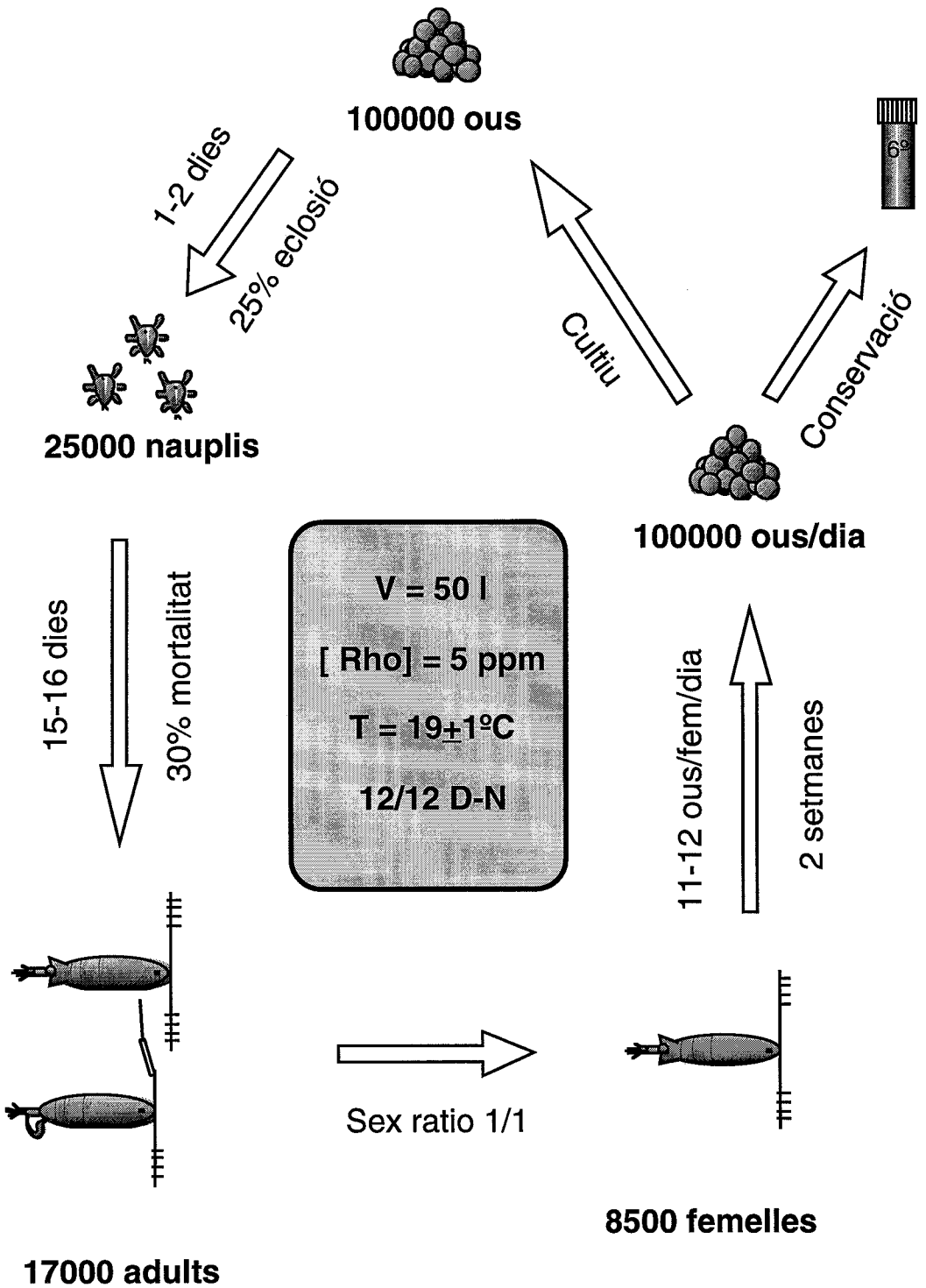


**Fig. 8.** Esquema del procediment habitual i resultats obtinguts en el transcurs d'un cultiu d'*Acartia grani*.

## BIBLIOGRAFIA

Alcaraz M., Paffenhöfer G. and Strickler R. (1980) Catching the algae: a first account of visual observations on filter-feeding calanoids. In: Evolution and ecology of zooplankton communities. Ed. by W.C. Kerfoot. University Press of New England, pp 241-248.

Alcaraz M. (1982) Zooplankton biomass and its relation to total particulate carbon and nitrogen in Northwest Africa. Rapp. P-v Réun. Cons. int. Explor. Mer. 180: 270-273.

Alcaraz M. (1983) Coexistence and segregation of congeneric pelagic copepods: spatial distribution of the *Acartia* complex in the Ria of Vigo (NW of Spain). J. Plankton Res. 5:891-900.

Alcaraz M. (1985) Vertical distribution of zooplankton biomass during summer stratification in the Western Mediterranean. In: Gibbs P.E. (ed.) 19th Eur. Mar. Biol. Symp. Proceedings. Cambridge University Press, Cambridge. pp 135-143.

Alcaraz M. (1988) Summer zooplankton metabolism and its relation to primary production in the Western Mediterranean. In: Minas H.J., Nival P. (eds.) Océanographie Pélagique Méditerranéenne. Oceanologica Acta. N° SP 9: 185-191.

Alcaraz M., Packard T.T. (1989) Zooplankton ETS activity and respiration in the Catalan Sea (Western Mediterranean). Scientia Mar. 53: 247-250.

Alcaraz M., Saiz E., Estrada M. (1994) Excretion of ammonia by zooplankton and its potential contribution to nitrogen requirements for primary production in the Catalan Sea (NW Mediterranean). Mar. Biol. 119: 69-76.

Ambler J.W. (1985) Seasonal factors affecting egg production and viability of eggs of *Acartia tonsa* Dana from East Lagoon, Galveston, Texas. Est. Coast. Shelf Sci., 20: 743-760.

