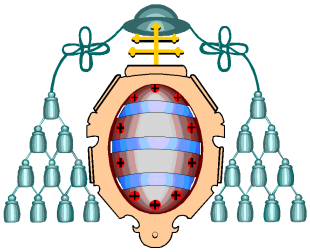

Universidad de Oviedo

Departamento de Biología de Organismos y Sistemas

Evaluación *in situ* de las tasas de ingestión de comunidades de apendicularias: impacto trófico e implicaciones ecofisiológicas

Ángel López-Urrutia Lorente

Tesis Doctoral **Julio 2002**

Universidad de Oviedo

Departamento de Biología de Organismos y Sistemas

Evaluación *in situ* de las tasas de ingestión de comunidades de apendicularias: impacto trófico e implicaciones ecofisiológicas

Memoria presentada para optar al grado

de Doctor en Biología por el Licenciado

Ángel López-Urrutia Lorente

Universidad de Oviedo

Departamento de Biología de Organismos y Sistemas

D. Tomás Emilio Díaz González, Director del Departamento de Biología de Organismos y Sistemas de la Universidad de Oviedo

AUTORIZA

la presentación ante la Comisión de Doctorado de la Memoria titulada: "**Evaluación *in situ* de las tasas de ingestión de comunidades de apendicularias: impacto trófico e implicaciones ecofisiológicas",** presentada por el Licenciado Ángel López-Urrutia Lorente para optar al grado de Doctor en Biología y realizada bajo la dirección del Doctor D. José Luis Acuña Fernández, considerando que ésta representa trabajo de Tesis.

Y para que así conste, expido el presente certificado.

Oviedo, Julio 2002

Fdo. Tomás Emilio Díaz González

Universidad de Oviedo

Departamento de Biología de Organismos y Sistemas

D. José Luis Acuña Fernández, Profesor Titular de Ecología del Departamento de Biología de Organismos y Sistemas de la Universidad de Oviedo

HACE CONSTAR que la presente Memoria, titulada "**Evaluación *in situ* de las tasas de ingestión de comunidades de apendicularias: impacto trófico e implicaciones ecofisiológicas**", ha sido realizada bajo su dirección en el Departamento de Biología de Organismos y Sistemas de la Universidad de Oviedo por el Licenciado **Ángel López-Urrutia Lorente**, y reúne las condiciones necesarias para que su autor pueda optar al grado de Doctor en Biología.

Oviedo, Julio 2002

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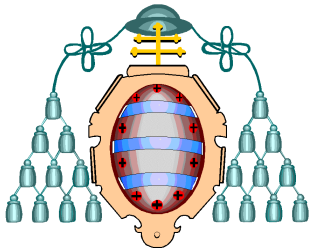

Universidad de Oviedo

Departamento de Biología de Organismos y Sistemas

In situ evaluation of the ingestion rates of the appendicularian community: trophic impact and ecophysiological implications

Ángel López-Urrutia Lorente

A thesis submitted for the degree of Doctor of Phylosophy in Biology **July 2002**

Evaluación *in situ* de las tasas de ingestión de comunidades de apendicularias: impacto trófico e implicaciones ecofisiológicas

Esta tesis doctoral se basa en el estudio de las tasas de ingestión de comunidades de apendicularias en ecosistemas marinos europeos, prestando una atención especial a la variabilidad estacional tanto de las tasas individuales sobre materia particulada total y material fitoplanctónico como de las densidades poblaciones. El estudio de la ingestión *in situ* nos ha permitido evaluar el impacto de estos organismos planctónicos como consumidores de la producción primaria y el desarrollo de ecuaciones para predecir sus tasas de ingestión a partir de variables ambientales. Los modelos existentes para describir las tasas de producción en estos organismos planctónicos han sido reevaluados y utilizados para estudiar el grado de limitación por falta de alimento en función de la biomasa corporal y de la temperatura ambiental. La combinación de medidas de la biomasa poblacional con los modelos para estimar las tasas de producción individuales desarrollados nos ha permitido estudiar la contribución de las apendicularias a la producción secundaria del mesozooplancton. Contrariamente a la visión tradicional de las apendicularias como importantes organismos en sistemas oligotróficos, nuestros resultados sugieren que su contribución al herbivorismo y producción del mesozooplancton es mayor en sistemas más productivos.

In situ evaluation of the ingestion rates of the appendicularian community: trophic impact and ecophysiological implications

This doctoral thesis is centered on the study of the ingestion rates of the appendicularian community in European marine ecosystems, with a particular emphasis on the seasonal variability of the ingestion rate of both total particulate material and phytoplankton prey and the population grazing impact. The study of the in situ ingestion rates has allowed us to evaluate the impact of these marine organisms as consumers of primary production and the development of predictive equations to estimate their ingestion rates from knowledge of environmental variables. The existing models to describe production rates in these organisms have been reevaluated and used to study the degree of food limitation as a function of individual body mass and habitat temperature. The combination of population biomass estimates with the models developed to estimate their individual production rates have allowed us to study the contribution of appendicularians to total mesozooplankton secondary production. Contrary to the traditional view of appendicularians as important organisms in oligotrophic ecosystems, our results suggest that their contribution to mesozooplankton herbivory and production is larger in more productive ecosystems.

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1. Introducción general

INTRODUCCIÓN GENERAL

La herbivoría ocupa un papel central en el ecosistema planctónico. El paradigma clásico sobre el flujo de carbono ha sido que la red trófica pelágica esta basada en la fijación fotosintética del carbono inorgánico disuelto a materia particulada por parte de organismos fitoplanctónicos de tamaño grande. El zooplancton representa el siguiente escalón en esta cadena trófica "clásica"; se alimenta de la biomasa vegetal y representa la mayor fuente de alimento para larvas y juveniles de peces (Cushing 1989). La idea de que los microbios (bacterias, microflagelados y protistas heterótrofos) constituyen un importante flujo de carbono en comunidades marinas se gestó durante los años 70 y cristalizó en la presentación por Azam et al. (1983) de lo que actualmente se conoce como el "bucle microbiano". Esta distinción entre sistemas en los que la producción está basada en fitoplancton de tamaño grande (cadena clásica) o en las

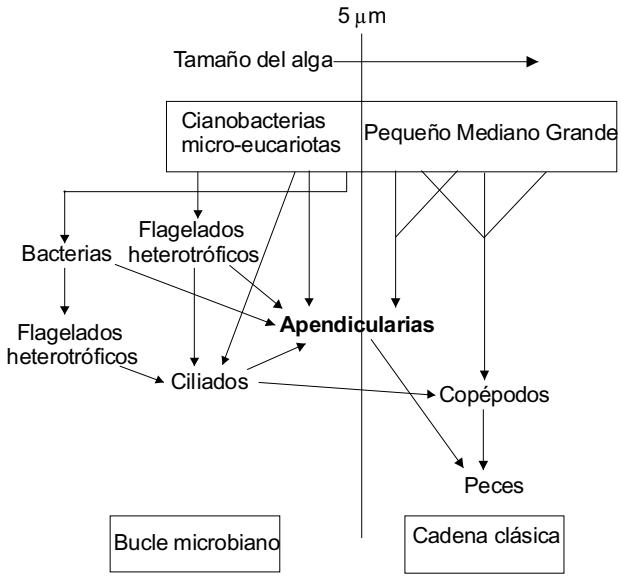

Figura 1. Distinción en la estructura trófica de los ecosistemas dependiendo del predominio del bucle microbiano o de la cadena clásica y representación de la posición incierta de las apendicularias entre ambos tipos de red trófica. (adaptado de Cushing 1989 y Gorsky and Fenaux 1998).

bacterias y el picoplancton (bucle microbiano) guía nuestras interpretaciones sobre la dinámica de los sistemas marinos pelágicos en la actualidad (Fig. 1). Generalmente, se considera que el bucle microbiano predomina en mares oligotróficos y en periodos en los que la columna de agua se encuentra estratificada durante el verano en mares templados mientras que la cadena trófica tradicional suele ser más importante durante las proliferaciones fitoplantónicas que suelen ocurrir en primavera y otoño en mares templados y en áreas de afloramiento (Cushing 1989).

El zooplancton es un grupo muy heterogéneo y juega un papel crucial en ambos tipos de cadena trófica (Fig. 1). En la cadena trófica clásica los copépodos representan una conexión eficiente en la transferencia de energía desde el fitoplancton relativamente grande (>5 µm de diámetro) hacia las larvas de peces. En el bucle microbiano, los protistas de tamaño pequeño se alimentan de bacterias y microflagelados y luego son consumidos por ciliados. Aunque el bucle microbiano implica una topología cerrada, suele ser parte de una red trófica de mayor tamaño donde copépodos y otros miembros de la cadena clásica se alimentan de ciliados y flagelados, lo cual representa un punto de unión con los niveles tróficos superiores. Por tanto, el zooplancton (protozoos y metazoos heterotróficos), a través del herbivorismo y la predación, ejercen un control sobre las tasas de producción del fitoplancton y de las bacterias heterotróficas. Además, el zooplancton influye sobre otros muchos procesos oceánicos importantes tales como los flujos de sedimentación hacia capas profundas o la regeneración de nutrientes.

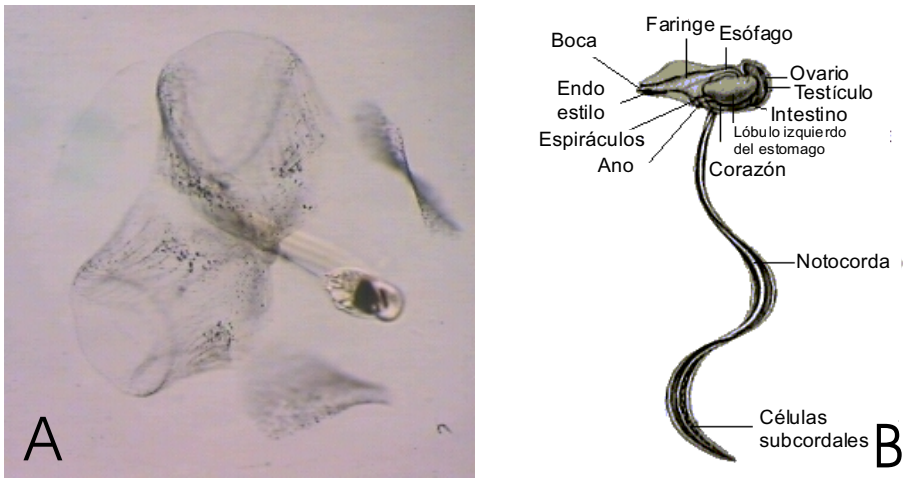

Figura 2. (**A**) Microfotografía de la apendicularia *Oikopleura dioca* alimentándose dentro de su casa. (**B**) Esquema mostrando las diferentes partes del tronco y la cola de una apendicularia (adaptado de Alldredge 1976).

Las apendicularias quedaron en una posición incierta en la revisión sobre la estructura de ecosistemas pelágicos hecha por Cushing (1989) (Fig. 1). Estos organismos mesozooplanctónicos empujan el agua a través de una complicada casa gelatinosa con filtros concentradores de alimento (Fig. 1). Este mecanismo de alimentación único les permite capturar partículas de un tamaño tan pequeño como bacterias e incluso coloides (Flood et al. 1992, Bedo et al. 1993). De este modo su estrategia de alimentación parece estar dirigida hacia las partículas de pequeño tamaño características del bucle microbiano aunque también son capaces de alimentarse de partículas de tamaño mayor (Alldredge & Madin 1982). Las apendicularias son lo

suficientemente grandes como para servir de alimento para larvas de peces y constituyen en particular la fuente de alimento preferente de larvas de peces planos (p.e. Shelbourne 1962, Last 1980). Esto hace que se considere a las apendicularias como un posible lazo de unión directo desde el bucle microbiano hasta los niveles tróficos superiores con una transferencia de energía más eficiente que a través de la cadena de protozoos heterotróficos – ciliados – copépodos en la que cada paso supone una pérdida de energía causada por los procesos respiratorios (Gorsky & Fenaux 1998).

A pesar de esta importancia potencial en las redes tróficas planctónicas nuestro conocimiento sobre su papel como consumidores y productores de materia orgánica particulada es todavía limitado, principalmente si consideramos que gran parte del conocimiento existente se ha obtenido en condiciones de laboratorio. Aunque la experimentación en el laboratorio es una herramienta útil para investigar el efecto potencial de diferentes factores ambientales, las condiciones experimentales suponen por definición una simplificación de la complejidad del ambiente natural y los factores elegidos durante el diseño experimental pueden tener poca relevancia en el campo (Peters & Downing 1984). Desde un punto de vista sinecológico, la determinación del impacto de la comunidad de apendicularias a escalas espacio-temporales relevantes es necesaria para evaluar su papel en las cadenas pelágicas marinas. Para evaluar el impacto poblacional es necesario multiplicar las repuestas fisiológicas individuales por estimas de abundancia o biomasa poblacional. Consecuentemente, el impacto ecológico de las apendicularias estará determinado no solo por su respuesta fisiológica frente a cambios ambientales sino también por los factores que controlan su variabilidad poblacional. Mientras que la abundancia del zooplancton depende de la abundancia de recursos alimenticios (Banse 1995), la probabilidad de que los copépodos encuentren en la naturaleza condiciones limitantes en la concentración del alimento y por tanto la importancia del control por el recurso es todavía fuente de debate (p.e. Huntley & Boyd 1984, Ikeda et al. 2001). Otros factores como fuerzas hidro-meteorológicas (p.e. Beaugrand et al. 2002), control por predación (p.e. Hopcroft & Roff 1998), mortalidad (p.e. Ohman & Hirche 2001, Hirst & Kiorboe 2002), competencia o incluso canibalismo (Ohman & Hirche 2001) han recibido una creciente atención como factores que pudieran controlar la dinámica de poblaciones del mesozooplancton.

El hecho de que las apendicularias son normalmente menos numerosas que los copépodos ha resultado en una relativa falta de información *in situ* sobre su papel como productores y consumidores de materia particulada y sobre los factores que controlan la variabilidad en su abundancia. Sin embargo, su posición única en la red trófica pelágica y sus altas tasas de crecimiento y producción que podrían compensar su menor biomasa poblacional sugieren que su impacto potencial podría ser significativo.

GENERAL INTRODUCTION

Herbivory occupies a central place in the plankton ecosystem. The classical paradigm has been that the carbon flux through the pelagic food web is based on the photosynthetic carbon fixation by large phytoplankton. Zooplankton is the next step in this "traditional" food chain; they feed on plant biomass and represent the most important prey for larval and juvenile fish (Cushing 1989). The realization that microbes and heterotrophic protists comprise a major trophic pathway in marine communities evolved during the late 70s and resulted in the formal presentation of what is known as the "microbial loop" (Azam et al. 1983). This distinction, depending on whether large phytoplankton ("classical" food chain) or bacteria and picoplankton (microbial loop) production are at the base of the food web, resulted in a dichotomy in the conceptual structure of marine pelagic ecosystems (Fig. 1). In the oligotrophic ocean and summer stratified waters in temperate seas the microbial food loop predominates while the traditional food chain is considered more important during the spring and autumn phytoplankton blooms in temperate oceans and in upwelling areas (Cushing 1989).

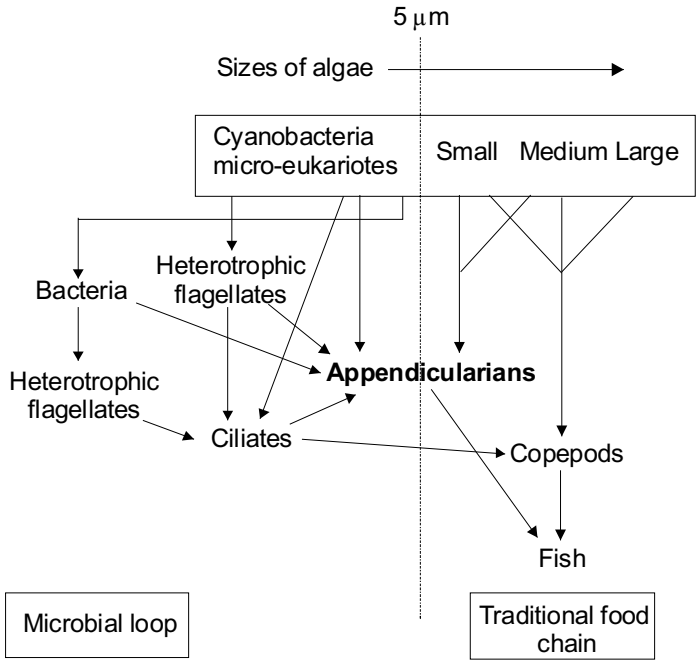

Figure 1. The structural difference between the microbial loop and the traditional food chain and the uncertain position of appendicularians between both types of food webs. (adapted from Cushing 1989 and Gorsky and Fenaux 1998).

The zooplankton is a widely heterogeneous group and plays a crucial role in both types of food chains (Fig. 1). In the classic food web, the copepods represent an efficient connection from the relatively large algae (>5 µm in diameter) to the fish. In the microbial loop, small protistan predators feed on bacteria and microflagellates and are in turn consumed themselves by ciliates. Although it involves a topologically closed food chain, the microbial loop is also usually part of a larger food chain where copepods and other members of the traditional food web feed on ciliates and flagellates, what represents a link with higher trophic levels. Therefore, the zooplankton (heterotrophic protozoans and metazoans) exert a grazing control on the rate of production by phytoplankton and heterotrophic bacteria. In addition, the zooplankton influence many other important oceanic processes such as sinking fluxes and carbon removal from upper layers and also sustain phytoplankton and bacteria by nutrient regeneration.

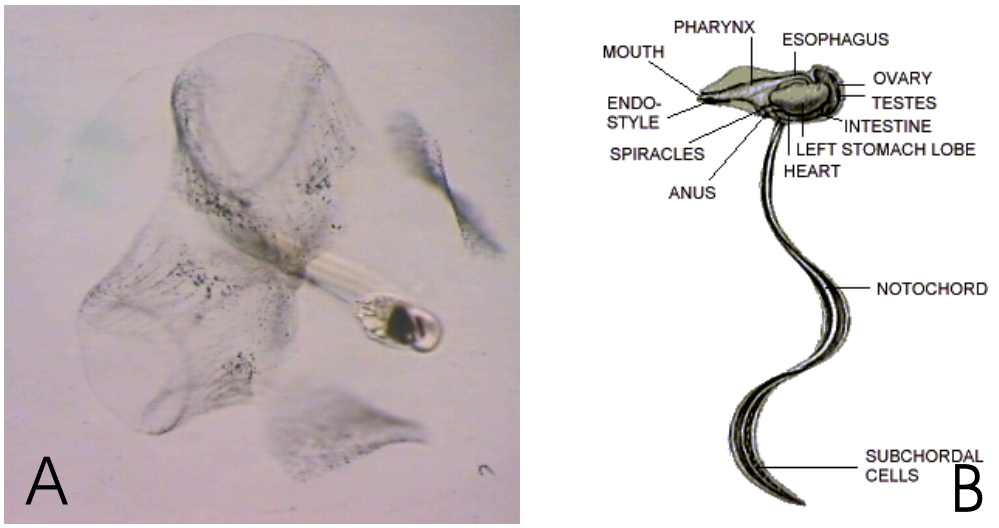

Figure 2. (**A**) Micrography showing the appendicularian *Oikopleura dioca* inside its filter house. (**B**) Schematic representation of the appendicularian trunk and tail adapted from Alldredge 1976).

Appendicularians remained in an uncertain position in the discrimination made by Cushing (1989) on the structure of pelagic ecosystems (Fig. 1). These mesozooplanktonic organisms pump water through a complicated gelatinous house with food concentrating filters (Fig. 2). This unique feeding mechanism allows them to thrive on particles as small as bacteria and colloids (Flood et al. 1992, Bedo et al. 1993), so their feeding strategy seems to be targeted towards the small particles characteristic of microbial-based ecosystems although they also feed on larger particles (Alldredge & Madin 1982). Appendicularians are large enough to be directly preyed by larval fish and constitute a preferential food source in particular for flatfish larvae (e.g. Shelbourne 1962, Last 1980). Therefore, it has been suggested that appendicularians act as a direct

by-pass from the microbial loop towards higher trophic levels, with higher transfer efficiency than the link through heterotrophic protozoa - ciliates – copepods where each step involves a respiratory loss term in the energy transfer (Gorsky & Fenaux 1998).

Despite this potential importance in planktonic food webs our understanding of their role as grazers and producers of organic matter is still limited; particularly if we consider that a major part of the current knowledge has been obtained under restricted laboratory conditions. Although laboratory experimentation represents a useful tool in investigating the potential effect of environmental factors, the experimental conditions represent by definition an oversimplification of the complex natural environment and the factors selected in the experimental design might have relatively low importance in the field (Peters & Downing 1984). From a synecological point of view, determination of appendicularian community grazing impact under relevant spatio-temporal scales is also needed to evaluate their role in pelagic food chains. To assess the population impact we necessarily have to multiply individual rates by abundance or biomass estimates. Consequently, the ecological impact of appendicularians will be determined not only by their physiological response to environmental factors but also by the factors controlling their population variability. While there is clearly a bottom-up control on zooplankton abundance (i.e. dependence on food supply, Banse 1995), the probability that copepods encounter food limiting conditions in nature and therefore the importance of the bottom-up control is still under debate (e.g. Huntley & Boyd 1984, Ikeda et al. 2001). Other factors like hydrometeorological forcing (e.g. Beaugrand et al. 2002), top-down control (i.e. predation Hopcroft & Roff 1998), mortality (e.g. Ohman & Hirche 2001, Hirst & Kiorboe 2002) competition or even cannibalism (Ohman & Hirche 2001) have received increasing attention as potential controllers of mesozooplankton population dynamics.

The fact that appendicularians are usually outnumbered by copepods has resulted in a relative lack of *in situ* information on their role as grazers and producers of organic matter and on the factors controlling their abundance (Calbet 2001). However their unique position in the pelagic food chain (Gorsky & Fenaux 1998) and their high production rates which could compensate their lower population biomass (Hopcroft & Roff 1998) suggest that their potential impact could be significant.

2. Objetivos, procedimientos, materiales y métodos utilizados

OBJETIVOS:

Nuestro objetivo con este trabajo es compensar el vacío existente en el conocimiento de la ecología de apendicularias a través de un enfoque observacional y de modelado a escalas espaciales y temporales relevantes en cuanto a la dinámica de los ecosistemas marinos pelágicos. Este objetivo general se traduce en los siguientes objetivos específicos:

-Comparar la variabilidad espacial y estacional de las densidades poblacionales de las especies de apendicularias presentes en mares europeos.

- Evaluar *in situ* el impacto de las apendicularias como consumidores de partículas y como productores secundarios.

-Determinar cuáles son los factores ambientales que explican una mayor parte de la variabilidad en sus tasas de ingestión y desarrollar ecuaciones predictivas.

-Explorar la importancia potencial del control por recurso sobre las tasas de crecimiento individuales de apendicularias y sobre su dinámica poblacional.

METODOLOGÍA GENERAL

La producción secundaria puede ser definida en un contexto amplio como el término que engloba los procesos por los cuales los animales se sustentan y propagan (Lehman 1988). En un sentido más restringido la producción secundaria suele interpretarse generalmente como crecimiento o incremento en biomasa corporal o en esfuerzo reproductor. Lehman (1988) clasificó las investigaciones ecológicas sobre la producción secundaria en tres categorías generales: taxonomía y zoogeografía, flujo de masa y energética y dinámica poblacional y estructura de comunidades. Estos diferentes acercamientos no son mutuamente excluyentes y pueden ser interpretados desde una perspectiva común. Los estudios sobre el flujo de masa y energética suelen estar enfocados generalmente desde un punto de vista holístico, mientras que en estudios de dinámica de poblaciones es más común un enfoque reduccionista basado en mecanismos a nivel del organismo individual. Verity & Smetacek (1996) avocaron la necesidad de un acercamiento más entrelazado sugiriendo que "probablemente sea más valioso entender porque el carbono fluye a donde lo hace que simplemente cuánto". Los factores que controlan el crecimiento de los organismos serán los responsables de la estructura de la comunidad y por tanto del funcionamiento del ecosistema marino, el cual determina el ciclo biogeoquímico. Finalmente, la biogeografía de las diferentes especies debería estar también controlada por los factores que regulan el crecimiento a un nivel individual (su adaptación a diferentes recursos o condiciones ambientales). Por tanto, el enfoque metodológico seguido en el presente trabajo esta probablemente más enfocado hacia la comprensión de los mecanismos que controlan a las poblaciones de apendicularias a un nivel de especie bajo condiciones *in situ*. La metodología seguida en nuestro trabajo sigue para ello tres enfoques generales que han dirigido parte de las investigaciones planctónicas en general y del zooplancton en particular durante las últimas décadas. Estos tres tópicos generales son: el nicho ecológico, el análisis empírico de las tasas de ingestión del zooplancton y la importancia del control por recurso alimenticio o limitación por el alimento de las tasas de crecimiento del zooplancton. Obviamente, no trataremos de reinventar las teorías y metodología existentes, sino adaptarlas a la biología particular de las apendicularias. El relacionar los resultados de investigaciones de campo en lugares concretos con generalidades sobre el papel de procesos específicos es esencial en ecología y los estudios comparativos realizados en varias localidades permiten determinar la importancia general de los

diferentes procesos estudiados (Thrush et al. 2000). Por lo tanto hemos intentado aplicar cada metodología en el campo durante un estudio estacional llevado a cabo desde Marzo de 1999 hasta Marzo de 2000 en cuatro ambientes contrastadamente diferentes: los fiordos noruegos, el oeste del Canal de la Mancha y los mares Cantábrico y Ligúrico (Fig. 1) en un total de ocho estaciones. Mientras que la dinámica estacional y los factores que afectan a la estructura de la comunidad fueron estudiados en todas estas localidades, las tasas de ingestión y el impacto que el consumo por parte de las diferentes especies de apendicularias tiene sobre el material particulado se investigó en una estación costera en el Canal de la Mancha y en un transecto de tres estaciones cruzando la plataforma continental en el Mar Cantábrico.

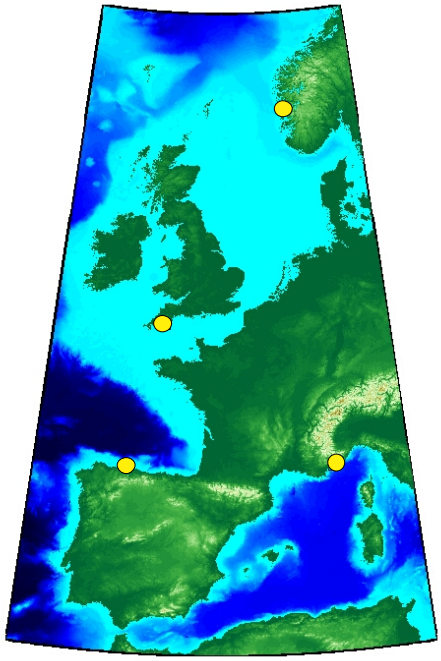

Figura 1. Mapa mostrando la posición de los sitios de estudio: el mar Cantábrico (NO de España), el Canal de la Macha, el mar Ligúrico en el Mediterráneo y los fiordos noruegos.

La información obtenida fue usada para desarrollar ecuaciones que permitan la predicción de las tasas de ingestión de las apendicularias. Estas ecuaciones fueron luego combinadas con una extensa compilación bibliográfica de diferentes aspectos de la ecología y fisiología de apendicularias para evaluar las condiciones bajo las cuales es probable que experimenten una limitación por falta de alimento. A continuación se llevara a cabo una introducción general (y lejos de ser extensiva) de cada metodología

mientras que un explicación más detallada en un contexto más concreto de la ecología de las apendicularias tendrá lugar en las secciones metodológicas de cada capítulo.

Análisis de la estructura de la comunidad.

Cada especie ocurre en un rango característico de hábitats y por tanto la composición biótica de las comunidades cambia a lo largo de gradientes medio ambientales, unas especies reemplazando a otras de una manera sucesiva en función de los cambios en el ambiente o a lo largo del tiempo sucesional. Los métodos usados en el análisis de la estructura de la comunidad han seguido dos enfoques generales: la clasificación y la ordenación. Los métodos de clasificación se basan en la asunción de que la comunidad consiste de entidades relativamente discretas mientras que los métodos de ordenación asumen que la comunidad consiste en un continuo ecológico. Sin embargo, estos dos enfoques no deberían ser considerados opuestos sino que deberían complementarse a la hora de ayudarnos a entender la estructura subyacente de la comunidad respondiendo a la misma pregunta desde puntos de vista diferentes. Por tanto, hemos usados ambos acercamientos en nuestro estudio de la dinámica estacional y de los factores ambientales que afectan a la estructura de la comunidad de apendicularias. Mientras que métodos multivariantes de agrupamiento caen por definición bajo la categoría de clasificatorios, el análisis de escalamiento multidimensional no métrico y el análisis de coordenadas principales están dentro de los métodos de ordenación. La teoría del nicho ecológico esta más cerca de la existencia de gradientes y continuos ecológicos (Austin 1985). Las dos metodologías de clasificación utilizadas han sido el agrupamiento cronológico en el estudio de la sucesión estacional (Legendre et al. 1985) y el agrupamiento por enlaces no jerárquicos usado en la determinación de grupos recurrentes de especies de apendicularias.

La sucesión estacional

El algoritmo de agrupamiento cronológico usado para caracterizar la sucesión de especies en la comunidad de apendicularias se corresponde con un modelo bien definido en el cual la sucesión ecológica ocurre de manera escalonada (Fig. 2). El resultado del agrupamiento cronológico consiste en una partición no jerárquica de la serie estacional en grupos homogéneos que no se solapan y que pueden ser interpretados desde una perspectiva ecológica como escalones o saltos en la sucesión. El grado de resolución en

el número de escalones detectados puede ser modificado alterando la conectividad (la proporción del número total de similaridades entre un objeto o grupo de objetos y un grupo adyacente que es necesaria para evaluar si ambos grupos podrían unirse) y la probabilidad α (el nivel de significación para rechazar la hipótesis nula de que dos grupos deben de unirse). Para detectar cuales son los los puntos de ruptura más importantes en una serie temporal es necesario usar un conjunto de combinaciones de valores de conectividad y probabilidad α, los pasos más importantes apareceran entonces como aquellos que ocurren en la mayoría de niveles de resolución usados (Legendre et al. 1985).

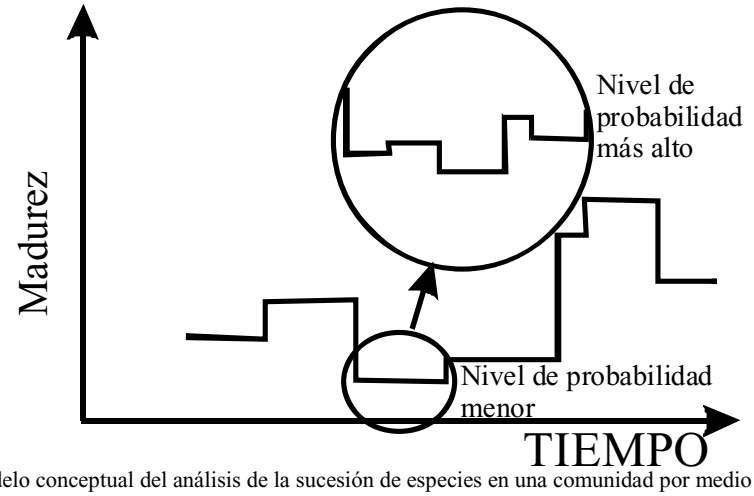

Figura 2. Modelo conceptual del análisis de la sucesión de especies en una comunidad por medio del agrupamiento cronológico. La sucesión puede ser analizada a diferentes grados de resolución, a niveles de probabilidad mayores se consigue detectar con más detalle las etapas o pasos de la sucesión (adaptado de P. Legendre, S. Dallot and L.Legendre 1985).

La teoría del nicho ecológico.

Hutchinson (1957) definió el nicho fundamental de una especie como el hipervolumen que delimita los puntos dentro del espacio ambiental multidimensional en los cuales una especie es capaz de existir de manera indefinida (Fig. 3). Debido a que los organismos de una especie actuarán de manera más eficiente y serán más abundantes en las partes optimas de su nicho, Levins (1968) sugirió que una mejor definición del nicho fundamental sería una medida del fitness de una especie en el espacio multidimensional (Fig. 3). Cuando una especie es excluida de parte de su nicho fundamental por interacciones bióticas tales como competencia, el hipervolumen reducido resultante es llamado nicho realizado. Este principio de la exclusión por competencia postulado por Hardin (1960) ha sido uno de los conceptos centrales en ecología (May & MacArthur 1972). Sin embargo, suele suceder, particularmente entre

organismos planctónicos, que especies con requerimientos similares coexisisten e incluso pueden agruparse en forma de asociaciones de especies co-ocurrentes (Fager & McGowan 1963). Hutchinson (1961) sugirió que esta observada coexistencia o aparente falsificación empírica del principio de exclusión por competencia podría explicarse como una consecuencia de la naturaleza cambiante del ambiente planctónico, la cual mantiene a la comunidad en equilibrio. La exclusión competitiva solo sería un principio aplicable cuando los cambios ambientales tardan en llevarse a cabo más

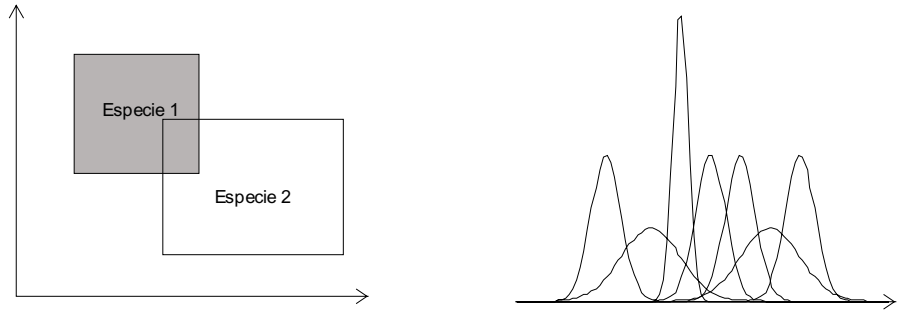

Figura 3. Representación de la teoría del nicho ecológico. Panel de la izquierda: el nicho fundamental de dos especies definido por dos variables en un espacio bidimensional. Supuestamente solo una de las dos especies es capaz de persistir en la intersección de ambos nichos fundamentales (modificado de Hutchinson 1957). Panel de la derecha: Conjunto de nichos unidimensionales: cada curva representa la función describiendo la utilización por parte de una especie de un recurso que varía de acuerdo con un gradiente representado por el eje x. (modificado de May et al. 1972).

tiempo que el periodo necesario para que una especie excluya a otra por competencia. Hutchinson (1961) sugirió que las especies que usan el mismo recurso limitante coexistirán cuando sus tiempos generacionales sean similares al tiempo que tardan en tener lugar los cambios ambientales. Los conceptos del nicho realizado y de la exclusión por competencia están en la base de cualquier estudio sobre la sucesión estacional y la estructura de la comunidad. El estudio del modo en que las diferentes especies responden a factores ambientales (el nicho realizado) debería permitir conocer que factores controlan los patrones observados en la estructura de la comunidad, las variaciones estacionales en la abundancia de las diferentes especies o los patrones biogeográficos en la distribución de cada especie. El estudio y la compresión de estos problemas debería permitirnos llegar a desarrollar modelos predictivos que puedan ser usados con posterioridad como herramientas hacia "una ecología más útil, rigurosamente científica y más informativa" (Peters 1991).

Asociaciones de especies

Una asociación de especies es un grupo recurrente de especies (Legendre & Legendre 1978) y representa un conjunto de especies que presentan respuestas similares

a las propiedades ambientales (Fager & McGowan 1963). Con el objetivo de determinar si existen grupos de apendicularias que muestran patrones de distribución espacio-temporal similares, hemos utilizado el agrupamiento por enlaces no jerárquicos desarrollado por Fager (1957). Esta técnica se basa en la formación de asociaciones de especies de acuerdo con la similitud en los patrones de presencia-ausencia de cada especie. Una vez reconocidas las diferentes asociaciones como grupos independientes, es posible relacionar las especies restantes (es decir, aquellas que no pertenecen a ninguno de los grupos detectados) con uno o varios de los grupos principales. Estas especies representan por tanto especies satélite reflejando la complejidad de la comunidad biológica (Venrick 1971; Legendre & Legendre 1998). Las especies que sean características de una etapa de la sucesión estacional deberían ser por tanto identificadas como un grupo recurrente y sus nichos superponerse de manera suficiente ya que sino no podrían ser detectadas como una asociación.

El estudio empírico de las tasas de ingestión del zooplancton.

La cuantificación del impacto herbívoro del zooplancton y la medida y desarrollo de ecuaciones para predecir sus tasas de ingestión han sido fuente de un gran número de investigaciones. Peters & Downing (1984) aplicaron técnicas de regresión múltiple para desarrollar ecuaciones que pueden ser usadas para predecir las tasas de ingestión de cladóceros y copépodos marinos. El desarrollo de estas ecuaciones requiere la previa compilación de una base de datos lo suficientemente extensa para permitir determinar que variables ambientales que explican una mayor parte de la variabilidad en las tasas medidas a diferentes escalas temporales y espaciales. La obtención de esta cobertura espacio-temporal para diferentes especies depende del desarrollo de una técnica de medida de las tasas de ingestión que sea de fácil aplicación y por lo tanto apropiada para el muestreo rutinario desde barcos. La fragilidad de las apendicularias dificulta la aplicación de los protocolos experimentales que tradicionalmente han sido utilizados con el zooplancton crustáceo. Las apendicularias son fácilmente perturbadas por las redes de plancton lo que ha causado que generalmente sus tasas de ingestión *in situ* hayan sido medidas con la ayuda de buceadores. Sin embargo, esta metodología no puede proporcional la cobertura espacio-temporal que buscamos durante este estudio. La técnica de los contenidos en pigmentos digestivos si cumple con nuestros requisitos, ha proporcionado gran parte de la información existente sobre la auto y sinecología del

zooplancton crustáceo y ha sido aplicada con existo en apendicularias (Acuña et al. 1999, Gonzalez et al. 2000). Esta técnica esta basada en la medida de la biomasa de presas autotróficas contenida en el sistema digestivo de los animales capturados *in situ* (es decir, de la cantidad de clorofila contenida en el digestivo; Fig. 4).

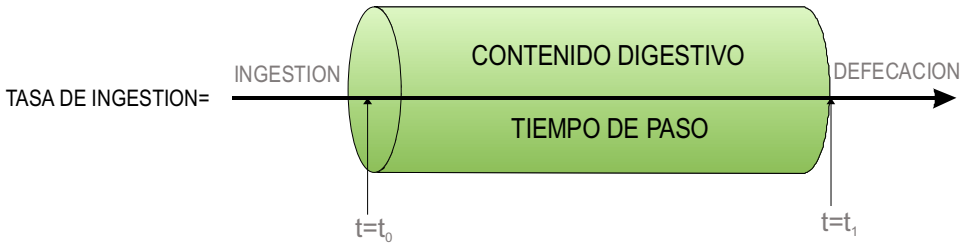

Figura 4. Representación esquemática de la técnica de los contenidos digestivos. La tasa de ingestión es calculada dividiendo la cantidad de alimento en el sistema digestivo del animal en un momento dado (el contenido digestivo) por el tiempo necesario para que una partícula de alimento pase a través del digestivo (el tiempo de paso).

Normalmente, esta técnica se aplica juntando varios animales y analizando su contenido en clorofila usando el método fluorométrico. Sin embargo, este procedimiento no permite separar el efecto de factores ambientales de efectos alométricos. La necesidad de juntar varios animales para su análisis se debe a que los niveles de detección de la técnica fluorométrica tradicional no son lo suficientemente bajos como para permitir la medida de contenidos digestivos individuales. Para conseguir medir los contenidos digestivos individuales en clorofila el nivel de detección puede ser reducido disminuyendo el volumen de extracción utilizado y analizando las muestras en tubos de ensayo especiales de un volumen reducido (Fig. 5). Otro problema

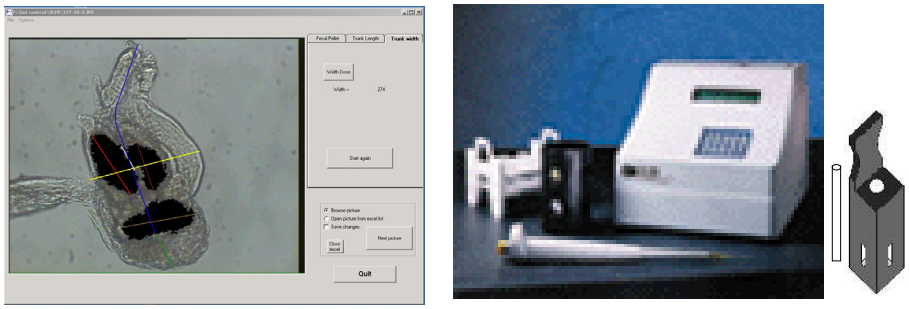

Figura 5. Las dos metodologías utilizadas para medir el contenido digestivo de las apendicularias. Panel de la derecha: software de análisis de imagen usado para medir la longitud y área de los paquetes de alimento que eran visualizados como áreas sombreadas en el interior del digestivo del animal. La longitud del tronco del animal era también medida. Panel de la derecha: fluorometro y adaptador para tubos de ensayo de pequeño volumen usados para medir el contenido en pigmentos.

con la técnica de los contenidos en pigmentos digestivos es que solo permite cuantificar la ingestión sobre presas que contengan clorofila y no sobre organismos heterotróficos o detritus (Bamstedt et al. 2000). Bochdansky & Deibel (1999) han demostrado que el volumen del material presente en el sistema digestivo de la apendicularia de aguas frías

Oikopleura vanhoeffeni puede ser usado como una aproximación para medir la cantidad total de material ingerido. Por tanto, hemos desarrollado un software de análisis de imagen para medir el volumen de alimento contenido en el interior del digestivo que permita obtener una estima del contenido total de material particulado (Fig. 5).

Para obtener una estima de la ingestión a partir de los contenidos digestivos estos tienen que dividirse por una medida del tiempo de paso del alimento obtenida experimentalmente. Sin embargo, debido a las dificultades en la recolección y experimentación con animales *in situ* explicadas anteriormente nos hemos visto obligados a utilizar un procedimiento alternativo basado en el uso de ecuaciones que permitan estimar el tiempo de paso en función de variables ambientales. Este acercamiento es una practica común en investigaciones de las tasas de ingestión en copépodos (Dam & Peterson 1988). Por tanto, hemos realizado experimentos en el laboratorio y desarrollado un método sencillo, preciso y no intrusivo que nos ha permitido medir el tiempo de paso por el digestivo y construir ecuaciones que puedan ser utilizadas para estimar el tiempo de paso en apendicularias.

