Ecology of the marine copepod genus *Oithona*

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UNIVERSITAT POLITÈCNICA DE CATALUNYA
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UPC
Departament d'Enginyeria Hidràulica,
Marítima i Ambiental

Dedico la tesi a la Bene i el Manuel, els meus pares
al Manel, el meu germà,
a la yaya Margarita,
to Claus,
a los pollastres,

Prologue

This thesis was mostly carried out at the Institute of Marine Sciences in Barcelona, under the supervision of Enric Saiz, during the period 2008-2012. The objective of this thesis was to obtain information regarding the ecophisioly and autoecology of the genus of small marine copepods *Oithona*, with a focus on its feeding and reproductive biology.

The thesis is presented in four chapters which correspond to four scientific articles, two of them published in high-ranking journals (*Limnology and Oceanography*, and *Marine Ecology Progress Series*), and the other two are manuscripts ready for submission. Three of the chapters of this thesis contain results obtained from fieldwork conducted abroad, and one is the result of laboratory work conducted in Barcelona.

During the realization of this thesis 3 short stays in connection with the research of the Ph.D. were conducted. The first stay (5 months) was in Greenland in 2010, under the supervision of Professor Torkel Gissel Nielsen. During this stay, fieldwork was conducted in two locations of west Greenland: Nuuk and Qeqertarsuaq; and in an oceanographic cruise in Godthåbsfjord on board R/V *Dana*. The second stay (3 months) was at the Australian Institute of Marine Science in Townsville in 2011, under the supervision of David A. McKinnon. During this stay fieldwork was conducted in the Great Barrier Reef during an oceanographic campaign on board R/V *Cape York*; and in a mangrove area in the Ross Creek. The third stay (2 moths) was at the Denmark Technical University (DTU) in Charlottenlund in 2012, under the supervision of Professor Torkel Gissel Nielsen.

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Abbreviations

C, concentration
C1, C2.., copepodite 1, copepodite 2,…
CI, confidence interval
Cil-C, ciliate concentration
CS, clutch size
CTD, conductivity, temperature, and depth
D, egg developmental time
d, day
Dino., dinoflagellate
Dino-C, dinoflagellates concentration
DR, daily ration
ED, egg diameter
EPR, egg production rate
Expt, experiment
F, clearance rate
GBR, Great Barrier Reef
GGE, gross growth efficiency
h, hour
HR, hatching rate
HT, hatching time
I, ingestion rate
I_{max}, maximum ingestion
ind., individual
IR, ingestion rate
IT, interclutch time
L, liter
MA, major axis regression
mL, milliliter
N1, N2… naupli 1, nauplii 2
OF, ovigerous females
OLS, ordinary least-squares regression
PL. prosome length
TF, total females

SD, standard deviation
SE, standard error
T, temperature
WSI, weight-specific ingestion rate
WSIR, weight-specific ingestion rate

Contents

Introduction

"…The white wave roared against its rocks; sad Oithóna sat on the coast. She looked on the rolling waters, and her tears descend. But when she saw Gaul in his arms, she started and turned her eyes away. Her lovely cheek is bent and red; her white arm trembles by her side. Thrice she strove to fly from his presence; but her steps failed her as she went."

(Oithóna; The Poems of Ossian)

Copepods are probably the most abundant metazoan on earth (Humes 1994), successfully distributed throughout oceans, nearshore habitats, and freshwater systems (Boxshall and Halsey 2004). Within zooplankton, copepods have an important role in the biogeochemical cycles recycling nutrients in the oceans, and are key organisms in trophic pelagic webs as link with higher trophic levels.

Although copepods have been deeply studied since the old days, our knowledge of their biology and ecology is still fragmentary. During years the methods used to collect zooplankton have caused a bias on our knowledge on the structure and functioning of plankton communities, neglecting the importance of their smallest fraction, including the small-sized species of copepods (Gallienne and Robins 2001; Calbet et al. 2001). Although in the early XX century the potential relevance of small copepods such as *Oithona* was already envisaged (Bigellow 1926), historically most of the research on copepods has been focused on the largest species. The difficult handling of small organisms, and the idea that larger copepods were more relevant in marine pelagic communities, probably contributed to the scarce interest devoted to the study of small copepods. The research focused on the smallest zooplankton has received a considerable impulse in the last decades, but still many aspects of the biology and ecology of small copepods are poorly known.

In this introduction I will briefly explain how, when and where the interest on plankton research started; and some basic aspects of zooplankton and marine copepods will be described. I will finish it by explaining some characteristics of the ecology and biology of the genus *Oithona*, and the reasons why this small-sized copepod has an important ecological role in marine plankton communities, and justify its scientific interest.

EARLY HISTORY OF MARINE BIOLOGY IN THE FRAME OF PLANKTON RESEARCH

Marine biology in the antiquity

Since ancestral times the sea has had an important influence in the human development. We should probably consider the fishermen and navigators from the old days as the first "marine biologists" for the simple fact of their observation and, in some way, comprehension of the seas. In times of the Greek philosophers, we can very likely find the first written references to creatures today called plankton. Anaximander of Mileto (610 B.C.), who is historically considered one of the first scientists, speculated about the origin of life in the seas. Aristotle (384-322 B.C.) described many marine forms, and in his writings some of the first specific references to marine life are found. There are many more examples of the relation of philosophers and naturalists from the old times with marine biology, but it is in the XVIII century, with the introduction of the binomial nomenclature funded by the Swedish Carl Linnaeus (1707-1778), when the systematic study of the marine organisms began. Before that time, however, the Dutchman A. van Leeuwenhoeck (1632-1723) was probably the first who noticed the existence of plankton organisms in a scientific way, when he examined water samples from the sea and lakes under his microscope (1677).

The early microbe observers: the description of plankton

The recognition of planktonic organisms started slowly, and further knowledge was only achieved a century after Leeuwenhoek's employment of the microscope (Mills 2012). O.F. Müller (1730-1784) described in his *Zoologiae Danicae Prodromus* (1776) animals from the Danish and Norwegian coasts, and introduced the dredge for biological sampling. Among the first planktonic organisms to be studied in detail were the boreal copepod *Calanus finmarchicus*, described by Bishop Gunnerus under the name *Monoculus finmarchicus* in 1770, and *Ceratium tripos*, a dinoflagellate described by O. F. Müller in 1777. Scientists of the XVIII century showed the way to the study of the sea on scientific modern bases, with a more evolved vision than that of the classic knowledge; however, it was during the XIX century when the bases of oceanography and marine biology were established.

The Irish J.V. Thompson was the first to make methodological use of (rudimentary) plankton nets in his studies of crab and barnacle larvae in 1816; but rudimentary systems for collecting plankton were used occasionally and in a casual manner by enthusiastic amateurs, earlier than that (Wimpenny 1966). Little was accomplished, however, until about 1846, when Johannes Müller introduced the plankton tow-net for use in extensive studies. With the introduction of the tow-net by Müller, plankton was the subject of a vast amount of investigations. Most of the investigations, however, were conducted to describe and classify organisms, until the quantitative study of plankton biology, as a scientific discipline, was born between 1870 and 1911 in a frame of practical concerns such as the economics of fisheries, together with the interest and curiosity of some of the scientists of that time.

Science and circumnavigating expeditions

The interest in marine natural history grew during the XIX century under the frame of large oceanographic expeditions, the establishment of biological stations, and the economical interests in fisheries. In the first years of the XIX century, the explorer of the Arctic William Scoresby recollected microscopic organisms (diatoms) in Greenlandic waters, which abundance was related to whales movements. In the first half of the century, Charles Darwin, during the *Beagle* voyage (1831-1836), made an important contribution to marine biology describing and classifying many marine species. However, the explorations by captain James Cook (1728-1779) on board *Endeavour* during the XVIII century, in great part contributed to the interest of Darwin and other contemporaneous scientists to marine biology.

Fig. 1. Drawing of HMS Challenger by John James Wild, the official expedition artist (http://www.nhm.ac.uk)

But it was Edward Forbes (1815-1854), a pioneer in the study of marine biology, who trying to understand the influence of the marine environment on the distribution of marine organisms involuntarily promoted the Oceanography as a scientific discipline. According to his "azoic theory" (1848), below 500 m life was not possible. In part to prove this theory (and, among other reasons, to evaluate the possibility for an efficient control of the British Empire by means of the telegraph), in the second half of the century (1872-1876) the first global oceanic expedition with exclusively scientific aims was launched on board the *H.S.M. Challenger* (Fig 1). The results of this expedition were recorded in 50 volumes, which were elaborated by the best specialists of the time; thanks to the research conducted on board the *Challenger*, studies on plankton reached a universal dimension. Although the *Challenger* is one of the most famous oceanographic expeditions from the XIX century, years before and after that trip other expeditions were conducted with relevant contributions to marine biology and oceanography. Among them, the Danish *Galathea* expedition (1845-1847); the German Plankton Expedition on *National* (1889) in the Atlantic Ocean; the Fridtjof Nansen's Arctic expedition on board *Fram* (1893-1896).

Marine biological stations

The success of the *Challenger* expedition contributed to increase the scientific interest in the sea. Simultaneously to the booming of oceanographic expeditions in the XIX century permanent laboratories near the sea (marine biological stations) were established through Europe to promote the study of the sea.

Some of the first biological stations were established in Scandinavia and France. One of the oldest stations was Kristineberg, a marine zoological station that started as a summer resort for naturalists like Professors Bengt Fries (1835) and Sven Lovén (1839), although until the 1870s was not established as a formal organization (Kofoid 1910). The station at Corcarneau (1859) has the distinction of being the first maritime laboratory founded in Europe; however, it was the laboratory founded in Kiel (1868), where the first marine laboratory, which devoted substantial attention to plankton, was established. It was actually in the sea

off Kiel where Müller made his first systematic tow-nettings (1845-55), and where his pupils, Haeckel and Anton Dohrn, received the knowledge and inspiration about plankton and marine life that were the foundation of the great contributions to marine biology these two were to make in later years (Mills 2012).

Although other laboratories were established in different countries (e.g. Sebastopol 1871; Roscoff 1871; Banyuls-Sur-Mer 1881; Villefranche Sur Mer 1882; St. Andrews 1884; Santander 1886; Woods Hole 1888; Plymouth 1888; Bergen 1892), foremost among the biological stations of the world, for its important contribution to biological science and its inspiring functioning, stands the zoological station at Naples (Fig. 2). In 1872 the German zoologist Anton Dohrn founded the Stazione Zoologica in Naples, with the idea of create an international scientific community with research facilities to conduct studies in marine biology. Dohrn's initiative was supported and funded by the German government and scientists such as T. H. Huxley or C. Darwin among others (Kofoid 1910).

The scientific network promoted in the Stazione Zoologica worked very well, and international research collaboration was born in Naples. The Prussian scientific, specialist in copepods, Wilhelm Giesbrecht (1854–1913) moved to Naples in 1880s, where he developed his most famous work, the monograph *Systematic und faunistik der pelagischen copepoden des golfes von Neapel und der angrenzenden meres-abschnitte* (1892) ("Systematics and faunistic of the pelagic copepods of the Gulf of Naples and neighbouring seas"), considered the first inventory of copepod species (Fig. 3).

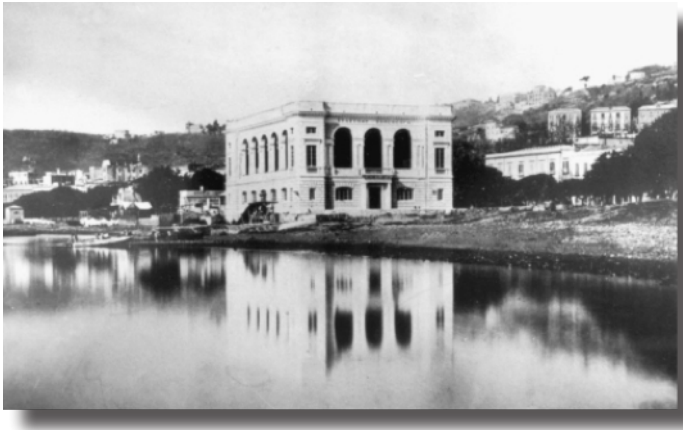


Fig. 2. Stazione zoologica at Naples (www.szn.it)

The establishment of the Stazione Zoologica at Naples made marine researchers aware of the advantages of coastal field stations as compared to huge expeditions. In Scandinavian countries like Norway, Sweden and Denmark, where fishing was an important economical activity at that time, governments favoured fisheries research, what undoubtedly contributed to the development of marine biology and quantitative plankton biology. In the same way, the Spanish Institute of Oceanography (1914) was born with the aim to study physical, chemical and biological characteristics of the sea to be applied in fisheries.

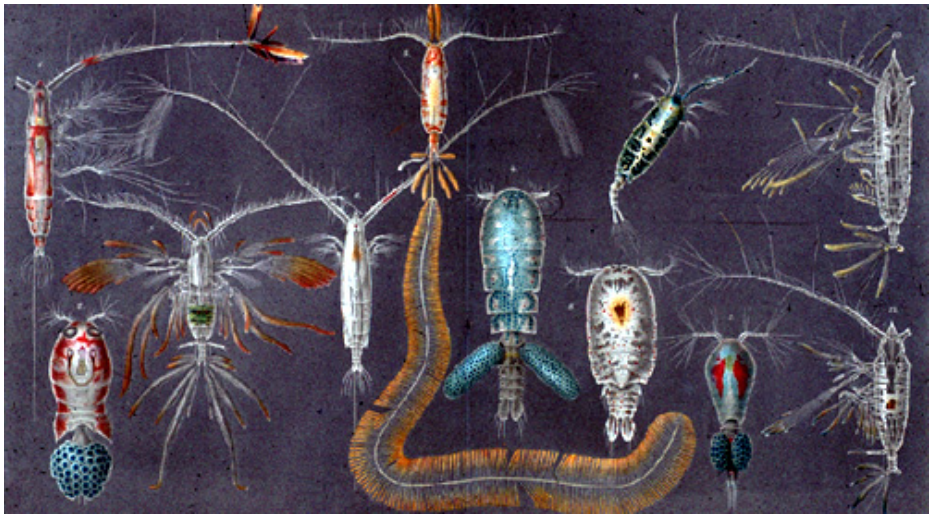


Fig. 3. Marine planktonic copepods *(Plate in Giesbrecht, 1892)*

The early development of quantitative plankton biology started in the Germany and Scandinavia, and Victor Hensen (1834-1924) is one the scientists with whom the story begins. Hensen, together with his co-workers Karl Brandt and Hans Lohmann created a new branch on marine biology between 1870 and 1911 at the University of Kiel, in many aspects focused to study the factors controlling the annual plankton production in temperate seas (Mills 2012). Hensen published the monograph "Über die Bestimmung des Planktons" in 1887, and by that time he had defined quantitative and chemical techniques for plankton work.