Ambler J.W. (1986) Effect of food quantity and quality on egg production of *Acartia tonsa* Dana from East Lagoon, Galveston, Texas. Est. Coast. Shelf Sci., 23: 183-196.

Andrew W.R.H. and Hutchings L. (1980) Upwelling in Southern Benguela Current. Prog. Oceanogr. 9: 1-81.

Anraku M. (1975) Microdistribution of marine copepods in a small inlet. Mar. Biol. 30: 79-87.

Antia N.J., McAllister C.D., Parsons T.R., Stephens K., Strickland J.D.H. (1963) Further measurements of primary production using a large-volume plastic sphere. Limnol. Oceanogr. 8: 166-183.

Armstrong D.A., Verheye H.M. and Kemp A.D. (1991) Short-term variability during an Anchor Station study in the southern Benguela upwelling system. Fecundity estimates of the dominant copepod, *Calanoides carinatus*. Prog. Oceanogr., 28: 167-188.

Attwood C.G. and Peterson W.T. (1989) Reduction in fecundity and lipids of the copepod *Calanus australis* (Brodskii) by strongly pulsed upwelling J. Exp. Mar. Biol. Ecol. 129:121-131.

Ayukai T. (1988) Egg production by the planktonic calanoid copepod *Acartia omorii* in Onagawa harbor during spring-summer. Bull. Plankton Soc. Japan, 35: 127-132.

Båmstedt U. (1983) RNA concentration in zooplankton: seasonal variation in boreal species. Mar. Ecol. Prog. Ser. 11: 291-297.

Båmstedt U. and H. R. Skjoldal. (1976) Studies on the deep-water pelagic community of Korsfjorden Western Norway. Adenosine phosphates and nucleic acids in *Euchaeta norvegica* (Copepoda) in relation to its life cycle. Sarsia 60: 63-80.

Båmstedt U. and H. R. Skjoldal. (1980) RNA concentration of zooplankton: relationship with size and growth. Limnol. Oceanogr. 25: 304-316.

Barange M. (1994) Acoustic identification, classification and structure of biological patchiness on the edge of the agulhas bank and its relation to frontal features. S. Afr. J. Mar. Sci. 14: 333-347.

Baudouin M.-F. and P. Scoppa. (1975) The determination of nucleic acids in freshwater plankton and its ecological implications. Freshwat. Biol. 5: 115-120.

Bautista B., Rodriguez V. and Jimenez F. (1988) Short-term feeding rates of Acartia grani in natural conditions: diurnal variation J. Plankton Res. 10: 907-920.

Bellantoni D.C. and Peterson W.T. (1987) Temporal variability in egg production rates of *Acartia tonsa* Dana in Long Island Sound. J. exp. mar. Biol. Ecol. 107: 199-208.

Berggreen U., Hansen B. and Kiørboe T. (1988) Food size spectra ingestion and growth of the copepod *Acartia tonsa* during development: implications for determination of copepod production. Mar Biol 99: 341-352.

Bollens S.M. and Frost B.W. (1989) Predator-induced diel vertical migration in a planktonic copepod. J. Plankton Res. 11: 1047-1065.

Bollens S.M. and Stearns D.E. (1992) Predator-induced changes in the diel feeding cycle of a planktonic copepod. J. Exp. Mar. Biol. Ecol. 156: 179-186.

Bollens S.M., Frost B.W. and Cordell J. (1994) Chemical, mechanical and visual cues in the vertical migration bohavior of the planktonic copepod *Acartia hudsonica*. J. Plankton Res., 16(5): 555-564.

Borchers P. and Hutchings L. (1986) Starvation tolerance, development time and egg production of *Calanoides carinatus* in the Southern Benguela Current. J. Plankton Res. 8:917-925.

Boucher J. (1984) Location of zooplankton population in the Ligurian marine front: role of ontogenic migration. Deep-Sea Res. 29: 953-965.

Boucher J. , Ibanez F., Prieur L. (1987) Daily and seasonal variations in the spatial distribution of zooplankton population in relation to the physical structure in the Ligurian Sea Front. J. mar. Res. 45: 133-173.

Burns C.W. (1985) The effects of starvation on naupliar development and survivorship of three species of *Boeckella* (Copepoda: Calanoida). Arch. Hydrobiol. Beih. 21: 297-309.

Buskey E.J., Mann C.G. and Swift E. (1986) The shadow response of the estuarine copepod *Acartia tonsa*. J. Exp. Mar. Biol. Ecol. 103: 65-75.

Buskey E.J., Mann C.G. and Swift E. (1987) Photophobic responses of calanoid copepods: possible adaptive value. J. Plankton Res. 9: 857-870.

Calbet A. , Alcaraz M., Saiz E., Estrada M. and Trepat I. (1996) Planktonic herbivourous food webs in the Catalan Sea (NW Mediterranean): Temporal variability and comparison of indices of phyto-zooplankton coupling based in state variables and rate processes. J Plankton Res. 18(12): 2329-2347.

Calbet A. and Alcaraz M. (1996) Effects of constant and fluctuating food supply on egg production rates of *Acartia grani* (Copepoda: Calanoida). Mar. Ecol. Progr. Ser. 140: 33-39.

Castellón A., Font J., Garcia-Ladona E. (1991) The Liguro-Provençal-Catalan current (NW Mediterranean) observed by Doppler profiling in the Balearic Sea. Scientia Mar. 54: 269-276.

Cervetto G., Gaudy R., Pagano M., Saint-Jean L., Verriopoulos G., Arfi R. and Leveu M. (1993) Diel variations in *Acartia tonsa* feeding, respiration and egg production in a Mediterranean coastal lagoon. Journal of Plankton Research, 15: 1207-1228.