La limitación por la concentración de alimento del crecimiento del zooplancton

Levins (1966) sugirió que solo dos de las tres propiedades buscadas en un modelo (generalidad, realidad y precisión) pueden ser mejoradas simultáneamente a expensas de una reducción en la importancia dada a la tercera propiedad. Por ejemplo, el modelo empírico de las tasas de filtración del zooplancton desarrollado por Peters & Downing (1984) maximiza la realidad y la precisión mientras que el modelo mecanístico de Lehman (1976) basado en teorías de forrajeo maximiza realidad y la generalidad. Este balance en las propiedades de los modelos ha llevado a dos diferentes enfoques a la hora de estudiar la importancia que la falta de alimento tiene sobre las tasas de crecimiento del zooplancton. La naturaleza de la pregunta ecológica fuerza a que ambos modelos traten de maximizar la realidad mientras que uno de los modelos sacrifica precisión el otro sacrifica la generalidad.

Modelo de balance metabólico

El primero de los enfoques es un modelo mecanístico o fisiológico y estudia la limitación por el alimento basándose en el balance metabólico del organismo. Este

acercamiento esta basado en las leyes cuantitativas generales del metabolismo y crecimiento desarrolladas en primera instancia por Bertanlanffy (1957) y adaptadas posteriormente por Huntley & Boyd (1984) a la biología particular del zooplancton. El crecimiento de un organismo, definido como el aumento en tejido somático y esfuerzo reproductor, esta determinado por la cantidad de la energía ingerida que queda sobrante una vez que los procesos metabólicos han sido satisfechos. Parte del material ingerido es asimilado mientras que los restos no digeridos son defecados (Fig. 6). De la energía asimilada una proporción servirá como combustible durante la respiración mientras que el resto es usado para el crecimiento en biomasa somática y órganos reproductores. Por tanto, un organismo morirá por inanición cuando la energía asimilada no sea suficiente

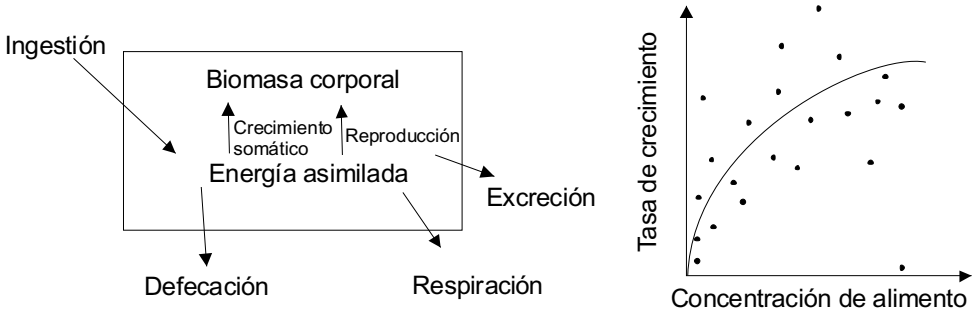

Figura 6. Los dos tipos de modelos usados en el estudio de la limitación del crecimiento del zooplancton por la falta de alimento: el modelo de balance metabólico (gráfico de la izquierda) y el modelo empírico (gráfico de la derecha).

para mantener el metabolismo basal. A concentraciones más altas de alimento la energía obtenida será mayor que el metabolismo de mantenimiento y el exceso de energía representará el potencial de crecimiento que será finalmente transformado en crecimiento una vez que los costes metabólicos del crecimiento (la acción dinámica específica o eficiencia del crecimiento) hayan sido completados. A medida que la concentración de alimento incremente más, el potencial de crecimiento también aumentará hasta un límite donde se haya alcanzado la tasa máxima de crecimiento (la cual estará determinada por el control térmico de las reacciones metabólicas). Usando parámetros, obtenidos empíricamente, que describan las relaciones existentes entre la respiración, ingestión y el límite superior para el crecimiento con la biomasa corporal, la temperatura y la concentración de alimento es posible construir un modelo para estudiar bajo qué condiciones el crecimiento estará limitado por la concentración de alimento (Huntley & Boyd 1984). Este acercamiento es por tanto un modelo mecanístico que trata de maximizar la generalidad pero a costa de una reducción en la precisión causada por el incremento en el error total del modelo inherente a la combinación de varios parámetros cada uno con su propio error (Lehman 1988).

Modelo empírico

El segundo enfoque ha sido un modelo empírico que fue desarrollado para copépodos primeramente por Huntley & Lopez (1992) y mejorado posteriormente por Hirst & Sheader (1997) y Hirst & Lampitt (1998). Esta basado en el estudio de la relación existente entre medidas de tasas de crecimiento *in situ* y factores bióticos (p.e. peso corporal) y abióticos (p.e. temperatura, concentración de alimento; Fig. 6). La no existencia de una relación entre las tasas de crecimiento y la concentración de alimento puede ser interpretada como una evidencia de que la concentración de alimento no es un factor limitante para el crecimiento del zooplancton o de que su efecto es no es significativo a la hora de explicar la variabilidad natural observada. Este tipo de modelo maximiza la precisión pero a costa de generalidad ya que no presta tanta atención a cuales son los procesos responsables de los patrones observados.

Ambos modelos posee sus propias ventajas y desventajas. En nuestro estudio en el capítulo 6 hemos utilizado ambos enfoques de manera conjunta ya que, aunque ambos tratan de responder a la misma cuestión ecológica, cada uno responde a otras preguntas interesantes. El modelo de balance metabólico permite comprender los mecanismos fisiológicos que funcionan a nivel del individuo mientras que el modelo empírico permite el desarrollo de ecuaciones que pueden ser utilizadas en combinación con medidas de biomasa poblacional para evaluar la contribución de las apendicularias a la producción secundaria del mesozooplancton.

OBJECTIVES:

Our aim with this work is to fill a gap in our understanding of appendicularian ecology through an observation and modelling approach at temporal and spatial scales that are relevant for the dynamics of the marine pelagic ecosystem. To achieve these general objective, our specific goals were:

- To compare the seasonal and spatial variability in the population densities of the appendicularian species present in European seas.

- To evaluate the *in situ* impact of appendicularians as particle grazers and secondary producers.

- To determine the environmental factors that explain most variability in their feeding rates and to develop predictive equations based on field information.

- To explore the potential importance of bottom-up control on appendicularian growth rates and population dynamics.

GENERAL METHODOLOGY.

Secondary production can be defined in a broad sense as the collective term for the processes by which animals sustain and propagate themselves (Lehman 1988). In a more restricted sense secondary production is usually interpreted as growth or increase in body mass or in reproductive output. Lehman (1988) classified ecological investigations of secondary production into three broad categories: taxonomy and zoogeography, mass flux and energetic and population dynamics and community structure. These approaches are not mutually exclusive and can often be interpreted from an interlinked perspective. Mass flux and energetic studies are usually tackled from a holistic perspective while organism-based reductionist studies are more common in population dynamics. Verity & Smetacek (1996) advocated the need for a more interlinked approach, suggesting that "it may be more valuable to understand why carbon flows where it does, rather than merely how much". The factors controlling the growth of organisms will ultimately determine the community structure and hence the functioning of the marine ecosystem, which drives the biogeochemical cycle. Finally the biogeography of different species would also be controlled by the factors regulating growth at the individual level (i.e. adaptation to different resources or environmental conditions). The approach taken in the present study is therefore probably more focused towards understanding the mechanistic factors controlling appendicularian species at the species level under *in situ* conditions. The general methodology used in our work follows three basic and important concerns that have driven some of the marine plankton research in general and zooplankton in particular over the last few decades. These three general topics are the ecological niche theory, the empirical analysis of zooplankton feeding rates and the importance of food-limitation or bottom-up control on zooplankton growth rates. Obviously we will not attempt to reinvent the wheel but try to adapt the existing methodologies and theories to the particular biological characteristics of appendicularians. Linking the results of localized field investigations to generalities about the role of specific processes is essential in ecology and comparative studies conducted at multiple locations enable the general importance of processes to be assessed (Thrush et al. 2000). We have therefore tried to apply each methodology to the field during a seasonal study carried out from March 1999 to March 2000 at four contrasting European environments: the Norwegian fjords, the western English Channel, and the Cantabrian and Ligurian Seas (Fig. 1) in a total of eight

stations. While the seasonal dynamics and factors affecting community structure was studied at all of these locations, the ingestion rates and grazing impact of different appendicularian species were investigated at a coastal station in the English Channel and on a transect of three stations across the shelf in the central Cantabrian Sea. Using

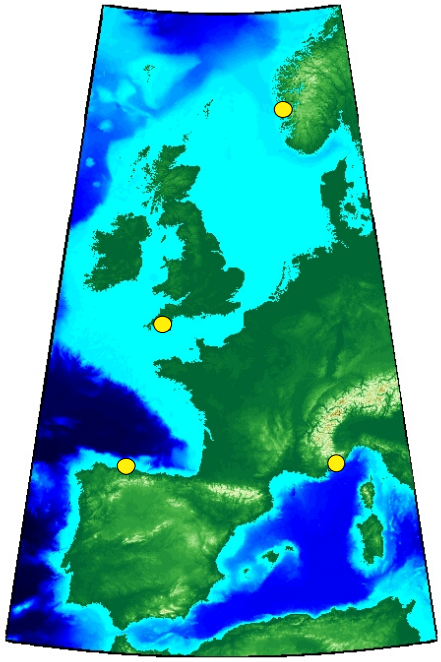

Figure 1. Map showing the locations under study: the Cantabrian Sea (NW Spain), the English Channel, the Ligurian Sea in the Mediterranean and the Norwegian fjords.

this information we have developed equations to predict appendicularian ingestion rates, and have combined these equations with an extensive review of previous reports on different aspects of appendicularian ecology to evaluate the conditions where they are likely to experience food limitation in nature. A general (and far from extensive) introduction to each of the general topics studied follows, while a more detailed explanation in the context of appendicularian ecology will be done in the subsequent methodology sections of each particular chapter.

Analysis of community structure

Each species occurs in a characteristic, limited range of habitats. The composition of biotic communities changes along environmental gradients and successive species replace each other as a function of variation in the environment or with successional time. There have been two general approaches in the study of community structure. Classification methods begin with the assumption that communities consist of relatively discrete entities while the ordination methods assume

that communities represent points along ecological continua. However, these two approaches should not be considered as opposite but they should complement one another in helping to understand the underlying structure by tackling the same question from different angles. In the chapter devoted to the study of the seasonal dynamics and environmental factors affecting appendicularian community structure, we have therefore used both ordination (non-Metric Dimensional Scaling and Principal Coordinate Analysis) and classification (clustering) techniques. The theory of the realized niche is more closely related to the existence of ecological continua and gradients (Austin 1985). The two clustering techniques used have been the chronological clustering in the study of seasonal succession and the non-hierarchical linkage clustering in the determination of recurrent groups of appendicularians.

Seasonal succession

The chronological clustering algorithm corresponds to a well defined model, in which the ecological succession proceeds by steps (Fig. 2). The output of the chronological clustering consists on a non-hierarchical partition of the seasonal series into non-overlapping homogeneous groups, which can be interpreted from an ecological perspective as successional stages. The degree of resolution in the number of stages detected can be varied modifying the connectedness (i.e. the proportion of the total number of similarities between an object or cluster and a neighbouring cluster needed to

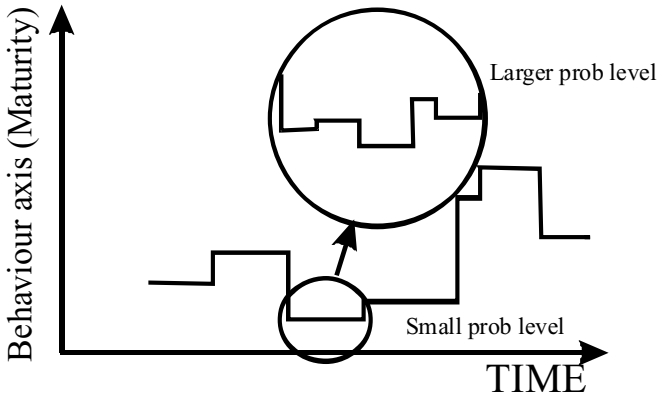

Figure 2. Conceptual basis for the analysis of the species succession within a community using the chronological clustering method. The community succession can be analyzed at different degrees of resolution, at larger probability levels the finer steps or stages become relevant. (After P. Legendre, S. Dallot and L.Legendre (1985)).

evaluate whether both groups should be fused into a single cluster) and the α-probability (i.e. the level to reject the null hypothesis that two groups should be fused together). Application of whole set of connectedness and α-probability levels allows

the most important breakpoints in the seasonal series to become obvious as those that occurred at most of the levels of resolution used (Legendre et al. 1985).

The ecological niche theory.

Hutchinson (1957) defined the fundamental niche of a species as the hypervolume that delimits the points in the multidimensional environmental space where a species will exist indefinitely (Fig. 3). Because a species will perform better and will be more abundant in optimal parts of its niche, Levins (1968) suggested that a better definition of the fundamental niche would be a measure of fitness in the

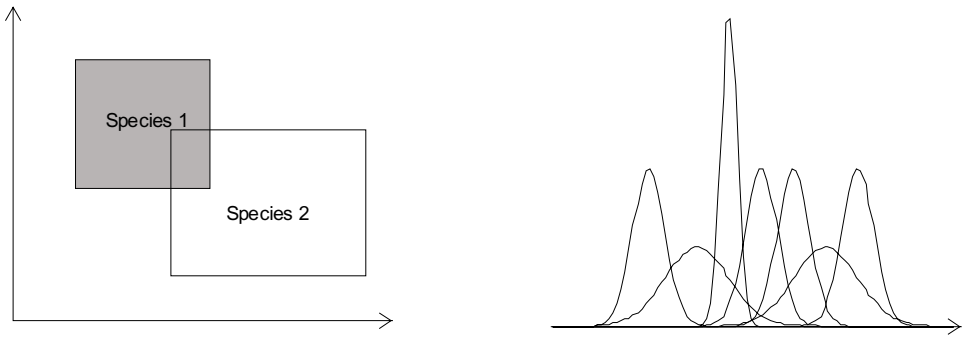

Figure 3. Ecological niche representation. Left panel: the fundamental niches of two species defined by two variables in a two-dimensional niche space. Supposedly only one species is able to persist in the intersection region. (Modified from Hutchinson 1957). Right panel: One dimensional array of niches: each curve represents the utilization function of the resource gradient represented in the x axis (Modified from May et al. 1972).

multidimensional space (Fig. 3). When a species is excluded from part of its fundamental niche by biotic interactions like competition, the reduced hypervolume is then termed the realized niche. This competitive exclusion principle postulated by Hardin (1960) has been one of the central concepts in ecology (e.g. May & MacArthur 1972). However it is often encountered, particularly amongst planktonic organisms, that different species with similar requirements coexist and can even be grouped into associations of co-occurring species (Fager & McGowan 1963). Hutchinson (1961) suggested that this observed coexistence or apparent empirical falsification of the competitive exclusion principle could be explained as a consequence of the changing nature of the planktonic environment, which keeps the community out of equilibrium. Only when the environmental change takes longer than the time needed for a species to exclude another by competition would the competitive exclusion principle be applicable. Hutchinson (1961) therefore suggested that species using the same limiting resource would coexist when their generation times are similar to the time required for

significant environmental changes. The concept of realized niche and competitive exclusion is at the base of any study on the seasonal succession and community structure. Understanding the shape and nature of the species' response (the realized niche) would allow understanding the factors controlling the observed patterns of community structure, the variations in abundance of species during the seasonal cycle (the seasonal succession) or the biogeographical patterns observed in species distributions. Ultimately this understanding should allow us to develop predictive models as future tools towards a "more rigorously scientific, more informative and more useful ecology" (Peters 1991).

Species associations

A species association is a recurrent group of co-occurring species (Legendre & Legendre 1978) and represents a group of species that have similar reactions to the properties of the environment (Fager & McGowan 1963). In order to determine whether groups of appendicularians show similar distribution patterns in space and time we used Fager (1957) non-hierarchical linkage clustering. This technique is based on the formation of species associations or recurrent group of species according to the similarity in their presence-absence patterns. In addition to the species association recognized as independent clusters, the remaining species (i.e. those that do not belong to any of the groups) can be associated to one or several of the main clusters using single linkage clustering. These species represent satellite or associate species reflecting the complexity of the biological community (Venrick 1971; Legendre & Legendre 1998). Those species characterizing a stage in the ecological succession would generally co-occur and they should therefore be perceived as associates or a recurrent group within a given taxocene (i.e. a set of species representing a taxonomic segment of a community, Legendre & Legendre 1998).

The empirical analysis of zooplankton feeding rates

The quantification of zooplankton grazing has been the subject of intensive research. Peters & Downing (1984) applied multiple regression techniques to develop equations that can be used to predict the feeding rates of cladocerans and marine calanoid copepods. The development of such predictive equations relies on the compilation of a dataset on feeding rates sufficiently large to detect the variables explaining most of the

natural variability on the measured rates at different spatial and temporal scales. Such spatio-temporal and interspecific coverage relies on a technique of easy application, and thus suitable for routine sampling from ships. The delicate nature of appendicularians represents a problem for the application of traditional experimental protocols used for crustacean zooplankton. Appendicularians are greatly disturbed by plankton nets, what

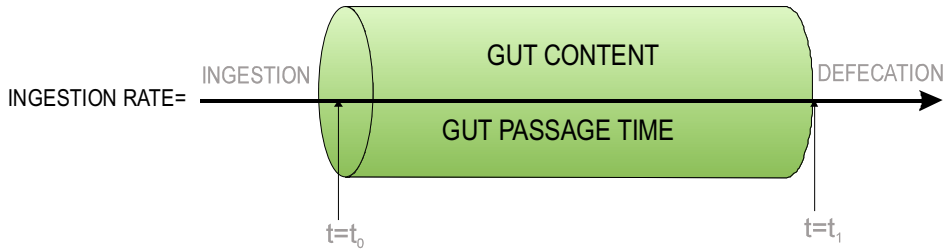

Figure 4. Schematic representation of the gut content technique. The ingestion rate is calculated dividing the amount of food in the gut of the animal at a given time (i.e. the gut content) by the time required for a food particle to pass through the gut (i.e. the gut passage time).

has caused that their *in situ* ingestion rates have been usually measured in the field with the aid of divers. However, this approach cannot provide the large spatio-temporal coverage at which our study is aiming. The gut chlorophyll content technique meets our requirements, it has provided much of the wealth of interesting auto and synecological information available on crustacean zooplankton and it has been already successfully applied to appendicularians (Acuña et al. 1999, Gonzalez et al. 2000). This technique is based on the measurement of the amount of biomass of autotrophic prey contained in the digestive system of the animals, i.e. the amount of chloropigments contained in the gut (Fig. 4). Division of gut contents by gut passage time allows calculation of the ingestion rate of autotrophic prey.

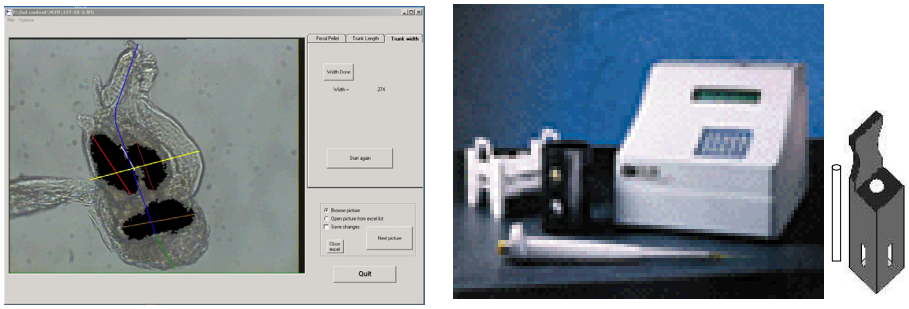

Figure 5. The two methodologies used to measure appendicularian gut contents. Left panel: image analysis software used to measure the length and area of the food parcels that were visualized as dark shades inside the gut of the animal. The animal length and width were also measured. Right panel: fluorometer and minicell adapter used to measure individual gut chlorophyll contents.

Usually, this technique is applied by pooling several animals from frozen samples collected at sea and by analysing their chlorophyll content using the standard fluorometric method. However, this approach does not allow separation of the effect of individual body size from that of environmental variables. The reason for this pooling of several animals is that the detection limits of the traditional fluorometric technique are not low enough to allow measurement of individual gut contents. To be able to measure gut chlorophyll contents at the individual level the detection limit can be decreased by reducing the extraction volume, what can be achieved by analysing the sample into special, small volume vials (Fig. 5). Another problem of the gut pigment technique is that it does only quantify the ingestion of chlorophyll bearing prey and not of heterotrophic organisms or detritus (Bamstedt et al. 2000). Bochdansky & Deibel 1999 have shown that the volume of food contained in the gut content of the cold water appendicularian *Oikopleura vanhoeffeni* can be used as a proxy for the total material ingested. We therefore have designed an image analysis software to measure the volume of food inside as an estimate of the gut content of total particulate material (Fig. 5).

The gut content is then divided by an experimentally derived gut passage time to obtain an ingestion rate estimate. However, the difficulties in experimentation with live animals explained above forced us to take an alternative approach consisting in the use of predictive equations to estimate the gut passage time as a function of environmental variables. This approach is also a common practice in copepod research (e.g. Dam & Peterson 1988). We have therefore used laboratory experimentation to develop an easy, accurate, and non-intrusive method to estimate gut passage time and to build a predictive model for the gut passage time in appendicularians.

The food limitation of zooplankton growth.

Levins (1966) suggested that only two out of three desirable properties of a model (generality, reality and precision) can be improved simultaneously while the third has to be sacrificed. For example, Peters & Downing (1984) empirical model of zooplankton filtering rates maximizes reality and precision while Lehman (1976) mechanistic model based on foraging theory maximizes reality and generality. This trade off has also lead to two different approaches when studying the importance of food limitation in controlling zooplankton growth rates. The nature of the ecological

question itself forces both approaches to try to maximize reality while one of the models sacrifices precision the other sacrifices generality.

Metabolic balance model

The first approach is a mechanistic or physiological model and studies food limitation based on the metabolic balance of an organism. This approach is based on the general quantitative laws in metabolism and growth first formulated by Bertanlanffy (1957) and later adapted to the particular biology of zooplankton by Huntley & Boyd (1984). The growth of an organism, defined as the increase in somatic tissue and reproductive output, will be determined by the amount of the energy ingested that is left after the metabolic requirements have been fulfilled. Part of the ingested food is assimilated while the non-digested remains are defecated (Fig. 6). A proportion of the energy assimilated is combusted during respiration while the rest is used to increase the biomass of somatic tissue and the reproductive organs. An organism will die of starvation when the energy assimilated is not sufficient to fulfil the basic metabolic requirements. At higher food concentrations the energy obtained is more than the basic or maintenance metabolism and the surplus energy represents the scope for growth that will be finally translated into growth once the metabolic costs of growth itself (i.e. the specific dynamic action or growth efficiency) have been fulfilled. As food concentration increases, the scope for growth would increase up to a limit where the maximum growth rate (determined in principle by the thermal control of metabolic reactions) is reached.

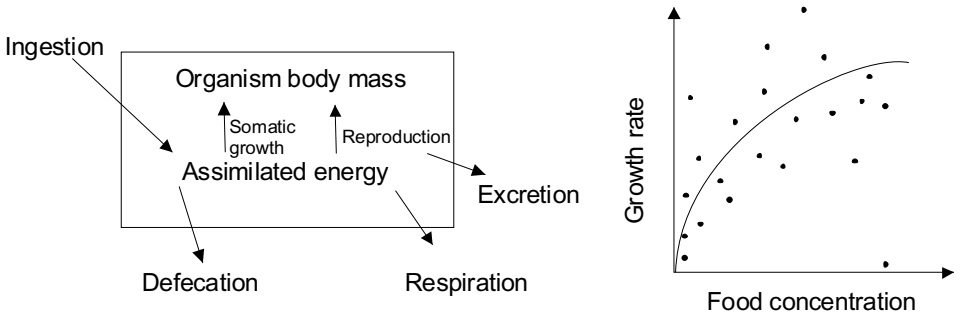

Figure 6. The two types of models used to study food limitation, the budgetary (left panel) and empirical (right panel) models of zooplankton growth .

Using empirically obtained values of the parameters describing the relationships between respiration, ingestion, assimilation and thermally controlled maximum growth with body weight, temperature and food concentration it is finally possible to build a model to study the conditions leading to food-limited growth (Huntley & Boyd 1984).

This is therefore a mechanistic approach which tries to maximize generality but at the cost of the precision due to the compounding error associated with the combination of several parameter estimates each one with its own error term (Lehman 1988).

Empirical model

The second approach has been an empirical model and was first developed by Huntley & Lopez (1992) and subsequently refined by Hirst & Sheader (1997) and Hirst & Lampitt (1998). It is based on the study of the relationship between *in situ* measured growth rates and biotic (e.g. body weight and food concentration) and abiotic (e.g. temperature) factors (Fig. 6). Therefore, if there is no relationship between the measured growth rates and food concentration, it can be interpreted that food concentration is not a limiting factor of zooplankton growth or its effect is insignificant in describing the measured natural variability. This type of model maximizes precision but at the cost of generality since it is not concerned about the underlying reasons for the patterns observed.

Both types of model have their own pros and contras. We have taken both approaches in parallel since, although both address the same ecological question, each one responds to other interesting ecological questions. The budgetary model allows understanding of the mechanisms working at the individual level while the empirical approach permits the development of predictive equations that can be used in combination with biomass measurements to estimate the contribution of appendicularian populations to total mesozooplanktonic secondary production.

3. Comparación de los ciclos estacionales de abundancia de apendicularias en cuatro ecosistemas costeros europeos

A comparison of appendicularian seasonal cycles in four contrasting European coastal environments

Angel Lopez-Urrutia, R. P. Harris, J. L. Acuña, U. Bämstedt, P. R. Flood, H. J. Fyhn, B. Gasser, G. Gorsky, X. Irigoien and M. Martinussen

(last eight authors in alphabetical order)

ABSTRACT

The European Union project EURAPP ("Impact of appendicularians in European marine ecosystems") represented an integrated European effort to elucidate specific biological and ecological aspects of appendicularians in the marginal seas of Europe. Within EURAPP, we have studied the seasonal variation in population densities and species assemblages from March 1999 to February 2000 in four contrasting European coastal environments: the Norwegian fjords, the western English Channel, and the Cantabrian and Ligurian Seas. The seasonal succession in the structure of the appendicularian community can be summarized into two distinct phases: a winter-early spring phase characterized by the presence of fritillarians prior to the onset of stratification or warming of the water column at the mixed water locations and a summer-autumn oikopleurid-dominated community. This summer phase can be subdivided into other two or three sub-steps depending on the dominant oikopleurid species. There was a positive relationship between the abundance of total appendicularians and chlorophyll concentration and a strong geographical influence on species composition, which was related with temperature. Three different appendicularian species associations were detected; the niche of each individual species was characterized by a unimodal response to temperature. Differences in temperature and to some degree in salinity explained to a considerable extent the seasonal and geographical distribution patterns detected. The close relationship between appendicularian species assemblages and physical environmental factors suggests their potential use as indicator species of climate changes or characteristic water masses.

INTRODUCTION

Appendicularians have received increasing attention by marine plankton ecologists due to their fast growth rates (e.g. Hopcroft et al. 1998), their ability to feed directly on small particles (e.g. Deibel & Lee 1992), their potential contribution to the biological pump through the excretion of filter houses and fecal pellets (e.g. Sato et al. 2001) and their contribution to the diet of the larvae of some commercially important fish species (e.g. Shelbourne 1962). Most recent studies have focused on their ecophysiology (e.g. Bochdansky & Deibel 1999, López-Urrutia & Acuña 1999, Acuña & Kiefer 2000). To translate physiological responses to population impact we need to multiply individual rates by abundance or biomass estimates and the variability of the latter is usually larger (e.g. Huntley 1996). The factors controlling the abundance and distribution of appendicularians are likely to be strong determinants on their potential role in marine ecosystems and are probably even less understood than the factors controlling their physiological rates.

There is already some information on the environmental factors controlling the seasonal and vertical changes in appendicularian species abundance and composition (e.g. Fenaux 1963, Shiga 1985, Acuña & Anadón 1992, Acuña et al. 1995). Although these studies have greatly improved our understanding of the seasonal dynamics of the appendicularian community their scope has been limited to the environmental

conditions and species present in each specific area. The European Union project EURAPP ("Impact of appendicularians in European marine ecosystems") represented an integrated European effort to evaluate the role of appendicularians in the marginal seas of Europe. Here, we present the analysis of EURAPP field work on the seasonal cycles of appendicularians at four contrasting European coastal environments:

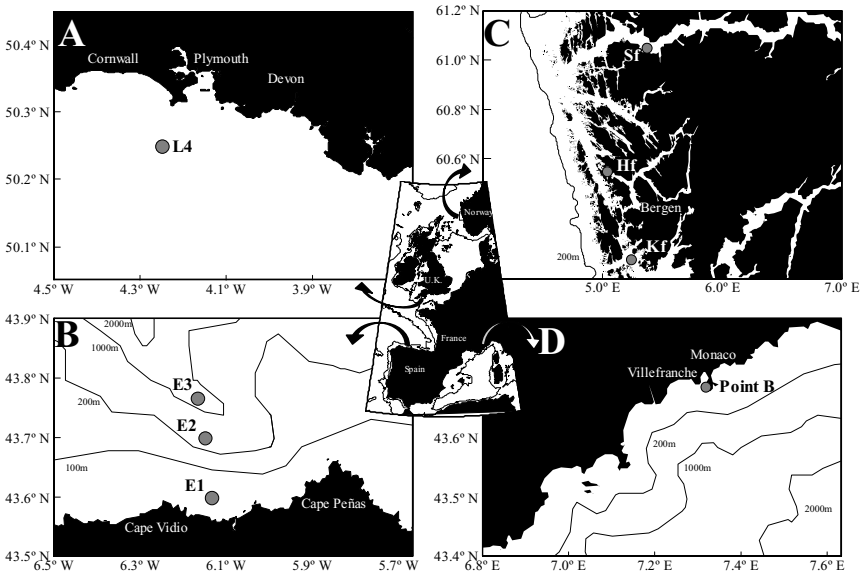

Figure 1. Map showing the study sites. (**A**) Station L4 in coastal waters of the English Channel, approximately 55 meters water depth. (**B**) Stations E1, E2, E3 in the Cantabrian Sea (NW Spain) 50, 120 and 1000 meters deep respectively. (**C**) Stations located in the Norwegian fjords: Sognefjörden (Sf), Herdlefjörden (Hf), and Korsfjörden (Kf) with main basin depths of 1250, 680 and 275 meters. (**D**) Point B in coastal waters of the Ligurian Sea at the entrance of the Bay of Villefranche with a water column depth of approximately 80 meters.

the Norwegian fjords, the western English Channel, and the Cantabrian and the Ligurian Seas. This paper represents an attempt to present from a comparative perspective the results obtained at each individual location in order to summarize the general changes in appendicularian seasonality and community succession. The wide range of environments covered would allow us to determine the role of environmental variables in shaping the niches of the different species and to provide a first evaluation on the concept of species associations within the appendicularian community.

MATERIALS AND METHODS

Data collection

Samples were collected from March 1999 to February 2000 at four European coastal environments at a total of eight stations: three Norwegian fjords (Herdlefjorden, Korsfjorden and Sognefjorden; Fig. 1 C), a coastal station in the western English

Channel off Plymouth (L4; Fig. 1 B), three stations on a transect across the shelf in the Cantabrian Sea (E1, E2 and E3; Fig. 1 A) and a station at the entrance of the Bay of Villefranche in the Ligurian Sea (Point B; Fig. 1 D). Although there is good overlap in the periods covered at each location, the sampling schedule varied between the different sites both in temporal coverage and frequency of sampling. Three stations were sampled on a weekly (Herdlefjorden, L4 and Point B), one on a biweekly (Korsfjorden) and four on a monthly basis (E1, E2, E3 and Sognefjorden). Sampling at Point B was carried out from January to December 1999 while at the stations in the Cantabrian Sea it was continued until May 2000.

The sampling protocols varied between sites, partly due to the need to maintain consistency with the long term plankton monitoring programmes at each location and also because the specific requirements of the particular physio-ecological studies carried out at each station. Appendicularian abundance and species composition were estimated from vertical hauls using a WP-2 net fitted with a 200 µm mesh size in the Cantabrian, Ligurian and English Channel stations and with a 90 µm mesh pore size at the Norwegian fjords. At Point B in the Ligurian Sea a modified large-volume non-filtering collector was used instead of the standard WP-2 codend. The vertical extent of the water column sampled was also different: down to 30 meters at Point B, 50 meters at L4 and E1, 100 meters at E2 and E3, and down to the basin at the stations in the Norwegian fjords (~275 meters in Herdlefjorden, ~680 meters in Korsfjorden and ~1250 meters in Sognefjorden). At Korsfjorden and Sognefjorden samples were depth-specific consisting of a surface tow covering the upper surface layer above the pycno or thermocline and then tows at depth intervals of 200 or 400 meters respectively down to the fjord basin. Samples were preserved in buffered glutaraldehyde in the Norwegian fjords and Ligurian Sea and flash frozen into liquid nitrogen at the Cantabrian and English Channel stations. Samples were counted and species identified by different scientists at each location. Although this is likely to introduce some inconsistencies in the data due to differing expertise, all the analysts were trained by Dr. Robert Fenaux during the First Workshop on the taxonomy of Appendicularia held in Villefranche during 1999.

In addition to the abundance and species composition estimates, at stations L4, E1, E2, E3, Sognefjorden and Korsfjorden, the trunk lengths (from mouth to upper

gonad end) of up to 30 individuals of each species present at a given date were measured to determine the species-specific size structure of the appendicularian population.

Water collected using 5-L Niskin bottles was filtered onto 25 mm GF/F filters, extracted in acetone and used for determination of chlorophyll concentration using the standard fluorometric procedure (U.S. Environmental Protection Agency Method 445.0). The depths sampled were: 0, 10, 20 and 30 meters at the Norwegian fjords, 10 meters at station L4, and 0, 10, 20, 30, 40, 50, 75 and 100 meters (or down to the bottom at the shallower stations) in the Cantabrian and Ligurian seas. At each location vertical profiles of temperature and salinity were obtained using a Sea Bird Instruments CTD in the Cantabrian and Ligurian Sea, a CTD probe developed for the Undulating Oceanographic Recorder (Aiken & Bellan 1990) in the English Channel and a miniCTD-204 in the Norwegian fjords.

Data analysis

The differences in the depth of the water column sampled and the mesh size used at each location cautions against making conclusive statements on the relative number of appendicularians collected at each location. To alleviate these differences and since almost all species were mainly present in the surface layer (see Chapter 5; Bamstedt et al. in preparation) appendicularian counts were transformed to number of individuals per square meter. Comparative plots of the seasonal changes in the abundance of the two major families of appendicularians (Oikopleuriidae and Fritillariidae) and the seasonal changes and vertical structure of temperature, salinity and chlorophyll concentration were examined to describe the general seasonal patterns at each location. The effect of the mesh size used on the appendicularian abundances recorded at each location was examined by comparing the size frequency distributions of the species which were present in the samples collected with both types of mesh. After this first evaluation, the data were analysed as described bellow for four specific purposes: to describe the seasonal succession of species within the appendicularian community, to evaluate the relationship between the major differences in the species composition at each location and their geographical position, to determine recurrent groups of appendicularian species and to describe the niches of each particular species.

Seasonal succession of species

At each location the seasonal succession of appendicularian species was examined using the chronological clustering method described in Legendre et al. (1985). This multivariate technique introduces a temporal contiguity constraint in the clustering algorithm in order to take into account the time sequence of sampling and is well suited to detect discontinuities in a time series and therefore to characterize the successional steps within a community (analyses were performed using the software CHRONO in the freely distributed R package by Legendre & Vaudor 1991). The output of the chronological clustering consists of a non-hierarchical partition of the seasonal series into non-overlapping, homogeneous groups, which can be interpreted from an ecological perspective as stages of a succession. The number of steps detected depends on arbitrary choices of the connectedness (i.e. the proportion of the total number of similarities between an object or cluster and a neighbouring cluster required to evaluate whether both groups should be fused into a single cluster) and α-probability levels (i.e. the probability to reject the null hypothesis that two groups should be fused together). The clustering routine was tried with a whole set of connectedness and probability levels and the most important breakpoints in the seasonal series then became obvious since they occurred at most of the levels of resolution (see Legendre et al. 1985 for a detailed methodological description). The choice of an index of similarity is a critical step in any multivariate analysis. For the chronological clustering analysis we selected the Bray-Curtis similarity coefficient (also known as Odum's or Steinhaus' coefficient, Legendre & Legendre 1998) that tends to give more importance to the most abundant species which are better sampled and generally also better identified. To counterbalance this tendency, we performed a squared root transformation of the species abundance, what down-weights the importance of the very abundant species so the less numerically dominant still play some role in determining the similarity. After the chronological clustering was performed and the major successional steps determined, the species that characterizing each stage were identified as those which together contribute more than 90% to the average similarity within each succession stage. This was evaluated using the similarity breakdown implemented in the PRIMER (Plymouth Routines In Multivariate Ecological Research) program SIMPER (similarity percentages, Clarke & Warwick 1994).

Geographical influences on appendicularian species composition.

The average number of individuals of each species was calculated for each site, square root transformed and standardized in order to remove the differences between locations in the total number of individuals collected. A measure of the similarity in the species composition between each pair of locations was then calculated using the Bray-Curtis similarity index. This similarity matrix was then analysed in two ways. First, a Non-Metric Multidimensional Scaling (MDS) was used to represent in a two dimensional space the distances between locations based on their respective species composition. Secondly, Mantel statistics were calculated to evaluate whether the distances between locations as determined by their species composition could be better explained by their geographic distances, by the similarity in their average temperature, salinity or chlorophyll concentration or by any combination of them. The Mantel statistic is basically a correlation coefficient between two similarity (or distance) matrices. In a similar way as a standard correlation coefficient or r^2 value give an estimate of the degree of relationship and proportion of explained variance between a dependent and independent variable, the Mantel statistics provides a measure of correlation but instead of between variables between similarity matrices. Accordingly, it is possible to select the descriptor or combination of descriptors that explain greater proportions of variance in the species composition similarity between sites.

Appendicularian species associations

A species association is a recurrent group of co-occurring species (Legendre & Legendre 1978) and represents a group of species that have similar reactions to the properties of the environment (Fager & McGowan 1963). In order to determine whether groups of appendicularians show similar distribution patterns in space and time we used Fager's (1957) non-hierarchical linkage clustering. This methodology was selected instead of the probabilistic clustering of Clifford & Goodall 1967 (see Legendre 1973 for an example) because it is based on presence and absence data, which was found more appropriate to take into account the variable effect of rare species. Krylov (1968) χ^2 probabilistic similarity index was used to calculate the level of affinity between each pair of species. The similarity between the species within a group had to be highly significant in order to accept formation of an association (χ^2 probability > 0.999). To evaluate the relationship between different environmental variables (temperature,

salinity and chlorophyll concentration) and the similarities between the presence-absence patterns of the different species, Mantel statistics (as described above) were calculated between the species χ^2 similarity matrix and similarity matrices based on the temperature, salinity and chlorophyll concentrations where each species was present.

Appendicularian species niche.

The concept of the niche used in this paper is based on the fundamental niche formalisation by Hutchinson (1957), as the hypervolume which defines the region in the multidimensional environmental space where a species will exist indefinitely. When a species is excluded from part of its fundamental niche by biotic interactions like competition, the reduced hypervolume is then termed the realized niche. Hutchinson (1957) recognized the limitation of his definition stating that "there will be however an optimal part of the niche with markedly suboptimal conditions near the boundaries", what lead Levins (1968) to define the niche as a fitness measure on an environmental space. Consequently, the term realized niche has been refined in the context of predictive habitat distribution modelling in terrestrial ecosystems as the qualitative response of the abundance of a species to environmental gradients (e.g. Austin et al. 1990). These distribution modelling studies use powerful statistical techniques to develop equations to predict the habitat distribution of a species (see review of methods in Guisan & Zimmermann 2000). However, the resulting predictive equations are difficult to interpret ecologically. Our intention was not to develop predictive equations but to describe the shape of the response of the abundance of individual appendicularian species to environmental properties. Therefore, we have used a different technique to parameterize the niche of each independent species. Considering a one-dimensional niche with temperature as the environmental gradient, there is usually a unimodal numerical response in the abundance of each appendicularian species (see Results and Essenberg 1922, Acuña & Anadón 1992). Therefore if we plot the average abundance of a species found at different temperature intervals, the bar chart obtained will resemble a probabilistic frequency distribution. This resemblance between the one-dimensional niche and a probabilistic function was first used by May & MacArthur 1972 who assumed in their theoretical analysis that resource utilization is normally distributed along the resource axis. However, a Gaussian response is not always the best approximation to the niche shape and it is often found that the response is skewed or

even bimodal. Therefore, we have used Johnson's (1949) systems of frequency curves to fit a curve to the abundance vs. temperature "histogram" of each species. Johnson's method is based on the fact that most probability frequency distributions can be transformed into a Gaussian distribution using the first four moments in the observed data (i.e. the mean, variance, skewness and kurtosis, for a detailed description of the method, see Johnson 1949, Elderton & Johnson 1969). Therefore the temperature niche of each species was parameterised using the mean (the optimal temperature), the variance or standard deviate (a measure of the niche breadth or eurythermality of that species) and the skewness and kurtosis (the asymmetry and peakedness of the niche shape around the optimum). The effect of other environmental variables in shaping the niche of each species was determined using this technique both on the raw data and on the residuals of the fitted temperature frequency distribution. The interaction between environmental variables in the determination of the niche was examined by two dimensional contour density plots of the appendicularian abundance as a function of combinations of environmental variables. The contour plots were calculated using a squared inverse distance interpolation.

RESULTS

Description of the environments studied.

The stations studied covered a wide range of environmental conditions from the nutrient poor and relatively warm station in the Ligurian Sea with chlorophyll concentrations lower than 1 mg m^{-3} throughout the year to the colder water stations in the Norwegian fjords where the spring phytoplankton bloom can reach chlorophyll concentrations of up to 15 mg m^{-3}.

The fjords are characterized by a strong halocline during most of the year in the upper water column related to river discharge (Fig. 2 E). In addition, a thermocline develops during spring and summer (approximately from May to November, Fig. 2 D). Sognefjorden ca. 80 km north of Bergen, is the deepest fjord in Norway, with the depth of the main basin reaching 1200-1300 m. Korsfjorden, just south of Bergen, consists of two arms, the outer one running WSW to the open sea, the inner one running SSE and connects to the outer open part of Hardangerfjorden by a sill at 450 m. The main basin is ca. 680 m deep and the sill towards the open coast in the west is 180 m.

Herdlefjorden, just north of Bergen, has the shallowest sill of the fjords studied with a depth of approximately 275 m and, compared to the other two fjords, is a more enclosed system.

In contrast, tidal mixing prevents any strong stratification during most of the year at station L4 (Fig. 2 D and E), which is located in coastal waters off Plymouth in the English Channel with a bottom depth of ca. 50m. There is a strong phytoplankton bloom in April-May dominated by diatoms and flagellates with chlorophyll concentrations up to 10 mg m^{-3} (Fig. 2 C) and also a summer dinoflagellate bloom dominated by *Gyrodinium aureolum*.