In summer of 1889 took place the first plankton expedition (on board *National*), organized by the Alexander von Humboldt Foundation, lead by Karl Brandt under the supervision of Victor Hensen.

The Kiel's school, in great part influenced by the theories of Hensen, brought to the recent biological oceanography discipline between 1880s and 1920s, knowledge of the production and chemistry of the ocean. Under Brandt's theory, the Kiel school provided the first model of how plankton abundance was controlled, including phytoplankton growth, the regeneration of nutrients (especially nitrogen), and the effect of herbivorous zooplankton in the plankton dynamics.

In the early 1900s, the development of a new group or research in oceanography in Plymouth Laboratory, highly contributed to provide new techniques for the determination of biological and chemical factors in controlling the plankton cycle. They attributed the inverse relationship between phytoplankton and zooplankton to grazing using physical and chemical approaches, and they also explained phytoplankton photosynthesis, nutrient uptake, and growth. At the same time, American scientists, mainly from Yale University, and later in Woods Hole Oceanographic Institution, worked in developing mathematical approaches on how phytoplankton and zooplankton might be controlled in the ocean.

In the XX century the number of scientists and studies dedicated to plankton research were numerous, and many of them will be mentioned in the next sections of this thesis, as part of the modern contributors to marine biology. This historical introduction is a personal summary of some of the relevant episodes in the history of biological oceanography, thus I truly apologize for the names of scientists, expeditions, and institutions that do no appear in the text.

PLANKTON, A WANDERING LIFE

We can broadly classify the organisms that inhabit marine environments in benthos, nekton and plankton. Those organisms that live in the bottom, or attached to it, are known as the benthos. The nekton are the free and active swimming forms, whereas the drifters of the oceans are known as the plankton. The term "plankton" was first used by Hensen in 1887 to describe drifting organisms in the water. The word derived from the Greek adjective *πλαγκτός - planktos*, meaning "errant", and by extension "wanderer" or "drifter". The German naturalist Ernst Haeckel (1834-1919) accepted the term "plankton" defined by Hensen, and defined the "benthos" (from the Greek ***βένθος***, "bottom or depth of the sea") and the "nekton" (from the Greek ***νηκτός***, "swimming").

The definition of plankton is vague because some planktonic animals are capable swimmers. However, in contrast with nekton organisms (e.g. fish), which are active swimmers, typically plankton cannot swim against the ambient flow and are carried away with currents. However, although exposed to the forces of turbulence and currents, almost all zooplankton species have developed some means to move, at least to change their vertical position within the water column.

The plankton community is formed by a large and complex group of organisms, such as bacteria, unicellular eukaryotes, and metazoans, which together are the base of marine life. The trophic interactions between primary producers and higher trophic levels in the ocean, was first described as a linear chain from phytoplankton to nekton. The classical view of marine food webs included the trophic interactions of the microbial levels when the microbial loop was described (Pomeroy 1974; Azam et al. 1983). Pomeroy (1974) highlighted the key role of microbes in ocean productivity, and some years later, Azam et al. (1983) introduced the term "microbial loop". After the discovery of the microbial loop, the classical view in which phytoplankton and marine plants were responsible of the primary production in the oceans changed, and bacteria were also included as a major group of primary producers.

The biogeochemical interactions in the plankton community are extremely complex (Fig. 4), and some trophic relationships are still not well described or understood. In some parts of the ocean the microbial food loop predominates (e.g. oligotrophic marine ecosystems), and in others the traditional food chain is more important (Cushing 1989).

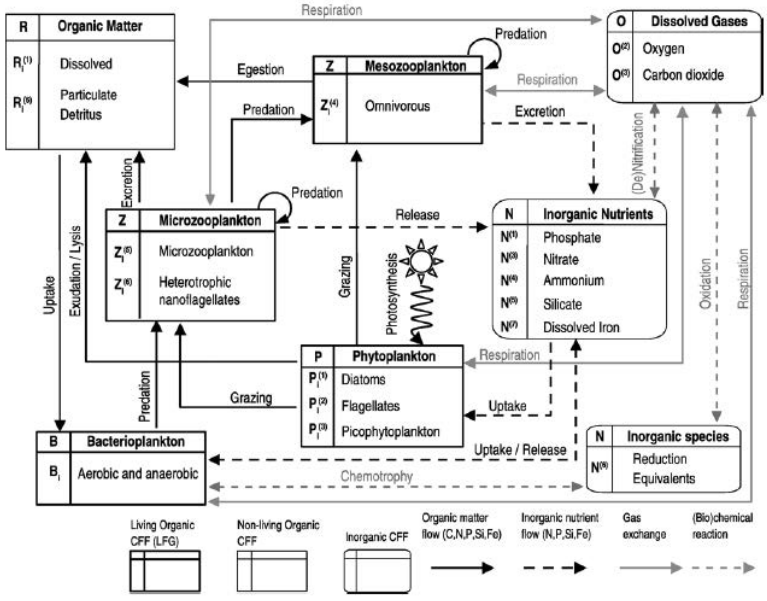


Fig. 4. Scheme of the state variables and pelagic interactions of the biogeochemistry model by Vichi et al. (2007)

Most of the planktonic organisms are small, often less than 1 mm, normally measured in terms of microns, and visible only with the aid of a microscope. But in the wide variety of plankton we also find animals with sizes up to metres (i.e. jellyfish) (Fig. 5). Groups of plankton can be defined according to different characteristics and several classifications have been proposed in the literature (Sieburth et al. 1978; Le Queré et al. 2005) (Fig. 6). In general terms, plankton can be classified according to their mode of nutrition as autotrophs and heterotrophs. Heterotroph organisms can be classified according to their diet in herbivorous, detritivorous, omnivorous, or carnivorous.

Heterotrophic plankton also include the osmotrophic bacteria, termed bacterioplankton. Mixotrophy, the combination of auto- and heterotrophy, is quite commonly found in flagellates and other protozoans like foraminiferans, radiolarians and ciliates; and a further distinction is made between obligatory and optional mixotrophy.

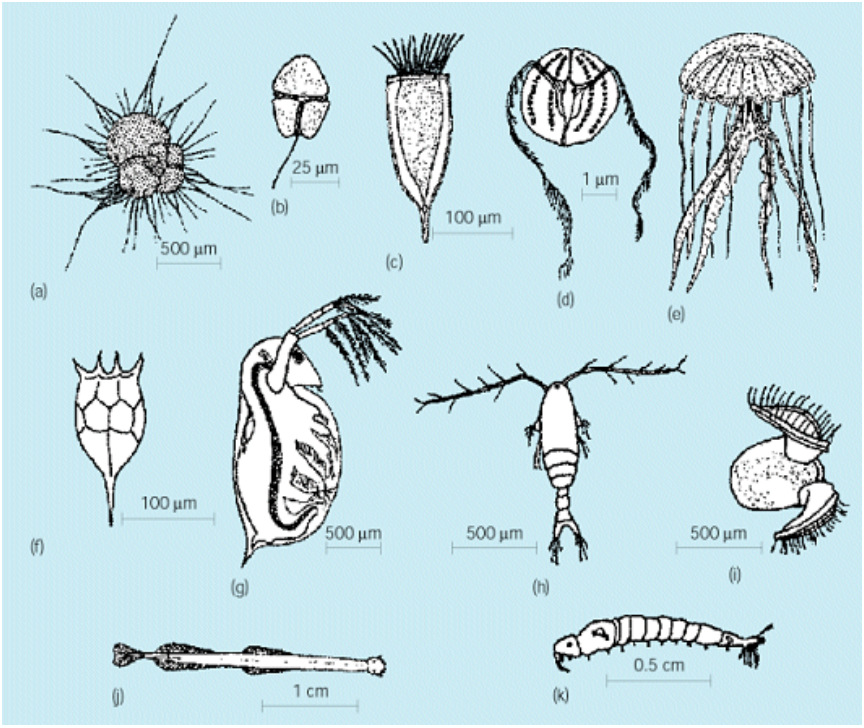


Fig. 5. Representative zooplankton. (*a*) Foraminifera. (*b*) Dinoflagellate. (*c*) Tintinnid ciliate. (*d*) Ctenophore. (*e*) Cnidarian (scyphozoan jellyfish). (*f*) Rotifer. (*g*) Cladoceran. (*h*) Copepod. (*i*) Gastropod veliger larva. (*j*) Chaetognath. (*k*) Insect larva (*Chaoborus*). (*Parts a and e from L. H. Hyman, The Invertebrates, vol. 3: Protozoa through Ctenophora, McGraw-Hill, 1940.*)

Zooplankton can be classified as pelagic or neritic in function of the marine area they inhabit; pelagic zooplankton live in open and deep ocean waters, whereas neritic zooplankton live in coastal and shallower waters. Some organisms occur in the plankton during only a part of their lives, this is the meroplankton; whereas most of the pelagic forms are holoplankton, they are planktonic during their entire lifespan (Raymont 1983).

Different variable factors determine the distribution of the plankton in the oceans. The intensity of the light, light/dark cycles, salinity, temperature, turbidity, currents and tides, nutrients, seasonal cycles, reproductive strategies, and predators, are factors that influence both the geographical and vertical distribution of plankton in marine ecosystems.

Zooplankton occupy a key position in the pelagic food web as they transfer the organic matter produced by the primary producers through photosynthesis to higher trophic levels such as pelagic fish stocks. The availability of zooplankton of the right size and at the right place and time during the first feeding period of fish larvae constitutes the famous match/mismatch hypothesis (Cushing 1990). Zooplankton grazing also determines the amount and composition of vertical particle flux, and contributes to the biogeochemical cycles in the sea (Fig. 7). It thus important, to increase our knowledge of aspects of zooplankton ecology to understand and predict the impact of environmental changes in fish stocks, but also to understand the function of biogeochemical cycles in the sea.

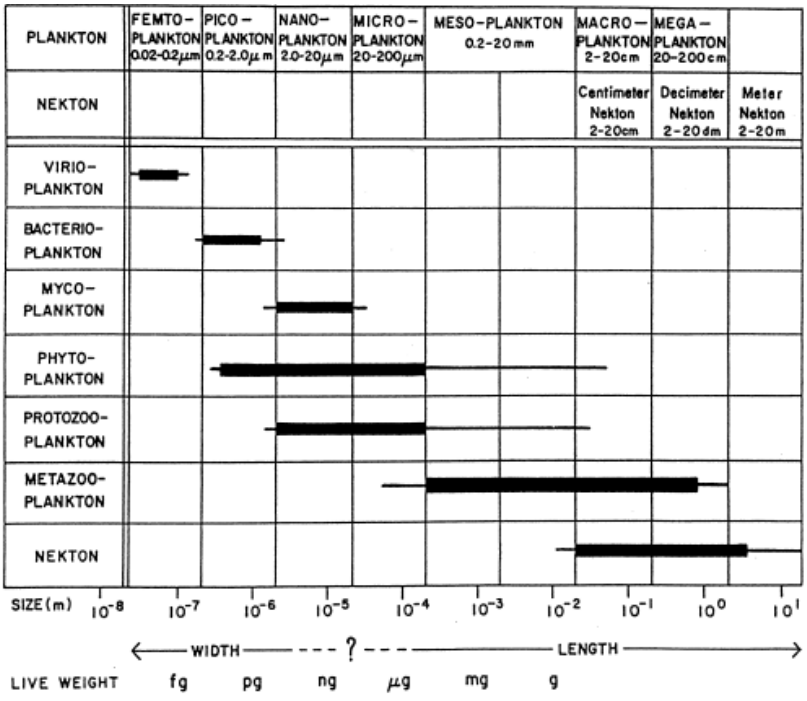


Fig.6. Distribution of different taxonomic-trophic compartments of plankton in a spectrum of size fractions, with a comparison of size range of nekton (Sieburth et al. 1978).

The trophic role and ecological significance of zooplankton communities depend on the diversity, behaviour and interaction of their species. Zooplankton communities are often dominated by so-called key taxa, which play the main role in transferring energy up to the food web, and exercising top-down control through grazing or predation. However, there are plankton communities with extremely high diversity in which might be difficult to determine key species, especially in oligotrophic environments. Research focused on environmental adaptations of predominant species is necessary to understand their ecological significance in forming the food web (Verity and Smetacek 1996).