Clemmensen C. (1993) Improvements in the fluorometric determination of the RNA and DNA content of individual marine fish larvae. Mar. Ecol. Prog. Ser. 100: 177-183.

Clemmensen C. (1994) The effect of food availability, age or size on the RNA/DNA ratio of individually measured herring larvae: laboratory calibration. Mar. Biol. 118: 377-382.

Conover R.J. and Corner E.D.S. (1968) Respiration and nitrogen excretion by some marine zooplankton in relation to their life cycles. J. Mar. Biol. Ass. U.K. 48: 49-75.

Cowles T.J., Olson R.J. and Chisholm S.W. (1988) Food selection by copepods: discrimination on the basis of food quality. Marine Biology 100: 41-49.

Cushing C.H. (1951) The vertical migration of planktonic organisms. Biol Rev. 26: 158-192.

Cushing, D. H. (1974) The natural regulation of populations. In: Harden Jones F. R. (ed.) Sea fisheries research. Elek Science, London, pp 399-412.

Cushing D.H. (1989) A difference in structure between ecosystems in strongly stratified waters and in those that are only weakly stratified. J. Plankton Res. 11: 1-3

Checkley D.M. (1980) The egg production of marine planktonic copepods in relation to its food supply: laboratory studies. Limnol. Oceanogr. 25:430-446.

Checkley D.M., Dagg M.J. and Uye S. (1992) Feeding excretion and egg production by individuals and populations of the marine planktonic copepods *Acartia* spp. and *Centropages furcatus*. J. Plankton Res. 14: 71-96.

Chisholm L.A. and Roff J.C. (1990) Size-weight relationships and biomass of tropical neritic copepods off Kingston, Jamaica. Mar. Biol. 106: 71-77.

Dagg M.J. (1977) Some effects of patchy food environments on copepods Limnol. Oceanogr. 22: 99-107.

Dagg, M.J. (1978) Estimated, in situ rates of egg production for the copepod Centropages typicus (Krøyer) in the New York Bight. J. Exp. Mar. Biol. Ecol. 34: 183-196.

Dagg M.J. (1985) The effects of food limitation on diel migratory behavior in marine zooplankton. Arch. Hydrobiol. Beih. Ergebn. Limnol. 21: 247-255.

Dagg,M.J. and Grill,D.W. (1980) Natural feeding rates of *Centropages typicus* females in the New York Bight. Limnol. Oceanogr., 25(4): 597-609.

Dagg, M. J. and Littlepage J.L. (1972) Relationships between growth rate and RNA, DNA, protein and dry weight in *Artemia salina* and *Euchaeta elongata.* Mar. Biol. 17: 162-170.

Daro M.H. (1985) Feeding rhythms and vertical distribution of marine copepods. Bull. Mar. Sci. 37: 487-497.

Davis C.S. and Alatalo P. (1992) Effects of constant and intermittent food supply on life-history parameters in a marine copepod. Limnol. Oceanogr. 37: 1618-1693.

Davis C.S. and Alatalo P. (1992) Effects of constant and intermittent food supply on life-history parameters in a marine copepod. Limnol. Oceanogr. 37:1618-1693.

Delgado M., Latasa M. and Estrada M. (1992) Variability in the size-fractionated distribution of the phytoplankton across the Catalan front of the north-western Mediterranean. J. Plankton Res. 14(5): 753-771.

Dumont H.J. (1972) A Competition-based approach of the reverse vertical migration in zooplankton and its implications, chiefly based on a study of the interactions of the rotifer *Asplachna priodonta* (Gosse) with several crustacea entomostraca. Int. Revue ges. Hydrobiol. 57(1): 1-38.

Durbin E.G. and Durbin A.G. (1978) Length and weight relationships of *Acartia clausi* from Narragansett Bay RI. Limnol Oceanogr 23:958-969.

Durbin E.G., Durbin A.G. and Campbell R.G. (1992) Body size and egg production in the marine copepod Acartia hudsonica during a winter-spray diatom bloom in Narragansett Bay. Limnol. Oceanogr. 37: 342-360.

Durbin A.G., Durbin E.G. and Wlodarczyk E. (1990) Diel feeding behavior in the marine copepod *Acartia tonsa* in relation to food availability. Mar. Ecol. Progr. Ser. 68: 23-45.

Duval W.S. and Geen G.H. (1976) Diel feeding and respiration rhythms in zooplankton. Limnol. Oceanogr. 21: 823-829.

Estrada M. (1979) Observaciones sobre la heterogeneidad del fitoplancton en una zona costera del mar Catalán. Inv. Pesq. 43(3): 637-666.

Estrada M. (1985a) Deep phytoplankton and chlorophyll maxima in the Western Mediterranean. In: Moraitou-Apostolopoulou M, Kiorstis V (eds.) Mediterranean marine ecosystems. Plenum Press, New York. pp 247-277.

Estrada M. (1985b) Primary production at the deep chlorophyll maximum in the Western Mediterranean. In: Gibbs PE (ed.) 19th Eur. mar. Biol. Symp. proceedings. Cambridge University Press, Cambridge. pp 109-121.

Estrada M. and Margalef R. (1988) Supply of nutrients to the Mediterranean photic zone along a persistent front. In: Minas HJ, Nival P (eds.) Océanographie Pélagique Méditerranéenne. Oceanologica Acta. N° SP. 9: 133-142.

Estrada MC, Marrase M, Latasa E, Berdalet, Delgado M and Riera T (1993) Variability of deep chlorophyll maximum characteristics in the Northwestern Mediterranean. Mar. Ecol. Prog. Ser., 92: 289-300.

Fara A., Berdalet E. and Arin L. (1996) Determination of RNA and DNA concentrations in natural plankton samples using Thiazole Orange in combination with DNase and RNase digestions. J. Phycol. 32: 1074-1083.