Stations E1, E2 and E3 in the Cantabrian Sea are located along a coast-ocean transect across the continental shelf (Fig. 1 B). Temperatures at these stations are higher than in the fjords and station L4 while chlorophyll concentrations are lower with maximum values close to 3 mg m^{-3} (Fig. 3 D and C). Thermal stratification starts in May and is less pronounced at the more coastal station E1.

Point B in the Ligurian Sea (NW Mediterranean, Fig. 1 D) is the most oligotrophic of the stations studied with chlorophyll concentrations remaining low throughout the year with reduced seasonal variation. Temperature reaches 26ºC at the surface during the period of stratification, which lasts from March until November (Fig. 3 D), and salinities are higher than at the other stations studied (Fig. 3 E).

Total appendicularian abundance.

The number of appendicularians collected was generally higher in the Norwegian fjords and Cantabrian sea, total appendicularian abundance at station L4 and Point B was relatively low (Fig. 2 A and 3 A, note different scale axis for each station). There was no correlation between total abundance and environmental temperature (Fig. 4 A) but there was a positive relationship between the abundance of appendicularians and chlorophyll concentration ($p<0.001$, $r^2=0.2$; Fig. 4 B). These statistics should be viewed with caution since they have not been corrected for the presence of autocorrelation in each individual seasonal series. The temporal autocorrelation (i.e. the correlation of a time series with itself) implies that each new value in the time series

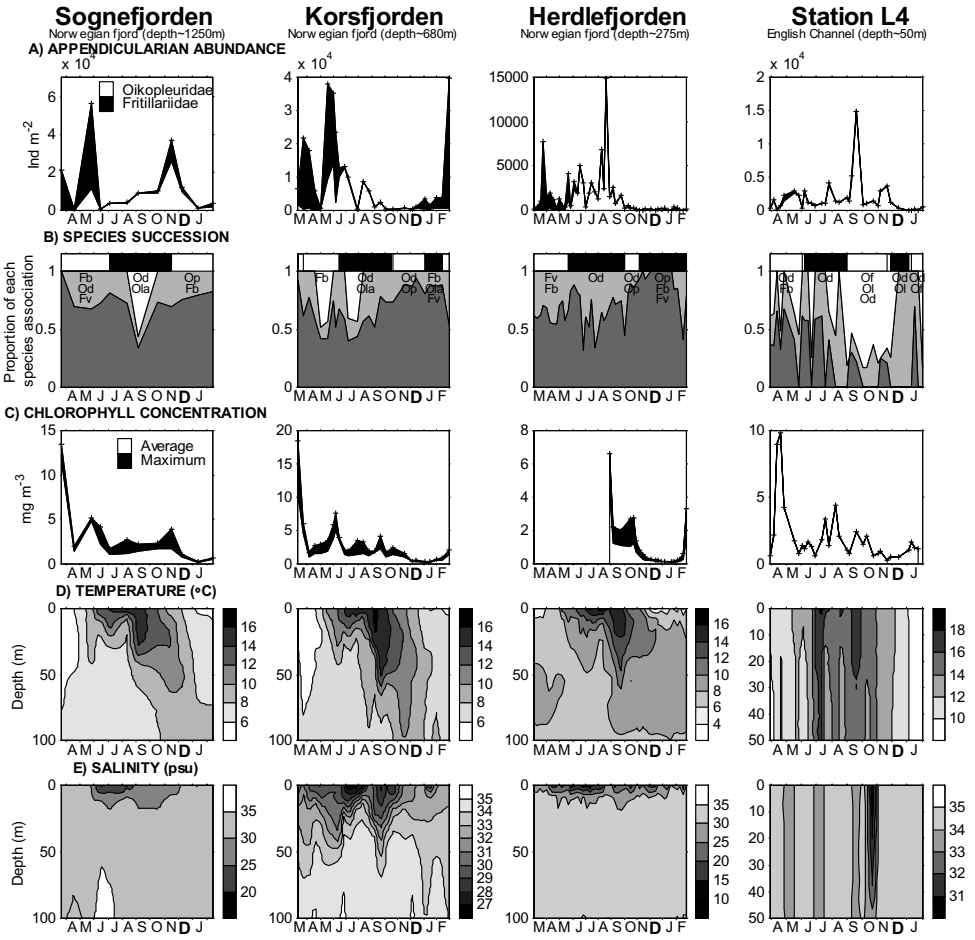

Figure 2. Seasonal variation at the stations in the Norwegian fjords and English Channel of (**A**) appendicularian abundance, (**B**) appendicularian species succession, **top black and white rectangles**: interval graph representing the discontinuities in the species seasonal succession (change in colour of the rectangles) as depicted by a chronological clustering on the Bray-Curtis similarity matrix calculated on the square root transformed species abundances, letters below each rectangle indicate those species which together contribute more than 90 % to the average similarity within each succession step (Fb, *Fritillaria borealis*; Od, *Oikopleura dioica*; Fv, *Fritillaria venusta*; Ola, *Oikopleura labradoriensis*; Op, *Oikopleura parva*; Of, *Oikopleura fusiformis*; Ol, *Oikopleura longicauda*, Fp, *Fritillaria pellucida*), **lower graph**: changes in the proportion of each species association (dark grey areas: *A.sicula*, *F.polaris*, *F. borealis*, *O.labradoriensis*, *O.parva* and *O.gorskii* group; light grey areas: *F.venusta* and *O.dioica* association and white areas: *F.formica*, *F.pellucida*, *O.cophocerca*, *O.fusiformis* and *O.longicauda* group), (**C**) average and maximum chlorophyll concentration over the upper 50 meters of the water column, (**D**) temperature and (**E**) salinity vertical contour plots. Note different x and y axis limits for each location, December 1999 is shown as a bold D.

doesn't represent a full degree of freedom since samples are not statistically independent (i.e. from the previous values in the time series we already have some knowledge of what the next value is likely to be). This autocorrelation results in underestimation of the p-value and overestimation of the r^2. Nevertheless, most of the relationship between the total abundance of appendicularians and chlorophyll concentration is due to the differences between locations (Fig 2 and 3 A and C) and therefore unaffected by autocorrelation within each time series but subject to the lack of methodological standardization in the sampling protocols.

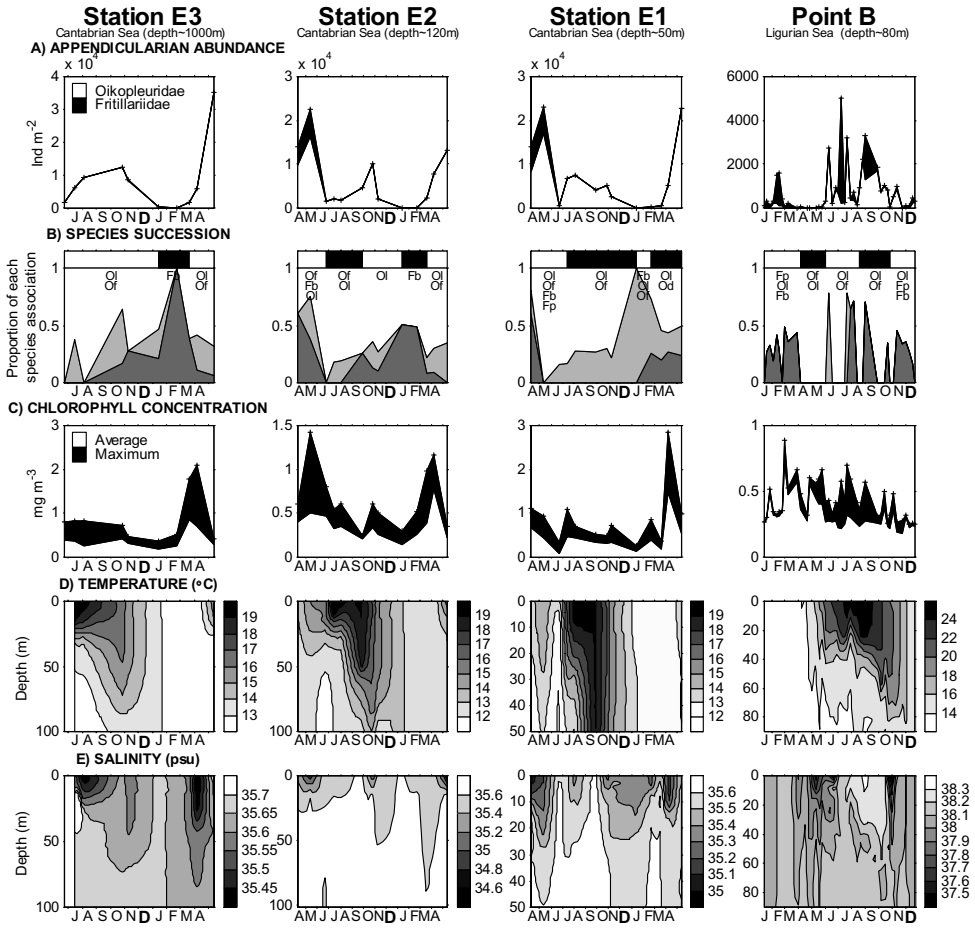

Figure 3. Same as Figure 2 but for the stations in the Cantabrian and Ligurian Sea.

Mesh size influence on appendicularian species size frequency distribution.

Each type of mesh was used at a different location and therefore the present analysis can not provide a direct comparison on the relative collection efficiency of each net type. Nevertheless, it should provide an indication of the consequences that the lack of standardization in the sampling design could have in the comparison of abundance estimates between locations. Both mesh sizes used resulted in an underestimation of the smaller size classes, what could explain the unimodal size frequency distribution of the appendicularian species collected (Fig. 5). If we accept that the mode in the histogram represents the minimum appendicularian size that is sampled efficiently, our data suggest that animals larger than 300 and 400 µm in trunk length were collected efficiently by the 90 (used in the Norwegian fjords) and 200µm mesh (used at the rest of locations) respectively, although these values varied between species probably due to

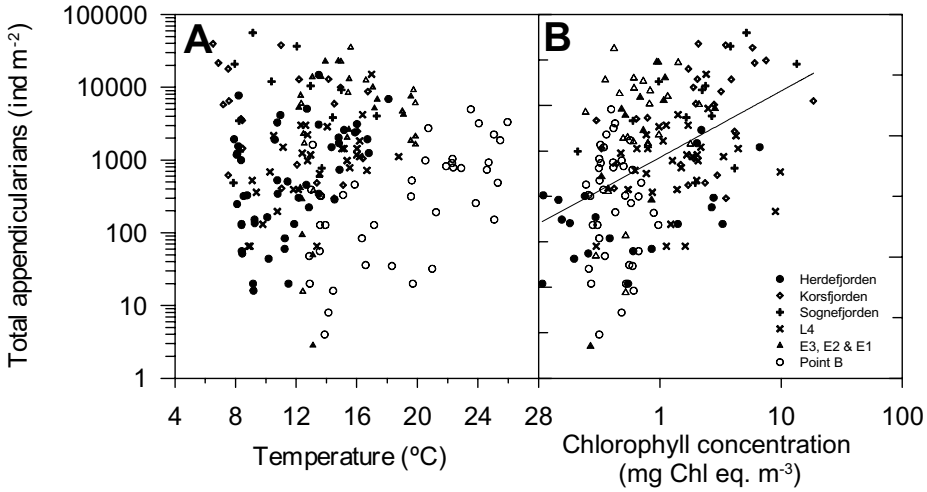

Figure 4. Relationships between total appendicularian abundance and (**A**) temperature and (**B**) chlorophyll concentration. Appendicularian abundance values represent the vertical integrated abundance over the whole water column sampled. Temperature and chlorophyll concentration represent the average and maximum over the water column sampled. Line in B shows the log-log least squares regression line ($\log_{10}$(Abundance)=3.03+0.913*$\log_{10}$(Chlorophyll), n=167; $F_{165,1}$=41.5; p<0.001, r^2=0.2, Note that the degrees of freedom could not be corrected for the autocorrelation at each station and therefore r^2 and p value could be erroneous).

the different trunk length morphologies (Fig. 5). Only sufficient numbers of *Oikopleura dioica* and *Fritillaria borealis* were measured with both types of meshes. At the Norwegian fjords, where temperatures are lower and therefore appendicularians larger,

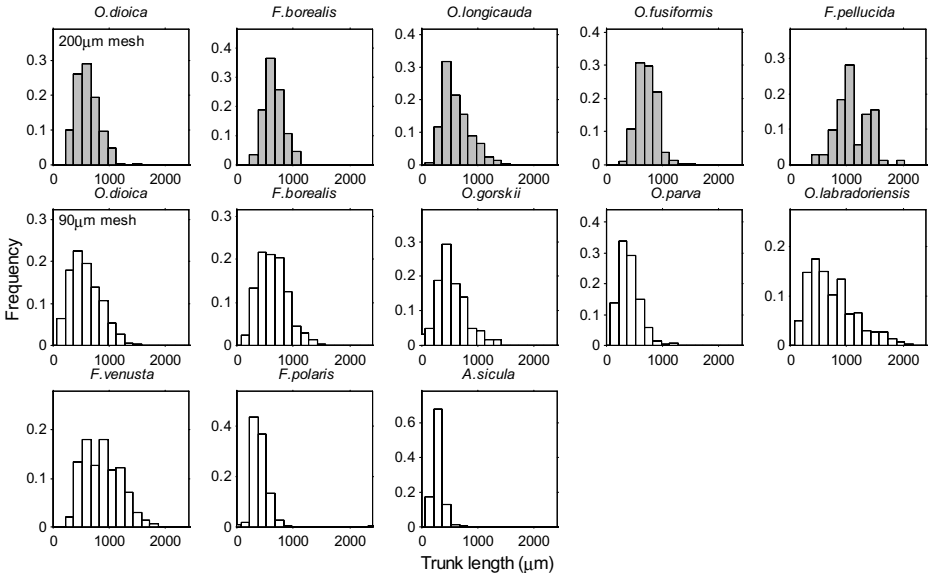

Figure 5. Size frequency distribution of the different appendicularian species. Data were separated depending on whether samples were collected using a 200µm (grey filled histograms, stations L4, E1, E2 and E3) or a 90µm (white filled histograms, Korsfjorden and Sognefjorden) WP-2 net. Only the histograms for those species where at least 60 individuals were measured are shown. To calculate the histogram frequencies, data were binned into 100µm trunk length (mouth to upper gonad end) intervals starting at 50µm.

the 90µm mesh collected a larger proportion of smaller appendicularians although still underestimating the population. Therefore there is a general bias towards an

underestimation of the total number of appendicularians, this bias being slightly less pronounced at the Norwegian fjords stations.

Seasonal succession of the appendicularian community.

The chronological clustering and similarity percentage analysis show that the appendicularian community seasonal succession can be summarized into two or three general stages. The first successional stage is characterized by the presence of fritillariids during the winter and spring months prior to the onset of stratification or warming of the water column at the mixed locations (Fig. 2 A & B and 3 A & B). In this first stage *Fritillaria borealis* was common to all the different locations along with *F.venusta* in the fjords or *F.pellucida* at the warmer locations (Fig. 2 A and 3 A). However, the degree of dominance of fritillariids during the winter months varied between stations and was stronger in the fjords (Fig. 2 A). A second successional stage is characterized by the dominance of oikopleuriids during the summer and autumn months (Fig. 2 A & B and 3 A & B). This main stage is subdivided into other two or three sub-stages depending on which species dominates (Fig. 2 A & B and 3 A & B). The summer-autumn stage was dominated by *Oikopleura longicauda* and *O.fusiformis* at the more temperate stations (E3, E2, E1 and Point B, Fig. 3 A) and by *O.dioica*, *O.parva* and *O.labradoriensis* in the fjords, with station L4 in an intermediate position where *O.dioica*, *O.longicauda* and *O.fusiformis* contributed more to the similarity within the autumn and summer successional stages.

Geographical influences on appendicularian species composition.

Table 1 shows the average percentage contribution of each species to the total number of appendicularians collected at each site. The number of species identified in samples from station L4 and the Cantabrian Sea is much lower (i.e. higher number of species with zero percentage) than in the fjords and Villefranche where the taxonomic expertise was higher. However it should also be noted that the species not recorded at stations L4, E3, E2 and E1 are generally present in either the fjords or Villefranche but usually not at both places, and that although some species could be misidentified other are morphologically distinctive enough to at least have been recognized as different species than the ones usually present. The differences between locations in the species which characterize each successional stage mentioned in the previous section, already

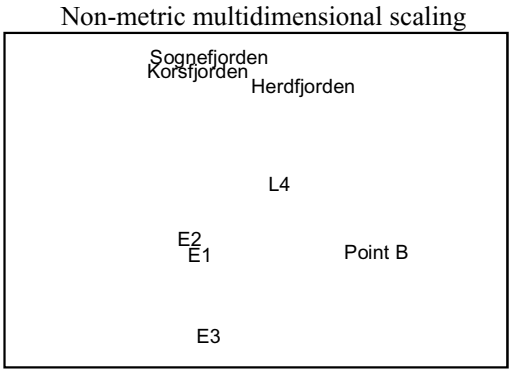

Figure 6. Non-metric Multidimensional Scaling on the Bray-Curtis similarity matrix based on the root transformed average species abundance at each location.

point to a geographical effect on the species composition. This is reflected by the Non-metric Multidimensional Scaling (nMDS) analysis performed on the species similarity between locations that resembles the geographical position of the stations (Fig. 6). The stress value was smaller than 0.01 indicating an almost prefect representation since the numerical iteration procedure used terminates when stress reduces below this value (MDS routine in PRIMER). Since there could be some methodological error in the pattern observed reflecting the differing degree of taxonomic expertise of the analysts who counted the samples at each location, we repeated the nMDS analysis using only those species that made up more than 90 % of the total appendicularian numbers and therefore less subject to misidentification (i.e. the eight first species in table 1). The

Table 1. Percentage contribution of each species to the total number of appendicularians collected at the different locations.

	Herdle-fjorden	Sogne-fjorden	Kors-fjorden	L4	E3	E2	E1	Point B	All locations
Oikopleura longicauda	0	0	0	14.52	54.45	48.80	54.26	43.65	26.96
Oikopleura dioica	36.44	25.05	27.19	61.29	0.13	0.64	12.24	0.05	20.38
Oikopleura fusiformis	0	0	0	13.60	34.77	45.30	28.27	19.28	17.65
Fritillaria borealis	16.75	35.55	23.38	10.59	10.58	5.14	3.20	4.28	13.68
Oikopleura parva	9.94	3.57	19.60	0	0	0	0	0	4.14
ikopleura labradoriensis	0.22	15.28	7.84	0	0	0	0	0	2.92
Fritillaria venusta	11.21	5.95	5.11	0	0	0	0	0	2.78
Fritillaria pellucida	0	0.30	0.02	0	0.07	0.11	2.04	18.02	2.57
Fritillaria polaris	5.23	6.02	1.62	0	0	0	0	0	1.61
Oikopleura gorskii	9.31	1.15	1.84	0	0	0	0	0	1.54
Fritillaria formica	0	0	0	0	0	0	0	8.67	1.08
Appendicularia sicula	3.28	1.66	2.44	0	0	0	0	0.19	0.95
Oikopleura cophocerca	0	0	0	0	0	0	0	4.22	0.53
Kowalevskia oceanica	0	0.01	0	0	0	0	0	0.77	0.10
Oikopleura albicans	0	0	0	0	0	0	0	0.43	0.05
Fritillaria haplostoma	0	0	0	0	0	0	0	0.19	0.02
Oikopleura vanhoeffeni	0	0.08	0.00	0	0	0	0	0	0.01
Fritillaria gracilis	0	0	0	0	0	0	0	0.07	0.01
Fritillaria tenella	0	0	0	0	0	0	0	0.07	0.01
Oikopleura rufescens	0	0.01	0	0	0	0	0	0.06	0.01
Fritillaria aequatorialis	0	0	0	0	0	0	0	0.04	0.01

resulting nMDS representation was very similar (not shown) indicating that the pattern detected is unlikely to be a spurious result due to lack of standardization.

According to the Mantel statistics the geographical distances explained 37.6% of the similarity in the species composition between sites while temperature explained 34.5% and neither chlorophyll or salinity nor any combination of the descriptor similarity matrices improved the variance explained. A partial Mantel test (similar to partial correlation coefficients) showed a strong intercorrelation between the geographical distances and temperature indicating that the geographical component is due mainly to temperature differences between stations.

Appendicularian species associations

Three different appendicularian species associations were detected by the non-hierarchical linkage clustering (Fig. 7). Most of the similarity in the presence-absence of the different species could be explained by temperature (44% of the variability as indicated by a Mantel statistic). The variance explained increased to 47% when including salinity and to 48% when including chlorophyll concentration. The relationship between the presence of each species and environmental temperature is summarized in Figure 7 B. The three main groups consisted of a warm water association (*F.formica, F.pellucida, O. cophocerca, O. fusiformis* and *O. longicauda*), a cold water association (*O.labradoriensis, O. parva, O. gorskyi, O. polaris, A. sicula, and F. borealis*) and a group formed by *F. venusta* and *O.dioica*. The remaining species could be associated by single linkage to the "warm" water group, except *O. vanhoeffeni* that was associated to the "cold-water" association and *O.rufescens* which was associated to all groups. These species represent satellite or associate species reflecting the complexity of the biological community (*sensu* Venrick 1971). The seasonal changes and differences between locations in the proportion of each species association, after the abundance of each species was standardized to zero mean and unit variance, show that the warm water group only appeared during the summer months at the northernmost locations (Fig. 2 B), their presence becoming more apparent farther south (Fig. 3B). An inverse trend was observed for the cold water association while the group formed by *O.dioica* and *F. venusta* did not show any clear pattern suggesting a more generalist characteristic.

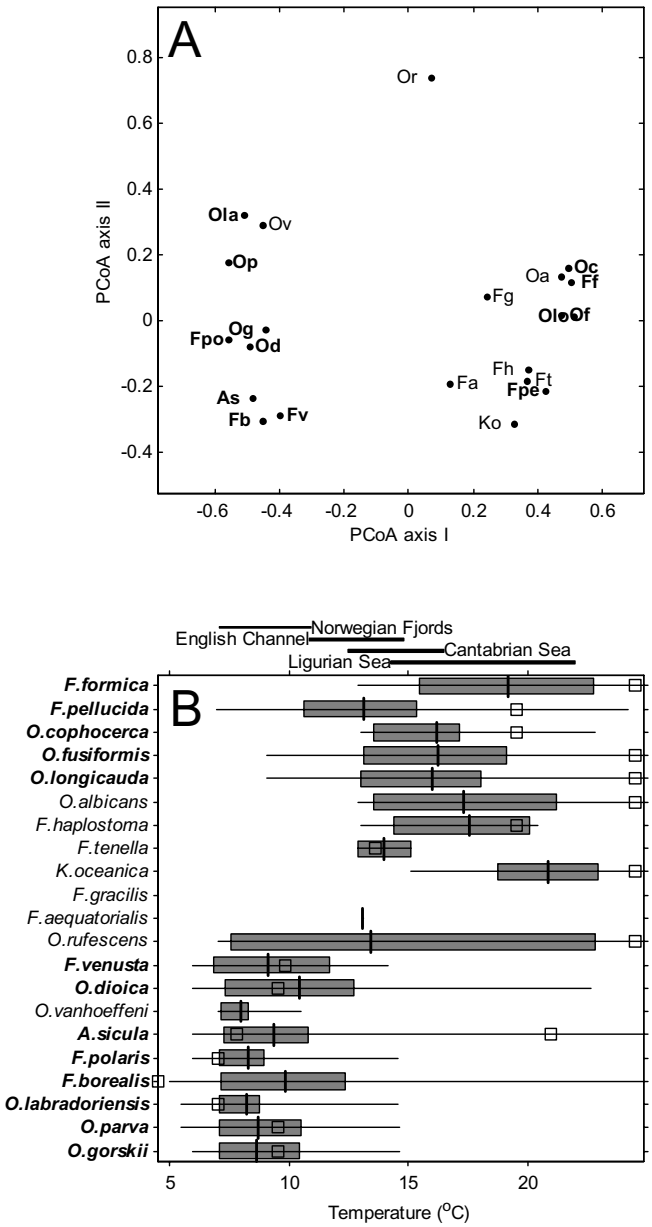

Figure 7. Appendicularian species associations identified using non-hierarchical complete linkage clustering. (**A**) Two dimensional principal coordinate ordination of the species, Axes I (abscissa) and II (ordinate) explain respectively 40 and 10% of the variability among species. Species names in bold represent those species grouped into an association (see B for the three species groups detected). Species names not on bold represent satellite species. Species names are abbreviated using the first letters of the genus and species name (or first two letters of the species name in case of conflict). (**B**) Species are ordered following their loadings of the first principal coordinate in A. Groups of species in bold represent recurrent associations. Horizontal lines represent the range of the temperatures where each species is present, vertical lines show the average temperature and grey rectangles the first and third quartiles calculated considering only the temperature values when that species was present. Open squares show the temperature where the maximum frequency of presences was found after the data was binned into two degree temperature intervals. Lines on top of the panel indicate the first and third quartiles of the temperatures at each of the locations studied.

Appendicularian species niches.

Most species showed a unimodal response to temperature, with an optimum temperature (Fig. 8) reflecting the species-specific patterns detected in the previous sections. The Johnson's (1949) frequency curves fitted summarize the shape of the response to temperature both in reflecting the niche optimum (mode, Fig. 8) and the eurythermality or niche breath (standard deviation, Fig. 8). However the fact that in

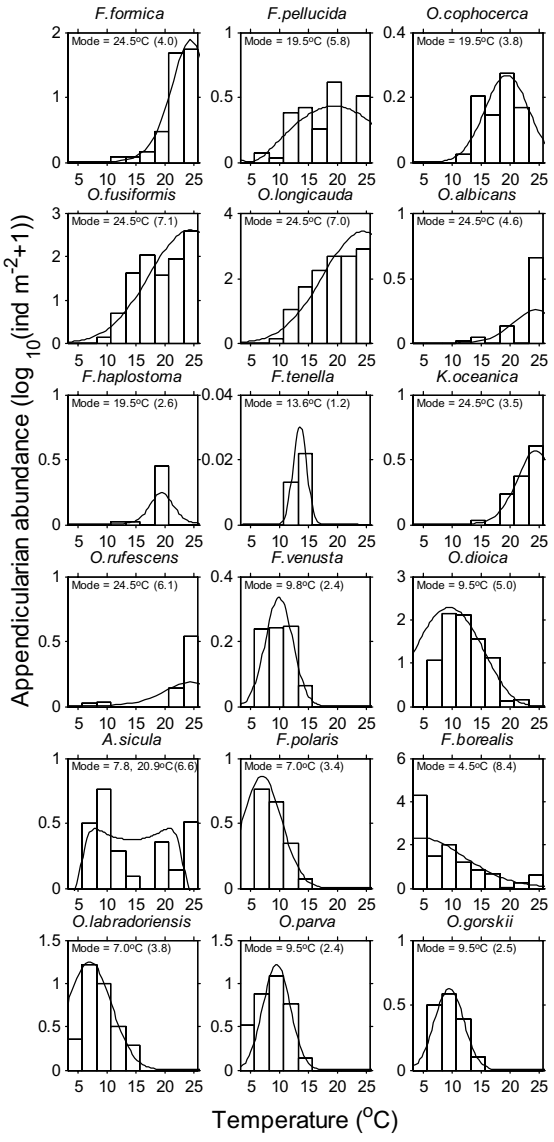

Figure 8. Relationship between appendicularian species abundance and environmental temperature. Species are ordered from top left to bottom right panels according to the first principal coordinate axis in Figure 4 A. Bar charts represent the mean values of the $\log_{10}$+1-transformed abundances observed within each temperature interval. Temperature intervals were arbitrary selected as 2ºC bins starting at 4ºC. Only those species with more than two presence values within at least two temperature intervals are shown. Lines represent the continuous functions fitted using Johnson's systems of frequency curves (see Methods for a detailed explanation). The optimal temperature for each species is presented as the mode of the frequency distribution and the degree of eurythermality or one-dimensional temperature niche breath is shown in brackets as the standard deviation of the fitted distribution.

some cases the temperature ranges at the stations sampled were not wide enough to cover the broad niches of some species (e.g. *F. formica* as a warm water and *F. borealis* as a cold water species) forced us to assume that the distribution was symmetrical for

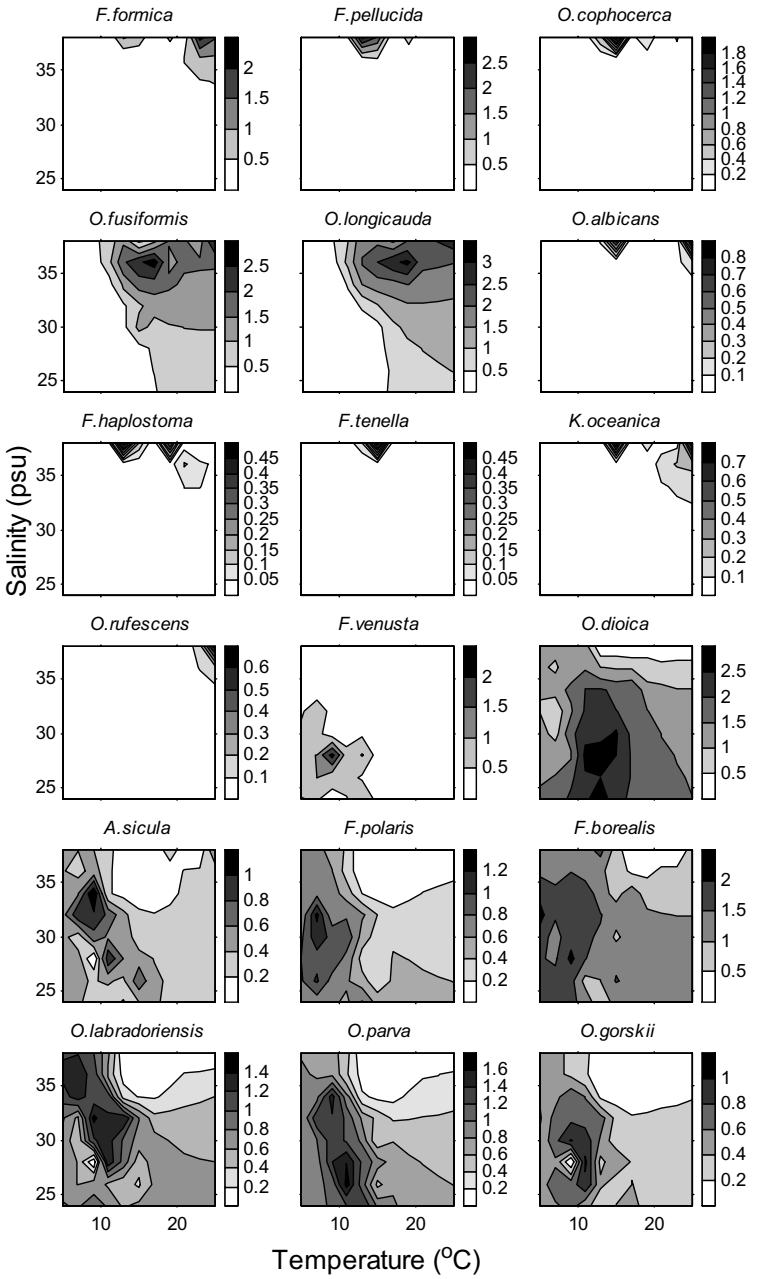

Figure 9. Relationship between temperature, salinity and appendicularian abundance. Graphs show the interpolated contour plots obtained using a squared inverse distance interpolation algorithm. Colour scales show the $\log_{10}+1$ transformed abundance corresponding to each line in the contour plot.

curve fitting purposes. Despite the relationship between total appendicularian abundance and chlorophyll we could not find any correlation between chlorophyll concentration and the abundance of each individual species. However, the interpolated contour plots of appendicularian species abundance vs. salinity and temperature (Fig. 9)

and histogram plots similar to those shown for temperature (using both the raw data and the residuals of the fitted temperature distributions, not shown) suggest that salinity should be possibly considered as the second most relevant variable in shaping the niche of each species.

DISCUSSION

The characterization of the successional stages within a community, the existence of recurrent groups of species and the realized qualitative niche of each individual species are closely interlinked ecological concepts. The first two methods begin with the assumption that communities consist of relatively discrete entities (i.e. classification methods) while the theory of the realized niche is more closely related to the existence of ecological continua and gradients (ordination method, Austin 1985). Each methodological approach (i.e. successional steps, species associations and ecological niche) represents an attempt to simplify the ecological complexity of the community. They should not be considered as opposed approaches but complement one another in helping to understand the underlying structure by tackling the same question from different angles. Those species characterizing a stage in the ecological succession would generally co-occur and be perceived as associates or a recurrent group within a given taxocene. Whether the environmental conditions in a successional stage lie within the niche of a species will determine its presence or absence and those species whose niches overlap sufficiently will form a recurrent group or species association.

Since the early studies by Essenberg (Essenberg 1922, 1926) the correlation between the number of appendicularians and habitat temperature has been described in a number of different environments (e.g. Fenaux 1963, Shiga 1985, Acuña & Anadón 1992, Acuña et al. 1995). The wide range of environments covered in our study has allowed us to provide a first description of the geographical differences in appendicularian species composition. At the species level appendicularian populations are mainly under thermal control, both the temperature optimum and the eurythermality are species specific, we have applied niche theory in an attempt to parameterize the patterns observed. The differences in the habitat described by temperature and to some degree salinity explained to a considerable extent the seasonal and geographical distribution patterns detected. Shiga (1985) arrived to a similar conclusion in a study of the vertical and seasonal distribution of appendicularians in Volcano Bay in Japan.

Acuña (1994) showed that the vertical distribution of the different species was also in close relationship to temperature. This thermal dependence could be a general characteristic of pelagic tunicates since it has also been reported for doliolids (Berner & Reid 1961) and to some degree in salps (Menard et al. 1994). Fenaux (1961) has shown that there is an ordered sequence in the numerical dominance of the different species through the seasonal cycle. Acuña & Anadón (1992) suggested as a working hypothesis that the overall abundance of appendicularians is dependent on primary production, while the relative abundance of the different species depends on the temperature. This hypothesis is supported by the correlation between the total number of appendicularians and chlorophyll concentration and the strong temperature dependent response in the abundance of each species. However the dominance, as a percentage of total individuals, is difficult to interpret when the species considered are dissimilar in biomass, activity, etc (Fager 1957). Therefore, the term "relative" should be considered with caution both in describing the seasonal sequence (e.g. Fenaux 1963) and in comparing the contribution of each species to the total number of appendicularians (e.g. our Table 1). For example, the fact that *O.labradoriensis* is generally less numerous than *O.dioica* (Table 1) does not say anything about their relative ecological relevance since their body size and therefore biomass are different (Fig. 5). Similarly the change in dominance of a particular species can be due to an increase in its abundance or a decrease in the abundance of some other species and we still know very little (if anything) about interspecific competitive exclusion processes to give dominance, even in biomass units, an ecologically sound meaning.

The development of quantitative descriptions of the species-specific response to environmental changes should not be considered only as a tool in autoecology research. Beaugrand et al. (2002) have used data collected by the Continuous Plankton Recorder (CPR) survey to show how global climate changes affect the organization of marine copepod communities in the North Atlantic. Unfortunately, appendicularians are not identified to species level by the CPR and therefore integrative studies like the EURAPP project are crucial in the quantification of appendicularian species-environment relationships. The close relationship between appendicularian species composition and physical conditions suggests that they could be greatly affected by changes in the environment and be used as indicator species of climate changes or characteristic water masses (Fenaux et al. 1998a).

Fenaux et al. 1998a pointed out the difficulties in appendicularian sampling both in the use of mesh sizes and the problems concerning distribution patchiness. The observed differences in the size frequency distribution of appendicularians collected with the 200 and 90 µm mesh plankton nets are similar to those observed by Fenaux & Palazzoli 1979 (between 200 and 53 µm meshes) and Capitanio et al. 1996 (between 200 and 90 µm meshes) with the larger mesh resulting in an underestimation of the smaller size classes. However, even with the smaller mesh there is still an underestimation suggestive of the need for new sampling approaches to improve our understanding of appendicularian population dynamics. Despite this underestimation the high appendicularian densities encountered during our study, particularly in the Norwegian fjords and Cantabrian Sea, are indicative of their potential ecological relevance.

4. Dinámica del flujo de alimento por el sistema digestivo de la apendicularia *Oikopleura dioica*

Gut throughput dynamics in the appendicularian *Oikopleura dioica*

Ángel López-Urrutia and José Luis Acuña

ABSTRACT

Oikopleura dioica is an excellent model to study food flow through the digestive system because of its transparency, non-motility and because fecal pellets move along the digestive system in an orderly sequence which can be easily timed. By observing fecal pellet circulation within the gut of healthy animals, we have concluded that the average number of fecal pellets inside the gut of *O. dioica* is 2.878±0.015 (mean±SE, n=43). Thus, gut passage time (GPT, min) can be estimated from the time interval between successive fecal pellets (DI, min fecal pellet^{-1}) as GPT=2.878DI. This establishes the basis to estimate GPT from simple fecal pellet production rate incubations, and is one way of determinating GPT without manipulating food concentration or quality, a major shortcoming of current techniques. In laboratory experiments, GPT of *O. dioica* was independent of body size. At 15ºC, GPT (min) decreased with increasing food concentration (FC, µg C l^{-1}) when the prymnesophyte *Isochrysis galbana* (4.5µm in size), the prasinophyte *Tetraselmis suecica* (10µm) or the chlorophyte *Chlorella sp.* (3µm) were used as food, according to the power function GPT= 29.4 FC$^{-0.245}$. There were no significant differences in GPT between algal types. The GPT of *O. dioica* exhibited a Q_{10} of 0.687 over a temperature range of 10 to 20ºC, independent of food concentration. Since the interaction between food concentration and temperature was not significant, GPT can be estimated as GPT= 51.67 * e$^{-0.0376\ T}$ * FC$^{-0.245}$.

INTRODUCTION

Current functional response models place special emphasis on the compensatory role played by digestive processes (Lehman 1976, Penry & Jumars 1987, Willows 1992). According to these models gut passage time (GPT, min) should respond to variations in the amount and quality of food to maximize net energy gain. Thus, GPT is a variable of paramount importance in the ecophysiology of marine organisms. Moreover, grazing rates of pelagic filter feeders have been calculated according to the gut pigment technique by dividing an *in situ* measurement of gut pigment content by an *in vitro* estimate of GPT (Madin & Cetta 1984). However, the task of measuring GPT in pelagic filter feeders by non-intrusive methods remains an elusive problem, and most current techniques rely on some important assumptions. For instance, the gut clearance (Mackas & Bohrer 1976) and gut filling (Head 1986) techniques assume that GPT is independent of ingestion rate. Exposure to filtered seawater in gut clearance experiments can be avoided by using surrogate particles which do not interfere with the pigment analysis and that are readily ingested (e.g. charcoal, Perissinotto & Pakhomov 1996), but this clearly influences the quality of the food offered. Use of radiolabelled food circumvents most of these caveats, but is hardly applicable during research cruises (Arashkevich 1977). Coloured markers, followed in their passage through the gut, are of common use for the transparent salps (Madin & Cetta 1984), at the cost of severely changing the quality of the food. Fecal pellet production experiments which involve measurement of the total amount of pigments egested do not have these shortcomings,

but rely on careful assessment of pigment degradation in gut and fecal pellets (Dagg & Walser 1987).

Appendicularians are not exempt from these methodological problems. Measurement of the GPT in these animals has relied on the use of marker particles (Acuña et al. 1994, Bochdansky et al. 1998, Acuña et al. 1999), which involves manipulation of food quality and/or concentration. Moreover, it is often hard to ascertain the precise moment at which the marker is being ingested, because the food has first to be concentrated in the external filter house. In addition, shipboard measurement of GPT in these delicate tunicates represents a technical challenge, necessitating the use of onboard cold rooms to conduct the observations, and of suspension systems to isolate the animals from the ship vibrations (Acuña et al. 1999). This has lead researchers to simply rely on literature values, obtained under unreported experimental conditions, to calculate grazing rates by the gut pigment technique (Alldredge 1981). Well-founded predictive equations are thus much in demand.

Oikopleurid appendicularians are transparent, non-motile animals in which food transit through the digestive system proceeds by an orderly sequence of fecal pellet translocations. Thus, appendicularians are ideal animals for the observation of gut throughput dynamics. Here we make use of these characteristics to propose a non-intrusive, observational method to measure GPT in *Oikopleura dioica*, and use this method to determine the influence of body size, food concentration, food type and temperature on the GPT. Last, we use this ecophysiological knowledge to build a predictive model for the GPT of *O. dioica*, which can be extrapolated to field conditions.

MATERIALS AND METHODS

Appendicularians and Phytoplankton cultures

Oikopleura dioica were collected from surface waters at the El Musel harbour in Gijón (N Spain), using plastic buckets, and quickly brought to walk-in controlled temperature rooms (set at the specified temperature ±0.5º C). Appendicularian cultures were initiated by placing healthy, wild-captured individuals inside their filter houses in 5000ml glass jars filled with 30µm filtered sea water which was continuously agitated

by means of an acrylic spiral paddle rotating at 10 rpm (Fenaux & Gorsky 1985). The animals were transferred to fresh, 30µm filtered seawater every two days.

The unicellular prasinophyte *Tetraselmis suecica* (10µm ESD, Equivalent Sphaerical Diameter), the prymnesophyte *Isochrysis galbana* (4.5µm ESD) and the unicellular chlorophyte *Chlorella sp.* (3µm ESD) were used as food for the experiments. Algae were cultured at 15º C in 1l bottles under 70 µEinstein white light, continuous aeration and 12:12 photoperiod. Only exponentially growing algae were used for the experiments.

Microscopic observation

Our data were collected by microscopic observation of individual *Oikopleura dioica* using an Olympus IMT-2 inverted microscope inside the controlled temperature room. To maintain the animal in a fixed position, a simple device was constructed using a plastic Petri dish (5.5 cm diameter, 1.5 cm height). A 1cm wide hole was made through the lid of the dish, allowing insertion of a cylindrical acrylic plastic tube 1.5 cm long that stood vertically on the Petri dish. Both Petri dish and plastic tube were filled with water at the target experimental temperature and food concentration. The animal was then transferred with an L-shaped wide bore Pasteur pipette from the 5l glass jar to the observation device, along with a certain amount of preconditioning food suspension. The activity of the animal was recorded with a SONY SSC Colour Video Camera fitted to the inverted microscope. The trunk length of the animal, defined here as distance between the mouth and the posterior edge of the stomach, was measured on the video screen to the nearest 45nm.

General experimental procedures

Algae for the experiments were centrifuged at 1500 R.C.F. (Relative Centrifugal Force) for seven minutes and resuspended twice on GF/F filtered sea water, and the final concentration of the stock solution determined with a Coulter multisizer II fitted with a 70 µm aperture tube. Organic carbon content of phytoplankton was estimated from cell volume using equations of Strathmann (1967). Because an appendicularian house lasts ca. 4 h, experimental animals were allowed to precondition in 5l of GF/F filtered sea water supplemented with algae up to the specified concentration for four hours, to ensure that they had enough time to expand a new filter house prior to

observation. We did not use replicate conditioning jars because preliminary experiments showed no significant effects of this random variable on GPT (data not shown). Prior to ANOVA and regression analyses, data were examined for homogeneity of variance. Tests of linearity were conducted prior to regression analysis with replication.