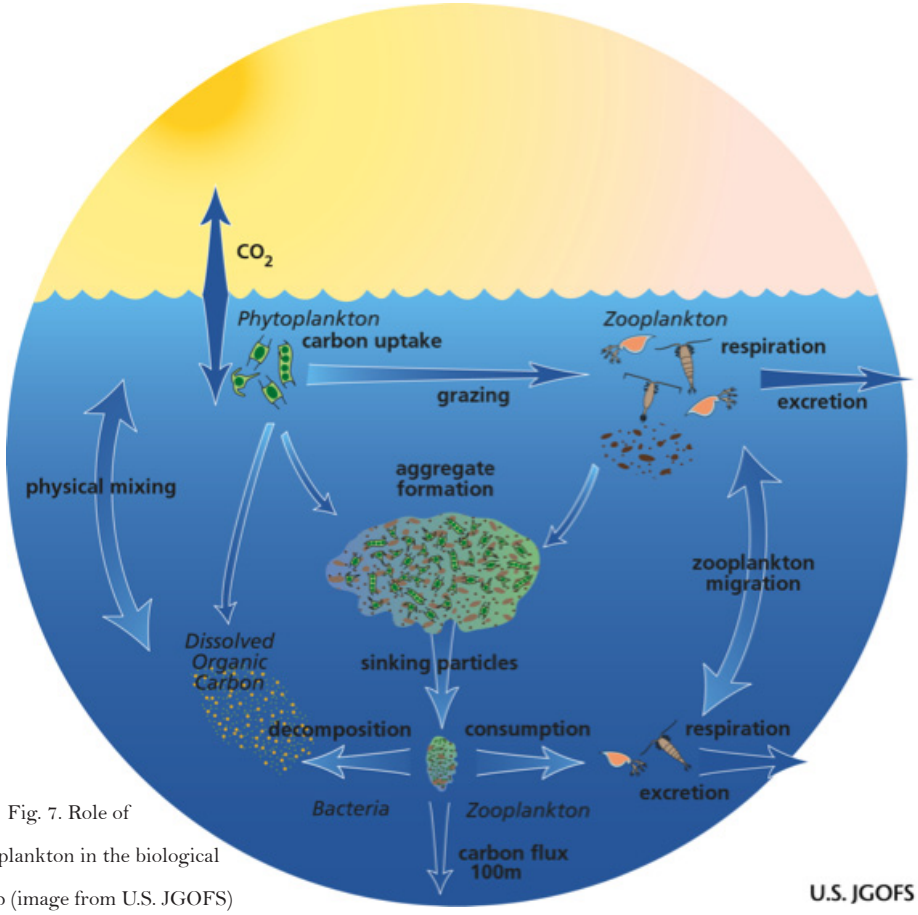


Fig. 7. Role of zooplankton in the biological pump (image from U.S. JGOFS)

MARINE COPEPODS, ERRANT ANIMALS OF THE OCEANS

The zooplankton constitute a extremely diverse group of organisms, including all the major and most minor phylum of the animal kingdom, if not as adults, then as larval stages. The subclass Copepoda (Class Crustacea, Phylum Artropoda), are by far the most abundant (metazoan) zooplankters in marine systems, contributing up to 50% of the abundance and biomass in the global oceans (Voronina 1998).

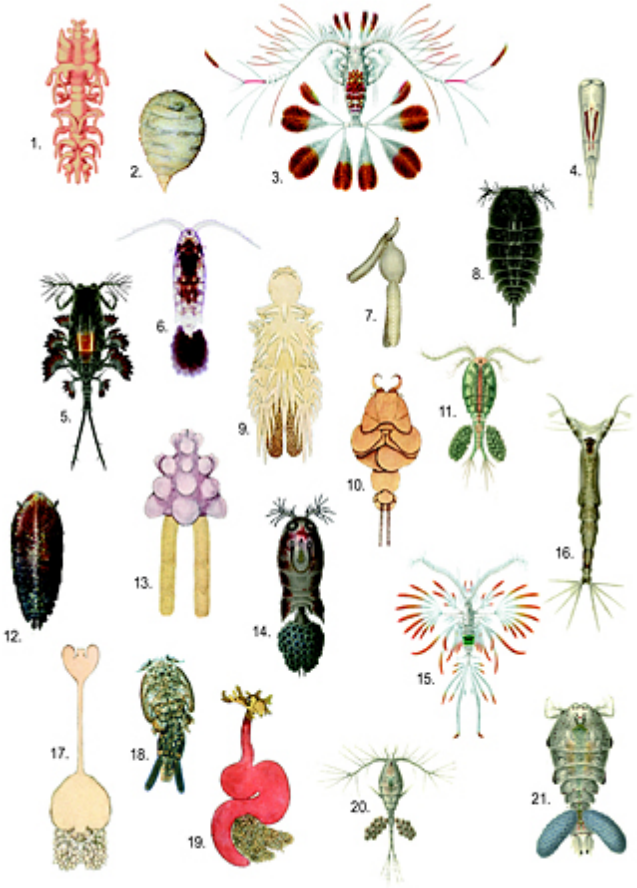


Fig. 8. Diversity of copepod forms. *1. Philichthys xiphiae 2. Sarcotaces sp. 3. Calocalanus pavo 4. Farranula rostrata 5.Copilia vitrea 6. Paracalanus parvus 7. Clavella adunca 8. Copilia quadrata 9. Chondracanthus zei 10. Phyllothyreus cornutus 11. Acanthocyclops vernalis 12. Sapphirina ovatolanceolata 13. Chondracanthus ornatus 14. Corycaeus obtusus 15. Euaugaptilus filigerus 16. Monstrilla longispinosa 17. Sphyrion lumpi 18. Caligus elongatus 19. Lernaeocera branchialis 20. Oithona nana 21. Sapphirina auronitens.* (Bron et al. 2011).

Copepods are an extremely ancient and extraordinarily diverse group (11500 species, Humes 1994) (Fig. 8), very likely having diverged from other arthropod taxa between 388-522 million years ago (Nishida 1985). We can find high diversity on morphologies, physiologies, life strategies and ecological distribution. Most copepods are small, active, free-swimming organisms, although there are numerous parasitic species to which this does not apply. Of the free-living species, some are of the open water, and are termed "pelagic"; whereas other species are bottom-living, and are termed "benthic". Copepods are very abundant in all kind of aquatic habitats, and at all seasons.

Giesbrecht (1893) established two groups of free-swimming copepods: *Gymnoplea* (meaning "naked abdomen"), in which the fifth segment of the cephalothorax remains a part of that division of the body (i.e. copepods with no appendix in the urosome); and *Podoplea* (meaning "feet on abdomen") in which this fifth segment, and any legs if present, are united with the abdomen (i.e. copepods with appendix in the urosome). Of the 9 current orders accepted by taxonomist, planktonic copepods fall into three of them: the Cyclopoida (which nowadays also include the former order Poecilostomatoida), the Harpacticoida (mainly benthonic, but with a few pelagic species), and the order Calanoida (Boxshall and Halsey 2004) (Fig. 9).

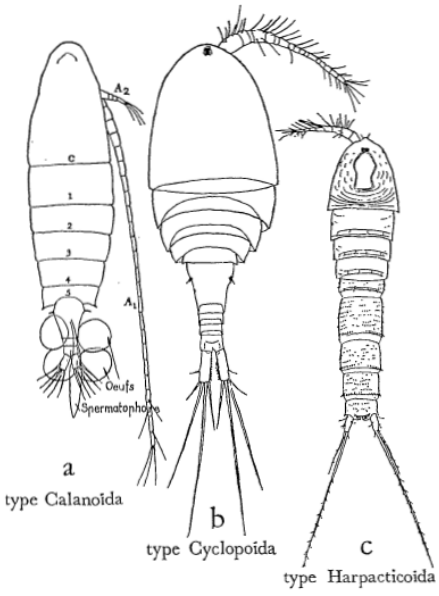


Fig. 9. External anatomy of the 3 main groups of marine pelagic copepods.

Marine copepods are important herbivores in the sea feeding on phytoplankton, but they are also able to carnivorously feed on protozooplankton and other copepods. Most fish and other marine organisms feed on copepods than on any other animal group; and juveniles of copepods (i.e. nauplii) are also important food items for the first-feeding larval stage of fish (Cushing 1989). Marine copepods are, therefore, very important organisms with an essential role in the pelagic ecosystem from a trophic point of view, as a link between the primary production and higher trophic levels (Calbet and Saiz 2005). Besides their trophic role, copepods characterize the secondary production of the sea, and they have a fundamental role in the CO_2 dynamics and other biogeochemical processes in the ocean (e.g. role of copepod grazing on biogeochemical cycling of dimethylsulfide, Lee et al. 2003 and references therein) (Fig. 10). The most important copepod outfluxes are excretion (dissolved matter) and faecal pellet production (particulate matter). Excretion offers inorganic nutrients that can be directly used by primary producers; whereas faecal pellets have an important role in the vertical transport of elements in the water column (Frangoulis et al. 2004).

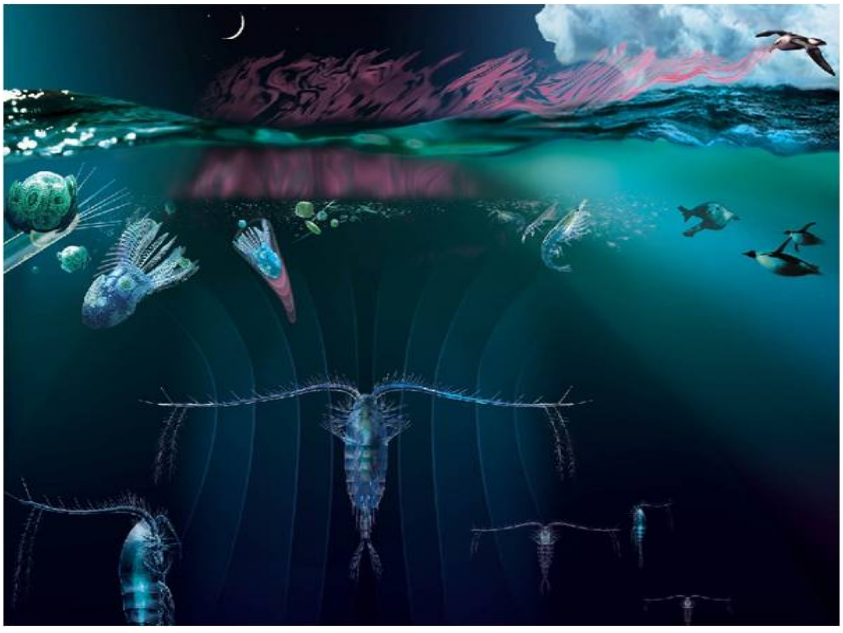


Fig. 10. Role of copepods in the biogeochemical cycling of dimethylsulfide
(Image by Glynn Gorick)

Adult external anatomy

Despite the many species of pelagic copepods that occupy different ecological niches, they are of strikingly similar shape (Huys and Boxshall 1991). It has been argued that the evolutionary success of the copepod shape is due to its superior escape ability (Verity and Smetacek 1996). Stalking predators are sense by the long antennae, and powerful paddle-shaped thoracic appendages enable the copepods to jump out of the way (Yen 2000).

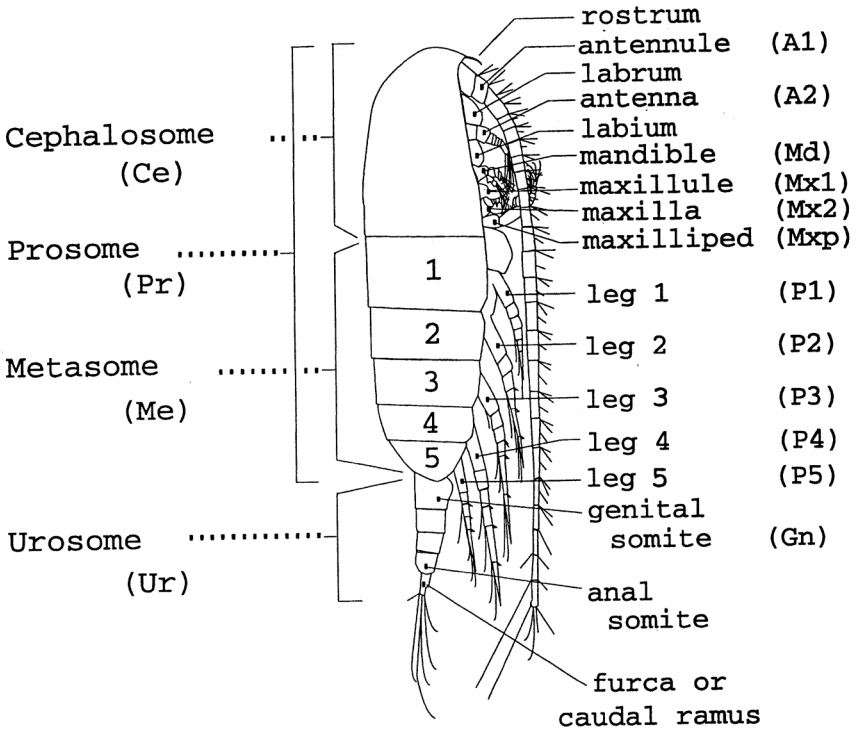
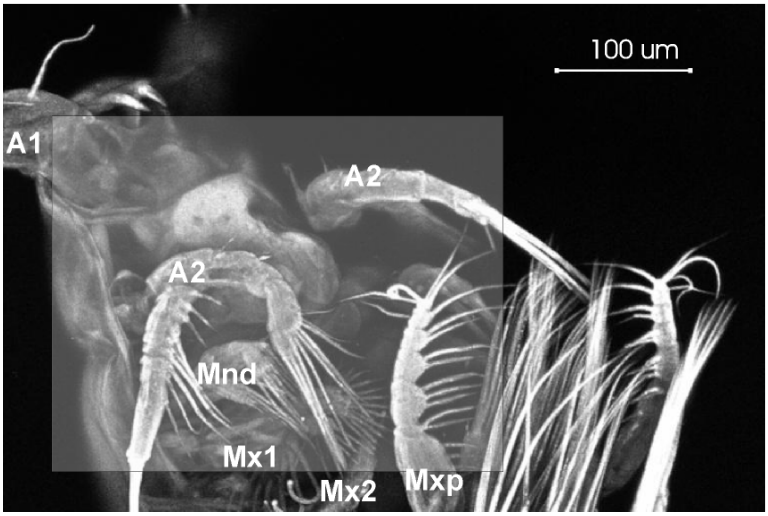


Fig. 11. External anatomy of a calanoid copepod (Mauchline 1998).

The external morphology of adult pelagic copepods consists in a body divided into segments of different sizes, no overlapped, although the head segments and the first thoracic segment are always combined. The body of the copepod is divided into several regions, the cephalosome, metasome, and urosome (Fig. 11). The **cephalosome** consists of 6 segments fused together (5 cephalic segments + first thoracic segment), all bearing limbs. The first pair of limbs in the cephalosome, the antennules (or antenna 1), are sensory; in the males of many orders one or both can be modified to grasp the female (called prehensile or geniculate) during the copulation (Fig. 12).