Folt C. and Goldman C.R. (1981) Allelopathy between zooplankton: a mechanism for interface competition. Science, 213: 1133-1135.

Fryd M, Haslund OH, Wohlgemuth O (1991) Development, growth and egg production of the two copepod species *Centropages hamatus* and *Centropages typicus* in the laboratory. J. Plankton Res. 13: 683-689.

Fryd M., Haslund O.H. and Wohlgemuth O. (1991) Development, growth and egg production of the two copepod species Centropages hamatus and Centropages typicus in the laboratory. J. Plankton Res. 13: 683-689.

Fuller J.L. (1937) The feeding rate of *Calanus finmarchicus* in relation to environmental conditions. Biol. Bull. mar. biol. Lab. Woods Hole 72: 233-246.

Guillard R.R.L. (1975). Culture of phytoplankton for feeding marine invertebrates. In W. L. a. C. Smith M.H. (Eds.), Culture of marine invertebrate animals. Plenum Press, New York. pp 29-60.

Hagen W. and Schnack-Schiel (1996) Seasonal lipid dynamics in the dominant Antarctic copepods: Energy for overwintering or reproduction? Deep-Sea Res. 43(2): 139-158.

Haney J.F. (1988) Diel patterns of zooplankton behavior. Bull. Mar. Science 43(3): 583-603.

Harris P. (1980) Temporal and spatial scales in phytoplankton ecology. Mechanisms, methods, models and management. Can. J. Fish. Aquat. Sci. 37: 877-900.

Hassett R.P., Duggins D.O. and Simenstad C.A. (1993) Egg production rates of the neritic copepod *Acartia tumida* Willey in the Aleutian Archipielago. Polar. Biol. 13: 515-523.

Hassett R.P. and Blades-Eckelbarger P. (1995) Diel changes in gut-cell morphology and digestive activity of the marine copepod *Acartia tonsa*. Mar. Biol. 124: 59-69.

Haury L.R., McGowan and Wiebe P.H. (1978) Patterns and processes in the time-scales of plankton distributions in J.H. Steel (ed.) Spatial Patterns in Plankton Communities. Plenum NATO Conference Series IV. Marine Sciences 3: 277-327.

Hay S. (1995) Egg production and secondary production of common North Sea copepods: field estimates with regional and seasonal comparisons. ICES J. mar. Sci. 52: 315-327.

Hay S.J., Kiørboe T. and Matthews A. (1991) Zooplankton biomass and production in the North Sea during the Autumn Circulation Experiment, October 1987-March 1988. Cont. Shelf. Res. 11: 1453-1476.

Hays G.C., Proctor C.A., John A.W.G. and Warner A.J. (1994) Interspecific differences in the diel vertical migration of marine copepods: The implications of size, color, and morphology. Limnol. Oceanogr. 39(7): 1621-1629.

Hirche H.J. (1989) Egg production of the Arctic copepod *Calanus glacialis*: Laboratory experiments. Mar. Biol. 103(3): 311-318.

Hirche H.J. (1990) Egg production of *Calanus finmarchicus* at low temperature. Mar. Biol. 106:53-58.

Holligan P.M., Harris R.P., Newell R.C., Harbour D.S., Head R.N., Linley E.A.S., Lucas M.I., Tranter P.R.G., Weckley C.M. (1984) Vertical distribution and partioning of organic carbon in mixed, frontal and stratified waters of the English Channel. Mar. Ecol. Prog. Ser. 14: 111-127.

Holligan P.M., Williams P.J. leB., Purdie D., Harris R.P. (1984) Photosynthesis, respiration and nitrogen supply of plankton populations in stratified, frontal and tidally mixed shelf waters. Mar. Ecol. Prog. Ser. 17: 201-213.

Holling C.S. (1959) Some characteristics of simple types of predation and parasitism. Can. Enthomol. 91 pp 385-398.

Hopkins T.L. (1985) Food web of an Antarctic midwater ecosystem. Mar. Biol. 89: 197-212.

Hopkins T.L. and Torres J.J. (1989) Midwater food web in the vicinity of a marginal ice zone in the western Weddell Sea. Deep-Sea Res. 36(4): 543-560.

Humes A. G. (1994) How many copepods? Hydrobiologia 292/293: 1-7.

Huntley M. and Escritor F. (1992) Ecology of *Metridia gerlachei* Giesbrecht in the western Bransfield Strait, Antarctica. Deep-Sea Res. 39(6): 1027-1055.

Huntley M.E. and Lopez M.D.G. (1992) Temperature dependent production of marine copepods: a global synthesis. Am. Nat. 140:201-242.

Hutchings L. (1981). The formation of plankton patches in the Southern Benguela Current. In F. Richards (Ed.) Coastal Upwelling.

Hutchings L., Verheye H.M., Mitchell-Innes B.A., Peterson W.T., Huggett J.A. and Painting S.J. (1995) Copepod production in the southern Benguela system. ICES J. mar. Sci. 52: 439-455.

Ianora A. and Buttino I. (1990) Seasonal cycles in population abundances and egg production rates in the planktonic copepods *Centropages typicus* and *Acartia clausi*. J. Plankton Res. 12: 473-481.

Ikeda T. (1974) Nutritional ecology of marine zooplankton. Mem. Fac. Fish. Hokkaido Univ. 22: 1-97.

Ikeda T. and Mitchell A.W. (1982) Oxygen uptake, ammonia excretion and phosphate excretion by krill and other Antarctic zooplankton in relation to their body size and chemical composition. Mar. Biol. 71:283-298.

Jazdzewski K., Kittel W. and Lotocki K. (1982) Zooplankton studies in the southern Drake Passage and in the Bransfield Strait during austral summer (BIOMASS-FIBEX. February-March 1981). Polish Polar Res. 3: 203-242.

Jónasdóttir S.H. (1994) Effects of food quality on the reproductive succes of *Acartia tonsa* and *Acartia hudsonica*:: laboratoryobservations. Mar. Biol. 121:67-81.