RESULTS

Measurement of gut passage time in *Oikopleura dioica*

The gut of *Oikopleura dioica* consists of a bilobate stomach, a vertical intestine, a median intestine and a rectum (Fig.1; Fenaux 1989). In a healthy animal inside its filter house, it is possible to see 2 (Fig. 1C & D) or 3 (Fig. 1B) pellets distributed among the vertical and median intestines and the rectum, plus a variable amount of food in the stomach (no food in Fig. 1B, some food in Fig. 1C and an almost fully formed fecal

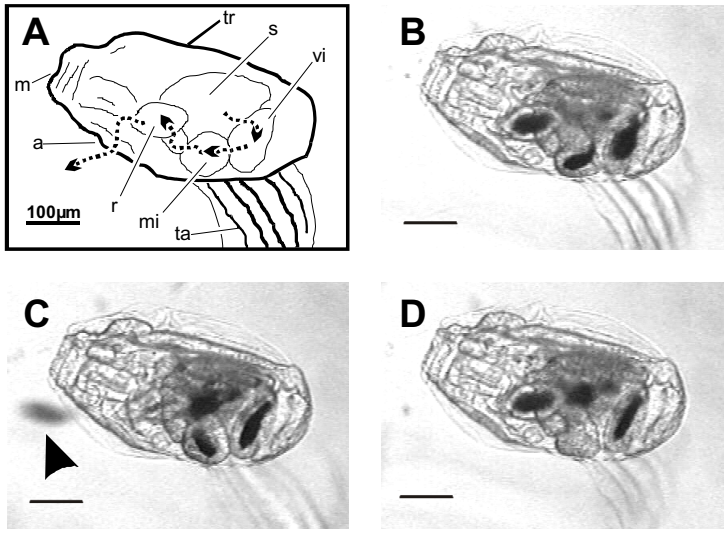

Figure 1. *Oikopleura dioica*. **A.** Sketch of the trunk showing the different parts of the digestive system, based in Fig. 1B. Letters indicate the anus, a; mouth, m; median intestine, mi; rectum, r; stomach, s; tail, ta; trunk, tr, and vertical intestine, vi. Dashed arrows indicate the path followed by the fecal pellets through the digestive system. **B, C & D.** Micrographies of the trunk of an actively filtierng animal. Animals for these video-images have been incubated in a dilute suspension of sepia ink; the ink is then ingested and incorporated into the fecal pellets which appear then heavily stained. The arrowhead in **C** signals a fecal pellet which has just been defecated. To understand how these different configurations are temporally linked, see Fig. 2 and results. These configurations corresponds to schematic representations in Fig. 2A, G, H and I (**B**), Fig. 2C and J (**C**) and Fig. 2D (**D**).

pellet in Fig. 1D). These different pellet configurations can only be understood by analysing the dynamics of food flow within the digestive system of *O. dioica* (Fig. 2). Particles captured in the pharyngeal filter enter the stomach continuously and are progressively compacted into a fecal pellet (Fig. 2B, C, D, E & F). Once formed, this fecal pellet passes into the vertical intestine (Figs. 1B & 2G) and is then sequentially

translocated to the median intestine and to the rectum (Fig. 2H & I) before its defecation (Fig. 2J). The movement of a fecal pellet through the digestive system is accompanied by a sequence of fecal pellet translocations in the preceding fecal pellets, which leave room for the following pellets (see Figs. 1 & 2). Under constant ambient conditions, this sequence is extremely precise, repetitive and can be accurately timed. It is possible to take advantage of this precise timing to develop an observational measurement of the GPT.

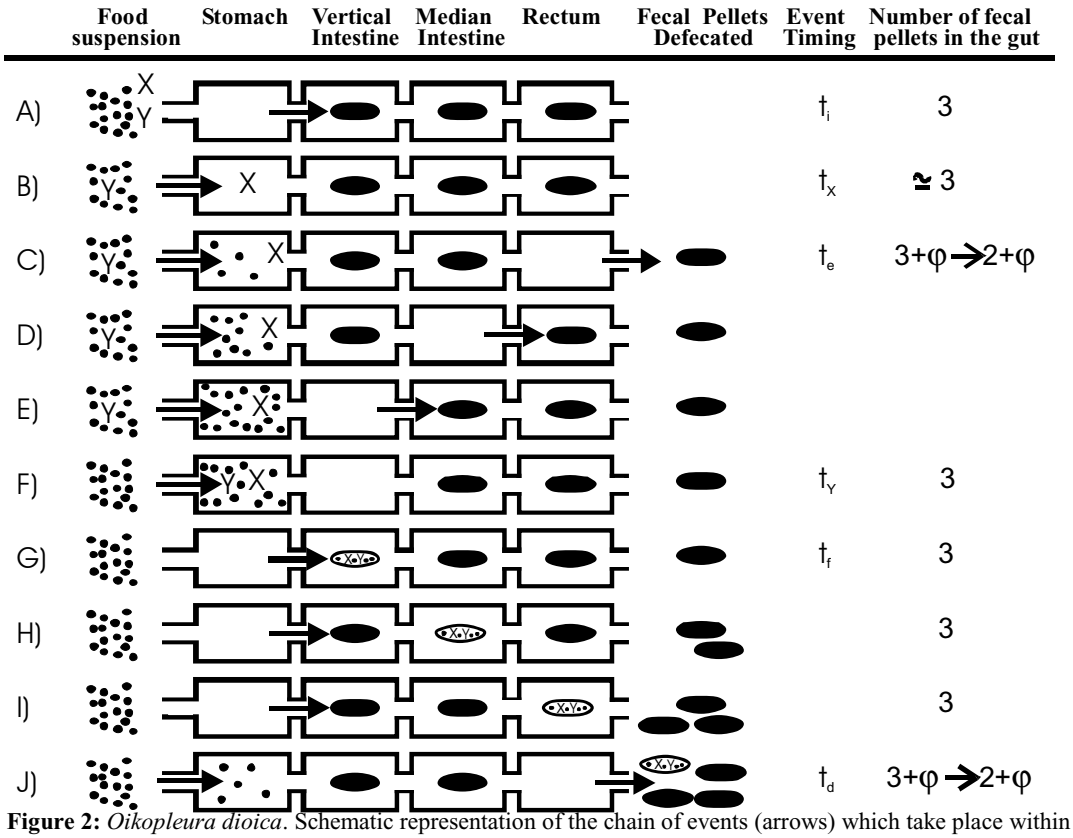

Figure 2: *Oikopleura dioica*. Schematic representation of the chain of events (arrows) which take place within the digestive system from incorporation of a particle into a fecal pellet to defecation. Events whose timing is pertinent to our measurements are assigned a time code under the heading "event timing" (see text); gut contents (fecal pellets) are indicated under the heading "Number of fecal pellets in the gut" (see text and Fig. 4). **A.** Translocation of a newly formed fecal pellet from the stomach to the vertical intestine. Two other pellets are present in the median intestine and the rectum (this corresponds to Fig. 1B). **B.** A new fecal pellet starts to form in the stomach. The first particle incorporated to that pellet is labelled X. **C.** The fecal pellet in the rectum is defecated. More particles, labelled as dots, are incorporated into the fecal pellet which is being formed in the stomach (this corresponds to Fig. 1C). Gut content passes from 3+φ to 2+φ pellets, where φ is the content of the stomach (in fecal pellets) during this moment. **D.** The fecal pellet in the median intestine is translocated to the rectum. More particles enter in the stomach (this corresponds to Fig. 1D). **E.** The fecal pellet in the vertical intestine is translocated to the median intestine. More particles accumulate in the stomach. **F.** The last particle, labelled Y, is incorporated into the fecal pellet which is being formed in the stomach. **G.** The newly formed fecal pellet is translocated form the stomach to the vertical intestine. This phase of the cycle is the same as A) above. **H.** This figure is a composite of several consecutive steps. The cycle starts over again and ends in a similar configuration as in A and G above, but with the fecal pellet with particles labelled X and Y located in the median intestine. **I.** Same as A), G) and H) above but with the labelled fecal pellet located in the rectum. **J.** Same as C) above but the labelled fecal pellet has just been defecated.

To measure the GPT of a particle we need a precise timing of the moment of ingestion and defecation of that particle. All particles in one fecal pellet are defecated simultaneously and this event can be visually timed (i.e. Fig. 1C, t_d in Fig. 2J). However, not all particles are ingested and incorporated into a forming fecal pellet at the same moment. A food particle that has been incorporated into a fecal pellet right at the beginning of its formation (i.e. particle X in Fig. 2B, incorporated at time t_x) spends more time within the digestive system than a particle incorporated at the end of the formation of the fecal pellet (particle Y in Fig. 2F, incorporated at time t_y). Thus we must look for a collective or average time of incorporation representative of the population of particles belonging to this fecal pellet, rather than considering the time of incorporation of any individual particle. Given a constant rate of stomach filling, the population, average time of incorporation to the fecal pellet is the arithmetic mean of t_x and t_y, or $\frac{t_y - t_x}{2}$. However, we observe fecal pellets and their translocations, not individual particles, so it is impossible to measure t_y and t_x. Because the animal has continuous feeding and it does not interrupt secretion of the pharyngeal filter, which keeps flowing along the esophagus while the pellet is translocated (pers. obs.), incorporation of the first particle into the fecal pellet (particle X at time t_x, Fig. 2B) and translocation of the preceding fecal pellet from the stomach into the vertical intestine (at time t_i, Fig. 2A) are consecutive events, and $t_x=t_i$. Similarly, incorporation of the last particle to the fecal pellet (particle Y at time t_y, Fig. 2F) and translocation of this fecal pellet into the vertical intestine (at time t_f, Fig. 2G) are consecutive events and $t_y=t_f$. This implies that the average time of incorporation to the fecal pellet will be given by $\frac{t_f - t_i}{2}$. It follows that the average gut passage time (GPT) can be calculated as the difference between the time of the defecation of a fecal pellet and the average time of incorporation of particles to that pellet, that is

$$GPT = t_d - \frac{t_f - t_i}{2} \qquad (1)$$

Estimation of the GPT from the defecation interval

Measurement of GPT as explained above involves observing individual animals from start of formation of a fecal pellet (t_i, Fig. 2A) to defecation (t_d, Fig. 2J). Even at

the fastest GPT achievable, observations by this technique take more than 6 minutes for each individual, so we looked for alternative, less time-consuming ways of measuring GPT.

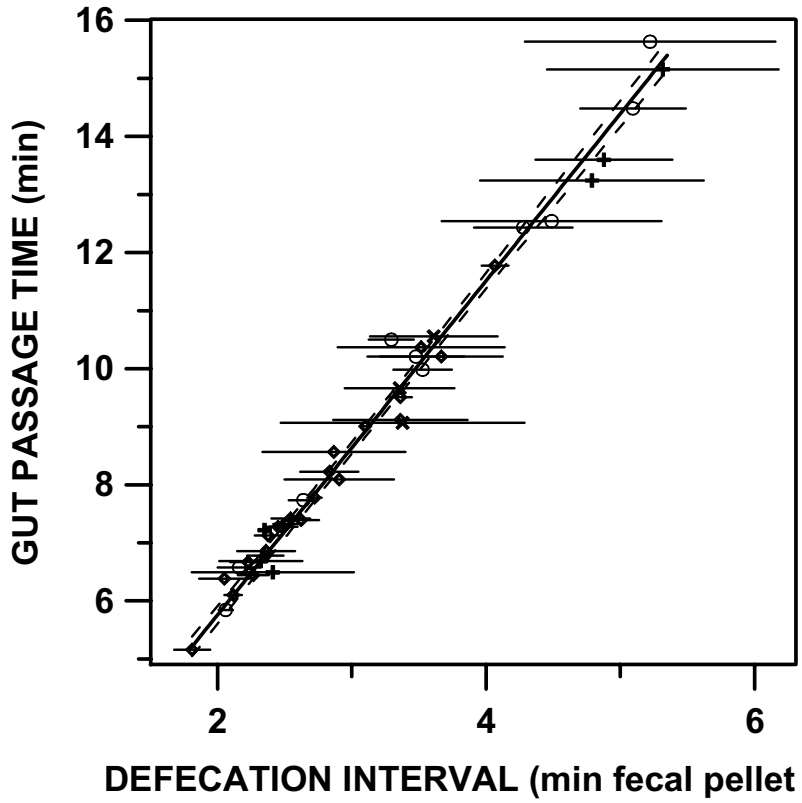

Figure 3. *Oikopleura dioica.* Plot of GPT (min) vs. DI (min fecal pellet^{-1}) for animals incubated at a range of food concentrations and food types and 15±0.5ºC. Empty circles, rhomboids and plus signs represent observations conducted on animals incubated at food concentrations of 60, 120 and 240µg C l^{-1} of *I.galbana* respectively*;* crosses correspond to observations conducted on animals incubated on unfiltered seawater. Horizontal solid lines represent standard deviations. The solid line is the Geometrical Mean Regression; dashed lines represent 95% confidence intervals. GPT (min) can be estimated from DI (min fecal pellet**-1)** according to the regression equation GPT= (2.878±0.015) * DI (mean±SE; r2=0.9825, n=43, F1,42=35793.87, p<0.001). The regression intercept was forced through zero because the intercept of an ordinary regression was not significantly different from zero (t=1.618, p=0.11).

Each complete observation of GPT involved observing the defecation of 4 fecal pellets, and consequently we also could measure 3 time intervals between consecutive fecal pellets (see Fig. 2). Thus we looked at the time interval between fecal pellets (DI, min fecal pellet^{-1}) as a proxy to estimate GPT, and compared the average of these three DI against GPT for a total of 43 individuals under contrasting ambient conditions (Fig. 3). Due to the existence of natural mutual variability in the observations, a Geometrical Mean Regression (GMR, Ricker 1984) was used instead of an ordinary least squares regression. There is a robust, linear relationship between GPT (min) and average DI (min fecal pellet^{-1}) with 0 intercept (Fig. 3) which indicates that DI is a constant proportion of GPT, i.e.

$$GPT = 2.878DI \qquad (2)$$

Since no significant differences were found between consecutive measurements of DI (ANOVA for repeated measures, $F_{2,\ 84}$=1.902, n=43, p=0.15, power of test$_{\alpha=0.05}$=0.615, 3 consecutive measurements for each animal) only one measure of DI was recorded per individual thereafter. The corresponding GPT was then calculated according to Eq. (2), which allowed us to reduce manipulation of animals and increase the number of observations per experiment.

Number of fecal pellets inside the gut of *Oikopleura dioica*

The coefficient 2.878 in the right hand term of Eq. (2) represents the average number of fecal pellets in the gut (i.e. GPT/DI). In fact, it is possible to obtain an independent measurement for the average number of fecal pellets (AFP, fecal pellet) inside the gut of an individual *Oikopleura dioica* by timing characteristic events in the fecal pellet formation and circulation. According to this analysis (Figs. 2 & 4) the number of fecal pellets inside the gut of *O. dioica* experiences oscillations between a

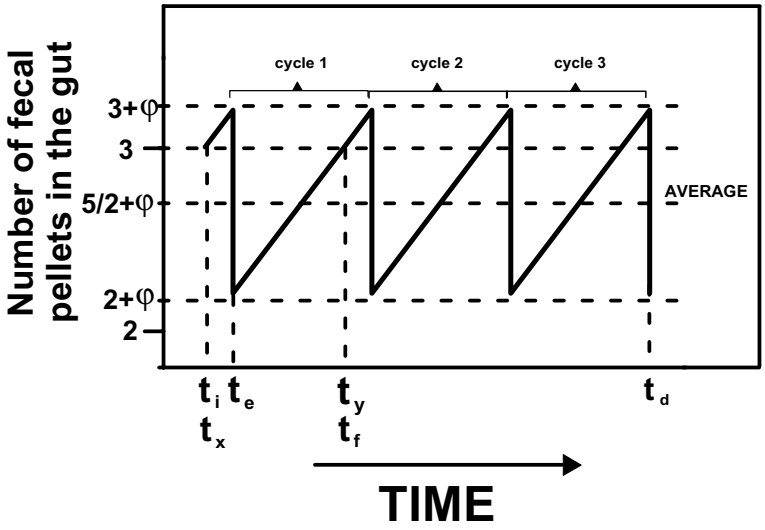

Figure 4: *Oikopleura dioica*. Temporal changes in the number of fecal pellets within the gut according to the diagram in Fig. 2 and assuming a linear increase in the stomach content. The stomach content when a fecal pellet is defecated (φ, fecal pellets) can be calculated as . Since the gut content oscillates between 3+φ and 2+φ in repeated cycles, the average gut content (AFP, pellets) within a single cycle will be given by the arithmetic mean between 2+φ and 3+φ, that is ; this mean value is also valid for any cycle and therefore for the time-integrated GPT.

minimum value of 2+φ and a maximum value of 3+φ, where φ (fecal pellets) is the stomach content when a fecal pellet is defecated (Figs. 1C, 2 C, J & 4), and can be calculated as:

$$\varphi = \frac{t_e - t_i}{t_f - t_i} \quad (3)$$

Since this cycle is repeated continuously, the AFP within each cycle will also be the time-integrated AFP in the gut of *Oikopleura dioica,* which should therefore be calculated as

$$AFP = \frac{5}{2} + \varphi \quad (4)$$

From our observations, and according to Eq. (3), the average value of φ was 0.368±0.013 (mean±SD, n=43). When inserted into Eq. (4), this value of φ yields an average number of fecal pellets of 2.868±0.013, which is close to the slope of the regression of GPT vs. DI (2.878; Eq. 2) and indicates us that we are right in accepting a 0 intercept for that equation.

Factors affecting gut passage time

Effect of body size

Expt. 1A. This experiment was designed to test for differences in GPT associated with body size through a linear regression approach. GPT of 24 animals of differing trunk length were observed at a concentration of 120 µg C l^{-1} of *Isochrysis galbana* and 15º C temperature. No significant effect of trunk length on GPT was detected, within a trunk length range from 0.25 to 1.22 millimetres (Fig. 5). However, the low power of the regression (i.e.1-$\beta_{\alpha=0.05}$=0.3974) and the low p-value ($F_{1, 23}$=3.307, p=0.082; n=25, r^2=0.126; Fig.5) suggest that we could be incurring a type II error, which warrants further confirmation of this result (see next experiment).

Expt. 1B. This experiment was designed to test for differences in GPT due to body size through an ANOVA approach. The idea was to incubate animals belonging to two cohorts of differing body size to test for differences in GPT between cohorts (i.e. between sizes). We used large (0.903±0.034 mm trunk length; ±SD, n=15) and small (0.242±0.061 mm; n=15) animals for the experiments, which were performed at 15º C

Table 1. Summary of ANOVA and ANCOVA experiments, including the sum of squares (SS), degrees of freedom (df), mean square (MS), F and p values. In all cases the dependent variable is gut passage time (GPT), except in experiment 3A where the dependent variable was the natural logarithm of GPT.

SOURCE OF VARIATION	SS	df	MS	F	p
EXPERIMENT 1B (two-way ANOVA: ind. Vars.= algal species & trunk length)					
Main effects	2.62	5	0.52	0.34	0.81
Algal species	1.29	2	0.65	0.42	0.66
Trunk length	0.22	1	0.22	0.14	0.71
Algal species * Trunk length	1.10	2	0.55	0.36	0.70
Model	2.62	5	0.52	0.34	0.88
Error	37.09	24	1.54		
Total residual	39.71	29	1.37		
EXPERIMENT 2 (rank ANCOVA: covariate= food concentration; ind. var.= algal species)					
Algal species	5952	2	2976	2.68	0.075
Error	85579	77	1111		
Total residual	91531	79	1158		
EXPERIMENT 3A (one-way ANCOVA: covariate= temperature; ind.var.= food concentration)					
Temperature	0.70	1	0.71	18.81	<0.001
Food Concentration	0.64	1	0.64	17.15	<0.001
Model	1.35	2	0.67	17.98	<0.001
Error	1.01	27	0.04		
Total residual	2.36	29	0.08		
EXPERIMENT 3B (two-way ANOVA: ind. vars.= algal species & temperature)					
Main effects	1.37	2	0.69	43.69	<0.001
Algal species	0.07	1	0.07	4.17	0.058
Temperature	1.31	1	1.31	83.2	<0.001
Algal species * Temperature	0.04	1	0.04	2.72	0.119
Model	1.42	3	0.47	30.03	<0.001
Error	0.25	16	0.02		
Total residual	1.67	19	0.09		

and a concentration of 100 µg C l^{-1} of either *Isochrysis galbana*, *Tetraselmis suecica* or *Chlorella sp.* Thus, we used a two-way orthogonal design with two levels of body size (large/small) and three levels of food type (3 algal species). This design allowed us to

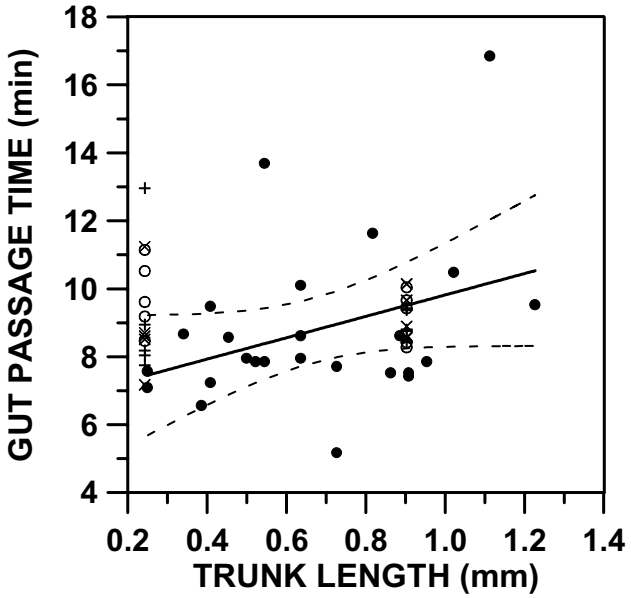

Figure 5. *Oikopleura dioica.* Plot of GPT (min) vs Trunk length (mm). Filled circles (•) represent data from expt. 1A. The solid line is the least square regression of GPT vs. Trunk length for that experiment (120µg C l^{-1}, *I.galbana*), dashed lines represent 95% confidence intervals. The slope of the linear regression was not significantly different from zero (r^2=0.13; n=43; $F_{1,23}$=3.31, p=0.082; power of the regression is 1-$\beta_{\alpha=0.05}$=0.40). Empty circles (**O**), crosses (**x**) and plus signs (+) represent data from expt. 1B (see Table 1 and Results). In that experiment, animals belonging to two distinct appendicularian size classes were incubated at 100 µgC l^{-1} of *I. galbana* (**O**), *Chlorella sp.* (**x**) and *T.suecica* (+).

test for the existence of interactions between trunk length and algal type, indicative of differences in the ability to capture or ingest phytoplankton of different size or taxonomic group between the small and large animals. There were neither significant effects of trunk length (p=0.71) or food source (p=0.66), nor significant interaction between these two factors (p=0.70, Table 1, Fig. 5).

Effect of food concentration and algal species

Expts. 2A, B and C. GPT was measured at five different food concentrations of *Isochrysis galbana*, *Tetraselmis suecica* and *Chlorella sp.* in three separate experiments at 15º C (Expts. 2A, B and C respectively) to test the effect of food concentration and algal type on GPT. The relationship between GPT and food concentration for each type of alga was fitted to a power relationship (Fig. 6A, B & C). We chose a power function to fit GPT *vs.* food concentration, because this model has been previously used for

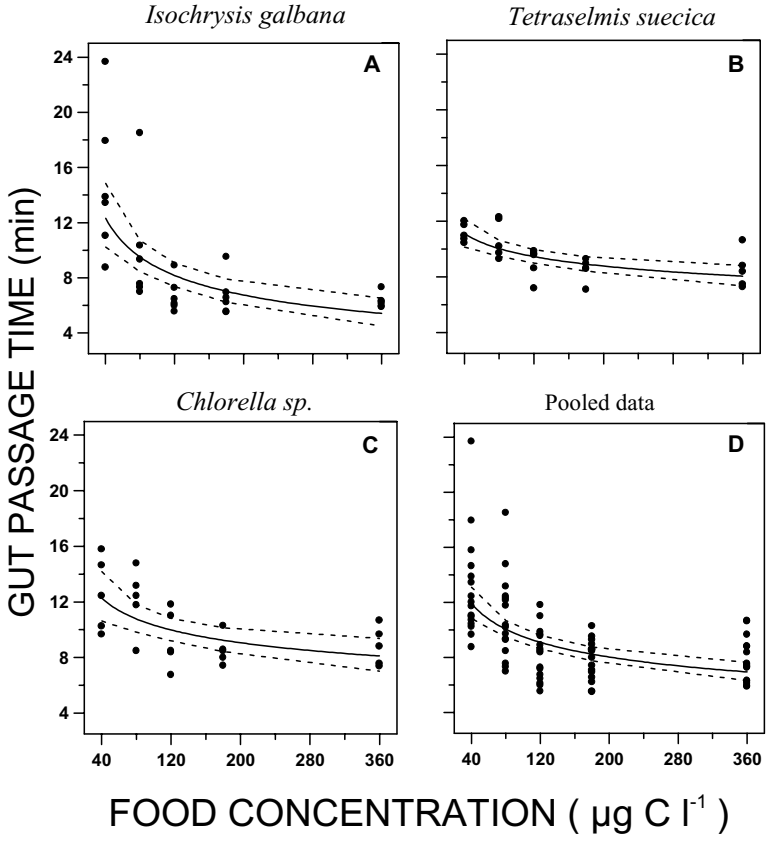

Fig. 6. *Oikopleura dioica*. Plot of GPT (min) vs Food Concentration (µg C l^{-1}) for animals incubated in A: *I.galbana*; B: *T.suecica*; C: *Chlorella sp.* and D: for all data pooled. Solid lines are the least square regressions for data fitted to a power model; dashed lines represent the 95% confidence intervals. Power regression equations between GPT (min) and Food Concentration (FC, µg C l^{-1}) are: GPT= (48.95±16.33) * $FC^{(-0.374\pm0.069)}$ (±S.E., r^2=0.51, n=30, $F_{1,28}$=29.52, p<0.001) for *I.galbana*; GPT= (19.038±3.057) * $FC^{(-0.146\pm0.033)}$ (±S.E., r^2=0.46, n=25, $F_{1,23}$=19.47, p<0.001) for *T.suecica*; GPT= (24.61±6.3) * $FC^{(-0.188\pm0.052)}$ (+/-S.E., r^2=0.36, n=25, $F_{1,23}$=12.72, p=0.0016) for *Chlorella sp.* and GPT= (29.4±5.1) * $FC^{(-0.245\pm0.036)}$ (±S.E.; r^2=0.37, n=80, $F_{1,78}$=45.97, p<0.001, Kolmogorov-Smirnov test for normality of residuals, Z=1.14, p=0.15) for the pooled data.

similar data in copepods Dagg & Walser 1987. Estimated slopes for the regression lines were significantly different from zero for all three algae ($p<0.01$, Fig. 6), which indicated that food concentration had a significant effect on GPT.

Next we compared the results of these three experiments to test for effects due to food type. Because variances in GPT between the three experiments were non homogeneous (Bartlett Chi-square test $B_{14}=26.497$, $p=0.02$), we used a Rank Analysis of Covariance (Quade 1967, cited in Huitema 1980). No significant effect of food type on GPT was detected ($F_{2,\ 77}=2.68$, $p=0.075$; Table 1), which is in agreement with the result obtained in expt. 1B above. Therefore, we pooled data from all three algae to estimate a power regression of GPT vs. food concentration, which explained 37% of the total variance in GPT (Fig. 6D). According to this equation, gut passage time at 15º C ($GPT_{15ºC}$, min) can be estimated from food concentration (FC, µg C l^{-1}) as:

$$GPT_{15ºC} = 29.4FC^{-0.245} \qquad (5)$$

Effect of temperature

Expt 3A. To test the effect of temperature, we observed the GPT of animals raised from egg to age 4 days at 10, 15 and 20±0.5º C and at 30 and 250 µg C l^{-1} of *Isochrysis galbana*. The idea was to estimate a Q_{10} value for the GPT, to determine if this estimated Q_{10} was different for the two algal concentrations and, if not different, to

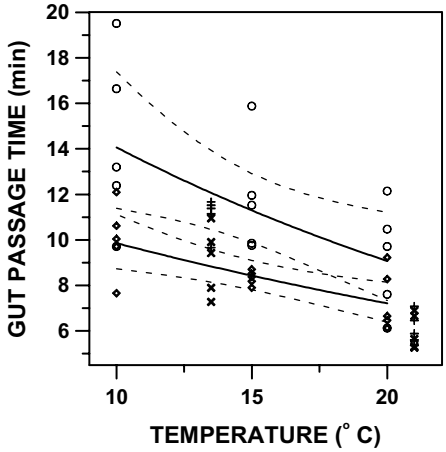

Figure 7. *Oikopleura dioica*. Plot of GPT (min) vs Temperature (T, ºC). Data at 10, 15 and 20ºC correspond to expt. 3A, in which the animals were incubated at concentrations of 30 (**O**) and 250 (◊) µg C l^{-1} of *I. galbana* (see Table 1 and Results). Solid lines represent least squares fits to an exponential model for data at 30 µg C l^{-1} (upper solid line, $GPT= 3.08\pm0.24\ e^{-0.044\pm0.015*T}$, ±S.E., $r^2=0.34$, $n=15$, $F_{1,13}=8.35$, $p=0.013$) and 250 µg C l^{-1} (lower solid line, $GPT= 2.60\pm0.13\ e^{-0.0313\pm0.0087*T}$; $r^2=0.50$, $n=15$, $F_{1,13}=13.02$, $p=0.003$). Dashed lines correspond to 95% confidence intervals for the regressions. Data at 13.5 and 21 ºC correspond to expt. 3B, in which the animals were incubated at a concentration of 60 µg C l^{-1} of *I. galbana* (+) and *Chlorella sp*. (**x**), see Table 1 and Results.

calculate a common Q_{10} valid for both concentrations. GPT (min) vs. temperature (T, ºC) data for each concentration were fitted to an exponential model following the Arrhenius equation, that is $GPT=ae^{cT}$, or ln GPT= lna+cT (Fig. 7). Regression slopes (i.e. c in the Arrhenius equation) were not significantly different for the different food concentrations (test of parallelism: $F_{1,\ 26}=0.52$, $p=0.48$), and the estimate for the common regression slope, which was significantly different from zero ($t_{27}=-4.337$, $p<0.001$), is c=-0.0376±0.0087 (mean±SE). However, there were significant differences in adjusted means among food concentrations (ANCOVA, $F_{1,\ 27}=18.81$, $p<0.001$; Table 1), which is in agreement with expts. 2A, B & C (Fig. 6). According to this result, GPT (min) can be estimated from knowledge of the environmental temperature (T, ºC) as

$$GPT = ke^{-0.0376T} \quad (6)$$

where k is a coefficient which depends on the food concentration. A common slope estimate of -0.0376 corresponds to a Q_{10} of 0.687.

Expt 3B. We wanted to know if different algal types might influence the response of GPT to temperature (i.e. the Q_{10}). Here we used a simpler design than that in expt. 3A, and incubated animals for measurement of GPT at 2 temperatures (13.5±0.5 and 21ºC±0.5) and 60 µg C l^{-1} of either *Isochrysis galbana* or *Chlorella sp.* No significant interaction was found between temperature and algal species used as food ($F_{1,\ 16}=2.71592$, $p=0.119$, $1-\beta_{\alpha=0.05}=0.34$, Table 1), which confirms that the Q_{10} value of 0.687 can be used for different types of food.

Prediction of GPT

According to the experiments above only food concentration and temperature significantly influenced GPT and expt. 3*A* confirmed that there was no interaction between these two factors. Here we use this knowledge to derive a simple equation to predict GPT from ambient temperature and food concentration. Eq. (6) requires knowledge of the exact value of a parameter k which is dependent on food concentration. Since the parameter c in Eq. (6) does not depend on food concentration, we can combine Eq. (5), which describes the relationship between GPT and food concentration (FC, µg C l^{-1}) as measured at 15º C, with Eq. (6) for a temperature of 15º C and solve for k to arrive at:

$$k = 51.67FC^{-0.245} \quad (7)$$

If k is substituted in Eq. (6) by the right-hand term of Eq. (7), Eq. (6) becomes:

$$GPT = 51.67FC^{-0.245}e^{-0.0376T} \quad (8)$$

This equation can be used to estimate gut passage time from knowledge of the concentration of available food (FC, in μg C l^{-1}) and ambient temperature (T, in º C).

DISCUSSION

The gut of *Oikopleura dioica*: a chemical reactor perspective

None of the chemical reactor types described by Penry & Jumars (1987) exactly describes the gut of *Oikopleura dioica*. This is important, since the choice of reactor determines the digestion strategy. While the intestine-rectum of *O. dioica* works like a plug flow reactor (i.e. a conveyor belt carrying fecal pellets, Fig. 2) the stomach works half way between a batch-reactor (i.e. it has pulsed outflow) and a continuous-flow, stirred tank reactor (i.e. it has continuous inflow, Fig. 2). Most planktonic filter feeders are similar in their continuous ingestion and discrete defecation, main differences arising in the way the fecal pellets are compacted. For example, only a proportion of the foregut contents are packed in a fecal pellet in copepods (Gauld 1957), while appendicularians pack the whole foregut (i.e. the stomach) content, which is thus emptied each time a fecal pellet is formed (Fig. 1B). Although we are far from explaining these differences, they clearly suggest that evolution has gone well ahead of chemical engineering in the design of optimal digestive strategies.

Measurement of GPT in *Oikopleura dioica:* implications for the estimation of grazing rates by the gut pigment technique

One of the characteristics of batch reactors with continuous filling is that different food particles have different gut residence times, defined as the time an individual element spends in the gut. Penry & Jumars (1987) defined gut passage time as the mean gut residence time, and recommended the use of gut passage time as a measure of gut throughput time (i.e. the time for one gut volume of food to be processed). Our newly developed method for the measurement of GPT in *Oikopleura dioica* is perfectly consistent with this definition. Previous determinations of GPT in

appendicularians have relied on the use of marker particles (Alldredge 1981, Acuña et al. 1994, Bochdansky et al. 1998, Acuña et al. 1999), and are therefore gut residence time measurements. Moreover, because our method is based on timing the defecation of two consecutive fecal pellets, it does not require manipulation of food quality or quantity. The existence of a relationship between defecation interval and gut passage time in appendicularians (Eq. 2) has been already pointed out by Bochdansky & Deibel (1999), who proposed that, for continuously feeding appendicularians, GPT should equal the amount of food in the gut (in fecal pellets) times the average defecation interval. Their estimates for GPT, after assuming a value of three as the approximate number of fecal pellets in the gut of *Oikopleura vanhoeffeni,* were very close to measurements using diatoms and cornstarch as markers. Recent shipboard observations conducted in the North Water polynya have confirmed that our observational method is also applicable to this species (Acuña et al. in press).

This method could also be a simple, accurate, and non-intrusive substitute to current gut clearance (Mackas & Bohrer 1976), gut filling (Head 1986), or radiotracer methods (Pond et al. 1995) to determine GPT in copepods. In a revision of data from (Arashkevich 1977) and (Arashkevich & Tseytlin 1978), Baars & Oosterhuis (1985) recommend the estimation of gut passage time from the time interval between egestion of consecutive fecal pellets, after calculating an average of 2.9 pellets inside the gut for four copepod species. The use of gut clearance rate experiments to estimate gut passage time in marine copepods has been criticised because of the unlikely assumption that gut clearance rate is independent of ingestion (see Dam & Peterson 1988 and references therein). Underestimation of grazing rates by the gut pigment technique when compared with other methods was explained by Peterson et al. (1990) as a consequence of using gut evacuation rate constants from evacuation experiments on filtered seawater as estimates of GPT. However, when GPT was estimated as twice the mean defecation interval, ingestion rates estimated by the gut pigment content technique were similar to values obtained by other techniques (Peterson et al. 1990).

Measurement of time interval between consecutive fecal pellets is conceptually linked to the measurement of fecal pellet production rates (the inverse of the time interval between consecutive fecal pellets). This suggests a different approach for the measurement of GPT, based on recording the number of fecal pellets produced within a

given time interval. Obviously this approach involves blind incubation rather than microscopical observation, and opens a simple avenue to conduct GPT measurements by incubation in thermo-insulated flasks onboard a ship, where availability of walk-in cold rooms may be limited. In a biogeochemical context, combination of these fecal pellet production rates with fecal pellet sinking velocities would allow the calculation of vertical pellet fluxes. Obviously these values would only represent maximum flux estimates as they do not take into account the possibility of fecal pellet recycling or the existence of non-feeding periods when building new filter houses.

A further implication of our observations regards the variability of *in situ* gut pigment content measurements. We have found clear evidence that the number of fecal pellets within the gut of *Oikopleura dioica* oscillates between 3+φ and 2+φ, with an average at $\frac{5}{2}+\varphi$ (Fig. 4), being φ=0.368. This means that the gut content (i.e. the gut pigment content) systematically varies between 135% and 65% of the average gut content. This variation is due to the mechanism of digestion of *O. dioica* and is independent of other intrinsic (i.e. body size) or environmental (i.e. temperature, food concentration and source) factors. When we capture animals from the field for the analysis of individual gut pigment contents we are taking snapshots of this oscillatory cycle; therefore these measurements are tied to a non-reducible variability that cannot be explained by regression on environmental variables or body size. In fact, regression models to predict the gut pigment contents of wild-captured oikopleurids explain only limited amounts of variance (Acuña et al. 1999). We see no reason why the copepod gut should differ in regard to this intrinsic variability (see Fig 4 A & B in Caparroy & Carlotti 1996 for a theoretical example), although gut pigments of copepods are seldom analysed individually.

Ecophysiology of GPT in *Oikopleura dioica*

We detected a non significant effect of body size on GPT, both through a regression experiment involving an even distribution of sizes or an ANOVA experiment in which two different cohorts of different size were compared (Fig. 5, Table 1). GPT seems to be unrelated to body size in other appendicularian species (Bochdansky et al. 1998), in some salps (Madin & Cetta 1984) and in copepods (Morales et al. 1990). This is interesting, because all effects of body size on ingestion rates are confined to gut

contents what greatly simplifies the task of building predictive models for GPT (i.e. Eq. 8).

As expected, GPT responded to temperature within the range of temperatures typical of surface waters off the Cantabrian coast (10 to 20ºC, Acuña & Anadón 1992). A Q_{10} value of 0.687 for GPT implies that its inverse, the gut evacuation rate constant (min^{-1}), has a Q_{10} of 1.46. This is lower than Q_{10} values for the gut evacuation rate constant for copepods (2.24, Dam & Peterson 1988) what indicates that feeding rates of the eurithermal *Oikopleura dioica* are less sensitive to temperature. It is also lower than the Q_{10} measured by Gorsky et al. (1987) for the respiration rate of *O. dioica* (1.96, after digitizing their figure 3 for calculation of a respiratory Q_{10} for body size-corrected data over the temperature range of 15 to 24 ºC). A Q_{10} close to 1.4 is what should be expected for the respiration rates of poikilotherms (Peters 1983).

GPT also varied within the range of suspended organic carbon concentrations typical of the coastal waters where *Oikopleura dioica* is present (Alldredge 1981), which confirms that a reduced food supply is in part compensated by increased digestion times in accordance with current digestion theory (Willows 1992). Bochdansky et al. (1998) found a low, non significant effect of food concentration on gut passage time in the cold water *Oikopleura vanhoeffeni*, while Dagg & Walser (1987) detected a clear response of GPT for the copepod *Neocalanus plumchrus* below a threshold chlorophyll concentration of 4 µg l^{-1}. Assuming a carbon:chlorophyll ratio of 40, this would correspond to 160 µg C l^{-1} which is close to our threshold at ca. 120 µg C l^{-1} (Fig. 6D).

Although we detected no significant effects of algal species on GPT, p values for these statistical tests were sufficiently close to 0.05 to take this result cautiously (Table 1; Expt. 1B, Expt. 2 and Expt. 3B). Moreover, the curve of GPT vs. food concentration for *Isochrysis galbana* had a markedly different aspect from that for *Tetraselmis suecica* and *Chlorella sp.,* which is consistent with differences between feeding functional response curves of *Oikopleura dioica* when feeding on these same algal species (Acuña & Kiefer 2000). The effect was small, however, when compared with the effects of temperature and food concentration, but we are probably not sampling the full range of particle qualities that *O. dioica* encounters in nature. More research is clearly needed in this regard.

Prediction of GPT in *Oikopleura dioica*

We found no significant interactions between food concentration and temperature on the GPT of *Oikopleura dioica* (Table 1). This indicated us that temperature had the same effect irrespective of food concentration, and that a combined model could be used to predict GPT from knowledge of food concentration and temperature (Eq. 8). The model does not require inclusion of any allometric terms, since we found non significant effects of body size on GPT (Fig. 5, Table 1). Thus, we recommend the use of Eq. (8) when no direct measurements of GPT are available. This relationship is based on laboratory experimentation which might not reflect actual field conditions, especially food type, and does not take into account the potential negative effect of large, non-ingestible diatom chains on ingestion rates (Acuña et al. 1999). Although further field validation of this model is clearly required, here we have established the basis for the measurement and prediction of gut throughput dynamics in appendicularians.

5. Estimación *in situ* de las tasas de ingestión e impacto trófico de las comunidades de apendicularias en aguas templadas: dinámica estacional en ambientes costeros y oceánicos

In situ feeding physiology and grazing impact of the appendicularian community in temperate waters: seasonal dynamics in coastal and oceanic environments

Ángel López-Urrutia, Xabier Irigoien, José Luis Acuña and Roger Harris

ABSTRACT

The physical and biological factors affecting the abundance and ingestion rates of different appendicularian species were investigated from April 1999 to May 2000 at a coastal station in the English Channel (L4) and on a transect of three stations across the shelf in the central Cantabrian Sea (E1, E2 and E3). Individual gut chlorophyll and gut food volume contents were used in parallel to determine the ingestion rates on chlorophyll containing prey and on total particulate material. Body size was the variable explaining most of the variability in gut contents. For most species, over 60% of the ingested material came from non-chlorophyll containing prey. Appendicularian community grazing impact was higher at the oceanic stations during early spring and autumn conditions, with maximum values close to 10 percent of the total phytoplankton biomass removed daily. *Oikopleura longicauda* and *Oikopleura fusiformis* were the species with the highest grazing impact. Over the whole period studied, appendicularians removed an average 8% of the primary production measured at station E2. Our results suggest that appendicularian populations are restricted to the surface mixed layer and that their grazing rates increase with increasing primary production. However, the percentage of the primary production removed by the appendicularian community decreases with increasing productivity, indicating that their grazing impact is relatively more important under oligotrophic conditions. This decrease in the grazing impact of appendicularians could be sufficient to explain similar patterns previously reported between total mesozooplankton grazing and primary production, since comparison of both relationships suggests that appendicularians could account for close to 40% of the total mesozooplankton grazing.