Fig. 12. Unidentified male calanoid copepod. (Photo: David Liittschwager)

The next pairs of limbs make up the feeding appendages; they are successively antenna (or antenna 2), mandibles, maxillules (or first maxilla), maxillae (or second maxilla), and the maxillipeds (in the first thoracic segment). The mouthparts can present modifications in segmentation and setae in relation with the feeding behaviour of the copepod (i.e. herbivorous, carnivorous) (Fig. 13).



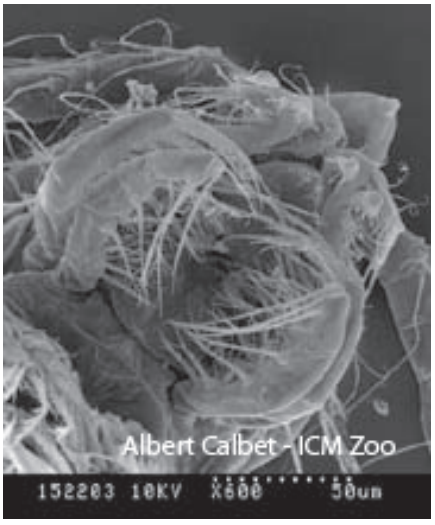


Fig. 13. *Left picture:* Laser scanning confocal micrograph of the calanoid *Skistodiaptomus oregonensis* mouthparts and feeding appendages. A1, first antenna; A2, second antenna; Mnd, mandible; Mx1, first maxilla; Mx2, second maxilla; Mxp, maxilliped; arrow points to mouth (Bundy and Vanderploeg 2002). *Right picture:* Detail of mouth appendages of the cyclopoid *Oithona davisae.*

The **metasome** consists of 5 segments (2-6 thoracic segments), which are partially or completely fused to the cephalosome. They are called pedigerous segments (P1-P5) as bear five pairs of swimming limbs (Fig. 14). The fifth pair of legs are often reduced or modified; heavily modified P5 legs are typical of many male copepods, which are adapted to deposit the spermatophore. The cephalosome and metasome together are sometimes referred as "prosome".

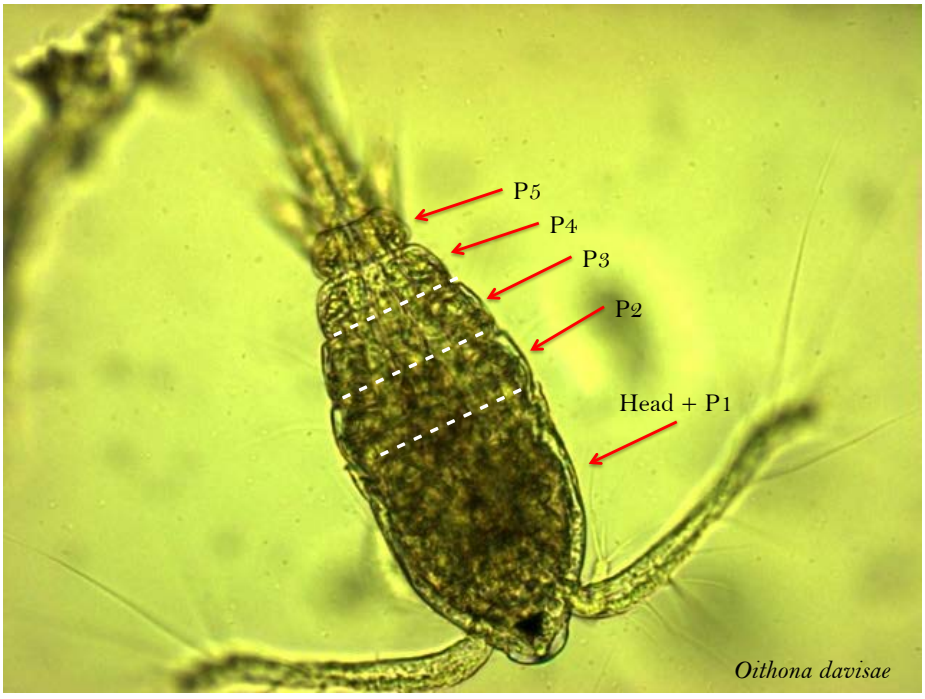


Fig. 14. Detail of the prosome of *Oithona davisae*. Head and pedigerous segments are shown.

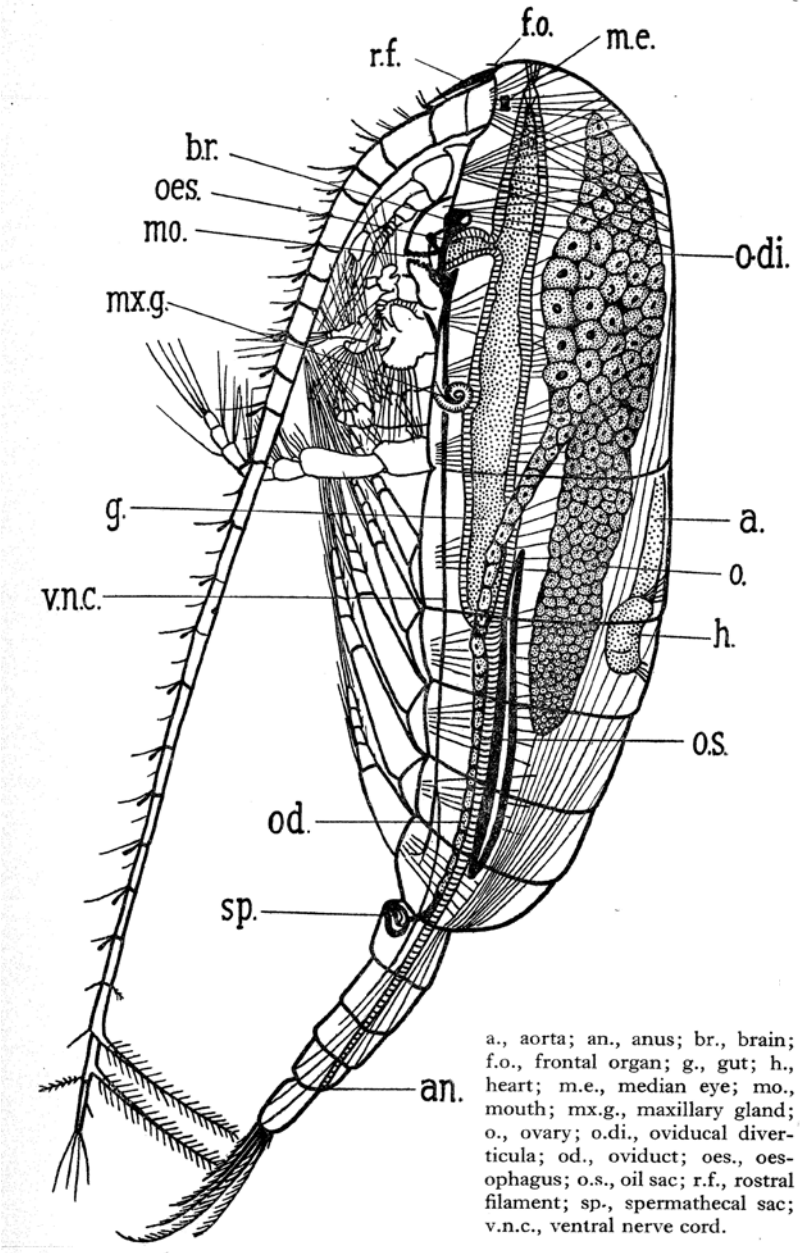
The **urosome** consists of the genital segment, abdomen and caudal rami. The genital segment in females is usually fused with the following segments. The segments after the genital one do not bear any limbs, and together are called the abdomen. The last abdominal segment bears the anal opening, and ends in two caudal rami.

A*dult internal anatomy*

Some of the early investigations on the internal anatomy of copepods to be considered are among others, the works of Hilton (1931), Lowe (1935), Marshall and Orr (1955), Park (1966), Blades and Youngbluth (1980). They refer to aspects of the skeletal-muscular, digestive, excretory, circulatory, reproductive, and nervous system.

In Fig. 15 is shown the internal anatomy of a calanoid copepod. The internal morphology, in a very simplified description, consists of an endoskeleton formed by tendinous and chitinous tissues, which serve as support for the muscles of the antennae and mouth, and for the attachment of the muscles. A short oesophagus connects the mouth with the main part of the gut; and an excretory system with a pair of maxillary glands. The heart is a small avoid body that lies dorsally beneath the junction of the second and third thoracic segments. The central nervous system consists of the brain and the ventral nerve cord; the brain nerves run to the eye and frontal organs. Both males and females have a single median gonad, with one and two conducts (oviducts) respectively, connecting the gonad to the genital opening on the firs urosome segment (Marshall and Orr 1955).

Fig.15. Internal anatomy of a *Calanus finmarchicus* female from the side (Marshall and Orr 1955) (Next page)



a., aorta; an., anus; br., brain; f.o., frontal organ; g., gut; h., heart; m.e., median eye; mo., mouth; mx.g., maxillary gland; o., ovary; o.di., oviducal diverticula; od., oviduct; oes., oesophagus; o.s., oil sac; r.f., rostral filament; sp., spermathecal sac; v.n.c., ventral nerve cord.

Life cycle

Most planktonic copepods present sexual reproduction, adult females and males are separate, and sexual dimorphism is frequently evidenced (Gilbert and Williamson 1983, Ohtsuka and Hay 2001). Copepod mating behaviour starts with the encounter, often followed by pursuit and capture, to achieve the final copulation (Buskey 1998) (Fig. 16). Females release chemicals, or can use hydromechanical signals to attract the males (Bagøien and Kiørboe 2005a,b); and males often present modifications on the antennule to grip the female. The fifth pair of legs in males are often adapted to deposit the spermatophore (i.e. container with sperm) in the female's genital opening, where the sperm is released in the seminal receptacle and egg fertilize (Ohtsuka and Hay 2001).

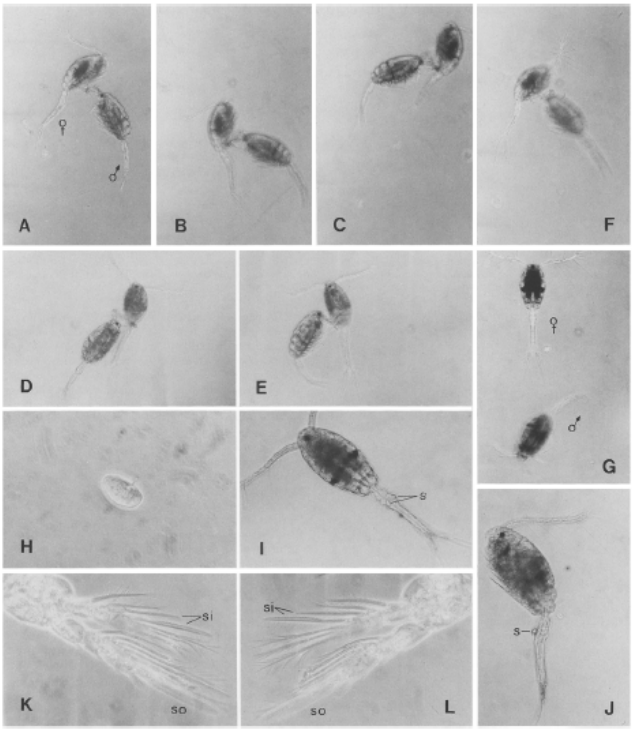


Fig. 16. Mating behaviour of the cyclopoid copepod *Oithona davisae* (Uchima and Murano 1988).

The larval stage of copepods consists of 6 naupliar and 6 copepodite stages, the copepodite VI is the adult (Fig. 17). The sequential stages are distinguished by the progressive development of the segmentation of the body, number and form of the appendages, and an increase in body size.. Eggs are sometimes carried in sacs attached to the body until they are ready to hatch, or they are released freely into the water once fertilised. When the egg hatches, a nauplius stage emerges, with the typical crustacean larvae form. The name *Nauplius* comes from one of the legendary Argonauts, and was given by O.F. Müller to the immature *Cyclops*, what he thought was the adult of distinct species.

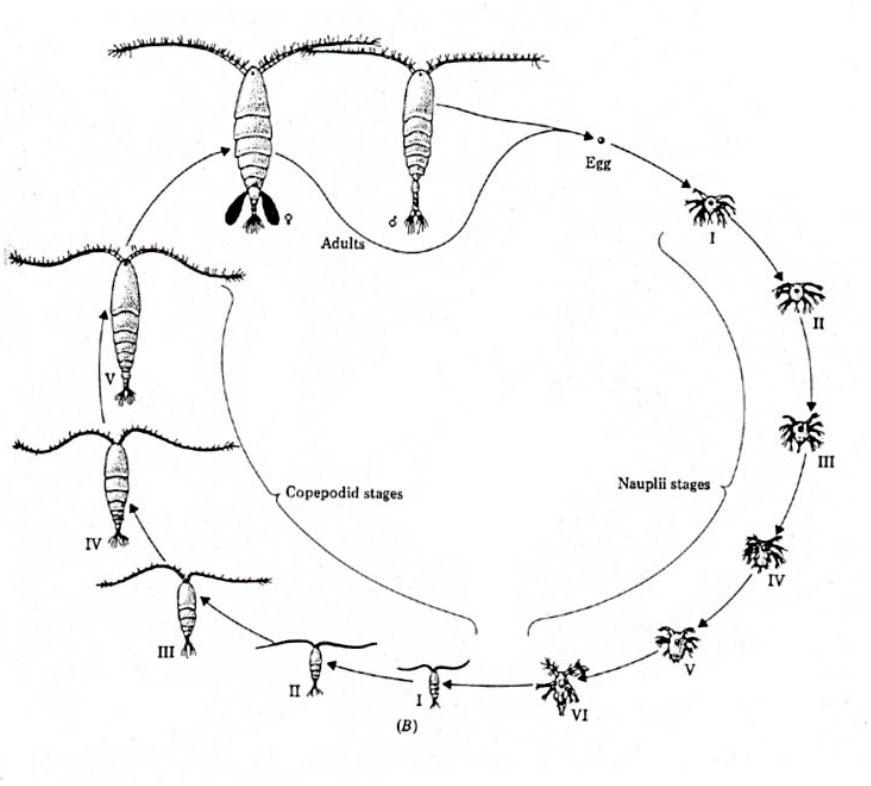


Fig. 17. Cycle of a calanoid copepod (after Nybakken, 1982).