Jónasdóttir S.H., Fields D. and Pantoja S. (1995) Copepod egg production in Long Island Sound, USA, as a function of the chemical composition of seston. Mar. Ecol. Prog. Ser. 119: 87-98.

Kierstead H. and Slobodkin (1953) The size of water masses containing plankton blooms. J. Mar. Res. 12: 141-147.

Kimmerer W.J. (1984) Spatial and temporal variability in egg production rates of the copepod *Acrocalanus inermis*. Mar. Biol., 78: 165-169.

King F.D., Cucci T.L., Townsend D.W. (1987) Microzooplankton and macrozooplankton glutamate dehydrogenase as indices of the relative contribution of these fractions to ammonium regeneration in the Gulf of Maine. J. Plankton Res. 9: 277-289.

Kiørboe T. (1989) Phytoplankton growth rate and nitrogen content: implications for feeding and fecundity in a herbivorous copepod. Mar. Ecol. Progr. Ser. 55: 229-234.

Kiørboe T. (1993) Turbulence, phytoplankton cell size, and the structure of pelagic food webs. Adv. Mar. Biol. 29: 1-72.

Kiørboe T. and Johansen K. (1986) Studies of a larval herring (Clupea harengus L.) patch in the Buchan area. 4. Zooplankton distribution and productivity in relation to hydrographic structures. Dana. 6: 37-51.

Kiørboe T., Møhlenberg F. and Hamburger K. (1985) Bioenergetics of the planktonic copepod Acartia tonsa: relation between feeding, egg production and respiration and composition of specific dynamic action Mar. Ecol. Progr. Ser. 26: 85-97.

Kiørboe T., Kaas H., Kruse B., Mohlenberg F., Tiselius P. and Aertebjerg G. (1990) The structure of the pelagic food web in relation to water column structure in the Skagerrak. Mar. Ecol. Progr. Ser. 59: 19-32.

Kiørboe T. and Nielsen T.G. (1994) Regulation of zooplankton biomass and production in a temperate, coastal ecosystem. 1. Copepods. Limnol. Oceanogr. 39: 493-507.

Kiørboe T., Møhlenberg F. and Riisgård,H.U. (1985) In situ feeding rates of planktonic copepods: a comparison of four methods. J. exp. mar. Biol. Ecol. 88: 67-81.

Kiørboe T., Munk P., Richardson K., Christensen V. and Paulsen H. (1988) Plankton dynamics and larval herring growth, drift and survival in a frontal area. Mar. Ecol. Prog. Ser. 44: 205-219.

Klein Breteler, W. C. M. (1980) Continuous breeding of marine pelagic copepods in the presence of heterotrophic dinoflagellates. Mar. Ecol. Progr. Ser. 2:229-233.

Klein Breteler W.C.H. and Gonzalez S.R .(1982) Influence of cultivation and food concentration on body length of calanoid copepods. Mar. Biol. 71: 157-161.

Kleppel G.S. (1992) Environmental regulation of feeding and egg production by *Acartia tonsa* off southern California. Mar. Biol. 112: 57-65.

Kleppel G.S., Davis C.S. and Carter K. (1996) Temperature and copepod growth in the sea: a comment on the temperature-dependent model of Huntley and Lopez. Amer. Nat. 148: 397-406.

Kleppel G.S., Holliday D.V. and Pieper R.E. (1991) Trophic interactions between copepods and microzooplankton: a question about the role of diatoms. Limnol. Oceanogr. 36: 172-178.

Kurbjeweit F. (1993) Reproduction and life cycles of dominant copepod species from the Weddell Sea, Antarctica. Ber. Polarforsch. 129: 1-238.

Laabir M., Poulet S.A. and Ianora A. (1995) Measuring production and viability of eggs in *Calanus helgolandicus*. J. Plankton Res. 17: 1125-1142.

Landry M.R. (1975a) Dark inhibition of egg hatching of the marine copepod *Acartia clausi* Giesbr. J. exp. mar. Ecol. 20:43-47.

Landry M.R. (1975b) The relationship between temperature and the development of life stages of the marine copepod *Acartia clausi* Giesbr. Limnol. Oceanogr. 20:854-857.

Landry M.R. (1975c) Seasonal temperature effects and predicting development rates of marine copepod eggs. Limnol. Oceanogr. 20:434-440.

Landry M.R. (1978) Population dynamics and production of a planktonic marine copepod *Acartia clausi* in a small temperate lagoon on San Juan Island Washington. Int. Revue ges. Hydrobiol. 63:77-119.

Latasa M., Estrada M. and Delgado M. (1992) Plankton-pigment relationships in the Northwestern Mediterranean during stratification. Mar. Ecol. Prog. Ser. 88: 61-73.

Le Fèvre J. (1986) Aspects of the biology of frontal systems. Adv. Mar. Biol. 23:163-299.

Le Fèvre J. and Frontier S. (1988) Influence of temporal characteristics of physical phenomena on plankton dynamics, as shown in North-West European marine ecosystems. In: Rothschild BJ (ed) Towards a theory on biological-physical

interactions in the world ocean. Kluwer Academic Publishers, Dordrecht. pp 245-272.

Le Fèvre J., Le Corre P., Morin P. and Birien J.L. (1983) The pelagic ecosystem in frontal zones and other environments off the west coast of Britanny. In: Proceedings of the 17th European Marine Biology Symposium. Oceanol. Acta SP. pp 125-129.

Legendre L. and Demers S. (1984) Towards dynamic biological oceanography and limnology (J. C. Stevenson Memorial Lecture). Can. J. Fish. aquat. Sciences 41: 2-19

Legendre L. and Le Fèvre J. (1992) Interactions between hydrodynamics and pelagic ecosystems: relevance to resource exploitation and climate change. S. Afr. J. Mar. Sci. 12: 477-486.