INTRODUCTION

Our knowledge on the importance of appendicularians in marine planktonic food webs is still limited (Calbet 2001). Their role in the classical herbivorous food web is uncertain due to their ability to feed on particles smaller than 5 µm (Cushing 1989). This nutritional adaptation has lead several authors to propose that appendicularians should play a significant role in microbial driven oligotrophic systems, where the majority of the particle biomass resides in the <5 µm size fraction (Deibel 1998, Gorsky & Fenaux 1998). However, the fact that reports of high appendicularian densities are mainly limited to highly eutrophic, neritic waters (e.g. Seki 1973, Uye & Ichino 1995, Nakamura 1998) is at odds with the notion, mostly based on laboratory experimentation, that they are microbial loop feeders. We are far from certain as to the exact role of appendicularians as grazers in pelagic food webs and measurements of appendicularian feeding at different temporal and spatial scales are needed to resolve these issues.

Although some information is available on factors affecting feeding rates under controlled laboratory conditions (e.g. Acuña & Kiefer 2000), this approach does not allow the determination of the relative importance of these factors in the field (Peters & Downing 1984). On the other hand, *in situ* information is particularly scarce, studies are

commonly restricted in time or space and information on the feeding rates of some abundant species is completely lacking. Wide spatio-temporal and species-specific coverage requires an easily applicable technique to estimate grazing that is suitable for routine sampling from ships. The gut chlorophyll content technique (Mackas & Bohrer 1976) meets these requirements, and has provided much of the wealth of interesting auto and synecological information available on crustacean zooplankton. This technique has two major drawbacks: the uncertainty about the degree of pigment degradation during digestion and its restriction to chlorophyll containing prey (Bamstedt et al. 2000). Although chlorophyll degradation in appendicularians has been found to be more consistent than in copepods (Bochdansky et al. 1998), appendicularians are known to feed on a wide range of non-autotrophic material (e.g. detritus Gerber & Marshall 1974, Dagg et al. 1996, heterotrophic flagellates Urban et al. 1992). Tackx et al. (1995) proposed a method to measure ingestion rates on total particulate matter based on the food volume in the gut instead of the gut chlorophyll content, and used it in combination with Coulter Counter measurements and bottle incubations to assess feeding selectivity by estuarine copepods. Bochdansky & Deibel (1999) also suggested that the volume of food in the gut of the cold-water appendicularian *Oikopleura vanhoeffeni* could be used to estimate its ingestion rate.

We have employed both approaches in parallel, gut chlorophyll and gut food volume content techniques, to determine the feeding rates of the different appendicularian species during a seasonal cycle at four different stations ranging from well-mixed, neritic locations to oceanic, seasonally stratified environments. In addition, we have explored the relationship between these feeding rates and physical and biological characteristics. We have then combined our ingestion rate estimates with appendicularian species abundance to determine the impact of appendicularian grazing on primary producers and on total particulate material.

MATERIALS AND METHODS

Data collection.

Samples were collected between April 1999 and February 2000 at weekly intervals from a coastal station in the English Channel (L4; Fig. 1 A), and on a monthly basis from April 1999 to May 2000 on a transect of three stations across the shelf in the

central Cantabrian Sea (E1, E2 and E3, Fig.1 B). At each station, vertical profiles of temperature (ºC), salinity (psu) and chlorophyll (µg l^{-1}) were obtained using a SBE 25

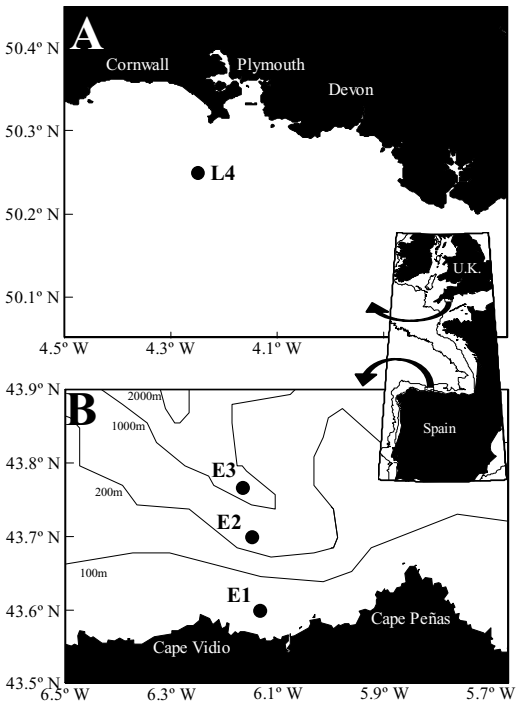

Figure 1. Study sites. (**A**) L4 in coastal waters of the English Channel, approximately 55 meters water depth. (**B**) E1, E2 and E3 in the Cantabrian Sea (NW Spain) 50, 120 and 1000 meters deep respectively.

CTD (Sea Bird Instruments) in the Cantabrian Sea and a CTD probe developed for the Undulating Oceanographic Recorder (Aiken & Bellan 1990) in the English Channel. Standard deviation of temperature (ºC) over the upper 50 meters of the water column was used as an index of thermal stratification (Valdes & Moral 1998) and the depth of the mixed layer was calculated using the formula in Sprintall & Tomczak (1992) assuming a temperature difference of 0.5ºC. Seawater collected from 10 m with a 5 L Niskin bottle was used for size-fractionated chlorophyll and for particle volume measurements. Two 250 ml water samples, one of them prefiltered using a 30 µm Nitex mesh, were filtered through 25mm Whatman GF/F filters to determine >0.7 and 0.7-30 µm chlorophyll concentrations (total and <30 fractions, µg l^{-1}) using the standard fluorometric procedure (U.S. Environmental Protection Agency Method 445.0). The fraction >30 µm, estimated as the difference between total and <30 µm fractions, was considered to represent non ingestible material (larger than the pore width of the house inlet filter: Flood & Deibel 1998). Total particle volume concentration (µm^3 ml^{-1}) in the size range 2-30 µm ESD (Equivalent Spherical Diameter) was measured using a Coulter Multisizer II fitted with a 70 µm aperture tube. The samples collected at 10m were

considered representative of the chlorophyll and particle volume concentrations over the whole surface mixed layer where appendicularians are mainly present (see Results and Fig. 2). There was no significant difference between the integrated total chlorophyll concentrations for the whole mixed layer obtained using data collected at 10 m depth intervals and those obtained assuming a constant chlorophyll concentration equal to that obtained from the 10m sample (data not shown). Primary production (mg C m^{-2} day^{-1}) was measured at station E2, using water collected at depths corresponding to 100% and 1% of surface irradiance, and to the chlorophyll maximum, following methods as described in Serret et al. (1999).

Vertical hauls using a 200 µm WP-2 net, from 50 meters at the shallow stations (L4 and E1, Fig. 1) and 100 meters in deep oceanic stations (E2 and E3, Fig. 1) to the surface, were used for determination of appendicularian abundance and for gut content analysis. After collection, the entire cod end was diluted into filtered seawater (Whatman GF/F), reduced to a known volume of water (usually 200 ml) and five 10 ml aliquots removed with a wide bore automatic pipette, placed in criovials and immediately frozen with liquid nitrogen.

In the laboratory, the frozen sample was taken out of the vial and placed in a Petri dish containing filtered seawater and up to 30-40 appendicularians were picked out as they detached from the thawing sample using a needle and a binocular microscope under dim light. Individuals were isolated onto a glass well slide with a drop of filtered seawater, photographed under an inverted microscope and individually transferred into 300 µl fluorometer minicells (Tuner Designs, Inc.) which were filled with 100 µl, chilled 90% acetone. Samples were extracted for 24 hours at –20ºC in darkness and chlorophyll concentration measured, using the standard fluorometric procedure, with a TD-700 fluorometer and a Minicell Adapter Kit (Turner Designs, Inc.). The remaining unfrozen aliquot was then counted to determine appendicularian densities.

The digitised images of the animals were used to determine the volume of food inside the gut of each animal, using a specifically designed image analysis software (available on request from Angel López-Urrutia). Food parcels were visualized as dark shades inside the gut of the animal and their length and area measured. Their volume was calculated assuming the food parcel to be a prolate spheroid if it was material already packed in a faecal pellet. When two faecal pellets overlapped, their area was

measured collectively and then divided by two, to avoid overestimation of the total volume. When a food parcel was material present in the stomach of the animal, its volume was also calculated using the formula for a prolate spheroid, but in the case of Oikopleuriid species, where the stomach is dorso-ventrally flattened, the value obtained was divided by three to avoid overestimation of the actual volume (Bochdansky et al. 1998). The gut food volume content (μm^3 ind^{-1}) was calculated as the sum of the volumes of all food parcels in the gut. The trunk length (TL, µm) of each animal was measured as the distance between mouth and distal gonad end.

The vertical distribution of appendicularians was studied at station E2 from June 1999 to April 2000 with depth-specific tows using a 200 µm WP-2 net and a Hydro-Bios net release system. The depth intervals were selected according to the structure of the water column as revealed by the chlorophyll, salinity and temperature vertical profiles from the CTD cast. If a subsurface chlorophyll maximum was observed then three depth intervals were used: surface layer (above the chlorophyll maximum), the chlorophyll maximum (from base to top of the chlorophyll peak) and deep layer (from 100 meters to the base of the chlorophyll maximum). Whenever no subsurface chlorophyll maximum could be detected just two depth intervals were used and the surface layer tow included the whole upper mixed layer determined on the basis of the presence of a thermo or halocline. Samples were processed and analysed for appendicularian species abundance in the same way as the depth-integrated tows described above.

Data analyses.

Multiple forward stepwise regression analyses were completed separately for each species, and on both gut chlorophyll and volume contents. The independent variables included trunk length, average temperature and salinity through the mixed layer, stratification index, concentration of ingestible particles (Chlorophyll <30µm and particle volume concentrations) and the concentration of chlorophyll in large non-ingestible >30µm particles. All dependent and independent variables were logarithmically transformed to linearize the relationships and to stabilize variances. Possible spurious results due to multicollinearity among the independent variables were examined by looking for significant correlations among them, and by deleting one or

more independent variables from the regression model and reanalysing the remaining data (Zar 1999).

To scale data for differences in body size between stations, ANCOVA analysis was used to obtain a body size corrected index for each location and date, equivalent to the gut content of an animal 1mm long (Acuña et al. 1999). This is preferred to weight specific rates due to the allometrical relationship between gut content and body size (Packard & Boardman 1987, Dam & Peterson 1991). Geometric Mean Regression (GMR; Ricker 1984) was used to obtain relationships that could be used to predict gut content from trunk length alone for each of the different species by pooling all data for each location and date.

Calculation of grazing rates

Individual ingestion rates (ng Chl ind^{-1} day^{-1} and μm^3 ind^{-1} day^{-1}) were calculated from gut chlorophyll and gut volume content measurements respectively in combination with gut passage times (GPT) estimated from temperature (T) and food concentration (FC) using the equation $GPT = 51.67e^{-0.0376T}FC^{-0.245}$ developed by López-Urrutia & Acuña 1999 for *Oikopleura dioica* (see Chapter 4). Food carbon concentrations required for this equation were estimated from chlorophyll concentrations in the <30µm fraction after assuming that the phytoplankton carbon to chlorophyll ratio (PhytoC:Chl) for the <30µm fraction was the same as the ratio for the total fraction. PhytoC:Chl ratios for each station and date were obtained from total chlorophyll measurements and estimates of phytoplankton carbon based on microscopic counts. Phytoplankton carbon biomass was estimated from phytoplankton species cell counts following the methods described in Holligan et al. (1984). The PhytoC:Chl ratios obtained at station E2 were used at stations E1 and E3. Although the available food concentration would be higher if we used total particulate organic carbon instead of phytoplankton carbon, we took this conservative approach to ensure that our measurements of ingestion rate were kept as underestimates. To calculate clearance rates we divided the ingestion rate estimates based on gut chlorophyll contents by the concentration of ingestible chlorophyll in the water and the ingestion rates based on gut volume contents by the particle volume concentrations. The percentage of the total chlorophyll and particle volume concentrations consumed by the appendicularian community on a daily basis was estimated by combination of appendicularian

abundances with individual ingestion rates (ng Chl ind^{-1} day^{-1} and μm^3 ind^{-1} day^{-1} respectively). The percentage of the primary production consumed was calculated using ingestion rate estimates at station E2 based on gut chlorophyll measurements and the PhytoC:Chl ratios. In order to calculate the proportion of the diet comprised of chlorophyll containing prey, gut content values obtained by both techniques were transformed into carbon units. Gut chlorophyll contents were transformed using the PhytoC:Chl ratios obtained as described above. To convert gut volume contents into carbon units we used total particulate organic carbon to volume ratios (POC:V) obtained from POC estimates and particle volume concentrations. At station L4, for each date sampled, POC measurements in the fraction <30µm were obtained from triplicate 250 ml aliquots taken at 10m depth, filtered onto 25mm GF/F filters and analysed using a Carlo-Erba Elemental Analyser Model NA 1500. In the Cantabrian Sea, POC concentrations were estimated from the chlorophyll <30µm fraction using the relationships in Legendre & Michaud 1999 (the equation for locations where depth is <300m for stations E1 and E2 and the equation for locations where depth >300m for station E3). POC:V ratios obtained through these approximation were in the range of those obtained from direct measurement of POC at L4 and those reported by Holligan et al. (1984).

RESULTS

Different environments studied

The locations studied (Fig. 1) ranged from station L4, which is shallow, eutrophic, mixed during most of the year and with a strong spring diatom bloom (Fig. 4 B, C), through stations E1 and E2 to station E3, which is deep, seasonally stratified and with low chlorophyll concentrations except during the spring bloom (Fig. 4 T, U). Although there may be differences due to geographical location (i.e. latitude) which are

Table 1. Descriptive statistics of the environmental variables, averaged over the whole study period, for each of the different locations (mean ±SD; number of observations). Particle volume is the concentration of particles in the size range 2-30µm Equivalent Spherical Diameter as determined with a Coulter Counter. Chlorophyll values represent the concentration of chlorophyll for the size fractions indicated. Salinity and temperature are the average values over the mixed layer. Temperature SD is the standard deviation of the temperature over the upper 50m of the water column, used as a simple index of water-column stratification.

Location	Particle volume 2-30µm (μm^3 ml^{-1} $X10^6$)	Chlorophyll 0.7-30µm (μg l^{-1})	Chlorophyll >30µm (μg l^{-1})	Salinity (psu)	Temperature (ºC)	Temperature SD (ºC)
L4	0.677 ±0.267; N=35	1.06 ±0.73; N=39	1.49 ±3.17; N=39	34.61 ±1.68; N=28	12.85 ±2.41; N=34	0.33 ±0.29; N=34
E1	0.49 ±0.40; N=10	0.444 ±0.241; N=13	0.30 ±0.54; N=13	35.482 ±0.147; N=13	14.67 ±2.59; N=13	0.50 ±0.69; N=13
E2	0.43 ±0.42; N=10	0.455 ±0.223; N=13	0.098 ±0.112; N=13	35.531 ±0.094; N=13	15.06 ±2.52; N=13	0.69 ±0.94; N=13
E3	0.281 ±0.074; N=7	0.454 ±0.181; N=10	0.21 ±0.39; N=10	35.590 ±0.083; N=9	15.09 ±2.83; N=9	0.73 ±1.09; N=9

overlooked using our environmental descriptors, average values of both food concentration and physical conditions are consistent with a coastal-oceanic gradient with increasing salinities and stratification and decreasing particle concentrations from L4 through stations E1 and E2 to station E3 (Table 1; Fig. 4).

Appendicularian abundance

Appendicularians were almost exclusively restricted to the upper surface mixed layer (Table 2, Fig. 2). Only in July 1999, some *Oikopleura dioica* and *O.longicauda*

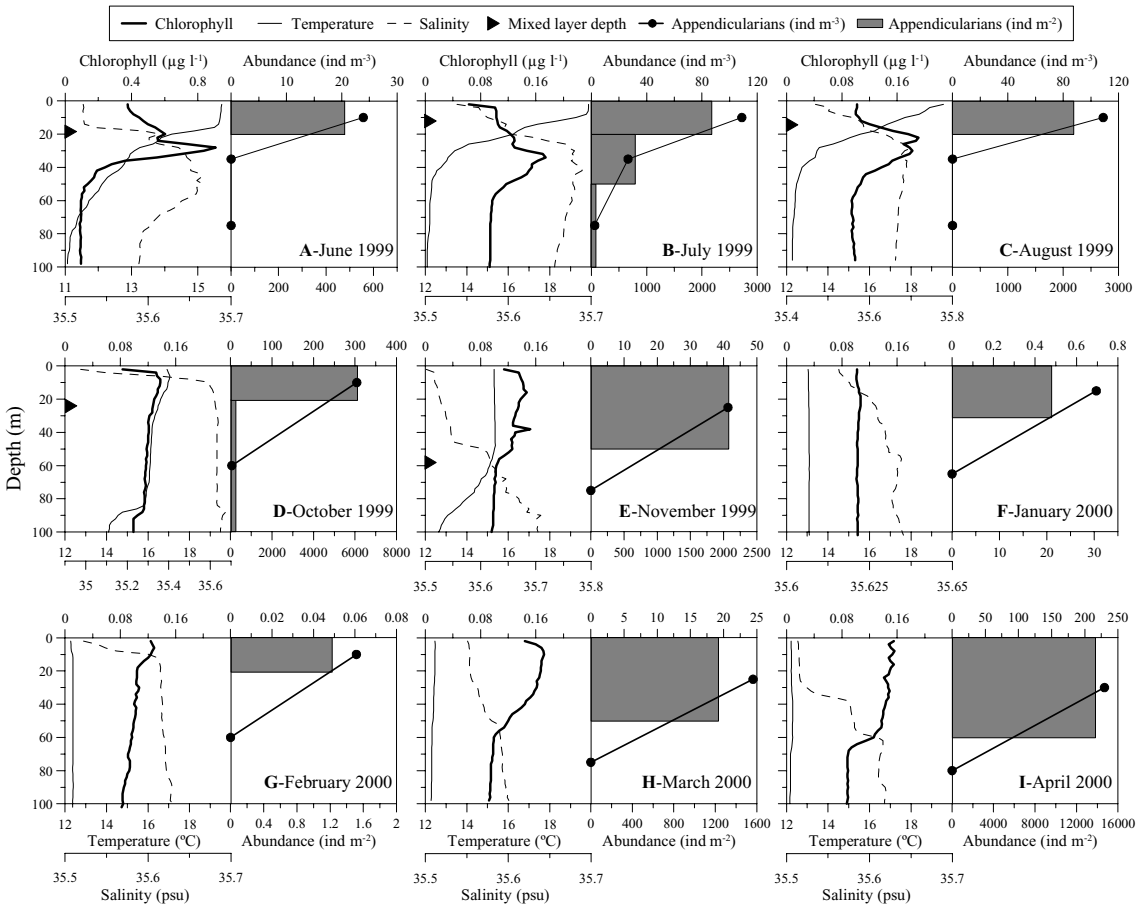

Figure 2. Vertical distribution of appendicularians at station E2. Left panels represent the temperature, salinity and chlorophyll vertical profiles from the CTD cast. Arrow heads on the depth axis represent the mixed layer depth calculated using the formula in Sprintall & Tomczak 1992 assuming a temperature difference of 0.5ºC. Appendicularian abundances are presented as individuals per square meter (lower axis, solid grey bars) and individuals per cubic meter (upper axis, solid circles and lines).

individuals were present in the subsurface chlorophyll maximum. The fact that appendicularians were generally present only in our surface samples, lead us to the preliminary conclusion that there could be a sampling bias due to our net release system, and that appendicularians present in the non-surface intervals were being undersampled. If this was the reason, then the number of appendicularians collected by the depth integrated sample from 100m to the surface should have been much higher

than the number collected by the surface tow. This was not the case and the

Table 2. Average number of individuals found at each depth interval at station E2 expressed as the percentage of the total number for each species collected through the whole water column. Values represent the mean (geometric - arithmetric) of the percentages obtained for each sampling date. Values in brackets represent the standard deviation of the percentages. Geometric means were calculated through a $\log_{10}+1$ transformation.

	Surface	Chlorophyll maximum	Deep
O.longicauda	75-87% (33)	3-23% (41)	1-3% (6)
O.fusiformis	98-98% (4)	1-4% (7)	0.2-0.41% (1)
O.dioica	46-83% (41)	9-50% (71)	0-0% (0)
F.pellucida	91-92% (12)		3-8% (12)
F.borealis	100-100% (0)		0 (0)

appendicularian species abundances obtained by the depth integrated sample were significantly correlated to the abundances in the surface layer (Fig. 3). The slope of the log-log relationship was not significantly different from 1 (p=0.3, Fig. 3), and the intercept was not significantly different than zero (p=0.09, Fig. 3) indicating that there was not a sampling bias and confirming our result that appendicularian populations were restricted to the upper surface layer. This result supports the selection of the 10 m depth sample, as opposed to a water column average, to estimate food concentrations and the use of the average temperature and salinity over the upper mixed layer as representative of the environment where appendicularians are living.

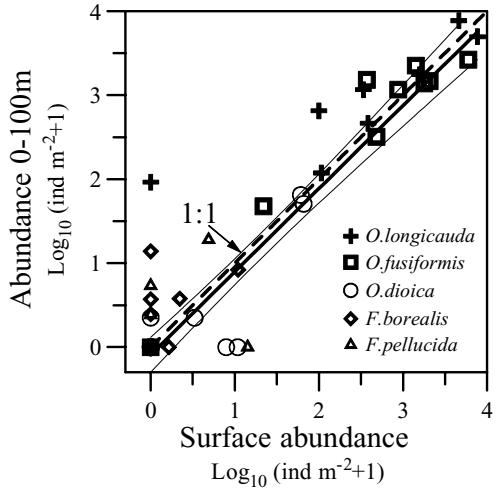

Figure 3. Relationship between the total number of individuals for each species collected using vertical tows from the bottom (100m) to the surface and vertical tows only through the surface layer (see Figure 2 for depth ranges). Dashed line represents the values where all individuals collected are in the surface layer (1:1 ratio). Solid line represents the GMR relationship obtained, which does not significantly depart from the 1:1 line (intercept=-0.09 ± 0.1 (SE), t-test for H_0: intercept=0, t_{43}=1.73, p=0.09; slope=0.989±0.055 (SE), t-test for H_0: slope=1, t_{43}=1.05, p=0.3). Thin solid lines represent the 95% confidence intervals for the regression estimates.

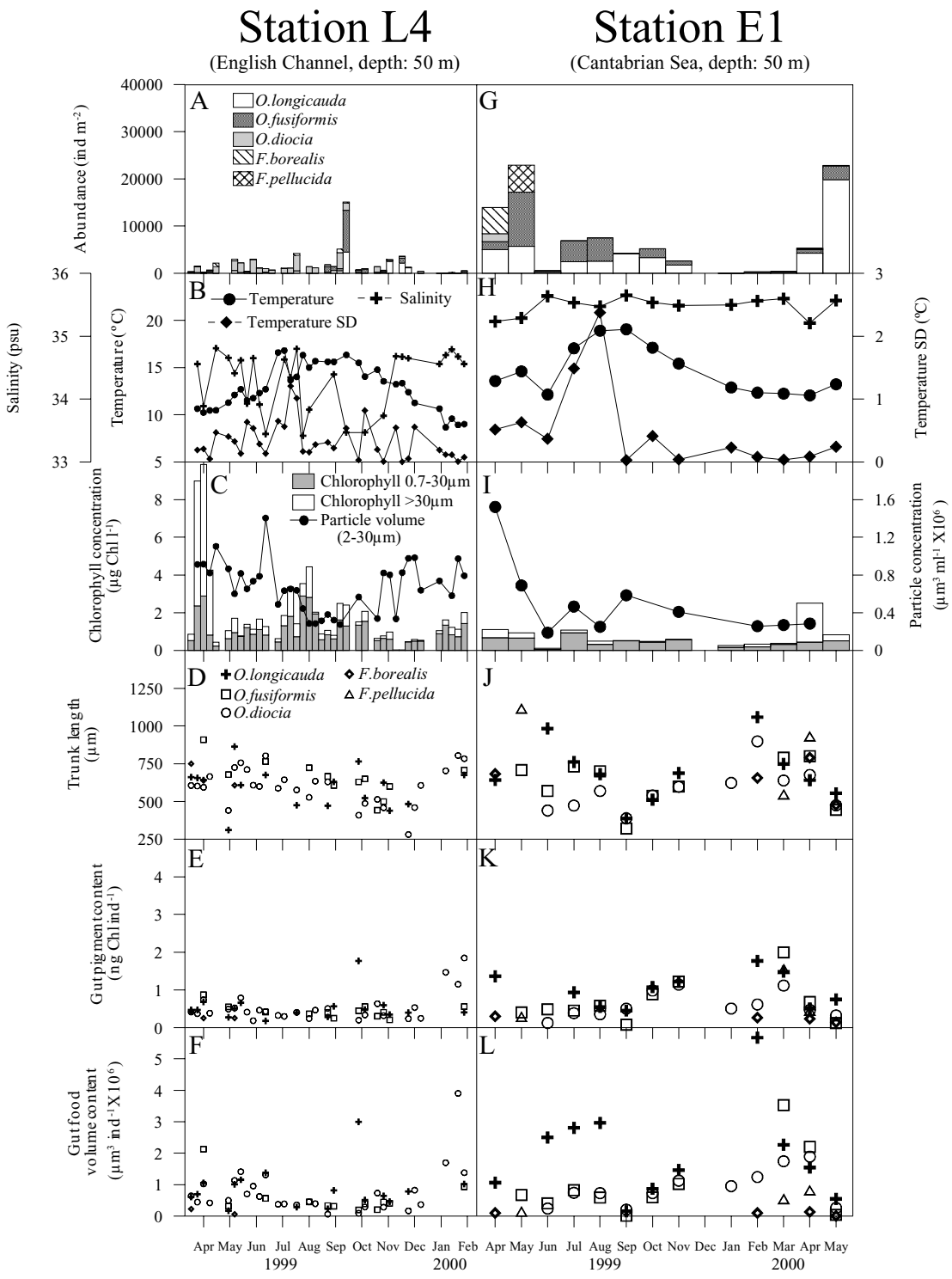

Figure 4. Seasonal variation of (**A, G, M, S**) appendicularian species densities, (**B, H, N, T**) salinity, temperature (averages for the mixed layer) and stratification index (temperature standard deviation of temperature over the upper 50m of the water column), (**C, I, O, U**) size fractioned chlorophyll and particle volume concentrations, (**D, J, P, V**) average appendicularian trunk length, (**E, K, Q, W**) average gut chlorophyll content and (**F, L, R, X**) average gut food volume content for the different appendicularian species at each of the stations studied: (**A-F**) L4, (**G-L**) E1, (**M-R**) E2 and (**S-X**) E3.

Variation in appendicularian abundance was markedly seasonal in the Cantabrian Sea with higher densities during spring and autumn (Fig. 4 G, M, S). At station L4, abundances were lower throughout the year (Fig. 4 A), except for an ephemeral peak of

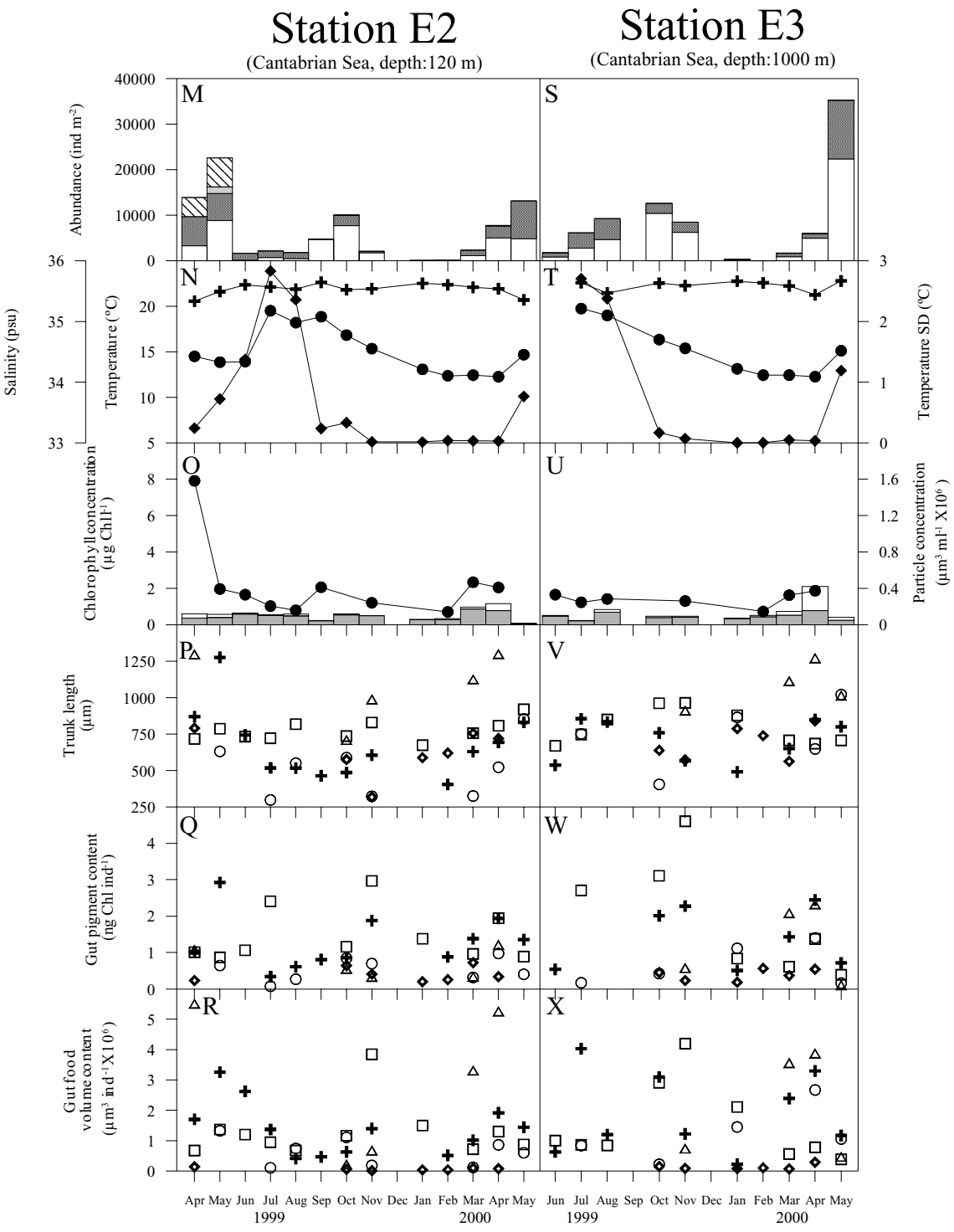

Figure 4. Continued

Oikopleura fusiformis in early autumn. *O. longicauda* and *O. fusiformis* were the dominant species on average for the four stations; *O. dioica* was the only species to show a clear gradient from neritic to oceanic locations, where it was less abundant (Table 3, Fig. 4). *Fritillaria pellucida* and *F. borealis* appeared in low densities mainly during early spring at stations E1 and E2 (Table 3, Fig. 4).

Table 3. Descriptive statistics of the different variables studied, averaged over the whole study period, for each of the different species and locations (mean ±SD and number of observations). For appendicularian abundance the number of non-zero values (presence) as well as the number of observations is shown. Trunk length is the distance between mouth and distal gonad end. Appendicularian gut chlorophyll content and gut food volume content represent the amounts of food inside the gut as obtained by the two separate techniques. In order to remove the effect of differing trunk length among the different locations, body size corrected values are also shown. These represent the gut content of an animal 1mm long as obtained by ANCOVA (see *methods* for details).

	Location	*O.longicauda*		*O.fusiformis*		*O.dioica*		*F.pellucida*		*F.borealis*	
Abundance	L4	359 ±878	37-18	416 ±1552	37-14	879 ±960	37-34	0	37-0	106 ±193	37-19
(ind m^{-2})	E1	3826 ±5174	13-12	2265 ±3219	13-11	165 ±456	13-12	443 ±1588	13-3	431 ±1544	13-5
	E2	2960 ±3020	13-12	2413 ±2717	13-11	124 ±407	13-8	8 ±21	13-4	810 ±2023	313-9
	E3	5313 ±6819	10-9	2809 ±3827	10-9	9 ±17	10-5	2 ±4	10-4	13 ±27	10-7
Trunk length	L4	570 ±187	84	640 ±132	67	614 ±188	271			699 ±83	5
(µm)	E1	539 ±196	86	625 ±173	30	619 ±215	76	920 ±146	18	710 ±183	15
	E2	590 ±224	131	792 ±153	85	574 ±206	34	1171 ±324	42	659 ±169	23
	E3	770 ±287	91	778 ±199	51	620 ±254	10	1172 ±245	11	628 ±167	42
Gut chlorophyll content	L4	0.474 ±0.356	84	0.441 ±0.279	67	0.469 ±0.333	271			0.359 ±0.172	5
(ng Chl-a eq ind^{-1})	E1	0.800 ±0.587	85	0.738 ±0.527	30	0.660 ±0.527	76	0.50 ±0.39	18	0.243 ±0.121	15
	E2	1.31 ±1.13	129	1.46 ±1.01	83	0.693 ±0.561	34	1.02 ±0.69	42	0.371 ±0.228	23
	E3	1.807 ±1.594	74	1.50 ±1.45	32	0.73 ±0.70	10	1.90 ±1.43	11	0.412 ±0.254	42
Body size corrected	L4	1.310 ±0.769	84	0.890 ±0.577	67	1.048 ±0.548	271			0.547 ±0.191	5
gut chlorophyll content	E1	2.35 ±1.57	85	1.53 ±0.96	30	1.47 ±0.96	76	0.64 ±0.80	18	0.397 ±0.202	15
(ng Chl-a eq ind^{-1})	E2	3.04 ±2.01	129	2.12 ±1.44	83	1.82 ±1.22	34	0.83 ±0.46	42	0.660 ±0.466	23
	E3	2.82 ±1.84	74	2.06 ±1.67	32	1.73 ±1.10	10	1.36 ±0.73	11	0.737 ±0.348	42
Gut food volume	L4	72.7 ±68.1	84	41.1 ±41.1	67	69.0 ±81.0	271			15.5 ±10.2	5
($10^4 µm^3$ ind^{-1})	E1	91 ±121	86	84 ±103	30	115 ±122	76	77 ±89	18	10.9 ±8.2	15
	E2	113 ±137	131	116 ±113	85	83 ±126	34	410 ±477	42	6.76 ±4.27	23
	E3	239 ±255	91	112 ±120	51	122 ±149	10	319 ±276	11	9.2 ±8.6	42
Body size corrected	L4	314 ±196	84	133 ±97	67	215 ±177	271			32.1 ±17.3	5
gut food volume	E1	319 ±213	86	271 ±213	30	324 ±218	76	119.6 ±125.0	18	21.0 ±11.5	15
($10^{-4} µm^3$ ind^{-1})	E2	373 ±255	131	219 ±192	85	293 ±206	34	144.4 ±112.1	42	16.6 ±8.9	23
	E3	364 ±230	91	198 ±144	51	312 ±243	10	145.2 ±72.6	11	23.9 ±12.5	42

Gut content variability

Methodological considerations

Ingestion rates estimated by the two separate gut content techniques represent underestimates since no correction was made for chlorophyll degradation or compression of food items when packed in faecal pellets. In addition, our estimated gut passage times ranged between 8.15 and 30 minutes, with an average of 14.34 minutes, well above previously reported values (8 min for *Oikopleura dioica* Alldredge 1981; 5.7 min *Fritillaria borealis* pers. obs.). Despite this conservative approach to avoid overestimation of grazing impact, the clearance rates derived by combination of our ingestion rate estimates with ambient food concentrations, were usually higher than the values reported in the literature (Fig. 5). Use of correction indices to account for pigment destruction (61-77%, Bochdansky et al. 1998) or material stuck in the filter house and not ingested (up to 80% of the material ingested, Acuña & Kiefer 2000), would have lead to unrealistically high clearance rates. Moreover, despite the

completely different approaches that we have followed to measure gut content and food concentration (chlorophyll *vs.* particle volume), both methods yielded similar clearance rates (Fig. 5; for *Oikopleura dioica*, log-transformed clearance rates by both methods were significantly correlated, r = 0.57, n=325, p<<0.001), which confirms that appendicularians are non-selective filter feeders and that our techniques for the measurement of grazing rates are mutually consistent. Even if we considered that

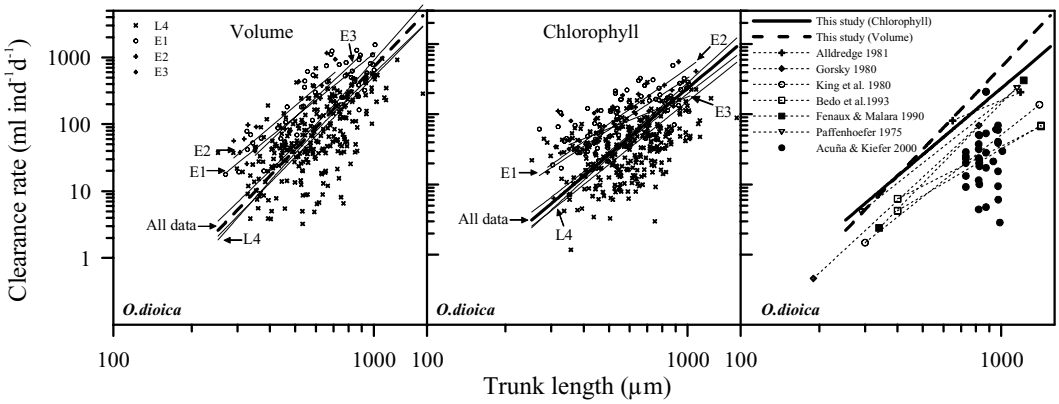

Figure 5. (**A**, **B**) Clearance rate versus trunk length relationships for *O. dioica* at each of the different locations studied (thin lines) and for data from all locations pooled (thick lines); (**A**) data were estimated from gut volume content and *in situ* particle volume concentration (CR (ml ind $^{-1}$ day^{-1})= 3.9 10^{-10} TL (µm) $^{4.08}$, n=325, r^2=0.36), (**B**) data were estimated from gut chlorophyll content and water chlorophyll concentration (CR (ml ind $^{-1}$ day^{-1})= 4.52 10^{-8} TL (µm) $^{3.29}$, n=391, r^2=0.18). Gut passage times estimated using equation in López-Urrutia & Acuña 1999 were used for both approaches. GMR was used in all cases. (**C**) Comparison of the clearance rate *vs.* trunk length relationships obtained in this study against clearance rate *vs.* trunk length relationships compiled by Deibel 1998 updated with data from Acuña & Kiefer 2000. All data represent particles cleared by the house and the animal together, except for our data and those of King et al. 1980 that represent the particles ingested by animals only.

appendicularians are able to feed on the full bacterial size range and we add to our Coulter Counter measurements typical bacteria volume concentrations in the area studied (e.g. 65000 µm^3 ml^{-1} at station L4, M. Zubkov personal communication), our estimates of clearance rates from particle volume concentrations and gut content volumes still remain higher than previously reported values and close to our estimates from chlorophyll measurements (data not shown). The only previously reported values showing clearance rates close to our estimates are those of Alldredge 1981 (Fig. 5), who conducted the only other *in situ* study. Whether this mismatch between laboratory and field estimates is due to improper sampling of the actual food concentrations in field studies (e.g. due to food patchiness) or to underestimation of appendicularian ingestion rates associated to laboratory experimentation needs further investigation.

Differences in gut contents between environments and season

The average gut content values obtained at the different stations for each species are summarized in Table 3. There is a consistent pattern of lower gut chlorophyll and

volume content values at the coastal (L4 and E1) compared to the oceanic stations (E2 and E3). However, trunk lengths were also shorter at the coastal stations, thus lower gut contents near the coast could be simply due to the smaller size of appendicularians, and not to fuller guts. To remove the effect of body size on gut contents, we calculated body-size corrected gut content values using ANCOVA, these values representing the gut content adjusted to that of an animal 1 mm long. The corrected values showed a more subdued pattern, although differences between shallow and oceanic locations still remained, indicating environmental effects that could not be explained by animal size. Amongst the different species, *Oikopleura longicauda* and *Oikopleura fusiformis* had the higher gut contents, particularly when the effect of trunk length was removed. There was no clear seasonal pattern at any of the locations in either gut chlorophyll contents or gut food volume contents, especially when compared to the differences between stations (Fig. 4), and if we take into account the seasonal changes in average body size (Fig. 4 D, J, P, V).

Multivariate analysis

The relationships of gut contents for each species with body size and different environmental factors were modelled using multiple regression analysis (Table 4). Particle volume concentration was excluded from the model because, when it showed significant effects (only two out of the ten models, data not shown), it presented substantial intercorrelation with the rest of the independent variables, leading to spurious results. In addition measurements of particle volume concentration are rarely available in oceanographic studies, which would have restricted the potential use of the predictor equations obtained. Body size was the variable explaining most variability (Table 4). After body size, salinity generally described most of the remaining variance (Table 4), which is consistent with the trend from diluted coastal environments with low gut contents to saline, oceanic stations where gut contents were higher (Tables 1 and 2). Gut contents increased with food concentration ($Chl_{<30}$, Table 4) and decreased with the concentration of large, non-ingestible particles ($Chl_{>30}$, Table 4). The effect of temperature was small and not significant for all species. However, gut contents showed a decreasing pattern with increasing stratification index ($T_{SD,}$ Table 4). The high degrees of freedom in our multiple regression analysis (Table 4) imply that we were able to detect and include in our regression models statistically weak effects, although their

magnitude and therefore importance as predictor variables was low (Table 4; Graham 2001). Also, because it was not possible to collect the same the number of

Table 4. Summary of multiple forward stepwise regression models of gut chlorophyll content (GCC, ng Chl-a eq ind^{-1}; left panels) and gut food volume content (GVC, μm^3 ind^{-1}; right panels) *vs.* trunk length (TL, µm; distance between mouth and distal gonad end), chlorophyll concentration for each size fraction ($Chl_{<30}$, $Chl_{>30,}$ µg Chl-a eq l^{-1}), average mixed layer salinity (Sal, psu) and temperature (T, ºC), and stratification index calculated as the temperature standard deviation of the upper 50 m of the water column ($T_{SD,}$ ºC). All variables were *log*-transformed before analysis, only those descriptors with significant partial correlation are shown (* significant, ** highly significant); the final *back*-transformed equations are included.