Copepods have developed a range of life strategies to survive in different environmental conditions, so species from tropical, temperate and high latitude environments present different life history patterns. Especially in high latitude environments, where there is a pronounced seasonality, copepods present overwintering strategies to ensure the survival of the populations. Among these strategies, two main forms are: 1) the presence of resting or diapause stages coupled with ontogenetic seasonal and vertical migration; and 2) the production of resting or diapause eggs, which reside in the mud of the seabed; strategy restricted to coastal and shelf species (Mauchline 1998 and references therein).

CYCLOPOIDS, THE LITTLE SUCCESSFUL COPEPODS

Within planktonic copepods, the order Cyclopoida is considered the most successful and abundant group of copepods in the world (Paffenhöfer 1993; Gallienne and Robins 2001). There are 12 pelagic families within the order (Razouls et al. 2005-2012), and among them, Oncaeidae and Oithonidae (Fig. 18) are the most common and numerous (Fish 1936; Deevey 1948; Marshall 1949). We can find them in all kind of habitats, including fresh and seawaters, epipelagic and mesopelagic zones of open oceans from tropical to polar seas, coastal waters, and estuaries (Burckardt 1913; Lindberg 1954; Ferrari and Bowman 1980; Nishida and Marumo 1982). Most of the species belonging to the family Oithonidae are characterized by small sizes, usually less than 1 mm in length, and we normally refer to them as small-sized pelagic copepods.

Cyclopoid have a high diversity of body forms (Fig. 19), however the typical adult is cyclopiform in both sexes (inverted-bulb form). The major articulation of the body occurs between the fourth and fifth pedigeorus somites, thus the first somite of the urosome bears the fifth pair of limbs (usually much reduced).

Phylum	Arthropoda
Subphylum	Crustacea
Class	Maxillopoda
Subclass	Copepoda
Order	Cyclopoida (Burmeister 1835)
Family	Oithonidae (Dana 1853)
Subfamily	Limnoithoninae (Kiefer 1928)
	Genus
	Limnoithona (Burckhardt 1913) → 2 species
	Oithoninae (Kiefer 1928)
	Genus
	Dioithona (Kiefer 1935) → 3 species
	Oithona (Baird 1843) → 46+ species

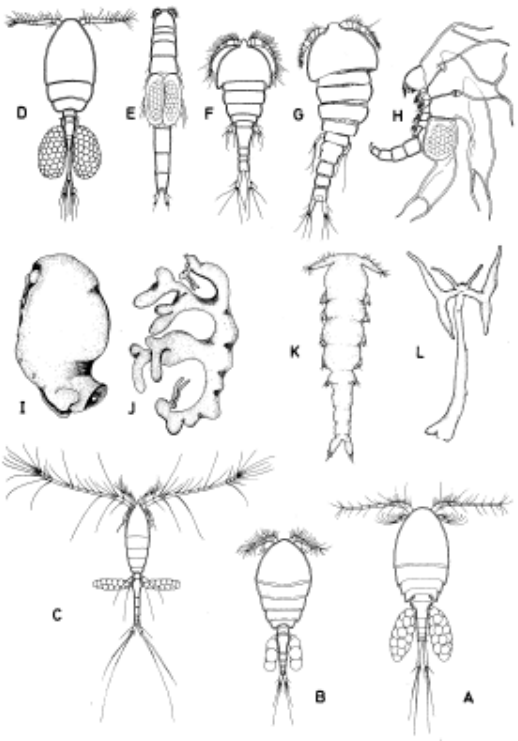


Fig. 19. Diversity of cyclopoid forms. A. Cyclopida. B. Cyclopinidae. C. Oithonidae. D. Thespesiopsyllidae. E. Ascidicolidae. F. Archinotodephyidae. G. Mantridae. H. Notodephydae. I. Chordeumiidae. J. Cucumaricolidae. K. Ozmanidae. L. Lernaeidae. (from Boxshall and and Halsey 2004)

Because of their great abundance in all marine habitats, they are considered a major component of the zooplankton. However, their abundance, biomass, and contribution to the secondary production of the oceans, have been underestimated for years due to the use of coarse mesh sizes (Galliene and Robins 2001; Calbet et al. 2001). Those plankton nets failed in efficient catching of small copepods, what caused an important bias of the actual contribution of cyclopoids to zooplankton abundance.

Despite the importance of cyclopoid copepods, especially in terms of abundance in marine waters worldwide, it is one of the less studied groups of copepods, and there are many unknown aspects of their role in marine ecosystems. Historically, the largest amount of studies on copepods have been focused on the largest species, mainly calanoids, because their important contribution to the secondary production in terms of biomass. This focus on large species of copepods is furthermore often justified for the easier manipulation of large organisms to conduct experiments, in contrast with the difficult handling in the lab of the smallest species (Fig. 20).

Fig. 20. From left to right: *Metridia longa* (~2.5 millimeters), Calanus glacialis (~4mm), *Calanus hyperboreus* (~7mm). The smallest, *Oithona similis* (0.5mm) is below the center. (Photo by Carin Ashjian,WHOI).

OITHÓNA, VIRGIN OF THE WAVE

Within marine cyclopoids, an outstanding group of copepods by their abundance and relevance is the genus *Oithona*. The genus *Oithona*, defined by W. Baird (1843), was born from the description of *Oithona plumifera* (Fig. 21) and *Oithona splendens* when he was investigating "insects" responsible of the luminescence of the sea (Baird 1843). "The Poems of Ossian" (J. Macpherson 1812) inspired him to use the name of Oithóna, what in gaelic means "virgin of the wave" (oi-, virgin; –thóna, wave). Soon thereafter, other scientists described some of the species that today are included in this genus, and which are focus of most of the studies: *Oithona similis* (Claus 1866), *Oithona brevicornis* (Giesbrecht 1891), *Oithona nana* (Giesbrecht 1893) (Fig. 22), among others. Some of the first descriptions of *Oithona* species coincided with the Golden Age of Copepodology in 1890s.

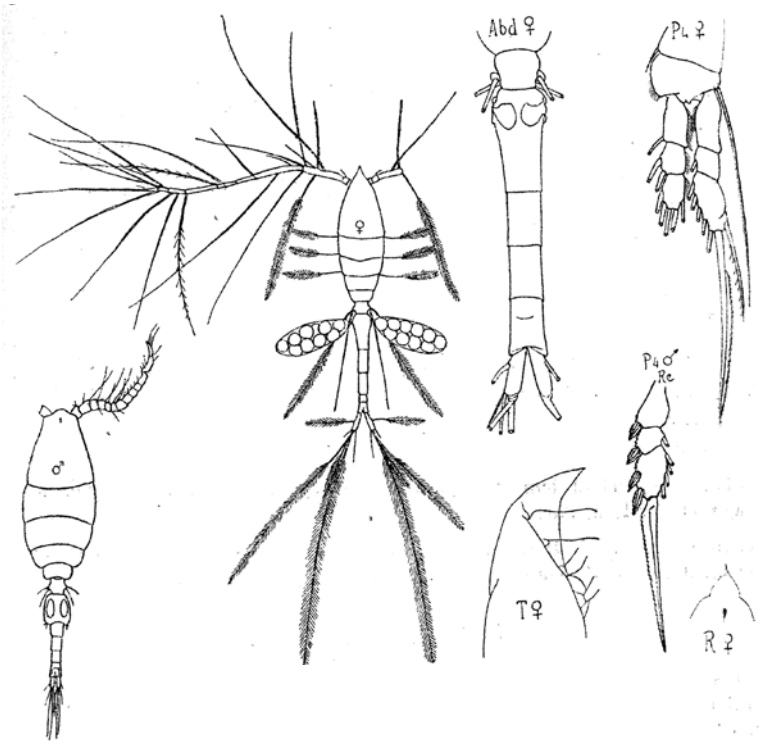


Fig. 21. *Oithona plumifera* (Baird) (from Rose 1933).

Some of the first investigations on pattern distribution and abundance of *Oithona* were conducted in North America. In the early XX century, Willey (1920) recorded the presence of *Oithona similis* in south of the Alaska Peninsula, in Bering Sea, and in several localities along the Arctic coasts of Alaska and Canada. Farran (1910) described this species as worldwide, whereas Willey (1920) described it as Arctic with southern extension. Bigelow (1926) reported *O.similis* as abundant as calanoid copepods in some periods of the year in Gulf of Maine, and noticed how coarse mesh sizes could affect the catching, and therefore the lack of listing in some of the studied areas. Bigelow also highlighted the wide range of temperature and salinity at which *O.similis* could bear in comparison with other marine planktonic copepod.

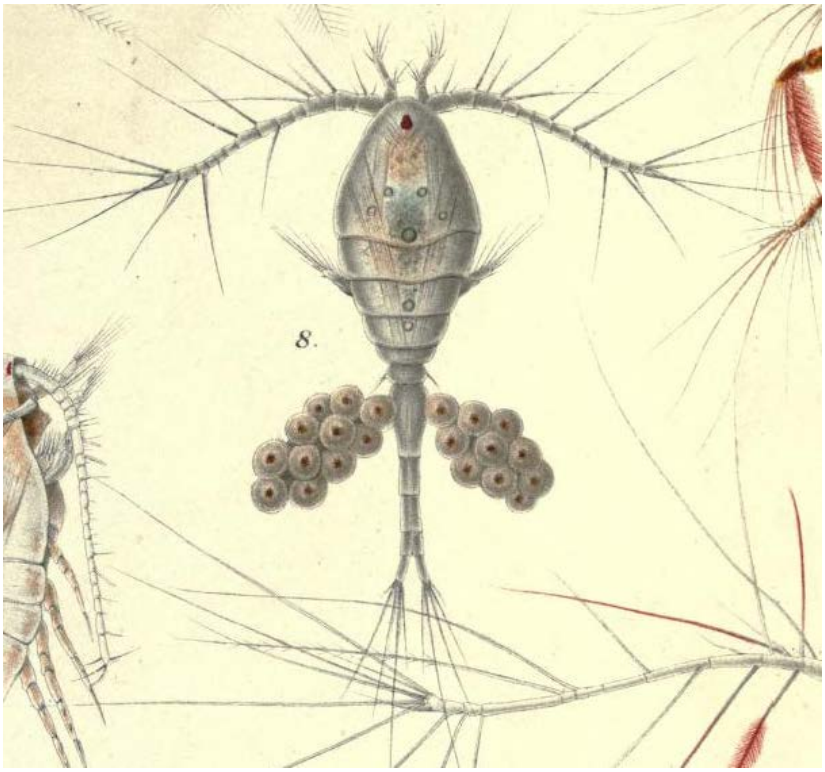


Fig. 22. *Oithona nana* (Pelag. Copepoda pp. 541, Giesbrecht 1892).

The recent interest on *Oithona*, in the base of its relevance in marine pelagic ecosystems (Gallienne and Robins 2001; Turner 2004), is therefore not something new. Bigelow (1926) already entitled *O. similis* "as one of the most abundant and ubiquitous copepods in the world". However, later on, only few studies focused on this group, although Evans (1977) noticed the independence of the abundance of *Oithona* from phytoplankton abundance, and pointed how the use of universal plankton nets (> 200 μm) resulted in wrong records of *Oithona* abundances throughout the literature. It has only been in the last decade that an increase in the number of studies focused on the genus *Oithona*, with the aim to have a better comprehension of its biology and ecological role.

"Is Oithona the most important copepod in the world's oceans?"

Oithona species are present in polar, temperate, subtropical, and tropical waters; in epipelagic and mesopelagic environments; in estuaries, mangroves, continental shelves and the open ocean (e.g. Bigelow 1926; Nielsen and Sabatini 1996; Atkinson 1998; Uye and Sano 1998; McKinnon and Klumpp 1998). They are present all year round, often showing a seasonal succession of species (Mazzochi and d'Alcalà 1995) as also occurs in calanoids (Jeffries 1962; Matthews 1969; Lee and Mcalice 1979). They are often the most abundant copepod, and in occasions capable to exceed the biomass of larger copepods in certain regions (Hay et al. 1991; Kiørboe and Nielsen 1994). This wide distribution and high abundance converts the genus *Oithona* of worldwide ecological importance in marine pelagic environments (Bigelow 1926; Nishida 1985; Gallienne and Robins 2001).

Oithona, a super ambush feeder

The number, morphology and distribution of appendages of *Oithona* define its ambush feeding behaviour (e.g. Paffenhöfer 1983; Kiørboe and Visser 1999). It has been described how non-moving and extended sensors (setae) are best suited for hydromechanical perception in those copepods with lack of feeding current and hardly move (Paffenhöfer 1998).