Legendre L., Le Fèvre J., Fortier L. (1993) Role of microphagous macrozooplankton in channeling biogenic carbon toward fish and/or the deep ocean. In: Guerrero R, Pedrós-Alió C (eds.) Trends in Microbial Ecology. Microbiological Spanish Society, Barcelona. p 431-434.

Longhurst A.R. (1976) Interaction between zooplankton and phytoplankton profiles in the eastern tropical Pacific ocean. Deep Sea Res. 23: 729-754

López M.D.G. and Hutley M.E .(1995) Feeding and diel vertical migration cycles of *Metridia gerlachei* (Giesbrecht) in coastal waters ot the Antarctic Peninsula. Polar Biol. 15: 21-30.

López M.D.G., Huntley M.E. and Lovette J.T. (1993) *Calanoides acutus* in Gerlache Strait, Antarctica. I. Distribution of late copepodite stages and reproduction during spring. Mar. Ecol. Prog. Ser. 100: 153-165.

Lorenzen G. J. (1971) Continuity in the distribution of surface chlorophyll. J. Cons. Int. Explor. Mer. 34: 18-23.

Mackas D. and Burns K.E. (1986) Poststarvation feeding and swimming activity in *Calanus pacificus* and *Metridia pacifica*.. Limnol. Oceanogr. 31: 383-392.

Mackas D.L., Denman K.L. and Abbott M.R. (1985) Plankton patchiness: Biology in the physical vernacular. Bull. Mar. Sci. 37: 652-674.

Madhupratap M. Nehring S. and Lenz, J. (1996) Resting eggs of zooplankton (Copepoda and Cladocera) from the Kiel Bay and adjacent waters (southwestern Baltic). Mar. Biol. 125:77-87.

Marcus N.H. (1988) Photoperiodic conditions food patchiness and fecundity. Bull. Mar. Sci. 43(3): 641-649.

Marcus N. H. and Lutz R. V. (1994) Effects of anoxia on the viability of subitaneous eggs of planktonic copepods. Marine Biology 121:83-87.

Margalef R. and Estrada M. (1987) Synoptic distribution of summer microplankton (algae and protozoa) across the principal front in Western Mediterranean. Inv. pesq. 51: 121-140.

Mayzaud P. (1973) Respiration and nitrogen excretion of zooplankton. II. Studies of the metabolic characteristics of starved animals Mar. Biol. 21:19-28.

Mayzaud P. (1976) Respiration and nitrogen excretion of zooplankton. IV. The influence of starvation on the metabolism and the biochemical composition of some species. Mar. Biol. 37: 47-58.

McAlice B.J. (1970) Observations on the small-scale distribution of estuarine phytoplankton. Mar. Biol. 7: 100-111.

McKinnon A.D. and Thorrold S.R. (1993) Zooplankton community structure and copepod egg production in coastal waters of the central Great Barrier Reef lagoon. J. Plankton Res. 15: 1387-1411.

Metz C. and Schnack-Schiel S.B. (1995) Observations on carnivorous feeding in Antarctic calanoid copepods. Mar. Ecol. Prog. Ser. 129: 71-75.

Miller, C.B., Johnson, J.K. and Heinle, D.R. (1977) Growth rules in the marine copepod genus *Acartia*. Limnol. Oceanogr. 22:326-335.

Mizdalzki E. (1988) Weight and length data of zooplankton in the Weddell Sea in austral spring 1986 (ANT V/3). Ber. Polarforsch. 55:1-72.

Mullin M.M. (1991) Production of eggs by the copepod *Calanus pacificus* in the Southern California sector of the California Current System. CalCOFI Rep. 32: 65-90.

Naganuma T. (1996) Calanoid copepods: linking lower-higher trophic levels by linking lower-higher Reynolds number. Mar. Ecol. Prog. Ser. 136: 311-313.

Nakata K. (1990) Abundance of nauplii and protein synthesis activity of adult female copepods in the Kuroshio Front during the Japanese sardine spawning season. J. Oceanogr. Soc. Japan 46: 219-229.

Nakata K., Nakano H. and Kikuchi H. (1994) Relationship between egg productivity and RNA/DNA ratio in *Paracalanus* sp. in the frontal waters of the Kurshio. Mar. Biol. 119: 591-596.

Nielsen T.G. and Hansen B. (1995) Plankton community structure and carbon cycling on the western coast of Greenland during and after the sedimentation of a diatom bloom. Mar. Ecol. Prog. Ser. 125: 239-257.

Nielsen T.G., Løkkegaard B., Richardson K., Bo Pedersen F. and Hansen L. (1993) Structure of plankton communities in the Dogger Bank area (North Sea) during a stratified situation. Mar. Ecol. Prog. Ser. 95: 115-131.

Nival S., Pagano M. and Nival P. (1990) Laboratory study of the spawning rate of the calanoid copepod *Centropages typicus*: effect of fluctuating food concentration. J. Plankton Res. 12:535-547.

Ohman M.D. (1990) The demographic benefits of diel vertical migration by zooplankton. Ecol. Monog. 60: 257-281.

Omori M. and Ikeda T. (1984) Methods in marine zooplankton ecology. John Wiley & Sons. New York

Ota A. Y. and Landry M. R. (1984) Nucleic acids as growth rate indicators for early developmental stages of *Calanus pacificus* Brodsky. J. Exp. Mar. Biol. Ecol. 80: 147-160.

Owen R.W. (1989) Microscale and finescale variations of small plankton in coastal and pelagic environments. J. Mar. Res. 47: 197-240.

Paffenhöfer G.A. (1970) Cultivation of *Calanus helgolandicus* under controlled conditions. Helgoländer wiss. Meeresunters. 20: 346-359.

Paffenhofer, G.A. (1979) Laboratory culture of marine holozooplankton and its contribution to studies of marine food webs. Adv. Mar. Biol. 16:211-308.