	Gut Chlorophyll Content (GCC; ng Chl-a eq ind^{-1})						Gut Volume Content (GVC; μm^3 ind^{-1})					
O.longicauda	$GCC=10^{-36.6}\ TL^{1.679}\ Sal^{20.5}\ T_{SD}^{-0.147}\ Chl_{>30}^{-0.011}$						$GVC=10^{-2.04}\ TL^{2.81}\ T_{SD}^{-0..087}$					
	($F_{3,332}$=133.3, R^2=0.56)						($F_{2,353}$=398.08, R^2=0.69)					
	b	SE	t			R^2 increase	b	SE	t			R^2 increase
Intercept	-36.6	±4.7	-7.84	**			-2.04	±0.28	-7.32	**		
TL (µm)	1.679	±0.098	17.21	**	1	0.42	2.810	±0.100	28.20	**	1	0.68
Sal (psu)	20.5	±3.0	6.77	**	2	0.08						
T_{SD} (ºC)	-0.147	±0.027	-5.35	**	3	0.04	-0.087	±0.027	-3.17	**	2	0.01
$Chl_{>30}$ (µg l^{-1})	-0.011	±0.023	-3.34	*	4	0.01						
O.fusiformis	$GCC=10^{-23.4}\ TL^{1.99}\ Sal^{11.36}\ Chl_{<30}^{0.41} Chl_{>30}^{-0.22}$						$GVC=10^{-3.1}\ TL^{3.04}\ Chl_{<30}^{0.183}\ Chl_{>30}^{-0.21} T_{SD}^{-0.078}$					
	($F_{4,184}$=37.13, R^2=0.45)						($F_{4,198}$=46.52, R^2=0.48)					
Intercept	-23.4	±4.3	-5.48	**			-3.10	±0.68	-4.59	**		
TL (µm)	1.99	±0.22	9.18	**	1	0.27	3.04	±0.24	12.81	**	1	0.43
Sal (psu)	11.36	±2.82	4.03	**	2	0.06						
$Chl_{<30}$ (µg l^{-1})	0.415	±0.075	5.57	**	3	0.05	0.183	±0.085	2.15	*	4	0.01
$Chl_{>30}$ (µg l^{-1})	-0.220	±0.048	-4.62	**	4	0.06	-0.210	±0.053	-3.96	**	2	0.03
T_{SD} (ºC)							-0.078	±0.032	-2.41	*	3	0.02
O.dioica	$GCC=10^{-4.13}\ TL^{1.29}\ Chl_{>30}^{-0.08}\ T_{SD}^{-0.20}$						$GVC=10^{-5.79}\ TL^{2.38}\ T_{SD}^{-0.233}\ Chl_{<30}^{-0.159}\ Sal^{-4.47}$					
	($F_{3,337}$=82.97, R^2=0.42)						($F_{4,336}$=93.99, R^2=0.53)					
Intercept	-4.13	±0.27	-15.29	**			5.79	±1.45	3.98	**		
TL (µm)	1.291	±0.097	13.26	**	1	0.31	2.38	±0.14	17.31	**	1	0.43
T_{SD} (ºC)	-0.198	±0.028	-7.11	**	2	0.10	-0.233	±0.039	-5.90	**	2	0.05
$Chl_{>30}$ (µg l^{-1})	-0.077	±0.021	-3.57	**	3	0.02						
Sal (psu)							-4.47	±0.91	-4.89	**	3	0.03
F.pellucida	$GCC=10^{-92}\ TL^{1.21}\ Sal^{57}$						$GVC=10^{-5.41}\ TL^{3.63}\ T_{SD}^{-0.44}$					
	($F_{2,68}$=13.60, R^2=0.28)						($F_{2,68}$=139.62, R^2=0.80)					
Intercept	-92	±35	-2.65	*			-5.41	±0.95	-5.68	**		
TL (µm)	1.21	±0.32	3.81	**	1	0.21	3.63	±0.34	10.80	**	1	0.75
Sal (psu)	57	±23	2.52	*	2	0.07						
T_{SD} (ºC)							-0.44	±0.10	-4.34	**	2	0.05
F.borealis	$GCC=10^{-3.85}\ TL^{1.2}\ Chl_{>30}^{-0.25}\ Chl_{<30}^{0.66}$						$GVC=10^{-2.54}\ TL^{2.61}$					
	($F_{3,78}$=10.93, R^2=0.30)						($F_{1,80}$=86.23, R^2=0.52)					
Intercept	-3.85	±0.69	-5.56	**			-2.54	±0.79	-3.22	*		
TL (µm)	1.20	±0.25	4.88	**	1	0.13	2.61	±0.28	9.29	**	1	0.52
$Chl_{>30}$ (µg l^{-1})	-0.253	±0.060	-4.19	**	2	0.08						
$Chl_{<30}$ (µg l^{-1})	0.66	±0.21	3.19	**	3	0.09						

appendicularians for gut content analyses at all the different locations or dates, the patterns depicted by the multiple regression analysis are less influenced by the situations where appendicularians were rare, and the number of animals analysed lower, than by those stations where a sufficient number of individuals could be collected for gut content measurement. To correct for this bias towards situations of high abundances of appendicularians, we performed the same multiple regression approach but instead of the raw data we used the average gut content values at each location, once the

differences in body size were corrected through ANCOVA analyses. Therefore, a corrected or adjusted mean was obtained for each station and date corresponding to the gut content of a hypothetical animal 1mm in trunk length. Through this approach

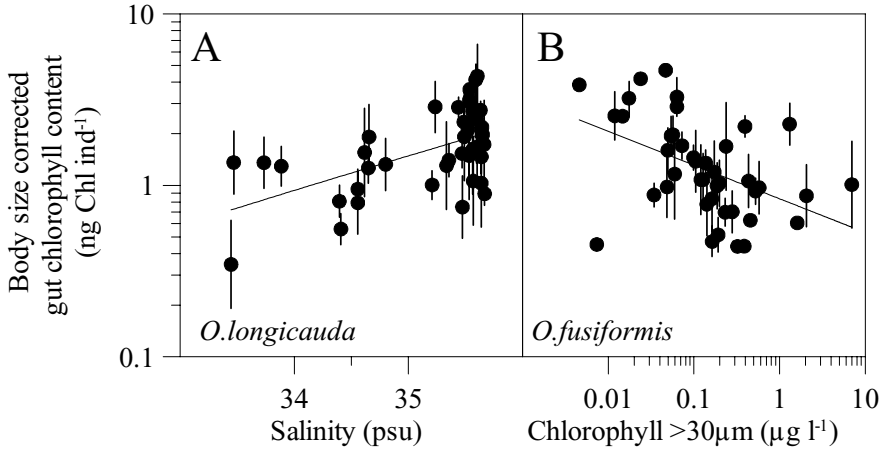

Figure 6. Average, body size corrected (to a standard trunk length of 1mm) gut chlorophyll contents (SGCC) *vs.* (**A**) salinity (Sal), for *Oikopleura longicauda* and (**B**) chlorophyll in particles larger than 30 µm ($Chl_{>30}$), for *O. fusiformis*. *Solid* circles represent the average gut chlorophyll content for each station and date sampled corrected through ANCOVA to that of an animal 1mm in trunk length. Lines represent least squares power regressions ($SGCC = 2.91\ 10^{-25}\ Sal^{16}$, r^2=0.3, $F_{1,41}$=17.47, p<0.001 for *O.longicauda*; $SGCC = 0.84\ Chl_{>30}^{-0.2}$, r^2=0.19, $F_{1,35}$=8.36, p<0.007 for *O. fusiformis*).

we also reduced the number of data points (and hence the degrees of freedom) and were able to detect those variables that have a strong statistical effect on the appendicularian gut contents. Only two significant relationships could be detected by the forward stepwise multiple regression approach: the gut chlorophyll contents of *O.longicauda* increased with increasing salinity (Fig. 6 A) and the gut chlorophyll contents of

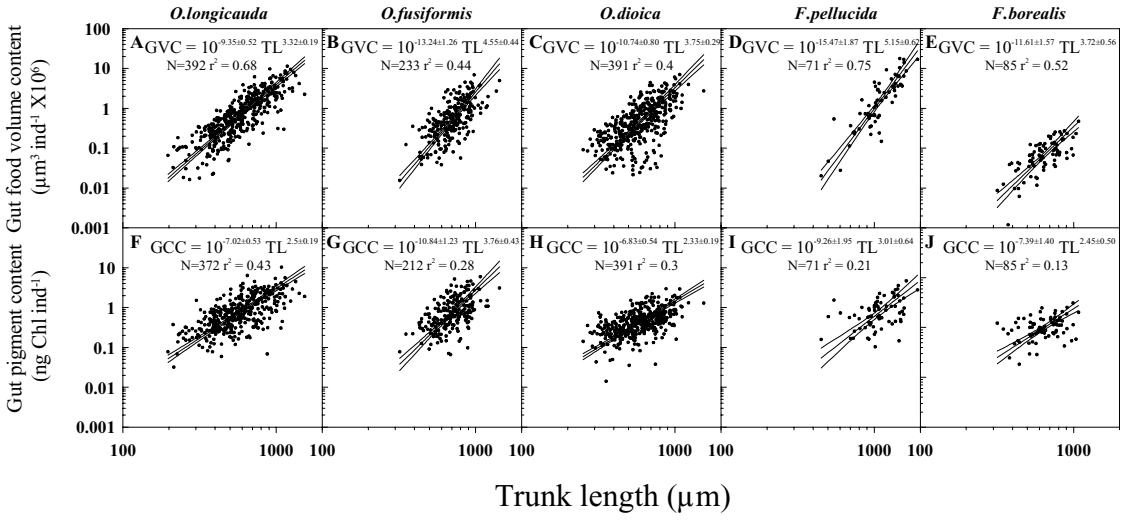

Figure 7. (**A-E**) Gut food volume content (GVC) and (**F-J**) gut chlorophyll content (GCC) as a function of appendicularian trunk length (TL) for the different species studied. Relationships were obtained by pooling data from all locations and dates, and calculating the GMR on the log_{10} transformed data. Equations show parameter estimates ± 95 % confidence limits, together with the number of data and r^2 values. Lines represent the regression line (thick lines) and 95 % confidence limits for the parameter estimates (thin lines).

Oikopleura fusiformis decreased with increasing chlorophyll concentration in the large fraction (Fig. 6 B). For the rest of species we detected no significant relationship with environmental variables. Therefore, we estimated simplified regression equations that used only the variable explaining most of the variability (i.e. trunk length). GMR power curves relating gut content and trunk length were highly significant and had exponents ranging from 3.32 to 5.15 for gut volume (Fig. 7 A-E) and between 2.45 and 3.01 for gut pigment content (Fig. 7 F-J). The higher allometric exponents for gut volume contents than for gut chlorophyll (Fig. 7, Table 4) mean that larger animals eat less chlorophyll containing prey per unit volume ingested than small animals. This has important implications for our initial objective of comparing the proportion of

Table 5. Average grazing impact expressed as the percentage chlorophyll and volume consumed on a daily basis for each of the species and locations studied. Values represent the geometric (GM, through a $Log_{10}+1$ transformation) and arithmetic means, the standard deviation (SD) and the maximum for each location. The percentage chlorophyll bearing prey represents the proportion of the total ingested material coming from autotrophic prey, after transformation of gut content volume into total carbon and gut chlorophyll into phytoplanktonic carbon. At L4 they were transformed using POC:Volume and Phytoplankton carbon: Chl ratios obtained at each date, values at E1, E2, E3 are dependent on the criteria used to obtain carbon conversion factors (see Methods for an explanation). Values represent the geometric (GM) and arithmetic means and the standard deviation of the estimates for each date and species.

		Percentage consumed								**% Chlorophyll bearing prey**		
		Chlorophyll				Volume						
		GM	Mean	SD	Maximum	GM	Mean	SD	Maximum	GM	Mean	SD
O.longicauda	L4	0.0158	0.0164	±0.0361	0.1450	0.078	0.092	±0.204	0.682	8.0	16.2	±23.5
	E1	0.72	0.92	±1.09	3.77	1.43	2.03	±2.42	7.63	10	29	±45
	E2	0.81	1.25	±1.79	5.04	0.76	1.33	±2.51	8.25	14.4	22.9	±16.8
	E3	0.94	1.51	±1.94	4.65	1.49	2.00	±1.71	4.00	15	21	±17
	All stations	0.35	0.60	±1.25	5.04	0.51	0.89	±1.75	8.25	27	21	±11
O.fusiformis	L4	0.01	<0.01		0.0553	0.0216	0.0226	±0.0458	0.1939	9.6	17.1	±19.3
	E1	0.31	0.38	±0.50	1.43	0.74	1.01	±1.19	2.93	13	27	±32
	E2	0.52	0.80	±1.48	5.33	0.51	0.60	±0.66	2.31	15.3	31.1	±48.7
	E3	0.72	0.97	±1.12	2.81	0.70	1.00	±1.36	3.59	19	20	±10
	All stations	0.21	0.33	±0.83	5.33	0.27	0.41	±0.82	3.59	30	23	±13
O.dioica	L4	<0.01	0.052	±0.064	0.249	0.159	0.176	±0.224	0.880	12.1	31.6	±59.3
	E1	0.01	<0.01		0.0397	0.039	0.041	±0.070	0.209	8	12	±16
	E2	0.014	0.016	±0.051	0.185	0.042	0.052	±0.168	0.560	9	14	±11
	E3	<0.01	<0.01		0.005	<0.01	<0.01		0.0194	3	5	±5
	All stations	0.03	0.03	±0.05	0.25	0.09	0.10	±0.19	0.88	47	24	±10
F.pellucida	L4	0	0		0.00	0	0		0.00			
	E1	0.03	0.04	;(±0.13);	0.4607	0.02	0.03	;(±0.09);	0.3239	18	33	±36
	E2	<0.01	<0.01		0.0073	0.01	0.01	;(±0.03);	0.0906	6	8	±7
	E3	<0.01	<0.01		0.0023	<0.01	<0.01		0.0094	6.9	7.2	±02.2
	All stations	0.01	0.01	±0.06	0.46	0.01	0.01	±0.04	0.32	21	15	±9
F.borealis	L4	<0.01	<0.01		0.02	0.00	<0.01		0.004	40	42	±16
	E1	0.02	0.03	;(±0.10);	0.37	0.01	0.01	;(±0.03);	0.09	60	125	±155
	E2	0.013	0.014	±0.048	0.174	0.00	<0.01		0.04	102	139	±121
	E3	<0.01	<0.01		0.00	0.00	<0.01		0.002	53	72	±56
	All stations	0.01	0.01	±0.05	0.37	0.00	<0.01		0.09	102	103	±66
All species	L4	0.082	0.084	±0.070	0.265	0.29	0.32	±0.26	0.94	43.3	24.5	±10.7
	E1	0.96	1.20	±1.15	3.87	1.95	2.82	±3.19	10.54	60	34	±13
	E2	1.14	1.92	±2.96	10.12	1.23	2.00	±3.31	11.11	67	40	±17
	E3	1.34	2.21	±2.56	6.13	2.08	3.00	±2.64	6.52	36	28	±15
	All stations	0.58	1.01	±1.90	10.12	0.91	1.50	±2.46	11.11	13	31	±53

autotrophic prey in the diet of appendicularians with the same ratio in the water suspension, because higher gut chlorophyll: volume ratios in a given location may be due not to a predominantly autotrophic diet, but to a numerical predominance of the smaller appendicularian size classes. However, visual inspection of data divided into size classes and multiple regression approaches including body size as an independent variable did not reveal any clear patterns (data not shown). Although the calculation of the proportion of the total ingested carbon coming from autotrophic prey depends strongly on the conversion factors used to transform data from chlorophyll and volume units to carbon values, our estimates show that over 60% of the gut contents of most species are of heterotrophic origin, with the exception of *Fritillaria borealis*, whose diet was more strongly dominated by autotrophic prey (Table 5).

Grazing impact on natural particles

Appendicularian community grazing impact on phytoplankton biomass was highest at the oceanic stations during early spring and autumn conditions with maximum values close to 10 percent of the total biomass removed daily (Table 5, Fig. 8). These values do not take into account material cleared but not ingested (i.e. attached to the filter house). *Oikopleura longicauda* and *Oikopleura fusiformis* were the most important species as particle grazers (Table 5).

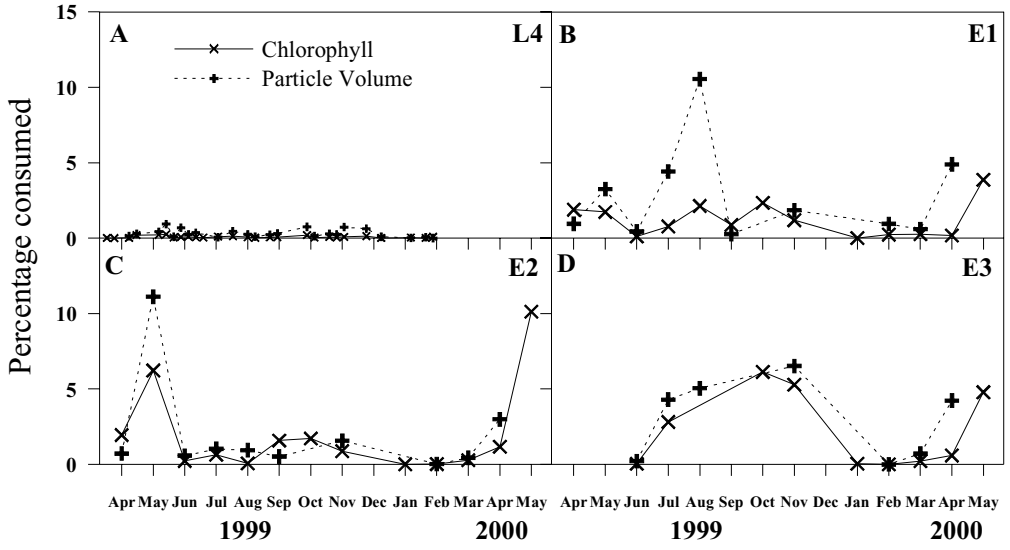

Figure 8. Seasonal variation of the percentage of the total chlorophyll concentration and particle volume concentration in the size range 2-30µm consumed by the appendicularian community on a daily basis, estimated with the gut chlorophyll and gut food volume techniques respectively at station (**A**) L4, (**B**) E1, (**C**) E2 and (**D**) E3.

The appendicularian community at station E2 consumed over 40% of the primary production per day during autumn when primary production was low and

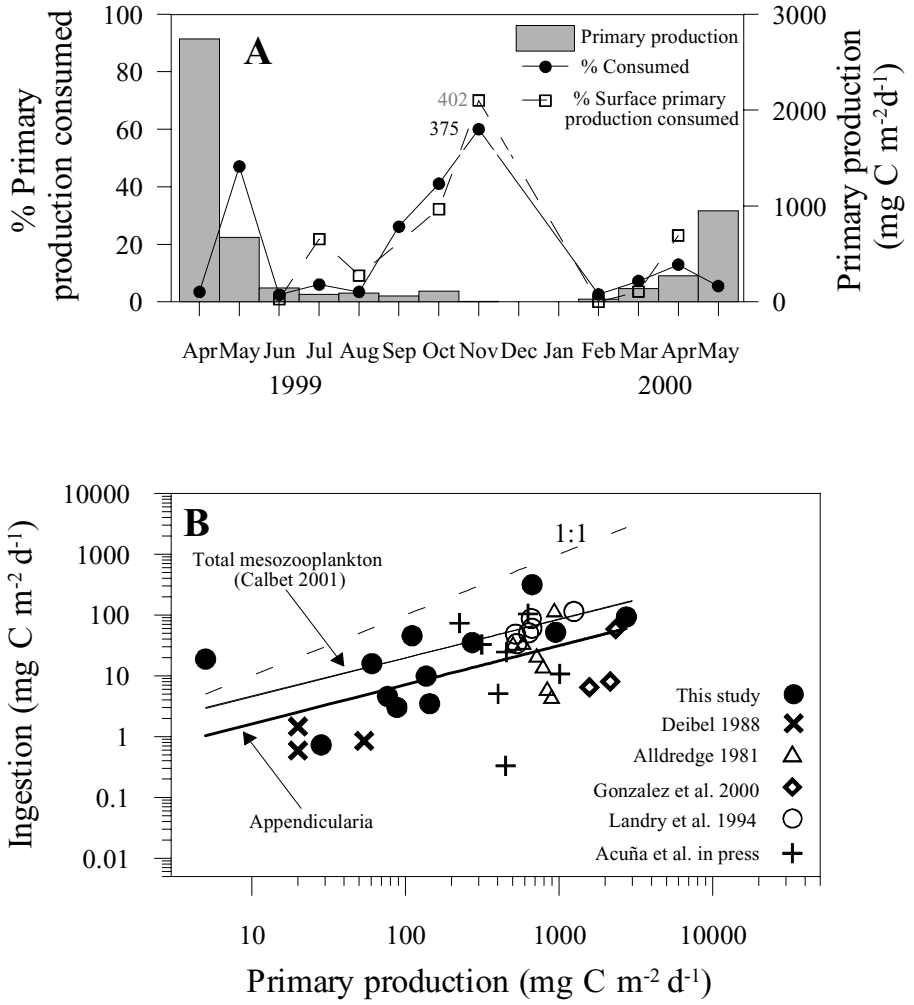

Figure 9. (**A**) Seasonal variation in primary production integrated over the whole photic layer and percentage of the primary production removed by the appendicularian community on a daily basis according to the gut pigment technique in combination with abundance Dashed lines and squares represent the estimates obtained using abundance data from the surface depth intervals (see Figure 2) and primary production integrated only through the surface layer sampled for the appendicularian tows. (**B**) Compilation of simultaneous measurements of appendicularian community ingestion rates (I_{app}) and primary production (PP). Data from this study represent values measured at station E2 integrated for the whole water column. Ingestion rate values in Gonzalez et al. 2000 were recalculated using their gut chlorophyll content data, uncorrected for pigment degradation, and gut passage times estimated using equation in López-Urrutia & Acuña 1999. Primary production in Alldredge 1981 was estimated from rates of increase and concentrations of ingestible particulate organic carbon (<15µm), assuming a water column depth of 30 meters (Alldredge per. comm.). Deibel 1988 ingestion rate values represent daily consumption of ingestible chlorophyll (<70µm). The thick line represents the least-squares, power regression obtained (I_{app} (mg C m^{-2} d^{-1}) = 0.37 PP (mg C m^{-2} d^{-1})$^{0.64}$; r^2=0.32; N= 38). The thin line represents the relationship between total mesozooplankton ingestion and primary production from Calbet 2001. Dashed line represents the values over which ingestion exceeds primary production.

appendicularia abundant (Fig. 9 A). During the spring bloom appendicularians consumed 3-40% and on a yearly basis appendicularians removed an average 8% (geometric mean) of the daily primary production. In order to compare our results with previous reports of *in situ* appendicularian grazing impact, we compiled data from publications reporting simultaneous estimates of appendicularian community ingestion

rates and primary production (Fig. 9 B). According to this compilation, appendicularian community grazing pressure increases with increasing productivity (PP), although the percentage of the primary production removed by appendicularians decreased with increasing productivity. The percentage of the primary production consumed averaged 8±26 % (Geometric mean±S.E.) in unproductive (PP < 250 mg C m^{-2} d^{-1}, sensu Calbet 2001), 4.6±2.5 in moderately productive (250 < PP < 1000 mg C m^{-2} d^{-1}) and 1.52±1.4 in highly productive (PP > 1000 mg C m^{-2} d^{-1}) ecosystems. The exponent of the least squares power relationship between appendicularian grazing and primary production was significantly lower than 1 (Fig. 9 B, exponent±S.E= 0.64±0.16, constant±S.E=0.37±0.34, t-test for exponent < 1, p=0.01) also suggesting that the relative impact (the percentage of the primary production grazed) was lower in more productive situations. This pattern is strikingly similar to that reported by Calbet (2001) in a recent review of total mesozooplankton grazing impact on primary production (Fig. 9 B). The exponent of the relationship between total mesozooplankton grazing and primary production obtained by Calbet (2001) was 0.64±0.082 (exponent±S.E), and the constant was 1.03 mg C m^{-2} d^{-1} (his Figure 1A). Although the standard errors of the regression estimates are generally high and therefore these equations should be used with caution, both slopes are equal and therefore if we divide the intercept of the appendicularian ingestion and primary production relationship (0.37) by the intercept obtained by Calbet (2001) for total mesozooplankton (1.03) we calculate that appendicularian grazing represents 36% of the total mesozooplankton grazing.

DISCUSSION

Introduction

Making concurrent use of several observational methods we have produced the most comprehensive set of *in situ*, species-specific data on the feeding ecology of appendicularian tunicates to date. Combination of our results with literature data has allowed us to evaluate their relative importance in planktonic food webs. These data suggest that appendicularian populations are restricted to the upper surface layers (Fig. 2), that their population grazing rates are higher under more productive conditions (Fig. 9 B), and that they account for a significant fraction of the impact of mesozooplankton on primary production (Fig. 9 B). Thus, appendicularians should not be neglected if we

seek an unbiased global view of the role of mesozooplankton as grazers of primary production (Fig. 9 B; Calbet 2001).

What controls feeding rates by appendicularians?

The ingestion rate values obtained in our study by both the gut pigment content and the gut volume content techniques were consistent and revealed similar patterns (Fig. 5). The variable explaining most of the variance in gut contents was appendicularian size (Fig. 7; Table 4). Once the effect of body size was removed, the amount of variability explained by the other variables was relatively small (Table 4). If a balance between complexity of the model and predictive power is to be achieved, body size should be the variable of choice (Table 4, Fig. 7), as it is the case for copepods (Morales et al. 1990) and cold-water appendicularians (Deibel 1988).

Next according to its explanatory power was salinity, which is consistent with the observed trend of increasing gut content from coastal to oceanic stations (Table 3 and 4). Sato et al. (2001) found an inverse relationship between house production and salinity under laboratory conditions. This could suggest that the euryhaline distribution of some appendicularians species may be achieved at the cost of metabolic performance, although there is little information on the direct effect of salinity on feeding rates. Temperature was a poor predictor of gut contents (Table 4). However, under stratified conditions gut content values were generally lower as indicated by the inverse relationship between the standard deviation of temperature over the upper 50 meters of the water column and the gut content values (Table 4).

We have detected an inverse relationship between the concentration of large non-ingestible particles in the water and appendicularian gut chlorophyll contents ($Chl_{>30}$, Table 4). A similar pattern has been previously reported for cold water appendicularians by Acuña et al. (1999) who suggested that large particles could clog the inlet filter present in the house of some appendicularian species, and therefore inhibit their ingestion rates. Sato et al. (2001) reported higher house production rates when large phytoplankton cells were offered to *Oikopleura dioica* in the laboratory. This would result in a higher proportion of animals expanding a new filter house, hence not feeding, in appendicularian populations exposed to high concentrations of large phytoplankton. The fact that gut chlorophyll contents of *Oikopleura longicauda,* which

lacks an inlet filter (Alldredge 1977), were also lower when large particle concentrations were high (Table 4), does not support an inlet filter clogging interpretation. Also, it should be noted that the correlation between gut chlorophyll contents and non ingestible particles was significant in three of the five species studied, while if we considered gut volume contents it was significant only in one of the species (Table 4). This suggests that relatively more non-chlorophyll containing prey is being ingested during periods of high concentrations of large particles, and that the decrease in gut chlorophyll contents could be a consequence of a change in the diet rather than a clogging effect. Whether this increase of non autotrophic prey is due to feeding on diatom exudates or on higher bacterial concentrations fuelled by phytoplankton exudates during bloom conditions (Sanders & Purdie 1998) needs further investigation.

Gut pigment contents in copepods usually increase up to one order of magnitude during phytoplankton blooms (e.g. Dam & Peterson 1991, Irigoien et al. 1998). We did not observe such an increase in the gut contents of appendicularians during our seasonal study and the correlation between food concentration and gut contents was generally low or not significant (Table 4). Acuña & Kiefer (2000) studied the functional response of *Oikopleura dioica* under laboratory conditions, and estimated half-saturation constants of 38, 177 and 290 $\mu g\ C\ l^{-1}$ (for the three different phytoplankton species they used as a food source). Such high concentrations were rarely encountered in our study (Fig. 4 C, I, O, U, Table 1) which suggests that the conditions were non-saturating and that we should expect higher gut contents with increasing food concentrations. However, this relationship could not be detected by our multiple regression approach suggesting that food concentration is not an appropriate predictor of appendicularian gut contents when the spatio-temporal coverage of the study is large. Nevertheless, during shorter periods or in localized areas, where the variability due to other factors should be smaller, food concentration could be an important variable affecting ingestion rates (e.g. Acuña et al. in press).

An alternative hypothesis would be that appendicularians were not food limited during our study and the seasonal increase in appendicularian population densities was related to changes in the physical conditions or a decrease in predatory pressure. This hypothesis is supported by the lack of relationship between appendicularian densities and gut contents (Fig. 4) and by the vertical distribution of appendicularian populations

which does not follow the depth distribution of their phytoplanktonic prey (Fig. 2). The association of appendicularians with surface layers has been previously reported by Shiga (1985) who considered *Fritillaria borealis*, *Oikopleura dioica*, *O.longicauda* and *O.fusiformis* as "surface dwellers". Fenaux et al. 1998b have also shown that the upper mixed layer contains numerous individuals of relatively few species whereas mid-depth waters contain few individuals belonging to many species. Longhurst et al. (1984) and Ashjian et al. (1997) found that cold water appendicularians exist preferentially in surface waters. The mechanism responsible for this vertical distribution (and for departures from this generalized pattern, see Acuña 1994, Deibel 1988 and *O. labradoriensis* in Shiga 1985) is unknown and our knowledge of appendicularian ecology is still too limited to determine whether it is a response to the physical environment, to the tendency for upward swimming when the animals abandon their filter houses (personal observations on cultured organisms, Alldredge 1982) or a mechanism to avoid predation or competition.

Our study has considered the most abundant appendicularian species in European temperate waters (Fenaux et al. 1998b), and for most of them we lacked any information on their trophic ecology. The equations provided in Table 4 and Figure 6 in combination with gut passage time estimates (López-Urrutia & Acuña 1999), should therefore provide a valuable tool to predict ingestion rates on phytoplankton and on total particle material by the most significant constituents of the appendicularian community in European waters.

The impact of appendicularians on phytoplankton and suspended particulate matter.

The role of appendicularians as grazers of particulate material has rarely been taken into account in mesozooplankton studies (Calbet 2001; but see Alldredge 1981, Deibel 1988, Knoechel & Steel-Flynn 1989, Landry et al. 1994, Gonzalez et al. 2000, Acuña et al. in press). During our study, the proportion of phytoplankton standing stock removed daily by the appendicularian community was generally low (Table 5, Fig. 8) and close to average values reported for copepods in similar environments (1-3% of the phytoplankton stock removed daily; Morales et al. 1991, Barquero et al. 1998). Appendicularian grazing impact on total particulate material was usually higher than on chlorophyll containing prey (Table 5, Fig. 8), probably because our Coulter Counter

measurements underestimated the actual food concentration by not taking into account the bacterial size fraction, that also contributes to the appendicularian diet (King 1982). The ingested bacteria would be taken into account by our gut volume measurements but clearly not by the Coulter Counter which only covered the 2-30 µm fraction and therefore our grazing rates on total particulate material represent overestimates. This result, together with the high proportion of non-autotrophic material in the diet of appendicularians, for most species over 60% of the ingested material (Table 5), shows the need to further investigate the role of bacteria in the feeding and grazing rates by appendicularians. The non-autotrophic size fraction is likely to contain other particulate material than bacteria, since appendicularians are also known to feed on inert material. Gerber & Marshall (1974) reported that 89% of the gut contents of *Oikopleura longicauda* were composed of detritus and Dagg et al. (1996) have shown that *O. dioica* is able to feed on fine-grained lithogenic material from river discharge. Except for the possible overestimation of the grazing rates on total particulate material explained above, our grazing rates should be considered as underestimates, since we did not take into account any possible pigment degradation in the guts, material stuck to the filter house and not ingested or the contribution of appendicularian early juveniles with trunk lengths smaller than 300µm which was probably underestimated as they are not properly sampled using a 200 µm net (Fenaux & Palazzoli 1979).

Calbet (2001) found that total mesozooplankton grazing rates increase as a power function of primary production. However, when he restricted his analysis to studies considering only copepods this relationship was not significant and he proposed that groups other than copepods are drivers of the primary production – grazing relationship. Our results support this hypothesis and suggest that appendicularians could play an important role in determining the correlation between mesozooplankton grazing and primary production (Fig. 9 B). Surprisingly, the groups that have been traditionally considered important grazers of primary production in oligotrophic ecosystems could be those responsible for the reduction in grazing pressure in unproductive environments. However, the increase in appendicularian grazing rates is less rapid than the increase in primary production (exponent of the least squares power relationship < 1, Fig. 9 B), and the proportion of the primary production consumed by appendicularians is lower in more productive conditions. The considerable proportion of the total mesozooplankton grazing that could be attributable to appendicularians (Fig. 9 B) and the high percentage

of primary production removed during unproductive periods (Fig. 9 A) suggest that their importance in marine ecosystems should not be neglected.

6. Limitación por el alimento, crecimiento y reproducción en apendicularias epipelágicas de aguas templadas

Food limitation, growth and reproduction in temperate epipelagic appendicularians (Tunicata)

Ángel López-Urrutia, José Luis Acuña, Xabier Irigoien, and Roger Harris

ABSTRACT

Through an extensive review of previous reports on different aspects of appendicularian ecology we have developed a comprehensive set of equations on the feeding, metabolic and growth rates of temperate epipelagic species. We have used these equations to study the conditions where they are likely to experience food limited growth. *Oikopleura dioica* has been the most studied appendicularian species; we have therefore used the equations developed for this single species to build a metabolic budgetary model. Our results suggest that large *O. dioica* are less likely to experience food limited growth than other mesozooplankton and that the growth during early development could be more limited by food concentration in the environment. The degree of food limitation will strongly depend on the assimilation efficiency of non-autotrophic material which comprises a great proportion of appendicularian diet. However, examination of direct measurements of growth rate at a wide range of food concentrations showed no significant relationship between food concentration and weight specific growth rates. Temperature alone explained more than 60% of the variance in appendicularian weight specific growth rates. Since there were no clear differences in the growth rates of different appendicularian species we have combined the available data to develop a temperature dependent equation to predict their weight specific growth rates. House production can represent a high percentage of the biomass produced by appendicularians; we have therefore modified the growth rate equation to take into consideration the expenditure in house secretion. Combination of this growth rate equation with biomass estimations has allowed us to evaluate their contribution to secondary production. Contrary to traditional views of appendicularians as important members of oligotrophic ecosystems, our results show that in the area considered their contribution to secondary production was higher during more productive conditions. Finally, we have developed equations to estimate reproductive allocation and the number of eggs produced by mature individuals that should provide a first step towards understanding the factors that control appendicularian reproductive biology and population dynamics.

INTRODUCTION

The importance of food availability in limiting zooplankton growth has been a long standing point of controversy (Ikeda et al. 2001). According to the approach followed by Huntley & Boyd 1984 the growth of zooplankton is limited under food concentrations (FC) between the maintenance (C_m) and the critical (C_c) food concentrations ($C_m < FC < C_c$). At food concentrations equal to C_m, the assimilated energy balances the minimum metabolic requirements and there is no energy left for growth. At concentrations higher than C_c, growth rates are maximal and therefore unaffected by an increase in food concentration. Most of the studies on the range of food concentrations where zooplankton growth is food limited have focused on copepods (Ikeda et al. 2001 and references therein). However, it is likely that other groups with different feeding strategies, such as gelatinous organisms, may differ in their response to food limitation. Acuña (2001), using a semi empirical approach through filtration theory and physiological allometry, estimated C_m values for the salp *Pegea confoederata* between 0.84 and 2.17 $\mu g\ C\ L^{-1}$ and suggested that the gelatinous

body of pelagic tunicates enables them to survive in nutritionally dilute environments. However, there is little information on the food concentrations under which pelagic tunicates reach their maximum growth rates (C_c). The clogging of the feeding structures when particle concentrations are high has been suggested as an important mechanism excluding pelagic tunicates from coastal ecosystems. Harbison et al. (1986) have shown that some salps lack a mechanism to expel food boluses that clog their pharyngeal filters when food concentrations are high and therefore can not benefit from the higher levels of food in neritic regions. In contrast, appendicularians discard their filter house and secrete a new one every few hours, even when food is scarce (Fenaux 1985). This raises the question as to whether, under oligotrophic conditions, appendicularian ingestion rates are able to support their high metabolic (Gorsky et al. 1987) and growth (Hopcroft & Roff 1995) rates, on top of the energy costs that results from discarding every few hours 15 percent of the body carbon in the form of filter houses (Sato et al. 2001).

In a recent study on appendicularian feeding physiology (see Chapter 5), we have developed equations to predict appendicularian ingestion rates. We have now used these equations to apply the Huntley & Boyd (1984) approach to appendicularians and determine the conditions where they are likely to experience food limitation in nature. The parameters on appendicularian growth and metabolic rates needed in the Huntley & Boyd (1984) approach were not readily available. We have therefore compiled published data on appendicularian metabolism, development time and growth, and combined them with biometrical measurements to develop equations to model appendicularian growth. Most of the available data is restricted to temperate epipelagic species, particularly *Oikopleura dioica*. Therefore, we have used the data for this single species to apply the Huntley & Boyd (1984) approach and tried to evaluate species differences when data for other species were available. In addition to the Huntley & Boyd (1984) approach, we have used a compilation of weight specific growth rates to study whether the direct measures of growth rate are related to food concentration. We have modified the weight specific growth rate equation to take into account the expenditure in house production and the semelparous reproductive strategy of appendicularians. Although the consideration of the reproductive biology is not needed in the Huntley & Boyd (1984) approach, the equations developed should provide a first step towards the estimation of the reproductive output of appendicularians in population dynamics models. Finally, we have used the temperature dependent growth rate

equation developed to evaluate, in combination with biomass measurements, the contribution of appendicularians to zooplankton secondary production.

MATERIALS AND METHODS

The Huntley & Boyd (1984) approach to study food limitation in zooplankton is based on the calculation of the difference between the energy assimilated and respiration, that is, the scope for growth

$$SG = \frac{\alpha \cdot I(W_b, T, FC) - R(W_b, T)}{W_b} \quad (1)$$

where SG is the appendicularian weight specific scope for growth (d^{-1}), α is the assimilation coefficient (dimensionless), I is the ingestion rate (μg C i^{-1} d^{-1}) as a function of body weight (W_b), food concentration (FC) and temperature (T), R is metabolism (respiration, μg C i^{-1} d^{-1}) as a function of body weight (W_b) and temperature (T) and W_b is the individual body weight (μg C).

There is a minimum or maintenance food concentration (C_m) at which assimilation balances respiration and there is no energy left for growth. C_m can be found by setting SG=0 in equation 1 and solving for FC. Above a critical food concentration (C_c), growth rates are not limited by the availability of food and attain a maximum which is determined only by temperature, the thermally defined upper limit to appendicularian growth (g_{Tmax}). C_c can be determined as the FC that satisfies equation 1 for SG = g_{Tmax}.

Therefore, determination of C_m and C_c requires knowledge of I(W, T, FC), R(W, T) and g_{Tmax}. We will first determine how appendicularian ingestion, respiration and growth rates are related to temperature, body weight and food concentration. As on most occasions species-specific data is still limited, we will evaluate these relationships for *Oikopleura dioica* since most of the key parameters are available only for this species. However, we will try to determine differences between appendicularian species when possible. Then, we will use the resulting equations to study the conditions under which *Oikopleura dioica* is likely to be food limited using the Huntley & Boyd 1984 approach. Finally, we will expand the growth rate equations developed to determine the reproductive allocation and the number of eggs produced by adult appendicularians and

Table 1. Symbols used throughout the text.

W_T	Total appendicularian weight (body, tail, gonads and cumulative carbon of houses produced to that point, µg C)
W_H	Weight of all the houses produced by an individual during its lifetime (µg C)
W_b	Appendicularian body weight (body, gonads, tail but not houses, µg C)
W_a	Adult appendicularian body weight (µg C)
W_e	Egg weight (µg C)
W_h	Body weight at hatching time (µg C)
W_s	Somatic body weight (trunk and tail but not gonads and houses, µg C)
TL	Trunk length, from mouth to distal gonad end (µm)
ω	Exponent of the weight-length relationship (dimensionless)
a	Coefficient of the allometric relationship between appendicularian weight and trunk length (complex dimensions depending on the weight-length allometric exponent (ω): µg C µg $C^{-\omega}$ i^{-1} d^{-1})
TL_s	Somatic trunk length, from mouth to distal end of the gut (µm)
TL_h	Trunk length at hatching time (µm)
g_T	Total appendicularian growth rates (d^{-1})
g_{Tmax}	Thermally defined upper limit for the total appendicularian growth rates (d^{-1})
g_b	Growth rates of appendicularian body (d^{-1})
g_s	Growth rate of somatic body (d^{-1})
SG	Scope for growth (d^{-1})
T	Habitat temperature (ºC)
FC	Food concentration (µg C)
C_m	Maintenance food concentration (µg C)
C_c	Critical food concentration (µg C)
b	Body growth allocation, ratio between gb/gT (dimensionless)
s	Somatic growth allocation, gs/gb (dimensionless)
D	Development time (d)
N_e	Number of eggs produced by a mature appendicularian
U_e	Proportional increase in the number of eggs produced if all gonad weight was converted into eggs
α	Assimilation constant (dimensionless)
I	Ingestion rate (IA, autotrophic prey, IT, total ingestion, µg C i^{-1} d^{-1})
GC	Gut content (AGC, autotrophic carbon, TGC, total carbon, µg C i^{-1})
GPT	Gut passage time (min)
R	Respiration rate (µg C i^{-1} d^{-1})
m	Respiratory allometric exponent (dimensionless)
k	Respiratory allometric coefficient (complex dimensions depending on the respiratory allometric exponent (m): µg C µg C^{-m} i^{-1} d^{-1})

we will apply the equations obtained to *in situ* conditions to evaluate the contribution of appendicularians to secondary production.

Ingestion.

The ingestion rates of most planktonic filter feeders equal their clearance rate multiplied by the concentration of food (Huntley & Boyd 1984). In appendicularians a

proportion of the particles cleared remain attached to the filter house and are not ingested (up to 80%, Acuña & Kiefer 2000). Therefore, estimation of their ingestion rates cannot be based on a simple product of clearance rates by food concentration, and this prompts modification of Huntley & Boyd (1984) approach. To obtain equations to estimate ingestion rates (I) as a function of body weight (W), food concentration (FC) and temperature (T) we have followed an approach based on the gut content technique. The gut content of appendicularians can be estimated from body size according to equations in Chapter 5. The gut passage time can be estimated from temperature and food concentration using the data in López-Urrutia & Acuña 1999 (Chapter 4). If we divide the gut content by the gut passage time, we obtain an estimate for the ingestion rate which depends on body size, temperature and food concentration.

In Chapter 5 we have shown that appendicularian gut content estimates based on total particulate material (TGC, µg C, estimated from gut content volume and volume to particulate organic carbon conversion factors) are higher than gut content estimates based only on autotrophic prey (AGC, µg C estimated from gut chlorophyll content and chlorophyll to phytoplankton carbon conversion factors), and that for most species over 60% of the material ingested comes from non-autotrophic food. The degree to which the non-autotrophic material is assimilated has crucial implications for the calculation of the assimilated energy obtained from the ingested food, and consequently on the determination of food limitation in appendicularians. However, there is only information on the assimilation efficiency of phytoplankton food (Gorsky 1980). Therefore, in order to provide a first evaluation of the importance of non-autotrophic material in appendicularian metabolic balance, we have used total and autotrophic gut contents to develop two separate equations to estimate ingestion rates on total particulate material (IT) and on autotrophic prey (IA). IA multiplied by the assimilation constant for phytoplankton (α) represents an estimate of the lower limit for the total assimilated energy (i.e. if the non-autotrophic material was not assimilated at all), while IT multiplied by the assimilation constant for phytoplankton (α) would represent the total assimilated energy if the non-autotrophic material was assimilated with the same efficiency as the autotrophic material.