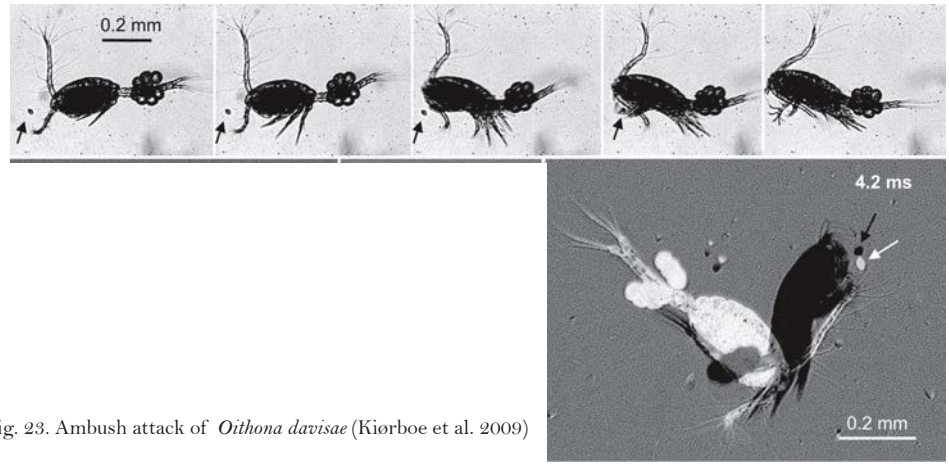


Fig. 23. Ambush attack of *Oithona davisae* (Kiørboe et al. 2009)

For cyclopoids like *Oithona* spp., which rarely move (Paffenhöfer et al. 1996), the initial motion perceived by juveniles or adults, originated from the potential food particle, often results in capture (Price 1988). Numerous setae (mechanosensors), some of them approaching the length of the prosome of *Oithona*, are located on the entire antennules (Fig. xx)

Oithona wait for the prey until they are in its threshold of (mechanical) detection, and in a rapid jumping attack approaches and captures them; the prey is captured by the 2nd maxillae and the maxillipeds that stretch out immediately after the forward lunge, completing the attack in few milliseconds (Kiørboe et al. 2009). This super fast performance is considered the key of the success of the feeding strategy of *Oithona* (Kiorboe et al. 2009).

To grow or to survive?

Oithona is small, has a quiescent swimming behaviour, and a fast escape performance, characteristics that might contribute to have low encounter rates and susceptibility to predators, and lower metabolic requirements than active "swimmers" copepods (Paffenhöfer 1993; Kiørboe 2013). These characteristics have been considered the explanation for the low mortality rates found for *Oithona* spp. in the few studies available in the literature (Eiane and Ohman 2004; Thor et al. 2008; Hirst and Ward 2008), where all developmental stages of *Oithona* spp. presented lower mortality rates than those of larger copepods.

It is believed that *Oithona* spp. present lower growth, feeding and egg production rates in comparison with larger calanoids (Paffenhöfer 1993). And this is considered the tradeoff of the life strategy of *Oithona* spp. to achieve high survival rates. However, how food resources, temperature, and body size affect the somatic growth and egg production of oithonids is still not well understood.

Few works have addressed the study of the development and growth of *Oithona* spp., either in the field (Uye and Sano 1998) or in the lab (Sabatini and Kiørboe 1994; Almeda et al. 2010). Some attempts have been conducted to investigate how somatic growth (Almeda et al. 2010) and egg production (Sabatini and Kiørboe 1994; Hopcroft and Roff 1998) are affected by body size and food availability. It is believed that growth and development of marine copepods are independent of body size (Hirst and Sheader 1997). Although this assumption may be true under optimal food conditions, the limiting effect of food and temperature in the growth of juveniles and adults of *Oithona* spp. is not clear. It has been reported that under optimal food conditions in the laboratory, the growth rate of juveniles (Almeda et al. 2010) and adults (Sabatini and Kiørboe 1994) are similar to equivalent-sized calanoids. However, the results of growth rates of *Oithona* spp. reported from field studies are contradictory.

In the same way, data on metabolic rates of *Oithona* spp. are very scarce in the literature in comparison with calanoids. A few studies have reported excretion (Hiromi and Ichisashi 1995; Atienza et al. 2006; Almeda et al. 2010) and respiration (Marshall and Orr 1966; Klekowski et al. 1977; Lampitt and Gamble 1982; Hiromi et al. 1988; Nakamura and Turner 1997; Castellani et al. 2005b; Almeda et al. 2010) rates of *Oithona* spp. Experiments conducted to determine respiration rates have confirmed the low metabolic requirements (Nakamura and Turner 1997; Castellani et al. 2005b) hypothesized by Paffenhöfer (1993).

In conclusion, low mortality rates are considered one of the clues of success of the ubiquitous distribution and abundance of *Oithona*, despite low rates of feeding, respiration and growth. However, other studies have attributed the success of *Oithona* spp. to their ability to feed on a wide range of prey (Lampitt 1978; Uchima 1988; González and Smetaceck 1994; Roff et al. 1995; Lonsdale et al. 2000).

Gourmet or rough feeder?

On the basis of raptorial mouthparts, laboratory feeding experiments, and gut content analyses, *Oithona* has been defined as carnivorous, herbivorous and omnivorous (Gauld1966; Turner 1986; Paffenhöfer 1993 and references therein). Nowadays it is widely accepted that *Oithona* is a carnivorous feeder, but this affirmation should be taken carefully since many aspects of the natural diet of *Oithona* are still unknown.

Oithona spp. has been reported to prefer motile to non-motile prey (Uchima and Hirano 1986; Svensen and Kiørboe 2000; Henriksen et al. 2007), selecting ciliates and dinoflagellates (Atkinson 1995; Castellani et al. 2005a; Atienza et al. 2006), and to be able to feed carnivorously (e.g. Marshall and Orr 1966; Lampitt 1978) and coprophagously (Gonzalez and Smetacek 1994). Nevertheless, phytoplankton, particularly diatoms (Hopkins et al. 1993, Atkinson 1996), and small flagellates (Calbet et al. 2001) have been occasionally reported to make up a large fraction of the diet of *Oithona* spp. Overall, many aspects of the diet of *Oithona* are not sufficiently known, what limits the understanding of their trophic role in marine pelagic webs.

Fecundity of Oithona

Several studies have examined reproductive characteristics of *Oithona* spp. in contrasted environments in the field, and in the lab (e.g. Eaton 1971; Sabatini and Kiørboe 1994; Uye and Sano 1995; Hopcroft and Roff 1998; McKinnon and Klumpp 1998; Nielsen et al. 2002; Castellani et al. 2005a; Ward and Hirst 2007; Dvoretsky and Dvoretsky 2009a). However, biological aspects of the fecundity of *Oithona* spp. are mostly unknown.

Field studies have evidenced the importance of variables such as temperature (Ward and Hirst 2007) and food availability (Castellani et al. 2005a) on the egg production of *Oithona*, but most of them did not investigate the efficiency of the egg production of *Oithona* spp. Most of the investigation on the seasonal variations of egg production throughout a period (Uye and Sano 1995; Dvoretsky and Dvoretsky 2009; Drif et al. 2010; Temperoni et al. 2011), are frequently focus on the most productive times of the year, creating a gap of information of how *Oithona* is reproducing in winter and unfavourable food conditions, information that is necessary to have a complete picture of the reproductive strategy of the genus.

The genus *Oithona* is considered to have relatively low weight-specific egg production rates in comparison to free-spawning copepods, and in general, within egg-carrying copepods, lower fecundity rates than similar-sized copepods (Sabatini and Kiørboe 1994; Bunker and Hirst 2004). However, the effect of body size, diet, food availability and temperature on fecundity rates of *Oithona* is not clear. Contradictory results of the investigations conducted on *Oithona* spp. have not clarified the weight of each factor on the fecundity of *Oithona* spp.

In general, free-spawning calanoids show very strong seasonal signals in both population abundance and reproduction rate, whereas *Oithona* spp. has been reported to maintain almost constant weight-specific egg production year-round (Sabatini and Kiørboe 1994; Temperoni et al. 2011), and relatively high population abundance when other copepod groups decline (Atkinson and Sinclair 2000; Lischka and Hagen 2005; Dvoretsky and Dvoretsky 2009).

Although different fecundity rates between cyclopoid and calanoid copepods have been attributed to different reproductive strategies, these differences in seasonality have been related to differences in the functional relationship between fecundity and food availability, rather than differences in reproductive strategies between calanoid and cyclopoids (Kiørboe and Sabatini 1994; Sabatini and Kiørboe 1994).

In conclusion, from the beginning of the XX century until now we have acquired quantitative knowledge on the relevant role of *Oithona* spp. in pelagic environments, in great part thanks to the application of appropriate methods of sampling. However, still many aspects of the biology and ecology of *Oithona* need to be investigated to explain which are the characteristics behind its life strategy to explain their success. Thus, our present fragmented understanding of the abundance, distribution, trophic ecology, population dynamics, growth, and role of *Oithona* spp. in biogenic fluxes impedes a proper understanding of the ecology of marine ecosystems.

Chapter 1

Effects of food concentration on the feeding and egg production rates of *Oithona davisae*

Based on the manuscript: Zamora-Terol, S. and Saiz, E. (2013) Effects of food concentration on egg production and feeding rates of the cyclopoid copepod *Oithona davisae*. *Limnology and Oceanography*, **58,** 376-387.

Abstract

Experiments to determine egg production and feeding rates of *Oithona davisae* were carried out under controlled laboratory conditions. From copepodite IV stage on, the animals were fed the heterotrophic dinoflagellate *Oxyrrhis marina* in a wide range of concentrations (from 10 μg C L^{-1} to 286 μg C L^{-1}), and adult females were daily monitored to study different aspects of their fecundity. Both clutch and egg-production rate increased with food concentration, with values from 8 to 20 eggs for the clutch size, and from 1.8 eggs to 6.3 eggs $female^{-1}$ d^{-1} for the egg production. In addition, to assess the efficiency of conversion of food intake into egg mass, two feeding experiments were conducted. Maximum weight-specific ingestion rates (< 80% body C d21) and the egg-production efficiency (16%) were lower than those reported for free-spawning calanoid copepods. The fact that satiating food concentrations for feeding and egg production of adult females of *Oithona davisae* were rather low suggests an adaptation to exploit oligotrophic environments, and might explain the ecological success of the genus in situations when food becomes limiting for other groups of copepods.

INTRODUCTION

It is widely accepted that small copepods (< 1 mm) are important, but poorly studied components of marine pelagic communities (Turner 2004). They were often neglected from samplings in past years until several authors (Paffenhöfer 1993; Gallienne and Robins 2001) warned about the bias due to use of coarse mesh sizes (> 200 μm) in plankton nets. This fact was actually not something new, because some studies from the early 20th century already pointed out the relevance of small copepods (Bigelow 1926; Fish 1936) and the need to use fine-mesh nets for correct estimates of zooplankton abundance (Evans 1973).

The marine cyclopoid copepods, and in particular the genus *Oithona*, have raised special interest in recent years due to its high abundance and ubiquitous presence (Uye and Sano 1998; Gallienne and Robins 2001) both in coastal and oceanic regions, and due to a range of distribution extending from polar to tropical latitudes (Nielsen and Sabatini 1996; McKinnon and Klumpp 1998). Besides their numerical dominance, *Oithona* also make up a significant fraction of the copepod biomass in some temperate areas (McLaren et al. 1989; Hay et al. 1991) and might be especially relevant at high latitudes in autumn and winter conditions when other copepods are not active in the upper layers (Kiørboe and Nielsen 1994; Nielsen and Sabatini 1996). Their described low metabolic rates (Lampitt and Gamble 1982; Nakamura and Turner 1997; Castellani et al. 2005b), coupled with an ambush feeding behavior and low motility, are considered the clue of their success (Paffenhöfer 1993) and of their capacity to maintain constant populations throughout the year (Lampitt and Gamble 1982; Sabatini and Kiørboe 1994).

Although new insights on the ecology of *Oithona* have been acquired in the past decade (Nielsen et al. 2002; Temperoni et al. 2011), knowledge on their vital rates is still very scarce. Some field studies report the feeding activity (Nakamura and Turner 1997; Castellani et al. 2005a; Atienza et al. 2006) and egg production (Ward and Hirst 2007; Dvoretsky and Dvoretsky 2009; Drif et al. 2010) of several *Oithona* species in different ecosystems. Moreover, very few laboratory studies have dealt with aspects related to their reproductive biology (Eaton 1971; Sabatini and Kiørboe 1994) and feeding responses (Drits and Semenova

1984; Saiz et al. 2003). This lack of studies on the ecophysiology of *Oithona* contrasts with the large amount of studies conducted on calanoid copepods on aspects related to feeding, growth, and egg production. In fact, to our knowledge, only Sabatini and Kiørboe (1994) have conducted laboratory experiments regarding the reproductive biology and growth of the congeneric species *Oithona similis*.

Here we present laboratory experiments done under controlled conditions to study the egg production and feeding rates of adult females of *Oithona davisae*. This species was originally distributed in coastal waters of the West Pacific Ocean, but presently it can also be found in coastal waters of the United States and Chile, the Black Sea, and in the Northwest Mediterranean as an invasive species (Razouls et al. 2005–2012). Our main goals were to study how food concentration affects the feeding and fecundity of O. davisae; to determine how clutch size and the fraction of ovigerous females vary with food availability; and finally, to assess their egg-production efficiency (gross growth efficiency, GGE). Such information on fecundity, growth rates, critical feeding thresholds, and maximum daily rations provides required information to better understand the life strategies within the genus and the species' capability to cope with environmental variability.

METHODS

General procedure

Experiments were conducted on adult females of *Oithona davisae* coming from a continuous culture kept in our laboratory at the Institut de Ciències del Mar (CSIC, Barcelona, Spain) since October 2000 (Saiz et al. 2003). Individuals of *O.davisae* were kept in 15 L transparent plexiglass cylinders (24 cm diameter) filled with 0.1-µm filtered seawater and grown under a 12 h light: 12 h dark cycle at 18ºC in a temperature-controlled room. The copepods were routinely fed the heterotrophic dinoflagellate *Oxyrrhis marina* (equivalent spherical diameter, ESD = 16 µm), which were at the same time fed the cryptophyte *Rhodomonas salina* (ESD = 8 µm) grown in f/2 medium (Guillard 1975).