Paffenhöfer G. A. (1993) On the ecology of marine cyclopoid copepods (Crustacea, Copepoda). J. Plankton Res. 15: 37-55.

Park C. and Landry M.R. (1993) Egg production by the subtropical copepod Undinula vulgaris. Mar. Biol. 117: 415-421.

Parrish K.K. and Wilson D.F. (1978) Fecundity studies on *Acartia tonsa* (Copepoda : Calanoida) in standardized culture. Mar. Biol. 46: 65-81.

Perissinotto R. (1992) Mesozooplankton size-selectivity and grazing impact on the phytoplankton community of the Prince Edward Archipielago (Southern Ocean). Mar. Ecol. Prog. Ser. 79: 243-258.

Peterson W. T., Tiselius P. and Kiørboe T. (1991) Copepod egg production, moulting and growth rates, and secondary production, in the Skagerrak in August 1988. J. Plankton Res. 13: 131-154.

Peterson W.T. and Kimmerer W.J. (1994) Processes controlling recruitment of the marine calanoid copepod *Temora longicornis* in Long Island Sound: Egg production, egg mortality, and cohort survival rates. Limnol. Oceanogr. 39: 1594-1605.

Peterson W.T., Painting S.J. and Hutchings L. (1990) Diel variations in gut pigment content, diel vertical migration and estimates of grazing impact for copepods in the

southern Benguela upwelling region in October 1987. J. Plankton Res. 12: 259-281.

Petipa T.S. (1958) The diurnal feeding rhythm of the copepod crustacean Acartia clausi Giesbr. Okeanologia, 435-437.

Piontkovskii S.A. and PetipaT.S. (1976) Quantitative description of the behavior of copepod *Acartia clausi* during feeding on algae. Soviet J. Mar. Biol., 2: 40-46.

Platt T., Dickie M. and Trites R.W. (1970) Spatial heterogeneity of phytoplankton in a near-shore environment. J. Fish. Res. Bd. Canada 27: 1453-1473.

Plourde S. and Runge J.A. (1993) Reproduction of the planktonic copepod *Calanus finmarchicus* in the Lower St. Lawrence estuary: relation to the cycle of phytoplankton production and evidence for a *Calanus* pump. Mar. Ecol. Prog. Ser. 102: 217-227.

Poulet S.A. and Ouellet G. (1982) The role of amino acids in the chemosensory swarming and feeding of marine copepods. J. Plankton Res. 4: 341-359.

Poulet S.A., Ianora A., Laabir M. and Klein Breteler W.C.M. (1995) Towards the measurement of secondary production and recruitment in copepods. ICES J. mar. Sci. 52: 359-368.

Rodriguez J., Jiménez F., Bautista B. and Rodriguez V. (1987) Planktonic biomass spectra dynamics during a winter production pulse in Mediterranean coastal waters J. Plankton Res. 9: 1183-1194.

Rodriguez V. and Jimenez F. (1990) Co-existence within a group of congeneric species of *Acartia* (Copepoda Calanoida): sexual dimorphism and ecological niche in Acartia grani J. Plankton Res. 12: 497-511.

Roff J.C., Kroetsch J.T.and Clarke A.J. (1994) A radiochemical method for secondary production in planktonic crustacea based on rate of chitin synthesis. J. Plankton Res. 16: 961-976.

Roman M.R., Ashton K.A. and Gauzens A.L. (1988) Day/night differences in the grazing impact of marine copepods. Hydrobiologia 167/168: 21-30.

Rosenberg R., Dahl E., Edler L., Fyrberg L., Granéli E., Granéli W., Hagström A., Lindhal O., Matos M.O., Pettersson K., Sahlsten E., Tiselius P., Turk V. and Wikner J. (1990) Pelagic nutrient and energy transfer during spring in the open and coastal Skagerrak. Mar. Ecol. Prog. Ser. 61: 215-231.

Runge J.A. (1980) Effects of hunger and season on the feeding behavior of *Calanus pacificus*. Limnol Oceanogr 25(1): 134-145.

Runge J.A. (1985a) Egg production rates of *Calanus finmarchicus* in the sea off Nova Scotia. Arch. Hydrobiol. Beih. Ergebn. Limnol. 21: 33-40.

Runge J.A. (1985b) Relationship of egg production of *Calanus pacificus* to seasonal changes in phytoplankton availability in Puget Sound Washington. Limnol. Oceanogr. 30: 382-396.

Runge J.A. (1987) Measurement of egg production rate of *Calanus finmarchicus* from preserved samples. Can. J. Fish. Aquat. Sci. 44: 2009-2012.

Runge J.A. (1988) Should we expect a relationship between primary production and fisheries? The role of copepod dynamics as a filter of trophic variability. Hydrobiologia 167/168: 61-71.

Saito H. and Taguchi S. (1996) Diel feeding behavior of neritic copepods during spring and fall blooms in Akkeshi Bay, eastern coast of Hokkaido, Japan. Mar. Biol. 125: 97-107.

Saiz E. (1994) Observations of the free-swimming behavior of *Acartia tonsa*: Effects of food concentration and turbulent water motion. Limnol. Oceanogr. 39(7): 1566-1578.

Saiz E. and Alcaraz M. (1990) Pigment gut contents of copepods and deep phytoplankton maximum in the Western Mediterranean. J. Plankton Res. 12(3): 665-672.

Saiz E., Alcaraz M. and Paffenhofer G.A. (1992) Effects of small-scale turbulence on feeding rate and gross-growth efficiency of three *Acartia* species (Copepoda: Calanoida). J. Plankton Res. 14: 1085-1097.

Saiz E., Rodriguez V. and Alcaraz M. (1992) Spatial distribution and feeding rates of Centropages typicus in relation to frontal hydrographic structures in the Catalan Sea (Western Mediterranean). Mar. Biol. 112:49-56.