We used body weight - trunk length relationships to convert appendicularian trunk length measurements (µm, mouth to distal gonad end) in Chapter 5 to body

weights (µg C ind^{-1}). Power relationships between gut carbon content and body weight were then obtained using Geometric Mean Regression (GMR) on log_{10} transformed gut carbon contents (GC, µg C) and body weights (µg C). Body weight-trunk length relationships were only available for *Oikopleura dioica*, for the rest of species they were obtained by compiling published data on simultaneous trunk length and dry weight measurements (Table 2). In those cases where the ash free dry weight was reported, it was back transformed to dry weight assuming an ash content of 10% dry weight (Hopcroft et al. 1998). We then transformed dry weight into carbon weight using the equation in Gorsky et al. (1988) and the relationships between trunk length and body weight were then obtained calculating the Geometric Mean Regression (GMR, Ricker 1984) on the log_{10}-transformed data. We took the same approach for *Oikopleura dioica*, in order to compare the equation for this species with previously published regressions obtained by direct measurement of carbon content. The relationship obtained for *Fritillaria pellucida* was used for *Fritillaria borealis,* since no data were available for this species.

Respiration.

To develop equations to predict the minimum carbon requirements for metabolism (R) as a function of temperature (T) and body weight (W_b), we used Gorsky et al. (1984) and Gorsky et al. (1987) data on the respiration rates of *Oikopleura dioica* and *Oikopleura longicauda*. After digitising their data, we transformed data on oxygen consumption units (µl O_2 i^{-1} h^{-1}) to carbon requirements for metabolism (R, µg C i^{-1} day^{-1}) using a conversion factor of 0.536 µg C µl O_2^{-1} and a respiratory quotient of 0.97 (Ikeda et al. 2000). Trunk length values were transformed to body carbon using the relationships obtained as described above.

Growth.

Calculation of C_c requires determination of the thermally defined upper limit for appendicularian growth (g_{Tmax}). Huntley & Boyd (1984) used a compilation of studies where the growth rates of copepods were measured under conditions of excess food to calculate a relationship between food saturated or maximum growth rates and temperature. Unfortunately, this relationship is not available for appendicularians. We have therefore compiled all published data on direct measurements of appendicularian

weight specific growth rates and analysed them in two ways. Firstly, the presence or absence of a relationship between growth rate and food concentration will provide a first and more straightforward indication of whether appendicularian growth rates are related to food concentration and therefore on the existence of food limited growth. Secondly, to draw a line that would describe the upper limit for growth at a given temperature (g_{Tmax}), we will follow a similar method to that described by Eppley (1972) for phytoplankton and, since the growth rates were not significantly related to the concentration of food (see Results), calculate g_{Tmax} as the upper 95% confidence limit for individual measurements of the exponential relationship between growth rate and temperature. House production can represent a high percentage of the biomass produced by appendicularians; we will therefore modify these growth rate estimates to take into account the expenditure in house production.

Body growth

The growth of the appendicularian body (trunk, gonads and tail but not houses) can be described by an exponential equation (Paffenhöfer 1975, Hopcroft et al. 1998) of the form

$$W_b = W_0 e^{g_b t} \quad (2)$$

where W_b is the animal body weight (trunk, tail and gonads, µg C) at time t_i, W_0 is the body weight at time t_0, t is the time interval (d, t_i-t_0) and g_b (d^{-1}) is the weight-specific instantaneous growth rate of the appendicularian body.

To generate a database on weight-specific growth rates, we have compiled all published laboratory or field measurements of g_b. These direct measures of g_b come from two different methodologies both based on the measurement of the increase in appendicularian body weight (derived from trunk length) with time. Hopcroft & Roff (1995) ; Nakamura et al. (1997) and Hopcroft et al. (1998) measurements are based on the creation of artificial cohorts from field collected animals which were then maintained in microcosm incubations with natural seawater. The growth rate was then calculated from the increase in the size frequency distribution of the appendicularian population with time. The second methodology is based on the creation of cohorts of appendicularians in laboratory culture and measurement of the increase in body weight with time taking subsamples of the cohort at different intervals from hatching to

spawning (e.g. Paffenhöfer 1975, see Table 2 for a complete list of references). When g_b was not provided by the original publication but trunk length and time measurements

Table 2. Summary of the studies used in the development of the different equations for the species studied and the variables used. α, assimilation constant; g, weight specific growth rate; g_b*, growth rates were not reported in the original study but were estimated from their data; GC, gut content; D, development time; GPT, gut passage time; Ne, number of eggs per mature animal; R, respiration rate; TL:TLs, trunk length *vs.* trunk length without gonad relationship. WTL, body weight *vs.* length relationship, W_H, house lifetime production.

Source	Species	Variables measured
Last 1972	*O.dioica*	Ne,
Paffenhöfer 1973	*O.dioica*	WTL, D, Ne,
Wyatt 1973	*O.dioica*	Ne
Paffenhöfer 1975	*O.dioica, F.borealis*	CR, WTL, D, g_b, Ne,
Alldredge 1976b	*O.fusiformis, O.longicauda*	WTL
Fenaux 1976a	*O.dioica*	D, g_b*, Ne,
Fenaux 1976b	*F.pellucida*	WTL, g_b*, Ne,
Gorsky 1980	*O.dioica*	D, g_b*, α
King et al. 1980	*O.dioica*	g_b, We
Alldredge 1982	*O.longicauda*	Ne
King 1982	*O.dioica*	D, g_b*
Fenaux & Gorsky 1983	*O.longicauda*	WTL, D, g_b*, Ne, ED
Gorsky et al. 1984	*O.longicauda*	R
Fenaux et al. 1986a	*O.dioica*	g_b*, ED
Gorsky et al. 1987	*O.dioica*	WTL, R
Gorsky et al. 1988	*O.dioica, O.longicauda, F.pellucida*	WTL
Hopcroft & Roff 1995	*O.dioica*	D, g_b
Uye & Ichino 1995	*O.dioica*	
Nakamura et al. 1997	*O.dioica*	g_b
Hopcroft et al. 1998	*F.borealis, O.longicauda, F.haplostoma, A.sicula, O.dioica*	WTL, g_b
Nishino & Morisawa 1998	*O.dioica, O.longicauda*	SE
López-Urrutia & Acuña 1999	*O.dioica*	GPT
Sato et al. 1999	*O.dioica, O.longicauda, O.fusiformis, O.rufescens, M.huxleyi, S.magnum, A.sicula, F.formica*	D
Acuña & Kiefer 2000	*O.dioica*	D
Sato et al. 2001	*O.dioica*	WTL, D, g_b*, W_H
López-Urrutia et al. *submitted*	*O.dioica, O.longicauda, O.fusiformis, F.pellucida, F.borealis*	GC, TL:TLs,

were available, we transformed trunk length into body weight using the relationships obtained as described above and calculated g_b as the slope of the relationship between $\log_e$ body weight and time (Equation 2). Temperature and food concentration were also compiled when available. When food concentration was not reported in total carbon units it was transformed from food volume assuming $1mm^3$ of phytoplankton to contain 200 µg C (Mullin et al. 1966) or from chlorophyll concentration using the equation in Legendre & Michaud (1999).

We have also compiled literature data on appendicularian generation or development time (D, both terms are similar in appendicularians as they are semelparous and fertilization and hatching occurs within few hours after spawning,

Nishino & Morisawa 1998). Generation time is the most important determinant of the rate of population growth (Gillooly 2000), we therefore studied the relationship between development time and temperature and food concentration. Most of the compiled development time measurements came from the same experiments used above for estimation of growth rates; therefore both datasets are not independent. When they were independent measures of development time an indirect approximated measure of g_b was obtained from the relationship

$$g_b = \frac{\log_e(\frac{W_a}{W_e})}{D} \quad (3)$$

where W_a is the body weight of mature animals (µg C) and W_e is the egg weight (µg C). Equation (3) is derived from (2) setting t_0 equal to time at egg and t equal to the developmental time. When data on development time were not accompanied by data on adult weight, we used maximum weight for that species reported by Alldredge 1976b. Egg weight was assumed to be constant for all appendicularian species and equal to the values reported by King et al. (1980) for *Oikopleura dioica* (0.0155 µg C) . The logarithmic transformation of body and egg weight in equation (3) implies that the choice of adult and egg body weights has little effect on the obtained growth rate estimate compared to differences in development time (Huntley & Lopez 1992). These estimates represent the only approximation available to the weight specific growth rate of some species (*Megalocercus huxleyi*, *Oikopleura fusiformis* and *Stegosoma magnum*). However, due to the assumptions to estimate the numerator in equation (3) and to the possible bias in growth rate estimates obtained following this equation (see Kleppel et al. 1996 for a detailed discussion) these data were maintained as independent estimates, separate from the dataset based on direct measurements of growth rate.

Incorporation of house production in appendicularian growth equations.

During house production a proportion of the total appendicularians weight (trunk, gonads, tail and house) is discarded periodically. This discarded carbon is not taken into account in the growth rate equations based on the measurement of the increase in body weight with time (Equations 2 and 3). However, house production can represent a high percentage of the biomass produced by appendicularians (Sato et al. 2001) and should therefore be included in their growth rate estimates. While house

production (discarding an old house and expansion of a new one) is an episodic event, house secretion (secretion of the mucous sleeve which covers the oikoblastic epithelium) is a continuous process (Fenaux 1985). This house secretion represents an energy compartment into which a proportion of the total growth is continuously devoted. Therefore, the exponential growth equation for appendicularians body (equation 3) can be rewritten for both body and house in the form

$$g_T = \frac{\log_e(\frac{W_T}{W_e})}{D} \quad (4)$$

where W_T is the sum of the mature individual body carbon (W_a, µg C) and the cumulative weight of all the houses produced by an individual during its lifetime (W_H, µg C), W_e and D as previously defined and g_T is the total weight specific growth rate. Unfortunately, data on the carbon weight of appendicularian houses is still scarce to calculate any direct relationships between g_T and temperature or food concentrations. Nevertheless, Sato et al. 2001 estimated values of W_H for *Oikopleura dioica* as well as the development time and weight of adult individuals. We can use his data to calculate g_T and g_b using equations 4 and 3 to estimate a proportion b of the total growth (house plus body) allocated to body growth ($b = \frac{g_b}{g_T}$). This value represents the integrated proportion over the period from birth to spawning and we assumed it was constant over development.

Reproductive allocation.

The Huntley & Boyd (1984) approach allows us to estimate the conditions under which the growth of appendicularians is food limited. From a population dynamics point of view it would also be interesting to determine the amount of energy invested in reproduction and the number of eggs produced by mature individuals. We shall therefore try to modify the equation that describes the growth of the appendicularian body (equation 2) to take into account what proportion of this growth goes into somatic tissue (W_s ; tail and trunk without gonads) and which part into reproductive tissue (gonads). For iteroparous copepods, it is assumed that once animals reach maturity most of their growth goes into egg production (Poulet et al. 1995). In contrast appendicularians are semelparous and gonad tissue starts to develop a few hours after

hatching. Gonads increase in size regularly until oocyte maturation begins (Fenaux & Gorsky 1983), then oocytes rapidly develop at the expense of accessory or nourishment cells (Last 1972, Nishino & Morisawa 1998) until spawning occurs and the animal dies. Because when the first filter house is produced (≈hatching) gonad growth has not yet started, the body weights with and without gonads are equal (W_h; Fenaux & Gorsky 1983). Therefore the increase in somatic tissue can be described by the equation

$$W_s = W_h e^{g_s t} \quad (5)$$

where W_s is the somatic body weight at time t_i (d), W_h is the body weight at hatching time (t_h), t is the time after hatching (t_i-t_h) and g_s is the instantaneous weight specific growth rate of somatic tissue. In the same way, equation 2 can be rewritten for t_0 equal to hatching time (t_h), as

$$W_b = W_h e^{g_b t} \quad (6)$$

where all variables are as previously defined. Solving equation 6 for t,

$$t = \frac{\log_e W_b - \log_e W_h}{g_b} \quad (7)$$

If t in equation 5 is substituted by the right-hand term in equation 7 then equation 5 becomes

$$\log_e W_s = \frac{g_s}{g_b} \log_e W_b + (1 - \frac{g_s}{g_b}) \log_e W_h \quad (8)$$

The body weight without gonads cannot be measured directly, so we have assumed that the intercept (a) and exponent (ω) of the weight-length relationships for somatic and total body were equal, therefore

$$W_b = aTL^{\varpi} \quad (9)$$

and

$$W_s = aTL_s^{\varpi} \quad (10)$$

This assumption satisfies that at hatching, when TL_s and TL are equal, W_b and W_s are also equal. If W_b and W_s in equation 8 are substituted by the right-hand terms in equations 9 and 10, equation 8 becomes

$$\log_e TL_s = \frac{g_s}{g_b} \log_e TL + (1 - \frac{g_s}{g_b}) \log_e TL_h \quad (11)$$

Therefore, the slope of the log-log relationship between trunk length without gonads (TL_s) and total trunk length (TL) can be used as an approximation of the proportion of the body growth which is allocated to somatic growth or somatic allocation ($s = \frac{g_s}{g_b}$).

The contribution of appendicularians to secondary production

To investigate the contribution of appendicularians to total secondary production we calculated the production of appendicularians and copepods using data in Chapter 5. Appendicularian production was obtained from measurements of body weights and *in situ* abundance, together with the temperature dependent growth rate equation developed in this study. We estimated the production of copepods using the Huntley & Lopez (1992) temperature dependent model and copepod biomass estimated as the difference between total mesozooplankton biomass and appendicularian biomass. Total mesozooplankton (>200µm) dry mass was not reported in Chapter 5 but it was available through the long term zooplankton monitoring programme in the Cantabrian Sea (their stations E1, E2, E3). Total mesozooplankton (>200µm) dry mass was measured following methods in Postel et al. 2000 and was converted to carbon units using a factor of 0.4 (Postel et al. 2000). The secondary production values obtained were compared with primary production measurements in Chapter 5 to determine the relationship between the production of appendicularians and primary production.

RESULTS

Conversion factors.

The body weight versus trunk length relationship obtained for *Oikopleura dioica* is similar to those obtained by Sato et al. (2001), King et al. (1980) and Gorsky et al. (1988). For comparison the equation obtained by Sato et al. 2001 is included in Figure

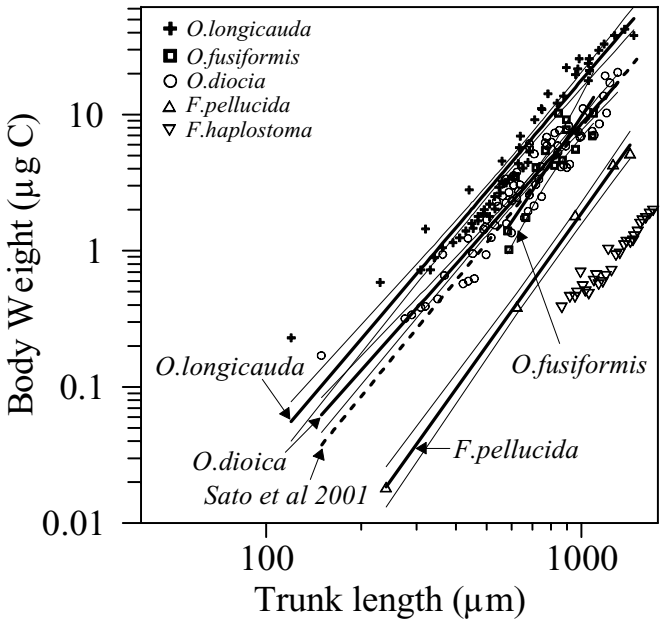

Figure 1. Body weight as a function of appendicularian trunk length for the different species studied using data obtained from the literature; see Table 2 for a list of references. Body weight was calculated from dry weight using the equation in Gorsky et al. 1988, when ash free dry weight was reported it was back transformed to dry weight assuming an ash content of 10% dry weight (Hopcroft et al. 1998). Relationships were obtained calculating the GMR on the log_{10}-transformed data. Lines represent the regression line (thick lines) and 95 % confidence limits for the parameter estimates (thin lines). Equations are summarized in Table 3; data for *Fritillaria haplostoma* are also included for comparison. For comparison with relationships obtained by direct measurement of the carbon content, the regression line obtained in Sato et al. 2001 for *Oikopleura dioica* is shown (dashed thick line).

1. The equations obtained (Fig. 1, Table 3) were therefore considered valid approximations and used to estimate body carbon from trunk length data.

Ingestion rates

After transforming data on appendicularian trunk length in Chapter 5 to carbon units we calculated GMR for each species using log_{10} transformed gut carbon contents (Autotrophic and Total, AGC, TGC, µg C) and body weight (W_b, µg C) data (Fig. 2 A and C). There were significant differences between the relationships obtained for each species (see Table 3 for equations) as indicated by the 95% confidence limits and an ANCOVA analysis on the least squares regression models (test of parallelism, $F_{4, 1162}=5.91$, $p<0.001$ for TGC and $F_{4, 1121}=1.89$, $p=0.11$ for AGC; ANCOVA, $F_{4, 1162}=17.29$, $p<0.001$ for TGC and $F_{4, 1121}=2.72$, $p=0.028$ for AGC). The high degrees of freedom in the ANCOVA means that the analysis is able to detect the effect of variables although the magnitude of such effect might be low (Graham 2001). The proportion of the total variance accounted for by differences between species was lower than 9%, while body weight accounted for over 40%. Therefore data for the different species were pooled and a general equation calculated (Fig. 2 B and D).

Table 3. Summary of the equations developed on the body weight (W_{eb}, µg C ind^{-1}) versus trunk length (TL, µm, from mouth to distal gonad end) and on the individual gut content versus body weight relationships. Data are presented in Figures 1 and 2 respectively. No data were available on the body weight of *Fritillaria borealis,* the equation for *F. pellucida* was used as an approximation. Equations show the parameter estimates ± SE, numbers in brackets represent the number of data used and the r^2 values.

	Body Weight (W_b, µg C)	Gut Content (Total Carbon; µgC ind^{-1})	Gut Content (Autotrophic Carbon; µgC ind^{-1})
O.longicauda	$10^{-6.91\pm0.10}\,TL^{2.72\pm0.28}$	$10^{-1.326\pm0.032}\,W_b^{1.283\pm0.042}$	$10^{-2.058\pm0.032}\,W_b^{0.941\pm0.040}$
	(54; 0.93)	(392; 0.58)	(372; 0.32)
O.fusiformis	$10^{-9.5\pm1.6}\,TL^{3.51\pm0.54}$	$10^{-1.17\pm0.041}\,W_b^{1.291\pm0.069}$	$10^{-2.01\pm0.039}\,W_b^{1.075\pm0.067}$
	(14; 0.72)	(233; 0.34)	(212; 0.17)
O.dioica	$10^{-6.84\pm0.27}\,TL^{2.59\pm0.10}$	$10^{-1.114\pm0.025}\,W_b^{1.358\pm0.056}$	$10^{-1.174\pm0.025}\,W_b^{1.174\pm0.053}$
	(72; 0.90)	(391; 0.58)	(391; 0.20)
F.pellucida	$10^{-9.45\pm0.23}\,TL^{3.241\pm0.081}$	$10^{-0.803\pm0.052}\,W_b^{1.52\pm0.098}$	$10^{-1.992\pm0.054}\,W_b^{0.91\pm0.10}$
	(5; 0.99)	(71; 0.71)	(71; 0.13)
F.borealis	*(F.pellucida)*	$10^{-1.16\pm0.052}\,W_b^{1.222\pm0.010}$	$10^{-1.52\pm0.056}\,W_b^{1.011\pm0.010}$
		(71; 0.71)	(85; 0.08)

López-Urrutia & Acuña 1999 (Chapter 4) obtained an equation to predict gut passage time from temperature and food concentration ($GPT = 51.67e^{-0.0376T}FC^{-0.245}$). Although this equation may be useful to provide estimates of GPT it would not allow saturation of the feeding rates unless the gut contents increase steadily with food concentration to compensate the slight decreases in GPT. To provide a more theoretically sound equation we fitted to the data in López-Urrutia & Acuña 1999 (Chapter 4) to a hyperbolic, inverse Michaelis-Menten model by iterative nonlinear regression using a Marquardt algorithm and obtained the relationship ($GPT = e^{-0.0376T}\,\frac{37.6+FC}{0.084FC}$, r^2=0.39). Therefore the relationship between gut content and body weight was divided by this equation to estimate gut passage time to obtain the ingestion rate of appendicularians as a function of temperature, body weight and food concentrations (Table 4). The equations for *Oikopleura dioica* presented in Table 4 were used for the evaluation of the Huntley & Boyd (1984) approach. The assimilation constant obtained from Gorsky (1980) was 0.61 (Table 4), close to the value reported for the cold-water appendicularian *Oikopleura vanhoeffeni* (0.67, Bochdansky et al. 1999) and for planktonic herbivores in general (0.7, Conover 1978).

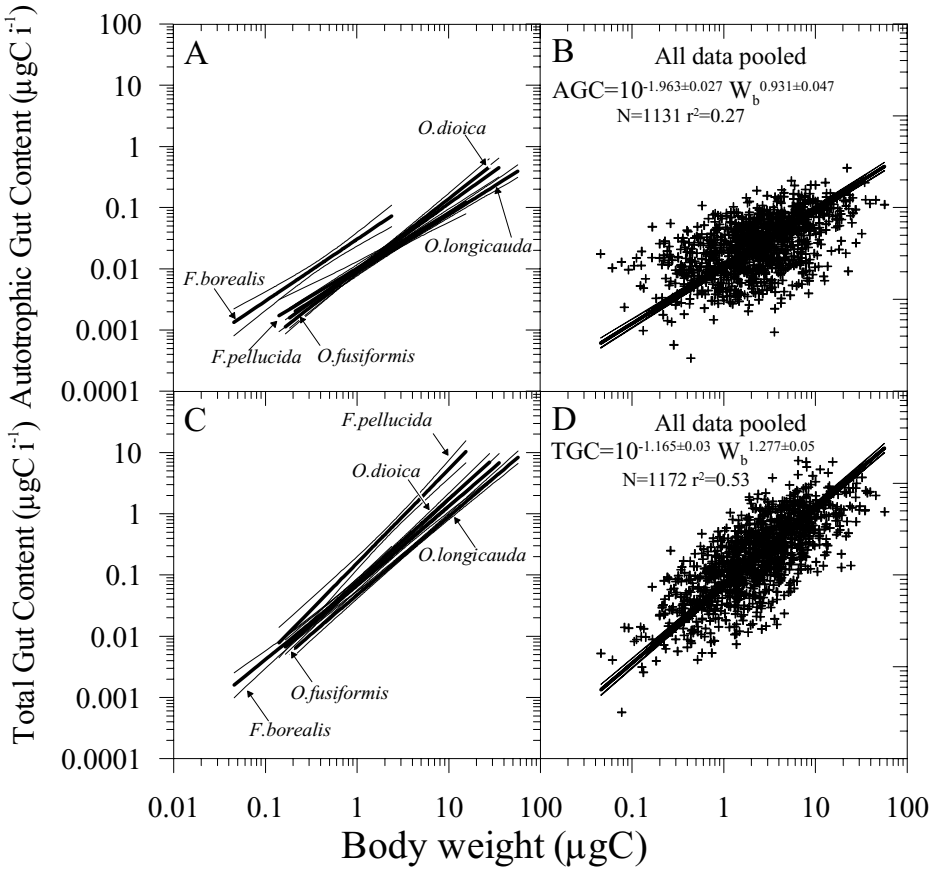

Figure 2. Relationships between gut content and body weight (W_b, µgC) based on data in López-Urrutia et al. *submitted*. Gut contents in **A-B** represent autotrophic material ingested (AGC, µgC i^{-1}, based on gut chlorophyll contents and chlorophyll to phytoplankton carbon conversion factors), while in **C** and **D** gut contents represent estimates of the total particulate carbon ingested (TGC, µgC i^{-1}, based on gut volume contents and volume to total particulate carbon conversion factors). (**A, C**) Comparison of the equations obtained for the different appendicularian species. Lines represent the GMR on the $\log_{10}$-transformed data and 95 % confidence limits for the parameter estimates (thin lines). Regression equations for each species are presented in Table 3. (**B, D**) Relationships between gut content and body weight obtained by pooling data for the different species. Equations show parameter estimates ± 95 % confidence limits of the GMR regression on the $\log_{10}$-transformed values (thick line). Thin lines represent 95% confidence intervals for the parameter estimates.

Respiration rates

Following Huntley & Boyd (1984), respiration was assumed to vary as a power function of body weight in the form

$$R = kW_b^{\,m} \quad (12)$$

where k and m are the respiratory allometric coefficient and exponent respectively and W_b the appendicularian body weight (see Discussion on the implications of assuming respiration to be independent of food concentration). This equation was fitted, using GMR, to data for each species and temperature. The 95%

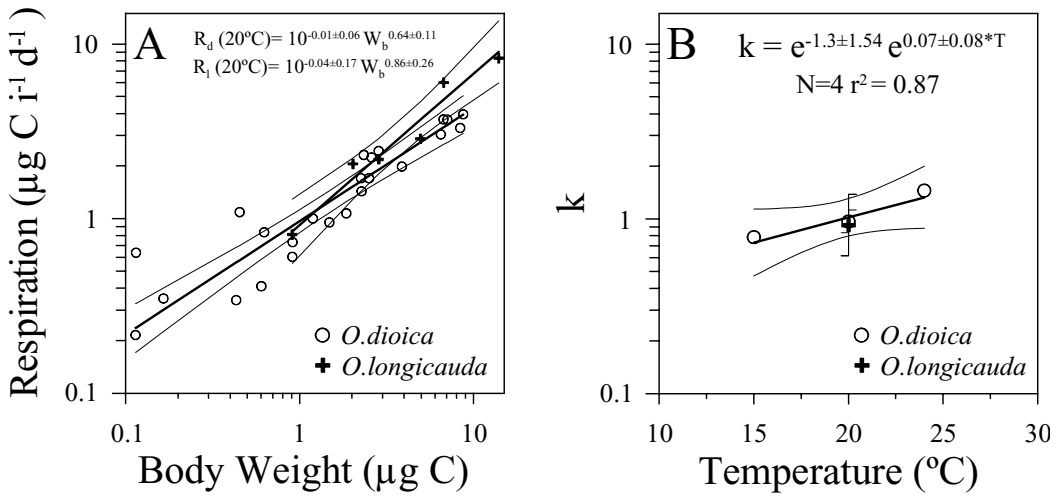

Figure 3. (**A**) Comparison of the relationships between respiration and body weight at 20ºC for *Oikopleura longicauda* (Rl; data from Gorsky et al. 1984) and *Oikopleura dioica* (Rd; data from Gorsky et al. 1987). Relationships were obtained by calculating the GMR on the $\log_{10}$-transformed data. Equations show parameter estimates ± 95 % confidence limits. Lines represent the regression line (thick lines) and 95 % confidence limits for the parameter estimates (thin lines). (**B**) Respiratory coefficient (k) as a function of temperature, vertical error bars represent the 95% confidence intervals for k estimates at each temperature and species. Equation shows parameter estimates, ± 95 % confidence limits, of the linear regression on the $\log_e$-transformed values on temperature (thick line). Thin lines represent 95% confidence intervals for the parameter estimates.

confidence intervals of the GMR estimates between respiration and body weight obtained for *Oikopleura dioica* and *O. longicauda* at 20 ºC overlap (Fig. 3 A) indicating no significant differences between the regression equations. Also, an ANCOVA analysis on the linear regression models showed no significant differences in the slopes (test of parallelism, $F_{1, 26}$=2.47; p=0.13) or intercepts ($F_{1, 27}$=2.63, p=0.12). While the allometric exponent (m) did not vary with temperature, the allometric constant k increased exponentially with temperature (Fig. 3 B). Combining the relationship between k and temperature (Fig. 3 B) with the average value for m resulted in the weight and temperature dependent equations presented in Table 4. The equation for *O. dioica* was used to estimate the minimum metabolic requirement for each individual as a function of its body weight and habitat temperature.

Body growth rates.

Our bibliographic search resulted in 49 measurements of weight specific growth rate; temperature was available for all estimates while food concentration was reported in 36 cases. Of these 49 growth rate values, 14 were obtained using the microcosm cohort methodology and 35 using laboratory cultured animals (see Methods for a detailed explanation), the lack of overlap in the temperature ranges where these values were obtained (Fig. 5 A) did not allow evaluation of the effect of different

methodologies and both types of data were pooled and analysed together. Temperature explained most of the variance in weight specific growth rates (r^2=0.61, Table 4). Food concentration was rejected as an explanatory variable by a forward stepwise multiple regression model with $\log_e$ transformed growth rates as dependent and temperature and $\log_e$ transformed food concentrations as independent variables. Also, examination of the weight specific growth rates adjusted to 15ºC (using the Q_{10} derived from the growth rate vs. temperature relationship in Table 4) did not reveal any changes in growth rate with food concentration despite the wide range of concentrations considered (21-13000 µg C l^{-1}, Fig. 5 B) suggesting that all the growth rate measurements compiled were under saturated food concentrations. Weight specific growth rates were also independent of adult appendicularian body weight (Power regression on growth rates adjusted to 15ºC *vs.* adult body weight, $F_{1, 33}$=0.56, p=0.46, data not shown). Therefore, a temperature dependent growth rate equation for the appendicularian body was calculated using all available data (g_b, Table 4 and Fig. 5 A). For comparison, the

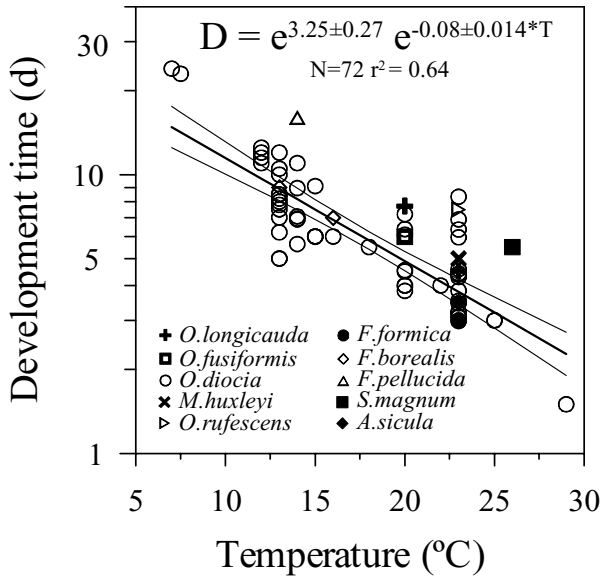

Figure 4. Plot of development time (D) versus temperature (T) for a compilation of available appendicularian literature data (see Table 2 for sources). Thick line represents the linear regression with $\log_e$-development time as dependent and temperature as independent variables based on data for all species (see Table 3 for the equation based on *Oikopleura dioica* data only). Thin lines represent 95% confidence intervals for the parameter estimates.

temperature and body weight dependent model of growth rate in copepods (Hirst & Lampitt 1998) is included in Figure 5 A, after substituting body weight in their multiple regression equation by 0.075 µg C (the minimum body weight in their data and therefore the higher weight dependent growth rate). Although most of the compiled weight specific growth rates were data for *O. dioica* (40 of the total 49) and measurements for other species are still too scarce to make any conclusive distinctions,

visual comparison did not reveal significant differences between species and the variability in the measured growth rates for different species was not markedly different from the variability observed within different measurements for *O. dioica* (Fig. 5). The temperature dependent growth rate equations obtained using the data for *O. dioica* and the data for the rest of species separately were not significantly different (test of parallelism, $F_{1,45}$=1.7, p=0.199, ANCOVA, $F_{1,45}$=1.8, p=0.183).

We compiled 71 measurements of development time; temperature was available for all measurements while food concentration was only reported in 52 cases. Development time was strongly dependent on temperature, while food concentration was rejected by forward stepwise regression models and by analyses of temperature corrected developmental times (data not shown, see the results above for growth rate for a similar description of the analyses performed). Combination of the developmental times (those independent from the laboratory experiments used in the calculation of

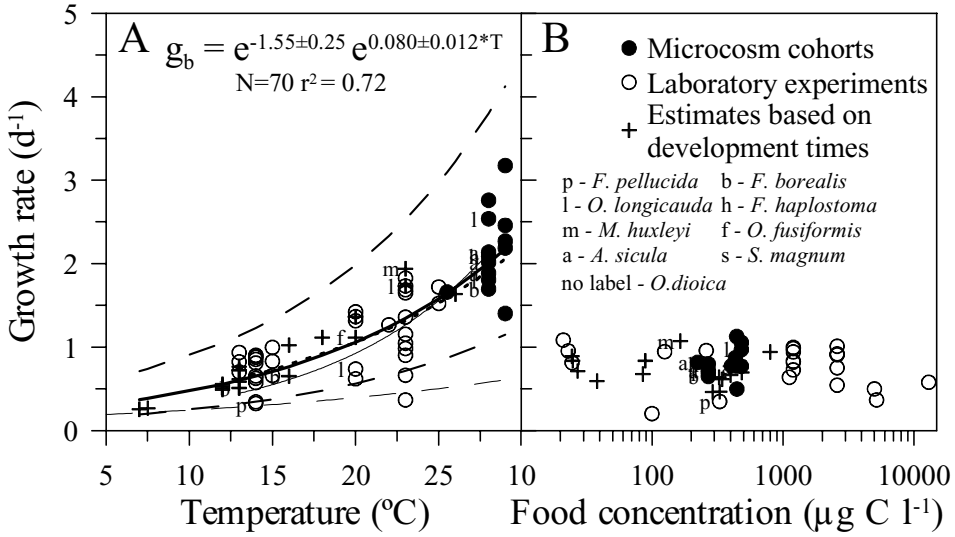

Figure 5. (A) Instantaneous weight-specific growth rate of the appendicularian body (g_b, d^{-1}) as a function of temperature (T). Solid circles represent data from microcosm incubations of natural populations. Empty circles represent growth rate estimates from laboratory experiments. Plus signs represent growth rate estimates based on development time in combination with adult and egg weight estimates (see Methods for detailed explanation). The equation shown represents the least squares regression (thick solid line) between $\log_e$ growth rate and temperature (± 95 % confidence limits for the parameter estimates) using all data from different methods and species. The thick dashed lines represent the 95% confidence limit of individual estimates for the regression obtained. The thick dotted line represents the relationship obtained using all data except those derived from development time (see Table 4 for the regression equation). Thin line represents the relationship obtained using data for all species except *O. dioica*. For comparison, the relationship obtained by Hirst & Lampitt 1998 for marine copepods is also included (thin dashed line), after substituting body weight in their multiple regression equation by 0.075 µg C (the minimum body weight in their data and therefore the higher weight dependent growth rate). (**B**) Plot of the weight specific growth rates of the appendicularian body (g_b, d^{-1}, adjusted to 15ºC using the equation obtained in A) versus food concentration.

growth rates, see Methods) with adult body weights and egg weight resulted in 21 estimates of weight specific growth rate using equation 3. The temperature dependent

equation obtained using this independent set of estimates was not significantly different from the relationship obtained from data on direct measurement of growth rate (test of parallelism, $F_{1, 66}$=3.97, p=0.051, ANCOVA, $F_{1, 66}$=3.08, p=0.08) suggesting that, when direct measurement of weight specific growth rates can not be obtained, estimates using equation 3 should provide a valid approximation. Combination of both datasets (direct measurements and indirect estimates through development time) resulted in the temperature dependent equation presented in Figure 5 A.

Table 4. Summary of the equations developed to estimate physiological and growth rates based on data for *Oikopleura dioica* and on all data available. Equations show the parameter estimates ± SE, numbers in brackets represent the number of data used and the r^2 values. *All equations were calculated by dividing the different gut content *vs.* body weight relationships by the same gut passage time equation based on data on *O. dioica*.

	Oikopleura dioica	**All data**
Total Ingestion * (IT,Total Carbon, µgC i^{-1} d^{-1}) =	$\frac{4.4*10^{-6}*FC*e^{0.0376*T}\ W^{1.231}}{37.6+FC}$	$\frac{4*10^{-6}*FC*e^{0.0376*T}\ W^{1.27}}{37.6+FC}$
Autotrophic Ingestion * (IA, Autotrophic Carbon, µgC i^{-1} d^{-1})=	$\frac{5.5*10^{-7}*FC*e^{0.0376*T}\ W^{1.358}}{37.6+FC}$	$\frac{6.4*10^{-7}*FC*e^{0.0376*T}\ W^{1.277}}{37.6+FC}$
Assimilation constant (α) =	0.61±0.22 (3)	----
Respiration (R, µgC i^{-1} d^{-1}) =	$e^{-1.33\pm0.34}\ e^{0.067\pm0.018*T}\ W^{0.5984\pm0.064}$	$e^{-1.30\pm0.36}\ e^{0.066\pm0.018*T}\ W^{0.665\pm0.069}$
Development time (D, d) =	$e^{3.29\pm0.13}\ e^{-0.085\pm0.007*T}$ (60; 0.70)	$e^{3.25\pm0.27}\ e^{-0.08\pm0.01*T}$ (72; 0.64)
Body Growth rate (g_b, d^{-1}) =	$e^{-1.28\pm0.21}\ e^{0.0678\pm0.010*T}$ (40; 0.53)	$e^{-1.43\pm0.19}\ e^{0.0741\pm0.0087*T}$ (49; 0.61)
Somatic growth allocation ($s=g_s/g_b$) =	0.933±0.035	0.9052±0.033 (5)
Body growth allocation ($b=g_b/g_T$) =	0.8196±0.0089 (6)	
Total Growth rate (g_T, d^{-1}) =	$0.338e^{0.0678*T}$	$0.292e^{0.074*T}$
Total growth rate upper limit (g_{Tmax}, d^{-1}) =	$0.71e^{0.0679*T}$	$0.49\,e^{0.08*T}$

House production and appendicularian growth rates.

Recalculation of data in Sato et al. (2001) using equations 3 and 4 resulted in a value for the body growth allocation (b) of 0.82 (Table 4). The data were too scarce to analyze the relationship of b with temperature, salinity, or food concentrations so we

assumed b to be a constant value. Therefore, the total production of appendicularians (g_T) was estimated by multiplying equations to estimate the body growth (g_b; Table 4) by a factor of 1.22 (1/b). Accordingly, the thermally defined upper limit for the *O. dioica* growth rate (g_{Tmax}) was obtained by multiplying by 1.22 the upper 95% confidence interval for individual estimates of the exponential relationship between g_b and temperature (Table 4). This g_{Tmax} represented the maximum growth rates of *O. dioica* as a function of temperature and was used in the Huntley & Boyd (1984) approach. The information available on the house production rates and carbon content of the houses of other appendicularian species is still limited, we assumed that the estimate of b obtained for *O. dioica* was representative for other species. Alldredge 1976a showed that the carbon content of discarded houses varies between species, however b depends also on the house production rate and the body growth rate so we could not transform the data in Alldredge 1976a to body growth allocation estimates.

Food limitation and the Huntley & Boyd 1984 approach.

The estimated C_m and C_c values for *O. dioica* based on ingestion of autotrophic and total carbon suggest that the degree of food limitation depends strongly on the extent to which the ingested non-autotrophic material is assimilated. The lack of knowledge of these assimilation rates prevents making any conclusive results on the concentrations where they would be likely to experience food limited growth. Also, the gut content values for autotrophic and total food are likely to represent underestimates since they do not take into consideration the possible chlorophyll degradation or compression of food particles in the gut which would lead to underestimation of the ingestion rates and overestimation of C_c and C_m (although this underestimation is likely to be small or absent, see Chapter 5 for a comparison with estimates of clearance rate for *O. dioica*). However, large *O. dioica* are less likely to experience food limited growth than other groups of marine zooplankton (Fig. 6) since, even if we consider that they are not able to assimilate non-autotrophic material at all and the under estimation of ingestion rates due to possible chlorophyll degradation in the gut, their C_m and C_c values lay below those estimated by Huntley & Boyd 1984 (Fig. 6 A). The growth during early development is likely to be more limited by the concentration of food in the environment (Fig. 6 A). The comparison of C_m and C_c values for both autotrophic and total ingestion with typical total and phytoplankton carbon

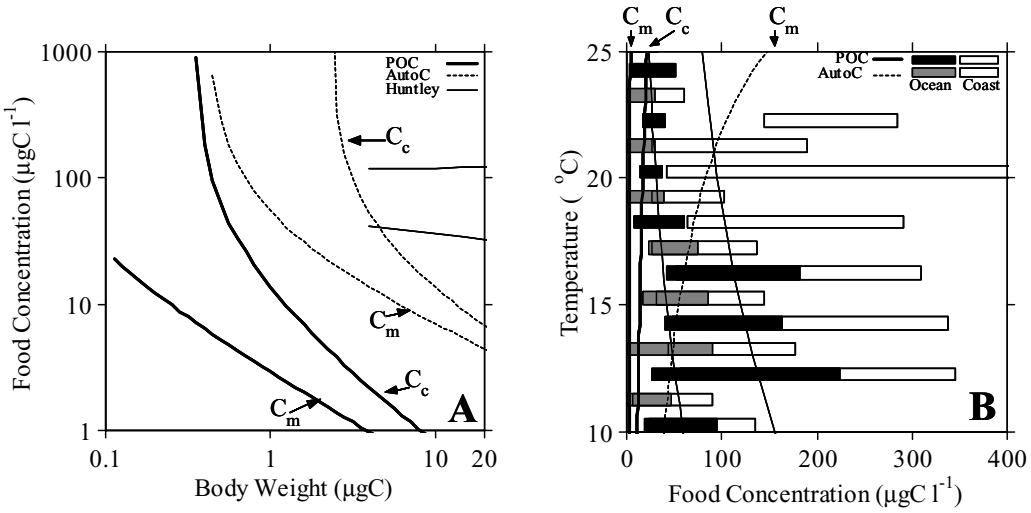

Figure 6. (**A**) The maintenance food concentration, C_m (where assimilation balances respiration), and critical food concentration, C_c (above which growth is not food limited) as a function of body weight at 15ºC for *Oikopleura dioica*. Dotted lines represent estimates obtained based on ingestion rates on autrophic carbon. Thick solid lines represent values based on ingestion rates on total particulate carbon assuming that all particulate material is assimilated with the same efficiency (0.61). Thin solid lines were calculated from the Huntley & Boyd 1984 equations for marine zooplankton assuming 0.4 µg C μg^{-1} dry weight. (**B**) Typical food concentrations in oceanic areas (>300m bottom depth, filled bars) and in coastal regions (<300m bottom depth, open bars) obtained using particulate organic carbon and chlorophyll data available through the U.S. National Oceanographic Data Center. Data were binned into 2ºC intervals and the first and third quartiles of the food concentrations within each bin were used as the ranges for the bars displayed. Horizontal bars starting with black filled segments represent data on total particulate organic carbon concentration (POC), those horizontal bars starting with grey filled segments represent autotrophic carbon concentration (AutoC) estimated from chlorophyll using the chlorophyll to autotrophic carbon relationship in Hewes et al. 1990. Lines represent the maintenance food concentration (C_m), and the critical concentration (C_c) as a function of habitat temperature for an *O. dioica* 1 µg C body weight. Dotted line represents estimates for *O. dioica* obtained based on ingestion rates on autrophic carbon. Thick solid lines represent values for *O. dioica* using ingestion rates on autrophic carbon. Thin solid lines represent values for marine zooplankton using equations in Huntley & Boyd 1984 for an animal 4 µg C (assuming 0.4 µg C μg^{-1} dry weight).

concentrations in oceanic and coast environments (Fig. 6 A), suggests that in coastal environments *O. dioica* would obtain enough energy to survive (C_m) from phytoplankton material alone, but under oceanic or oligotrophic conditions the assimilation of non-autotrophic material would be a key requirement if they are able to survive.