In order to examine the effect of food concentration on the egg production, a cohort of *Oithona davisae* was created and fed with different concentrations of *Oxyrrhis marina*. Figure 1 shows a schematic outline of the followed procedures. To begin the cohort, the stock culture of *O.davisae* was fed ad líbitum (i.e., > 1200 cells mL^{-1}) with *O.marina* during 24-48 hours to maximize the egg production of the adult females. Females were carefully concentrated on a submerged 132-µm sieve, and then transferred into a new cylinder filled up with filtered (0.1 µm) seawater for 24 hours to allow the hatching of the eggs. After that period, the females were removed from the cylinder using a 100-µm submerged in seawater, and the recently hatched nauplii (NI) were fed ad líbitum until they reached the copepodite IV-V stage (CIV-CV). Then the culture was split into 6 aliquots and transferred into new cylinders filled with filtered seawater, in which copepods were kept under starvation for approximately 48 h in an attempt to homogenize the past well-fed conditions experienced in the stock cultures. After that time, food concentration in each cylinder was adjusted to the desired concentrations of *O. marina* (nominally 50, 100, 200, 400, 800, or 1200 cells mL^{-1}; Table 1).

Table 1. *Oxyrrhis marina*. Nominal initial food levels (in cells) and the corresponding actual average concentrations (in cells and biomass) of prey in the two feeding experiments conducted.

	Nominal concentration	Actual average concentration	
	(cells mL^{-1})	(cells mL^{-1})	(μg C L^{-1})
Expt 1	50	32 ± 1.0	10 ± 0.3
	100	63 ± 1.0	20 ± 0.4
	200	157 ± 5.0	51 ± 1.8
	400	318 ± 4.0	104 ± 1.5
	800	672 ± 4.0	219 ± 1.3
	1200	880 ± 6.0	287 ± 1.9
Expt 2	50	33 ± 1.0	10 ± 0.3
	100	72 ± 1.0	21 ± 0.3
	200	150 ± 5.0	44 ± 1.4
	400	288 ± 9.0	85 ± 2.6
	800	614 ± 11.0	181 ± 3.3
	1200	969 ± 22.0	286 ± 6.5

Food concentrations were daily checked using a Multisizer III particle counter (Beckman Coulter) and adjusted to keep them constant during the experimental time. The suspension in 2/3 of the cylinder was renewed every second day to ensure a fresh supply of food. The females were kept in acclimatization to the respective food levels and daily checked until the presence of the first egg sacs was observed (approximately after 72 h). Since that moment, egg production was monitored (Fig. 1, *see* below). All the incubations and experiments were conducted in a controlled-temperature room at 18 ± 1ºC.

Monitoring of egg production in the cohort

During the experimental period we determined the percentage of ovigerous females, the clutch size, and the size of both females and eggs in the 6 cylinders with the different concentrations of *Oxyrrhis marina*. A sample (approximately 250 mL) was daily taken from each cylinder (except day 6) and females were inspected under the stereomicroscope, and dead animals removed (< 1%, mostly males). Afterwards, the samples were preserved in 1% buffered-formaldehyde for later assessment of the percentage of ovigerous females, and also to estimate clutch sizes by dissecting the egg sacs. The water volume in the cylinders was kept constant by adding either the corresponding fresh suspension of *O.marina* or filtered (0.1 µm) seawater.

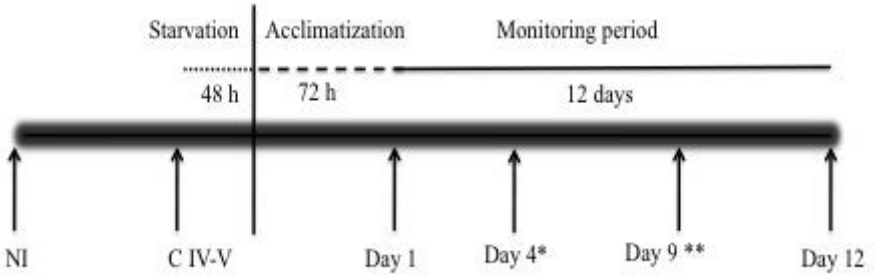


Fig. 1. Schematic representation of the experimental design. One asterisk (expt 1) and two asterisks (expt 2) indicate when the feeding experiments were carried out.

Feeding and egg production experiments

Two feeding and egg production experiments were conducted during the experimental period at different reproductive phases. The first experiment was carried out on day 4, when females carrying eggs were not yet present at the lowest food condition (50 cells mL^{-1}); the second experiment was carried out on day 9, when in all food levels ovigerous females were most abundant.

Feeding rates were obtained by incubating adult females of *Oithona davisae* in bottles (treatment bottles) with the different suspensions of *Oxyrrhis marina* (Table 1) and measuring the change in prey concentration relative to the bottles with the same food suspensions but with no copepods (control bottles) after the incubation time. For each food level, 3 treatment, 3 control, and 2 start bottles (time 0) were prepared. The culture of *O. marina* used to prepare the prey suspensions was not fed for 48 hours before the experiment started to ensure that only *O. marina* was offered as prey. Prey suspensions were prepared by filtering the stock culture using a 10-µm mesh (*O. marina* squeezes through the 10-µm mesh) to remove detrital material, and then by diluting the prey stock with filtered (0.1 µm) seawater to achieve the corresponding food concentrations (Table 1). A nutrient mixture (15 µmol L^{-1} NH_4Cl and 1 µmol L^{-1} Na_2HPO_4) was added to each food suspension to compensate for nutrient enrichment due to copepods excretion.

Adult females were removed from the cylinders and carefully concentrated on a submerged 100-µm mesh sieve, rinsed in filtered seawater, and then picked out and transferred to the corresponding food level treatment bottles. The food levels 50, 100, and 200 cells mL^{-1} were conducted in 310 mL Pyrex bottles, with respectively 24, 33, and 40 adults females each; and the higher food levels 400, 800, and 1200 cells mL^{-1} were conducted in 130 mL Pyrex bottles, with respectively 27, 31, and 34 adult females each. The bottles were completely filled, and plastic film was put over the mouth of the bottle to prevent the presence of air bubbles during the incubation.

Food concentrations at time 0 of the experiment were checked by preserving 50 mL of each start bottle in 2% acid Lugol's solution, which were later counted under an inverted microscope. Bottles were placed on a plankton wheel (0.2 revolutions per minute, end over end) at a fixed temperature (18 ± 1ºC) in controlled light conditions (12 h light : 12 h dark) during the incubation. After the incubation time (approximately 24 h) the females were checked under the stereomicroscope to ensure they were alive and they swam normally (97-100% of females were viable). Copepods were then transferred to small vials and fixed in 1% buffered-formaldehyde for later sizing and counting of both females and egg sacs. From each control and treatment bottle a subsample of 75 mL was preserved in acid Lugol's solution (2% final concentration) and subsequently settled and counted under an inverted microscope for determination of *Oxyrrhis marina* concentration at the end of the experiments.

Clearance and ingestion rates were determined for each food level according to Frost's equations (Frost 1972), after verification that prey growth rates in treatment bottles were statistically different from those in the control ones (*t*-test at the 0.05 significance level). Clearance rates were fitted to an exponential decay function as follows:

$$F = F_{max} e^{-\lambda C} \qquad (1)$$

where F_{max} is the maximum clearance, C is the concentration of food and l is the decay rate.

Ingestion rates were fitted to a type II functional response model using the Ivlev (1961) equation as follows:

$$I = I_{max}(1-e^{-\alpha C}) \qquad (2)$$

where I_{max} is the maximum ingestion rate, C is the food concentration and a is the rate at which ingestion approaches the maximum rate. The critical or saturating food concentration (K_s), i.e., the threshold concentration below which feeding is food limited, was calculated as the food concentration at which ingestion equals 95% of the maximum ingestion rate (Almeda et al. 2010).

For the females corresponding to the feeding experiments, the percentage of ovigerous females was determined, and their mean clutch size was quantified by dissecting all egg sacs present. The average population egg production rates (EPR, eggs female^{-1} day^{-1}), computed for the ensemble of ovigerous (i.e., carrying egg sacs) and non-ovigerous (i.e., not carrying egg sacs) females, was calculated using the egg-ratio method according to the following equation, modified from Uye and Sano (1995),

$$EPR = CS \times OF / TF \times IT \quad (3)$$

where CS is clutch size (eggs female^{-1}), OF is the number of ovigerous females present in the sample, TF is the total number of females, and IT is the interclutch time (days). We have used the interclutch time determined by Uye and Sano (1995) instead of the hatching time (as can be found elsewhere in the literature, Sabatini and Kiørboe 1994; Castellani et al. 2005*b*) because the interclutch time for *Oithona davisae* is longer than the embryonic time at the corresponding experimental temperature (respectively, 2.5 and 1.8 days, Uye and Sano 1995).

Weight-specific rates were estimated from size measurements and carbon content-size relationships from the literature. In the case of *Oxyrrhis marina* biovolume estimates from the Multisizer Counter were converted into carbon using the conversion factor 0.123 pg C µm^{-3} for this dinoflagellate provided by Pelegrí et al. (1999). The carbon weight of both eggs (C_{egg}, µg C) and females (C_{female}, µg C) of *Oithona davisae* were estimated by taking digital pictures with a camera attached to an inverted microscope, using a 40x magnification for the females and 100x for the eggs. For each experimental condition at least 200 eggs and 20 females were measured from the digital pictures by using the image processing program ImageJ (W.S. Rasband, ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, http://imagej.nih.gov/ij/, 1997-2011).

The egg diameter (ED, μm) was estimated as equivalent circular diameter from the area measurements in ImageJ, and then the egg carbon content was calculated using the equation given by Uye and Sano (1995) for *Oithona davisae*:

$$C_{egg} = 5.32 \times 10^{-8} \times ED^{3.04} \quad (4)$$

where C_{egg} is the egg carbon content (μg C) and ED is the egg diameter (μm).

For adult females, since we expected differences in individual biomass among the populations grown under different food concentrations, reflected as differences in length but also in width, we first estimated their individual biovolume (μm^3) at each food level by using the minor and major axis of the cephalothorax to build an ellipsoid. Then using only the major axis, homologous to the prosome length (PL, μm), we estimated carbon content of the females (C_{female}, μg C) for all conditions using the equation given by Uye (1982) for this copepod species:

$$C_{female} = 1.26 \times 10^{-4} \times PL^{1.31} \quad (5)$$

Finally, we computed an average carbon content : biovolume factor, $6.74 \cdot 10^{-8}$ μg C μm^{-3}, which was used in all calculations to estimate the female biomass from the biovolume measurements.

The efficiency of conversion of ingested food into egg biomass (gross growth efficiency, GGE) of adult females of *Oithona davisae* was calculated as the slope (in %) of a major axis (MA) fitting (i.e., Model II linear regression) between weight-specific ingestion (WSIR) and egg production (SEPR) rates. We chose that model instead of ordinary least-squares regression fit (i.e., Model I) because both variables contain error, i.e., they were not controlled by the researcher. Nevertheless, as GGE is typically calculated as the slope of Model I linear fit (Castellani et al. 2005*b*; Almeda et al. 2010), we also applied this fitting method to obtain values comparable to other studies.

RESULTS

Reproductive activity of the females

The time for the first appearance of ovigerous females in the population varied inversely with food concentration (Fig. 2). Females carrying egg sacs contributed approximately 40% of the population on day 2 in the highest food concentrations (800 and 1200 cells mL^{-1}), whereas

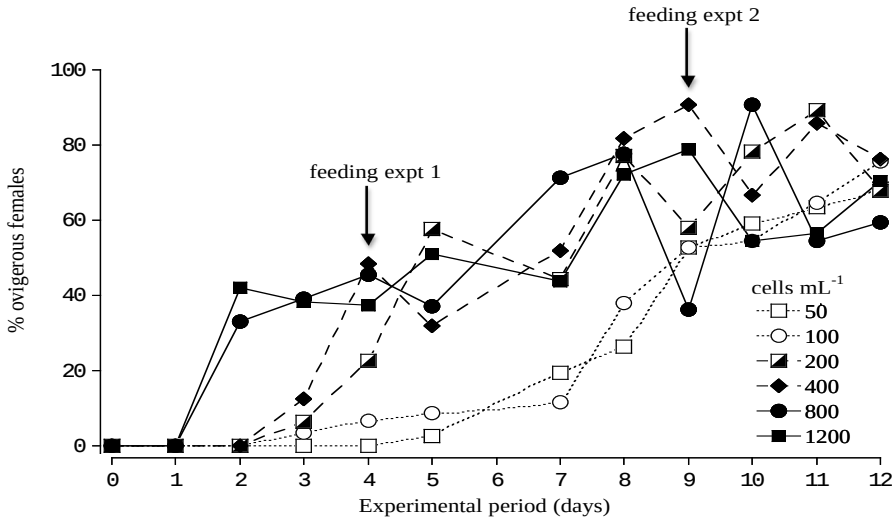


Fig. 2. Temporal development of ovigerous females (%) present in the population under different food levels (nominal concentrations, cells mL^{-1}). Days when the feeding experiments were carried out are indicated.

on the medium (200 and 400 cells mL^{-1}) and low (50 and 100 cells mL^{-1}) food concentrations that percentage was only achieved after respectively 5 and 8 days of the experimental period (Fig. 2). Close to the end of the experimental period the proportion of ovigerous females in the population achieved similar values in all food treatments (≈ 75%, Fig. 2). The monitoring during the experimental period was discontinued after day 12 since the females population in the cylinders started to decline (data not shown), both because of the daily removal of individuals from the cylinders and the earlier mortality in the populations kept at the highest food levels.

Feeding and egg production rates

Both feeding experiments provided similar rates (Fig. 3). Clearance rates found in this study declined as a function of food concentration (Fig. 3A), and the values (average ± SE) ranged from 7.3 ± 0.39 to 0.63 ± 0.06 mL swept clear female^{-1} day^{-1} at the lowest and highest food concentration, respectively. Ingestion rates increased asymptotically when increasing food concentration, showing saturation food concentrations (K_s) of 249 cells mL^{-1}, and maximum ingestion rates of 737 cells female^{-1} day^{-1} (Fig. 3B).

In terms of weight-specific rates, daily ration (DR) varied from 30% of body carbon ingested per day at the lowest food concentration tested, to maximum values of 80% body C day^{-1} (Fig. 3C). The saturation food concentration of *Oxyrrhis marina* in terms of carbon was 56 µg C L^{-1}.