Sapienza C. and Mague T. H. (1979) DNA polymerase activity and growth rates in *Artemia salina* nauplii. Limnol. Oceanogr. 24: 572-576.

Schnack-Schiel S.B. and Hagen W. (1995) Life-cicle strategies of *Calanoides acutus*, *Calanus propinquus,* and *Metridia gerlachei* (Copepoda: Calanoida) in the eastern Weddell Sea, Antarctica. ICES J. Mar. Sci. 52(3-4): 541-448.

Smith S.L. (1990) Egg production and feeding by copepods prior to the spring bloom of phytoplankton in Fram Strait, Greenland Sea. Mar. Biol. 106: 59-69.

Sreekumaran Nair S.R., Devassy V. P. and Madhupratap M. (1992) Blooms of phyyoplankton along the west coast of India associated with nutrient enrichment and the response of zooplankton. Science of the total environement,Suplement 1992 pp 819-828.

Stearns D.E. (1986) Copepod grazing behavior in simulated natural light and its relation to nocturnal feeding. Mar. Ecol. Progr. Ser. 30: 65-76.

Stearns D.E., Tester P.A. and Walker R.L. (1989) Diel changes in the egg production rate of *Acartia tonsa* (Copepoda Calanoida) and related environmental factors in two estuaries. Mar. Ecol. Prog. Ser. 52: 7-16.

Steele J. H. (1978) Some coments on plankton patches. In J. H. Steele (Eds.) Spatial patterns in plankton communities. Plenum Press, New York pp 1-20.

Stommel H. (1963) Varieties of oceanographic experience. Science 139: 572-576.

Støttrup, J.G., Richardson, K, Kirkegaard, E. and Pihl, N.J. (1986) The cultivation of *Acartia tonsa* Dana for use as a live food source for marine fish larvae. Aquaculture 52:87-96.

Støttrup J.G. and Jensen J. (1990) Influence of algal diet on feeding and egg-production of the calanoid copepod *Acartia tonsa* Dana. J. Exp. Mar. Biol. Ecol. 141: 87-105.

Strass V. (1992) Chlorophyll patchiness caused by mesoscale upwellind at fronts. Deep-Sea Res. 39(1): 76-95.

Strickler J.R. (1977) Observation of swimming performances of planktonic copepods. Limnol. Oceanogr. 22: 165-169.

Sutcliffe W. H. J. (1970) Relationship betwen growth rate and ribonucleic acid concentration in some invertebrates. J. Fish. Res. Bd. Can. 27: 606-609.

Tester P.A. (1985) Effects of paternal acclimation temperature and egg-incubation temperature on egg-hatching time in *Acartia tonsa* (Copepoda: Calanoida). Mar. Biol. 89:45-53.

Tester P.A. and Turner J.T. (1990) How long does it take copepods to make eggs? J. Exp. Mar. Biol. Ecol. 141: 169-182.

Tiselius P. (1992) Behavior of *Acartia tonsa* in patchy food environments. Limnol. Oceanogr. 37: 1640-1651.

Tiselius P., Nielsen T.G., Breuel G., Jaanus A., Korshenko A. and Witek Z. (1991) Copepod egg production in the Skagerrak during SKAGEX, May-June 1990. Mar. Biol. 111: 445-453.

Tsuda A. (1994) Starvation tolerance of planktonic marine copepod *Pseudocalanus newmani* Frost. J. Exp. Mar. Biol. Ecol. 181: 81-89.

Uye S.I. (1982) Length-weight relationships of important zooplankton from the Inland Sea of Japan. J. Oceanogr. Soc. Japan 38: 149-158.

Uye S.I. (1991) Temperature-dependent development and growth of the planktonic copepod *Paracalanus* sp. in the laboratory. Bull. Plankton Soc. Japan, Spec. Vol. : 627-636.

Uye S. and Sano K. (1995) Seasonal reproductive biology of the small cyclopoid copepod *Oithona davisae* in a temperate eutrophic inlet. Mar. Ecol. Prog. Ser. 118: 121-128.

Uye S. and Shibuno N. (1992) Reproductive biology of the planktonic copepod *Paracalanus* sp. in the inland Sea of Japan. J. Plankton Res. 14: 343-358.

Vaqué D. Marrasé C. and Guixa N (1994) Trophic dynamics of microbial communities in an oligotrophic sea. ASLO/PSA Joint Meeting

Verheye H.M., Hutchings L., Huggett and Painting S.J. (1992) Mesozooplankton dynamics in the Benguela ecosystem, with emphasis on the herbivorous copeods. S. Afr. J. mar. Sci. 12: 516-584.

Vives F (1963) Distribution annuelle verticale du zooplankton sur une station de l'Adriatique meridionale. Acta Adriatica, 7(7): 3-72.

Walsh J.J. (1976) Herbivory as a factor in patterns of nutrient utilization in the sea. Limnol. Oceanogr. 21(1): 1-13.

Ward P. and Shreeve R. (1995) Egg production in three species of Antarctic Calanoid Copepods during an austral summer. Deep-Sea Res. 42(5): 721-735.

White J.R. and Roman M.R. (1992) Egg production by the calanoid copepod *Acartia tonsa* in the mesohaline Chesapeake Bay: the importance of food resources and temperature. Mar. Ecol. Prog. Ser. 86: 239-249.

Wiebe P.H. (1970) Small-scale spatial distribution in oceanic zooplankton. Limnol. Oceanogr. 15: 205-217.

Williams R. and Robins D.B. (1982) Effects of preservation on wet weight, dry weight, nitrogen and carbon contents of *Calanus helgolandicus* (Crustacea: Copepoda). Mar. Biol. 71: 271-281.

Yentsch C.S. and Menzel D.W. (1963) A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. Deep-Sea Res. 10: 221-231.