Reproductive allocation.

Shiga (1976) reported the existence of inflexion points in the relationships between the lengths of various body parts of *Oikopleura labradoriensis*, and suggested that they corresponded to different maturity stages. Fenaux (1963) could define inflexion points in *Oikopleura dioica* and *Fritillaria pellucida* but not in *O. longicauda* or *O. fusiformis.* However, we could not detect any of these inflexion points in the trunk length without gonads vs. trunk length relationship for any of the species we considered (Fig. 7). We have therefore taken a different approach and considered maturation as a

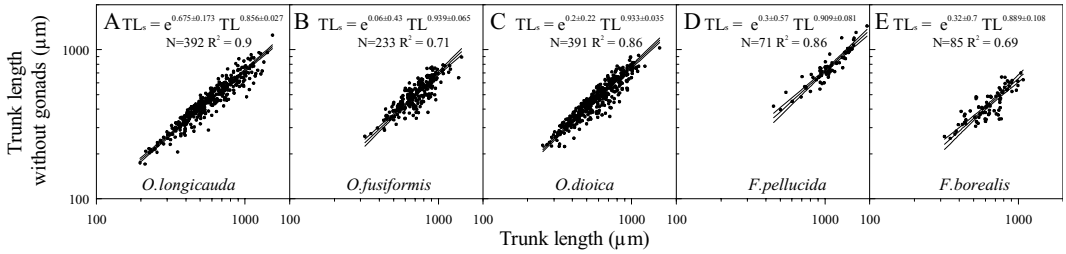

Figure 7. Relationships between trunk length without gonads (TL_s) and total trunk length (TL) for each of the species studied in López-Urrutia et al. *submitted*. Relationships were obtained by calculating the GMR on the $\log_{10}$-transformed data. Equations show parameter estimates ± 95 % confidence limits. Lines represent the regression line (thick lines) and 95 % confidence limits for the parameter estimates (thin lines). The allometric exponent represents the somatic growth allocation, the ratio between instantaneous growth rates for somatic tissue and instantaneous growth rates for the animal body (see methods for explanation).

continuous process without discrete transitions or developmental stages which better suits the lack of any metamorphic or distinct morphological steps in the life cycle (but see Shiga 1976 for an alternative approach). The somatic allocation is therefore constant during the whole life cycle of an individual (Fig. 7). On average for all species 90% of the total body instantaneous growth rate corresponded to somatic growth ($s \approx 0.9$).

Equation 5 can then be rewritten in its most expanded form as

$$W_T = W_e e^{s g_b D} e^{(1-s) g_b D} e^{(\frac{1}{b}-1) g_b D} \quad (13)$$

where W_T is the total carbon produced by an animal during its lifetime for a given egg weight (W_e), somatic allocation (s), body growth allocation (b), body instantaneous growth rate (g_b) and development time (D). If we sequentially remove from right to left the two exponential terms on the right-hand side of equation 13, we obtain the equations to calculate the weight of an adult appendicularian body (W_a) and the somatic tissue of an adult individual (W_s) respectively. The total lifetime house production ($W_H=W_T - W_a$) and weight of the gonads of a mature individual ($W_g=W_a – W_s$) can be calculated by difference of the values obtained by the respective equations. Using the equations to estimate g_b and D as a function of temperature (Table 4) and estimates for b (see previous section) and s (Figure 7) equation 13 can be used to predict the weight of the different body compartments of an appendicularian during its lifetime. For example the equation for gonad weight at the end of development (reproduction time) would be

$$W_g = W_e e^{s g_b G T} (e^{(1-s) g_b G T} - 1) \quad (14)$$

The compilation of the number of eggs produced by mature individuals resulted in 62 measurements for *O. dioica* and 21 for *O. longicauda* (see Table 2 for references), the number of eggs produced depends strongly on the trunk length of the mature animal (Paffenhöfer 1971, Alldredge 1982, Fig. 8). Data in Wyatt (1973) were not included in our analyses since Last 1972 studied in detail the process of egg maturation (on the same samples from which Wyatt 1973 obtained his number of eggs per female data) showing that most of the animals analysed by Wyatt (1973) were not fully ripe individuals. Last (1972) showed that close to 56% of the initial number of ova fail to develop into eggs during the last stages of maturation. Therefore Wyatt (1973) data represent overestimates of the actual number of eggs produced.

We used the empirical relationships in figure 8 to evaluate whether it was possible to formulate a theoretical equation that would allow calculation of the average number of eggs produced by an appendicularian population at a given temperature. First, dividing both terms in equation 14 by the weight of an egg (W_e) we obtain

$$\frac{W_g}{W_e} = e^{sg_bGT}(e^{(1-s)g_bD} - 1) \quad (15)$$

where $\frac{W_g}{W_e}$ represents the number of eggs produced by a mature animal times the gonad weight which does not result in production of eggs (expressed in number of eggs equivalents, i.e. one plus the proportional increase (U_e) in the number of eggs produced if all gonad weight was converted into eggs,) that is

$$\frac{W_g}{W_e} = N_e(1+U_e) \qquad (16)$$

Combining equations 15 and 16 we arrive at

$$N_e = \frac{1}{1+U_e} e^{sg_bD}(e^{(1-s)g_bD} - 1) \quad (17)$$

If we replace g_b and D with the temperature dependent equations for *O. dioica* (Table 4), the estimate of s for *O. dioica* and assuming that U_e represents the proportion of ova that fail to mature (56% following Last 1972), that is, assuming that the weight

of the epidermic cover of the gonads and remains of nourishment cells is close to zero, equation 17 can be approximated as $N_e = 375e^{-0.1144T}$ which yields a theoretical approximation (based on a number of assumptions to estimate s and U_e) of the average number of eggs produced by an *O. dioica* population at a given temperature (i.e. the number of eggs produced by an adult *O. dioica* of average trunk length for that temperature). In the same way, if instead of replacing g_b and D in equation 17 by its

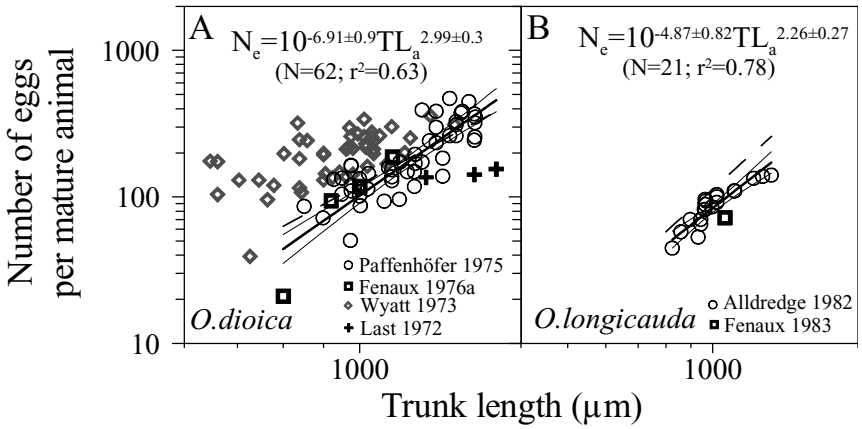

Figure 8. Relationship between the number of eggs (N_e) produced as a function of mature animal trunk length (TL) for (**A**) *Oikopleura dioica* and (**B**) *O. longicauda* using bibliographic data. Relationships were obtained calculating the GMR on the $\log_{10}$-transformed data. Equations show parameter estimates ± 95 % confidence limits. Lines represent the regression (thick lines) and 95 % confidence limits for the parameter estimates (thin lines). Dashed lines represent the relationships obtained based on the growth rate equations developed in this study and based on the assumptions for the calculation of the somatic allocation and the percentage of the total gonad weight which does not become eggs. Trunk lengths without gonads from Alldredge 1982 were transformed to total trunk lengths using the relationship for *Oikopleura longicauda* obtained from our data (the inverse of the equation in Fig. 7 A). Data from Wyatt 1973 were not included in the calculation of the regression equation as they do not represent fully ripe individuals and therefore show higher numbers of eggs and shorter trunk length (following the detailed analysis by Last 1972 on the same samples).

temperature dependent equations, we replace the product g_b*D by $\log_e (W_a/W_e)$ (Equation 3) and then W_e by the egg weight (0.0155 µg C) and W_a by the inverse of the weight trunk length relationship, we obtain an equation that can be approximated by $N_e = 2\times10^{-7} TL_a^{2.9}$ which provides a theoretical approximation of the number of eggs produced as a function of adult trunk length (TL_a). For comparison with the real number of eggs produced, the previous equation is plotted in figure 8 (A) together with the empirical equation from the bibliographic compilation. The same approach taken for *O. longicauda* using an egg weight of 0.03 µg C (calculated from the egg volume reported by Fenaux & Gorsky 1983 and the egg volume to carbon ratio of *O. dioica*, 2.96 10^{-8}) and an ovary :testis weight ratio of 1 yields the equation $N_e = 1\times10^{-7} TL_a^{2.97}$ which is presented in figure 8 (B).

Evaluation of the contribution of appendicularians to secondary production.

The appendicularian and copepod production rates obtained through combination of biomass estimates with temperature dependent growth rate models (equation in Table 4 for appendicularians and Huntley & Lopez (1992) equation for copepods) suggest a direct relationship between appendicularian and copepod production (Fig. 9 A). The percentage contribution of appendicularians to total mesozooplankton production (appendicularian plus copepods) increased with increasing primary productivity up to values close to 32.7% (Fig. 9 B).

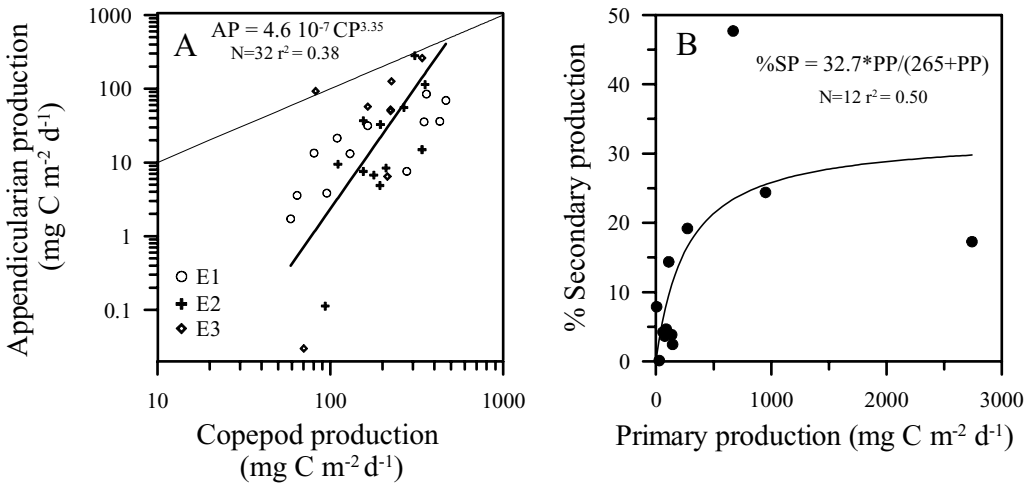

Figure 9. (**A**) Relationship between appendicularian community production (AP) and copepod production (CP). Appendicularian production was estimated using individual biomass from trunk length measurements and species densities provided in López-Urrutia et al. *submitted* in combination with the temperature dependent growth rate model developed in this study (see Table 4). Station E1 (depth ≈ 50 m, 43º 36' N 6º 8'W), station E2 (depth ≈ 120 m, 43º 42' N 6º 9'W), station E3 (depth ≈ 1000 m, 43º 46' N 6º 10'W). Copepod production was calculated using the Huntley & Lopez 1992 model for temperature dependent growth of copepods, and copepod biomass estimated as the difference between total mesozooplankton biomass and appendicularian biomass. Thin line represents the values at which appendicularian production equals copepod production. (**B**) Estimated percentage of the total secondary production attributable to appendicularians (%SP), using estimates as described above, as a function of primary production at station E2 (PP). Line represents a Michaelis-Menten model fitted by iterative nonlinear regression using a Marquardt algorithm.

DISCUSSION

We are far from understanding the factors that control appendicularian populations and their importance as secondary producers. Some of the key parameters in appendicularian physiology are only known for a few species and many data are restricted to experimental information from laboratory cultures of *Oikopleura dioica*. We lack knowledge on the metabolic, growth rates and developmental times of many species, particularly the cold water and deep oceanic appendicularians. Contrary to this

lack of information, our compilation of the available data suggests that the generally high metabolic and growth rates of appendicularians mean that their contribution to secondary production could be potentially important and that such information is needed.

Do appendicularians experience food limitation?

Due to this lack of data, our attempt to determine whether these high growth rates are likely to be limited by the availability of food using the Huntley & Boyd (1984) approach has been restricted to *O. dioica*. Our results suggest that *O. dioica* would only experience food limited growth under oligotrophic conditions and early development. Although determination of food limitation in copepods is still a source of discussion (Hirst & Lampitt 1998) our results suggest that appendicularians are generally less likely to experience food limitation than copepods (Fig. 6). The low maintenance food concentrations are in agreement with filtration theory and physiological allometry studies (Acuña 2001), which indicate that gelatinous organisms are adapted to survive in dilute environments. Appendicularians lack any selection mechanism by which they could favour ingestion of high quality food so they will be more affected by changes in the composition of the available food than other zooplankton that have evolved selective feeding strategies. The fact that the total food carbon concentrations in nature are almost always above the theoretical maintenance minimum does not rule out the possibility of resource limitation through differences in the assimilation efficiency of different types of food. Although we have attempted a first quantification of the importance of non-autotrophic *vs.* phytoplankton food (Fig. 6), differences in the phytoplankton or heterotrophic community composition and assimilation efficiencies of different food sources could have important implication for appendicularian growth rates and population dynamics. For example, in salps Andersen (1986) found that assimilation in *Salpa fusiformis* did not depend on algal concentration but was greater with a flagellate than with diatom diet. The high protein catabolism encountered in larvaceans, as indicated by the high protein absorption efficiencies reported by Bochdansky et al. (1999) in *Oikopleura vanhoeffeni,* high levels of protease activity encountered in *O. dioica* (Bedo, A and Harris, R *unpublished*) and high O:P and N:P metabolic quotients in *O. dioica* (Gorsky et al. 1987), could indicate that appendicularians would not depart from other zooplankton in having their production

limited by the availability of nitrogen (proteins; Checkley 1980). Another important physiological variable which was not considered by Huntley & Boyd (1984) and that we could not account for is the specific dynamic action or modification of the metabolic rates associated with changes in the feeding rates. Respiration is likely to vary with ingestion rate and therefore to be a function not only of the body weight but also of the food concentration. The respiration rate values in Gorsky et al. (1987) used for the application of the Huntley & Boyd (1984) approach were measured under filtered (30μm) natural seawater and therefore we do not know how much they would vary under different feeding conditions. The number of parameters in the physiological or budgetary method developed by Huntley & Boyd (1984) and the compounding error associated in the difference between assimilation and respiration in equation 1 (Lehman 1988) clearly show that a better understanding of food limitation can be obtained through the study of the relationship between direct measurements of growth rate and food concentration (Huntley 1996). The fact that there was no significant relationship between food concentration and the measured weight specific growth rates does suggest that growth rates in appendicularians are not controlled by food concentration but does not rule out the possibility of resource limitation due to changes in food quality.

The result that *Oikopleura dioica* is more likely to experience food limitation during early development (Fig. 6 A) is due to the allometric exponent of the ingestion vs. body weight relationship being higher than the allometric exponent for respiration (Table 4). In a comparison of faecal pellet volume and body weight of different zooplankton groups by Uye & Kaname (1994), appendicularians were the only group to have an allometric exponent greater than 1 (1.14). Paffenhöfer (1975) also obtained allometric exponents of 1.19 and 1.24 for the ingestion rate of *O. dioica*. These values are higher than those for calanoid copepods and cladocerans (Peters & Downing 1984) that are closer to the general allometric exponent of 0.75. Whether this high allometric exponent of appendicularian ingestion rates is due to experimental error, or that the gelatinous strategy of appendicularians could also distort their allometric relationships, needs further investigation. However, our result that juveniles are more likely to experience food limitation is supported by observations on laboratory cultures of *Oikopleura dioica* where juveniles did not survive at concentrations of food less than 60 μg C l^{-1} while larger animals grew at food concentrations of 40 μg C l^{-1} (K. King. unpublished, quoted from King 1982).

Conditions below C_m (when the assimilated energy is less than the metabolic demands) would result in the rapid extinction of an appendicularian community, as appendicularians do not store lipids (Deibel et al. 1992) and no dormant stages have been reported. This limited storage capacity, and the high metabolic activity of appendicularians even during starvation (Gorsky et al. 1987) suggests that appendicularians resemble small, tropical and neritic zooplankton in being in quasi-steady state with their environment (i.e. they grow quickly and die quickly and the ratio of production to biomass is higher than for large copepods; Conover 1968).

We are far from understanding the mechanisms that control appendicularian population dynamics. Factors other than food quantity or quality could play a significant role in controlling appendicularian communities (e.g. predation Hopcroft & Roff 1998). The average number of eggs by produced *Oikopleura dioica* at 9ºC is 167 (the average temperature and number of eggs in Figure 8 A). For an population of *O. dioica* to be maintained in steady state conditions, the number of eggs that reach maturity should be 2 (assuming a 1:1 sex ratio, Fenaux et al. 1986b). Therefore, an average 98% mortality from egg to spawning is needed for a steady state population at this temperature. Paffenhöfer (1973) measured an approximate 45% mortality from hatching to spawning in isolated culture conditions. Much of this natural mortality occurs during the period from hatching to the start of feeding. Paffenhöfer (1975) mainly attributes it to failure in unfolding the first filter house. Other factors such as encounter rates of adult individuals, egg sinking, fertilization and hatching success or predation have been rarely taken into account and they could be important factors controlling appendicularian populations.

The contribution of appendicularians to secondary production

Our compilation of data on appendicularian growth rates has allowed us to develop a temperature dependent model to estimate the production rate of temperate epipelagic species when data in appendicularian biomass and temperature are available (Table 4; Fig. 9). The high individual growth rates of appendicularians compared to those of copepods (Fig. 9 A; Hopcroft & Roff 1998) suggest that both the relative composition of mesozooplankton and the contribution of different groups to the total biomass, should be taken into account if reliable estimates of secondary production are to be obtained. Comparing the individual growth rates and development times obtained

in this study with those of other groups of zooplankton (Hirst & Sheader 1997, Gillooly 2000) suggests that appendicularians significantly depart from the models developed for crustacean zooplankton. For example, at 15ºC the development time of an appendicularian is 7.73 days (using equation in Table 4), the weight specific growth rate is 0.88 d^{-1} (using equation in Table 4) and the adult body weight is 15.25 µg C (using equation 14 with an egg weight of 0.015 µg C). The development time for crustacean zooplankton of similar adult body weight is 21 days (Gillooly 2000) while the weight specific growth rate for copepods is 0.06 d^{-1} (Hirst & Lampitt 1998). Therefore, appendicularian growth rates are close to one order of magnitude higher than those of copepods of similar body weight. The fact that copepods generally dominate numerically and in biomass mesozooplankton samples does not have to translate directly into their dominance of the secondary production and our results suggest that groups other than copepods (appendicularians in this particular study) should not be disregarded when estimating mesozooplankton production (Fig. 9 A).

Huntley & Lopez (1992) central concern still remains irrefutable, the natural variability in biomass is greater than that of growth and in order to increase the precision of our estimates of production we should improve our knowledge on the sources of this variability in biomass. We could extend this conclusion to the fact that estimates of production would increase in precision by studying the variability in the relative contribution of different groups to the total biomass, and combining these data with "group-specific" temperature dependent models. Application of such an approach to our estimates of appendicularian and copepod biomass shows that appendicularians could represent an average 10 percent of the total mesozooplankton production and, what it is more relevant, their proportional contribution is not constant (Fig. 9 B) and can reach values close to 40% in productive environments. The fact that the appendicularian contribution to secondary production during our study increased with increased productivity suggests that their role in oligotrophic environments needs re-evaluation.

7. Discusión general

DISCUSIÓN GENERAL

Nuestra percepción sobre la estructura de las redes tróficas marinas ha seguido una tendencia general de incremento en la complejidad de los modelos usados para resumir los procesos en los océanos. Los ecólogos marinos han pasado de la relativamente sencilla cadena clásica diatomeas- copépodos- peces hacia un énfasis en la heterogeneidad de la comunidad microbiana y sus posibles consecuencias en los flujos de carbono oceánicos (e. g. Azam 1998). También ha crecido la complejidad con respecto al papel de los diferentes miembros del zooplancton y su importancia relativa. Con el "descubrimiento" del bucle microbiano llegó el reconocimiento de que el control predador del zooplancton de pequeño tamaño era generalmente más importante que el ejercido por los copépodos. Aun así, los copépodos siguen siendo considerados los consumidores más importantes en aquellas ocasiones en las que domina la cadena clásica. Aunque la estrategia de alimentación de los copepodos no les permite alimentarse directamente del pico y nano-plancton que domina los procesos en la base del bucle microbiano, su capacidad de ingerir flagelados heterotróficos y ciliados sugiere que su papel controlando los procesos heterotróficos que dominan durante las fases oligotróficas pudiera ser más importante de lo que en principio se hubiera pensado (Calbet 2001). El trabajo realizado durante esta tesis sugiere que el papel del mesozooplancton en la cadena trófica pelágica es también más complejo debido a que grupos diferentes de los copépodos pueden, al menos durante periodos concretos, jugar un papel importante como consumidores y productores secundarios.

Nuestras medidas de las tasas de ingestión *in situ* de diferentes especies de apendicularias sugieren que éstos animales podrían ser responsables de una parte significativa del impacto herbívoro del mesozooplancton. Las apendicularias pueden tener un efecto importante sobre los productores primarios alcanzando valores de herbivoría por encima del 400 por cien de la producción primaria diaria. Sin embargo, este alto impacto herbívoro se restringe a los meses de otoño cuando la producción primaria era baja y la población de apendicularias densa. Para determinar si este patrón representa un punto anómalo o un patrón general será necesario realizar más estudios estacionales. Landry et al. (1994) también observaron que el impacto herbívoro de las apendicularias en el suroeste de California era mayor durante los meses de otoño. Nuestra compilación de valores publicados de medidas simultaneas de ingestión de

apendicularias y producción primaria (Capítulo 5) sugiere la existencia de un patrón general según el cual la proporción de la producción primaria consumida por las apendicularias es menor en condiciones más productivas. Sorprendentemente, y a pesar de que este patrón confirma la visión tradicional de que, como herbívoros, las apendicularias son más importantes en sistemas menos productivos (Gorsky & Fenaux 1998), nuestros resultados también sugieren que las apendicularias podrían ser al mismo tiempo los organismos responsables de la reducción del herbivorismo total del mesozooplancton en sistemas oligotróficos observada por Calbet (2001). Esto último queda puesto de relieve por el hecho de que, según nuestras medidas, la contribución de las apendicularias a la producción total del mesozooplancton es mayor cuanto mayor es la producción primaria, lo que también sugiere que su papel relativo en sistemas oligotróficos debería ser re-evaluado. Calbet (2001) sugirió que la contribución de fuentes de alimento alternativas (organismos heterotróficos) a la dieta del mesozooplancton debería ser mayor en situaciones poco productivas y por tanto permitirles mantener una producción más alta que si dependieran tan solo de productores primarios como fuente de alimento. Desafortunadamente, los métodos usados en el presente trabajo nos han permitido obtener sólo una visión muy general de la importancia del consumo por parte de apendicularias sobre los diferentes compartimentos de carbono particulado. Sin embargo, el alto porcentaje de material no autotrófico en la dieta de las apendicularias (Capítulo 5) sugiere que podrían ser importantes no sólo como herbívoros sino también como consumidores de detritus y microbios (p.e. King et al. 1980, Dagg et al. 1996). Recíprocamente, la asimilación de material no autotrófico podría ser decisiva en el grado de limitación por el alimento de las poblaciones de apendicularias y por tanto en la importancia del control por recurso (Capítulo 6). La compilación bibliográfica de medidas directas de tasas de crecimiento de apendicularias obtenidas en un rango amplio de concentraciones de alimento no mostró ninguna relación entre tasa de crecimiento y concentración de alimento (Capítulo 6). Estos resultados sugieren que debería ponerse una mayor atención en la importancia del control por predación de las poblaciones de apendicularias. La dicotomía en la estructura de los ecosistemas pelágicos puesta de relieve por Cushing 1989 está exclusivamente basada en cascadas tróficas marinas (como puede deducirse de que las flechas de su esquema conceptual se dirigen solo hacia abajo, ver la Figura 1 de la introducción general). Aunque esta sencilla división ha sido útil a la hora de resumir y generalizar la importancia de los diferentes constituyentes de la red trófica

pelágica, el numero de líneas de unión cruzando entre las dos cadenas, la heterogeneidad de las estrategias alimenticias dentro de cada compartimiento y la cada vez mayor evidencia de la importancia de procesos descendentes (predación, reciclado de nutrientes) apoya la visión de Verity & Smetacek (1996) de que este acercamiento pudiera ser demasiado simple y autolimitado.

El grado hasta el que los patrones observados durante nuestro estudio pudieran ser generalizados requerirá más estudios sobre la variabilidad espacial y temporal del consumo herbívoro y producción de las apendicularias a escalas espaciotemporales mayores que las consideradas durante nuestro trabajo. En este sentido, el desarrollo de ecuaciones predictivas basadas en la información obtenida *in situ* ha sido siempre considerada como una herramienta útil para estimar el impacto del consumo y las tasas de producción poblacional del mesozooplancton (p.e. Peters & Downing 1984, Huntley & Lopez 1992). Las ecuaciones desarrolladas durante nuestro estudio (Capítulos 5 y 6) podrían por tanto usarse en combinación con estimas de biomasa o abundancia de apendicularias para evaluar su importancia a escalas espacio-temporales a las cuales medidas directas no sean practicables.

Huntley & Lopez (1992) sugirieron que, ya que la variabilidad natural en biomasa es mayor que la de las tasas de crecimiento individual, para aumentar la precisión de nuestras estimas de producción deberíamos mejorar nuestro conocimiento de las causas de variabilidad en biomasa. Las ecuaciones desarrolladas para la estima del crecimiento de los diferentes compartimientos del cuerpo de las apendicularias y su reproducción deberían servir como un primer paso hacia el desarrollo de modelos poblacionales que en último término permitieran mejorar nuestro conocimiento sobre los mecanismos que controlan las poblaciones de apendicularias. Hemos usado estas ecuaciones para evaluar el papel de control por limitación de recurso en apendicularias. Tanto el acercamiento basado en balances metabólicos como el enfoque empírico sugieren que las apendicularias están generalmente menos limitadas que otros grupos del mesozooplancton. El hecho de que durante etapas tempranas del desarrollo la concentración de alimento podría ser más limitante y de que el grado de limitación dependerá en gran manera de la eficiencia de asimilación del material no autotrófico sugiere la necesidad de evaluar en más detalle la importancia que tienen, a la hora de controlar la dinámica de poblaciones de las etapas tempranas del desarrollo de

apendicularias, la naturaleza y el grado de eficiencia en la utilización de los diferentes tipos de alimento.

La explicación hecha por Levins (1966) sobre el balance entre precisión y generalidad en los modelos ecológicos no es sólo valida para el modo de estudiar la limitación por el alimento sino también para los diferentes modelos que pueden desarrollarse para estudiar la dinámica poblacional en apendicularias. Mientras que los modelos poblacionales basados en crecimiento individual favorecen la generalidad al basarse en los mecanismos responsables de los procesos, otro tipo de modelos poblacionales podrían estar basados en el desarrollo de ecuaciones predictivas empíricas, es decir, basados en la observación de cambios en poblacionales naturales, lo que favorecería un aumento en la precisión de las estimas. Nuestro estudio de los cambios estacionales y geográficos en la abundancia de las diferentes especies de apendicularias (Capítulo 3) apoya las ideas previas acerca del importante control ejercido por las propiedades termohalinas, es decir, por la estructura física de la columna de agua, sobre los ciclos de abundancia estacional y la biogeografía de las distintas especies (p.e. Essenberg 1922, Fenaux 1963, Shiga 1985, Acuña et al. 1995). La descripción de estas relaciones basándose en la teoría del nicho ecológico (Capítulo 3) debería proporcionar la base para un enfoque alternativo en los modelos de cambios poblacionales. Los modelos de predicción de la distribución del hábitat han recibido una atención creciente en ecología terrestre y caen dentro de este tipo de modelos centrados en realidad y precisión (ver la revisión de Guisan & Zimmermann 2000). Estos modelos están basados en la combinación de ecuaciones predictivas para modelar el nicho de una especie con sistemas de información geográfica (GIS) describiendo la variabilidad geográfica de esos factores ambientales. El reciente desarrollo de atlas oceánicos mundiales de salinidad y temperatura (p.e. Levitus & Boyer 1994) podrían ser usados como GIS oceánicos que en combinación con ecuaciones describiendo el nicho ecológico de cada especie podrían representar un punto de inicio para el desarrollo de modelos predictivos de la distribución del hábitat del zooplancton. Estos modelos podrían proporcionar los mapas de distribución a gran escala necesarios para evaluar la importancia potencial de las apendicularias como productores secundarios. Junto con ecuaciones predictivas de las tasas de ingestión y producción de apendicularias podrían proporcionar un enfoque interesante para el desarrollo de estimas que pudieran ser validadas con futuras medidas de tasas poblacionales *in situ*.

GENERAL DISCUSSION

Our perception of marine pelagic food webs has followed an increasing trend in the degree of complexity of the models used to summarize ecological and biogeochemical processes. Marine ecologists have gone from the relatively simple diatom-copepod-fish classic food chain to the recent recognition of the heterogeneity of the microbial community and its potential consequences on ocean carbon fluxes (e. g. Azam 1998). This increased complexity has also affected our understanding of the role of zooplankton and the relative importance of its different members. With the "discovery" of the microbial loop came the realization that the grazing control by small zooplankton was generally more important than the control exerted by copepods. Nevertheless, copepods are still considered the most important consumers when the classical food chain dominates. Although the feeding strategy of copepods does not allow them to feed directly on the pico and nano-sized organisms that dominate the base of the microbial loop, their ability to feed on heterotrophic flagellates and ciliates suggests that their role controlling the heterotrophic processes that prevail during oligotrophic phases could be more important than it was originally thought (Calbet 2001). The work done during this thesis suggests that the role of mesozooplankton in the pelagic food chain is also more complex since groups other than copepods can, at least during particular periods, play a significant role as grazers and secondary producers.

Our measured appendicularian *in situ* grazing rates (Chapter 5) suggest that they could account for a significant fraction of the grazing impact of mesozooplankton on primary production. Their grazing impact can represent up 400 percent daily removal of the primary production. However, this high grazing impact was restricted to the autumn months during a sporadic population development matching an oligotrophic period when primary production was low. More seasonal studies on different areas will be required to determine whether this pattern represents an outlier or a general pattern. Landry et al. (1994) also observed that the grazing impact by appendicularians was higher during the autumn months in the Southern California Bight. Our compilation of published values reporting simultaneous estimates of appendicularian community ingestion rates and primary production (Chapter 5) suggests a general pattern where the proportion of the primary production consumed by appendicularians is lower during

more productive conditions. Surprisingly, and despite the fact that this relationship supports the traditional view of appendicularians as more important grazers of primary production in oligotrophic ecosystems (Gorsky & Fenaux 1998), our results suggest that appendicularians could at the same time be those responsible for the reduction in the mesozooplankton grazing pressure in unproductive environments observed by Calbet (2001). Our estimates of appendicularian production have shown that their contribution to total mesozooplankton production increases with increased primary productivity also suggesting that their relative role in oligotrophic ecosystems should be re-evaluated. Calbet (2001) suggested that the importance of the contribution of alternative prey (i.e. heterotrophic organisms) to mesozooplankton diet should be higher in unproductive communities and it would alow them to maintain higher production rates than of they would depend only on primary producers as sources of food. Unfortunately, the methods used during this thesis allowed us to obtain only a very general view of the importance of appendicularian grazing on the different particulate carbon pools. However, the high percentage of the appendicularian diet consisting of non-autotrophic material (Chapter 5) suggests that they could be important not solely as herbivores but as grazers of the detrital and microbial compartments (e.g. King et al. 1980, Dagg et al. 1996). Inversely the assimilation of non-autotrophic diet could be decisive for the degree of food limitation in appendicularian populations and therefore for the importance of the bottom-up control. The bibliographic compilation of direct measurements of appendicularian growth rates obtained at a wide range of food concentrations showed no significant relationship between food concentration and weight specific growth rates (Chapter 6). These results suggest that more attention should be placed to the importance of top-down forces in controlling appendicularian populations. Cushing (1989) dichotomy on the structure of pelagic ecosystems is exclusively based on trophic cascades (as can be see from the downward-only arrows in his conceptual scheme; see Figure 1 in the introduction). Although this simple division has proven useful in summarizing and making generalizations on the importance of the different constituents of the pelagic food web, the number of crossing links between both food chains, the heterogeneity in the feeding strategies within each general compartment and the increasing evidence on the importance of top-down processes supports Verity & Smetacek (1996) contention that the approach could be too simplistic and self-limiting.

The degree to which the patterns observed during our study could be generalized will require further studies on the spatial and temporal variability of appendicularian grazing and production at higher resolution scales than those considered during our work. In this regard, the development of predictive equations based on *in situ* information has always been viewed as a tool to allow determination of the population grazing impact and production of mesozooplankton (e.g. Peters & Downing 1984, Huntley & Lopez 1992). The equations developed during our study (Chapters 5 and 6) could therefore be used in combination with appendicularian biomass or abundance estimates to evaluate their importance at spatio-temporal scales at which direct *in situ* measurements would not be practically feasible.

Huntley & Lopez (1992) suggested that, since the natural variability in biomass is greater than that of growth, we should improve our knowledge on the sources of this variability in biomass in order to increase the precision of our estimates of production. The equations developed to estimate the growth rates of the different compartments of the appendicularian body and their reproductive output should provide a first step towards the development of population dynamics models that would ultimately allow understanding the mechanisms that control appendicularian populations. We have used these equations to evaluate the importance of bottom-up control of appendicularian populations. Both the budgetary and the empirical approaches suggest that appendicularians are generally less likely to experience food limited growth than other mesozooplankton. The fact that during early development appendicularians could be more limited by food concentration and that the degree of food limitation will strongly depend on the assimilation efficiency of non-autotrophic material suggests the need to further evaluate the nature and the efficiency of utilization of different prey and the factors controlling the population dynamics of early life stages.

Levins (1966) explanation on the trade-off between precision and generality in ecological models is not only valid for the approaches followed in the study of food limitation (Chapter 6) but also for the type of models that could be develop to predict appendicularian population dynamics. While population models based on individual growth rates would favour generality and the understanding of mechanisms, the development of predictive equations based on the observed population changes would emphasize more the precision of the obtained estimates. Our study on the seasonal and

geographical changes in the abundance of appendicularian species supports the previously observed patterns of a close control of the appendicularian species seasonal cycles and geographical distributions by thermohaline properties, that is, by the physical structure of the water column (Essenberg 1922, Fenaux 1963, Shiga 1985, Acuña et al. 1995). The description of these relationships through an approach based on niche theory should provide the basis for an alternative method to model population changes. Predictive habitat distribution models have received increasing attention in terrestrial ecosystems and they lay within the latter type of models centred on reality and precision (see review by Guisan & Zimmermann 2000). The Predictive habitat distribution models are based on a combination of predictive equations to model the niche of a species as a function of environmental factors with geographical information systems (GIS) describing the geographical variability of those environmental variables. The world ocean atlases of salinity and temperature that have been recently developed from *in situ* and satellite observations (e.g. Levitus & Boyer 1994) could be used as oceanic GIS and, in combination with equations describing the ecological niche of each species, could constitute a starting point for the development of predictive plankton habitat distribution models. These models could therefore provide the large scale abundance distribution maps required to evaluate their potential importance as secondary producers. In combination with predictive equations of appendicularian grazing and production rates (Chapters 5 and 6), they should represent an interesting approach to develop estimations that could then be validated with future *in situ* measurements.

8. Conclusiones general

Los resultados obtenidos en éste trabajo nos permiten formular las siguientes conclusiones:

La sucesión estacional en la estructura de la comunidad de apendicularias puede ser resumida en dos fases distintas: una fase de invierno y comienzo de primavera caracterizada por la dominancia de especies del género *Fritillaria* y una fase de verano-otoño caracterizada por especies del género *Oikopleura*. Existe una relación positiva entre la abundancia total de apendicularias y la concentración de clorofila, mientras que la composición específica de la comunidad tiene un importante componente geográfico relacionado con la temperatura.

Se detectaron tres asociaciones de especies de apendicularias. El nicho de cada especie está caracterizado por una respuesta unimodal a la temperatura. Las diferencias ambientales en temperatura y hasta cierto grado en salinidad explican una proporción considerable de la variabilidad geográfica y estacional observada. La estrecha relación entre las asociaciones de especies y factores ambientales físicos sugiere el uso potencial de las apendicularias como indicadores de cambios climáticos o masas de agua.

El tiempo de paso por el digestivo (GPT, min.) en *Oikopleura dioica* puede ser estimado a partir del intervalo entre la defecación paquetes fecales sucesivos (DI, min. paquete fecal^{-1}) mediante la ecuación GPT=2.878DI. Esto establece la base para la estimación del tiempo de paso a partir de sencillos experimentos de producción de paquetes fecales, y representa un modo de determinación del tiempo de paso sin la necesidad de manipular la concentración de alimento. El tiempo de paso se puede estimar a partir de la temperatura y la concentración de alimento como $GPT= 51.67 * e^{-0.0376\ T} * FC^{-0.245}$.

El tamaño corporal fue la variable que explicó la mayor parte de la variabilidad en los contenidos digestivos. Para la mayoría de las especies una gran parte (generalmente por encima del 60%) del material ingerido proviene de alimento no autotrófico.

El impacto herbívoro de las apendicularias aumenta con la producción primaria. Sin embargo, la proporción de la producción primaria que es consumida por la

comunidad de apendicularias es mayor en ambientes más oligotróficos. Puesto que las apendicularias pueden ser responsables del 40% del herbivorismo del mesozooplancton esto podría ser suficiente para explicar los patrones previamente detectados para el total del mesozooplancton.

Nuestros resultados sugieren que los adultos de *Oikopleura dioica* son menos susceptibles a la limitación por el alimento que otros grupos del mesozooplancton. El grado de limitación dependerá fuertemente de la eficiencia de asimilación del material no autotrófico. Sin embargo, no se encontró ninguna relación significativa entre las tasas de crecimiento y la concentración de alimento.

La temperatura explica más del 60% de la variabilidad en las tasas de crecimiento específicas de las apendicularias. No se encontró ninguna diferencia clara entre tasas de crecimiento de las distintas especies. Por lo tanto, hemos desarrollado una ecuación que puede ser usada para predecir las tasas de crecimiento de apendicularias en función de la temperatura.

Contrariamente a la visión tradicional de las apendicularias como organismos más importantes en sistemas oligotróficos, nuestros resultados muestran que, en el área considerada, su contribución relativa a la producción secundaria total es mayor en sistemas más productivos.

Se han desarrollado ecuaciones para la estima del número de huevos producidos y la inversión reproductora, que suponen un primer paso hacia el desarrollo de modelos poblacionales y la comprensión del los factores que controlan la biología reproductora de las apendicularias.

GENERAL CONCLUSIONS

The results obtained during this work allow us to formulate the following conclusions:

The seasonal succession in the structure of the appendicularian community can be summarized into two distinct phases: a winter-early spring phase characterized by the presence of the genus *Fritillaria* prior to the onset of stratification or warming of the water column at the mixed water locations and a summer-autumn community dominated by species of the genus *Oikopleura*. This summer phase can be subdivided into other two or three sub-phases depending on the dominant oikopleuriid species. There was a positive relationship between the abundance of total appendicularians and chlorophyll concentration and a strong geographical influence on species composition, which was related with temperature.

Three different appendicularian species associations were detected, the niche of each individual species being characterized by a unimodal response to temperature. Differences in temperature and to some degree in salinity explained to a considerable extent the seasonal and geographical distribution patterns detected. The close relationship between appendicularian species assemblages and physical environmental factors suggests their potential use as indicator species of climate change or characteristic water masses.

Gut passage time (GPT, min) can be estimated from the time interval between successive fecal pellets (DI, min fecal pellet^{-1}) as GPT=2.878DI. This establishes the basis to estimate GPT from simple fecal pellet production rate incubations, and is one way of determinating GPT without manipulating food concentration or quality, a major shortcoming of current techniques. Gut passage time can be estimated from food concentration and temperature as $GPT = 51.67 * e^{-0.0376\ T} * FC^{-0.245}$.

Body size was the variable explaining most of the variability in gut contents. For most species, over 60% of the ingested material came from non-chlorophyll containing prey.

Appendicularian grazing rates increase with increasing primary production. However, the percentage of the primary production removed by the appendicularian community decreases with increasing productivity, indicating that their grazing impact is relatively more important under oligotrophic conditions. Since appendicularians could account for 40% of the total mesozooplankton grazing, this could explain similar patterns previously reported between total mesozooplankton grazing and primary production.

Our results suggest that large *Oikopleura dioica* are less likely to experience food limited growth than juveniles or other mesozooplankton groups. The degree of food limitation will strongly depend on the assimilation efficiency of non-autotrophic material which comprises a great proportion of appendicularian diet. However, examination of growth rates measurements obtained at a wide range of food concentrations showed no significant relationship between food concentration and weight specific growth rates.

Temperature alone explained more than 60% of the variance in appendicularian weight specific growth rates, and there were no clear differences in the growth rates of different appendicularian species. Therefore, we have developed a temperature dependent equation to predict their weight specific growth rates.

Contrary to traditional views of appendicularians as important members of oligotrophic ecosystems, our results show that in the area considered their relative contribution to secondary production was higher during more productive conditions.

We have developed equations to estimate the reproductive allocation and the number of eggs produced by mature individuals that should provide a first step towards understanding the factors that control appendicularian reproductive biology and population dynamics.

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