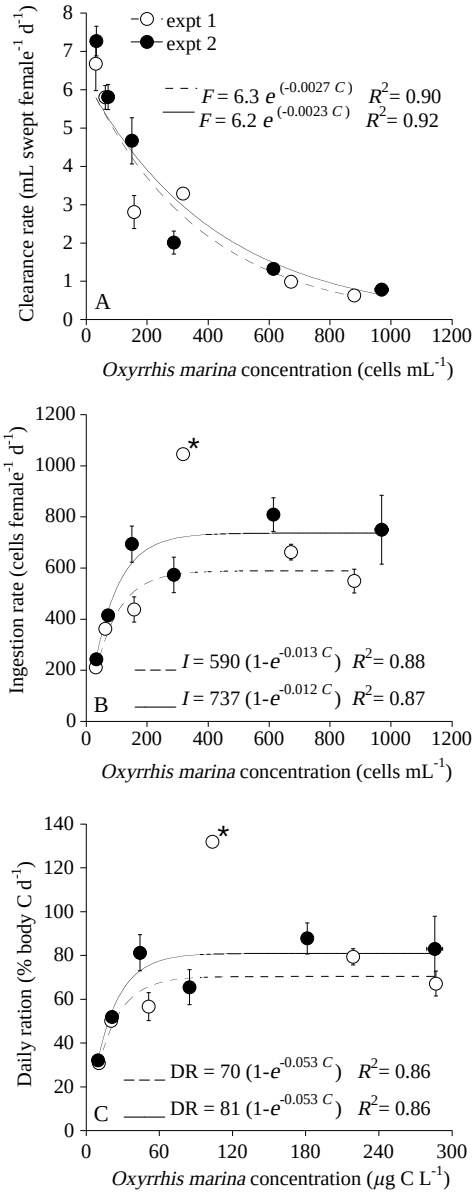


Fig. 3. *Oithona davisae* feeding rates as a function of food concentration (*C*). (A) Clearance rate (*F*, mL female^{-1} d^{-1}). (B) Ingestion rates (*I*, cells female^{-1} d^{-1}). (C) Weight-specific ingestion rate, as percentage of body carbon ingested per day (daily ration, DR, %). Mean values (n = 3) and standard errors (SE) are shown. Exponential decay and Ivlev's equations fits are also shown. Symbols marked with an asterisk correspond to extreme values not used in the equation fitting.

The effect of food concentration on the fecundity of *Oithona davisae* is shown in Fig. 4. Differently to what happened with ingestion rates, it is observed that although maximum values are similar between both experiments, the rate at which both clutch size and egg production reached those maximum values (the *a* parameter in the Ivlev's fits) are notably different.

Females from expt 2 reached maximum egg production rates at lower food levels than those from expt 1, and we interpreted that fact as if the young females from expt 1 did not have enough time to mature their gonads. For that reason we will only refer to egg production values from expt 2.

Both clutch size (eggs female^{-1}, both sacs together; Fig. 4A) and egg production rate (eggs female^{-1} day^{-1}; Fig. 4B) increased asymptotically with increasing food concentration. Clutch size varied in average from 8 eggs to maximum values of 20 eggs (Fig. 4A), and although the females produced eggs at the lowest food concentration (10-20 µg C L^{-1}) they never reached the clutch size of the highest food levels. The egg production rates (eggs female^{-1} day^{-1}) found in this study varied from 1.8 ± 0.07 to 6.3 ± 0.14 (Fig. 4B). It is important to notice that clutch size was only computed for ovigerous females, whereas the egg production rate is computed on a population basis, i.e., also includes non-ovigerous females. The critical concentration of food at which saturating values (K_s) for egg production (EPR) were found was 81 μg C L^{-1}, with egg production rates of approximately 5-6 eggs female day^{-1} above this threshold.

The weight-specific egg production rates (SEPR, µg C_{egg} µg C_{ind}^{-1} day^{-1}) are shown in Fig. 4C. Values (avg. ± SE) ranged from 0.04 ± 0.002 to 0.12 ± 0.003 µg C_{egg} µg C_{ind}^{-1} day^{-1}. Maximum weight-specific egg production rates corresponded to those females carrying the largest clutch sizes (20 eggs on average).

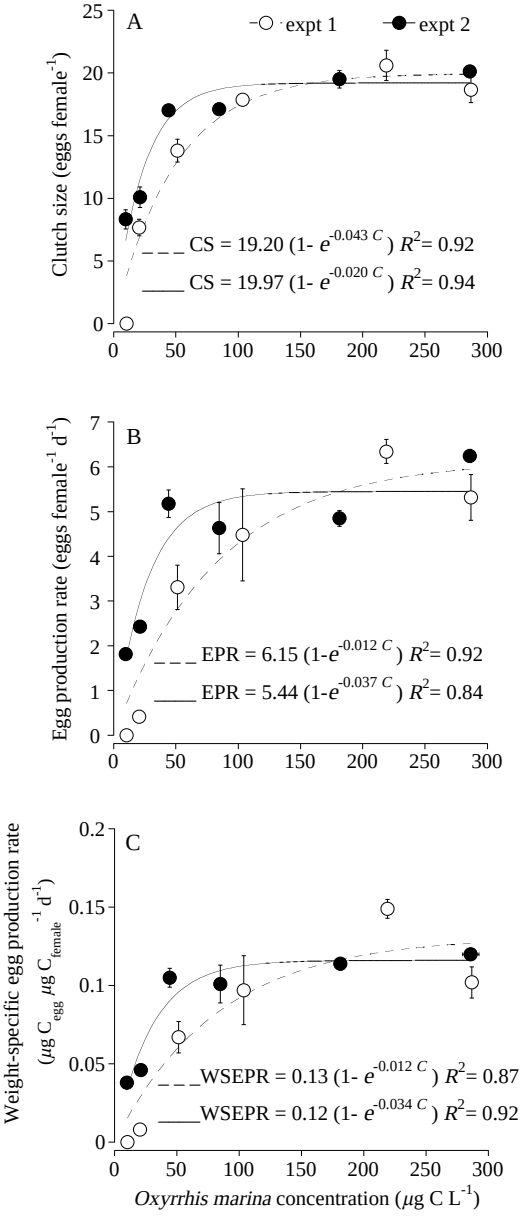


Fig. 4. Relationship between fecundity of Oithona davisae and food concentration (C) in terms of: (A) mean clutch size (CS); (B) egg production rate (EPR); (C) weight-specific egg production rate (SEPR). Mean values and SE (n = 3) are shown. Ivlev's fits are displayed in the graphs.

For consistency to what we mentioned above about a likely delay in the maturation of gonads in young females from expt 1, fact that would lead to an overestimation of the egg production efficiency (gross growth efficiency, GGE) (Fig. 5), we only used expt 2 for the assessment of GGE. After running the two different regression models for the estimation of the GGE, we found that both major axis (MA) and ordinary least-squares regression (OLS) fittings gave the same results (MA slope: 0.16, 95% confidence interval: 0.081-0.232; and OLS slope: 0.16, 95% confidence interval: 0.080-0.230). Therefore, we will use that value of the slope (in percentage) obtained for further discussion, i.e., 16% (Fig. 5).

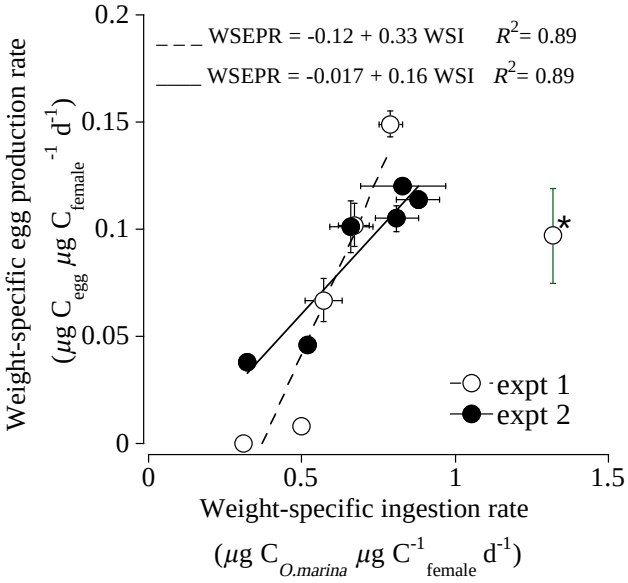


Fig. 5. Relationship between weight-specific egg production (SEPR) and weight-specific ingestion (WSIR). Mean values and SE (n = 3) are shown. Model II regression (major axis) fits to the data are also shown. Symbol marked with an asterisk corresponds to the outlier not taken into account for the fit.

Egg and female size

Figure 6 shows the relationship between female size and female body weight with food level. Females grown at higher food concentrations were larger in prosome length and width (Fig. 6A), which resulted in an asymptotic increase in body weight from approximately 0.22 to 0.26 µg C female^{-1} as food availability increased (Fig. 6B).

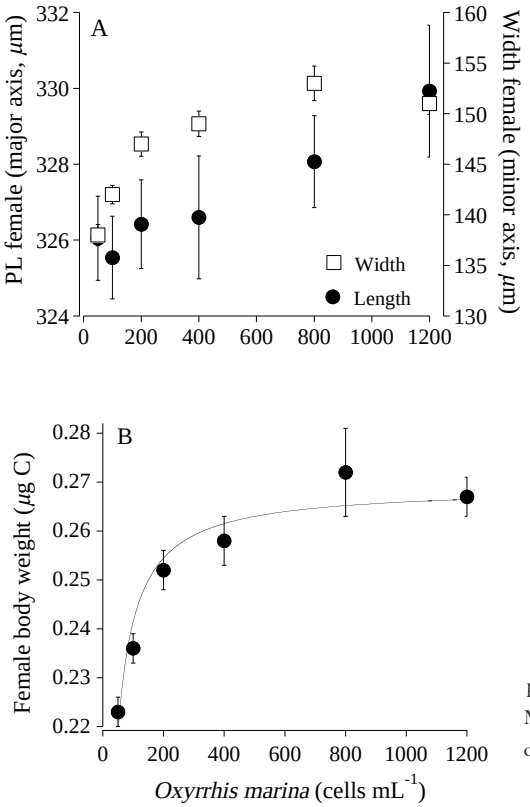


Figure 6. Relationship between (A) food concentration (*C*) and female prosome length (PL) and width. (B) Michaelis-Menten relationship (R^2 = 0.94) between food concentration and female body weight (BW). Mean values and SE are shown.

On the other hand, the mean diameter of the eggs (ECD ± SE) varied from 41.8 ± 0.18 to 46.9 ± 0.24 µm, and was positively related to clutch size (R^2 = 0.67, Fig. 7A). There was also a positive relationship between clutch size and female body weight (Fig. 7B), but this relationship is spurious because it was mediated by the fact that the heaviest females corresponded to the populations grown under the highest food concentrations (Fig. 6B).

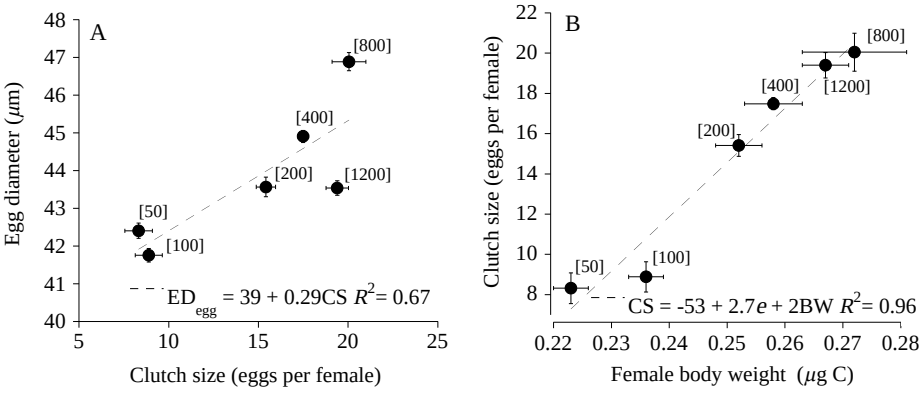


Fig. 7. Relationship between mean clutch size and (A) egg diameters (ED, μm), and (B) female body weight (BW, μg C). Mean values and SE are shown. The corresponding nominal concentrations (cells mL^{-1}) for each data point are also indicated.

DISCUSSION

Feeding rates

Clearance rates found for *Oithona davisae* decreased exponentially with food (Fig. 3A), with maximum rates (≈ 7 mL female^{-1} day^{-1}) similar to the ones found in other studies for the same species (6 mL female^{-1} day^{-1}, Saiz et al. 2003). In situ maximum clearance rates for other *Oithona* species have been reported to be higher than those found in the present study (36 mL female^{-1} day^{-1}, Atienza et al. 2006; 23 mL female^{-1} day^{-1}, Castellani et al. 2005*b*). But in most of the cases the experiments were run with larger species, different temperatures, and natural seawater was used instead of a monospecific diet, making the comparison difficult. However, when only dinoflagellates were considered on in situ clearance rates of *Oithona*, those were lower or on the same range of our values (1.7-3.1 mL female^{-1} day^{-1}, Nakamura and Turner 1997; 0.6-10.1 mL female^{-1} day^{-1}, Castellani et al. 2005*b*; 6-7 mL female^{-1} day^{-1}, Atienza et al. 2006).

The ingestion rates of *Oithona davisae* followed a Holling type II functional response (Holling 1959), with almost identical curves in both feeding experiments (Fig. 3B,C). The maximum feeding rates (ingestion and clearance) found in this study were similar to those

reported for adult females and higher than those reported for males and early stages (Saiz et al. 2003; Kiørboe 2008; Almeda et al. 2010; *see* table 2) of the same species. However, the functional pattern found here (type II) for adult females is different from the one reported by Almeda et al. (2010) for early stages of *O.davisae*, which exhibited a type III functional response with feeding threshold from 153 to 235 cells mL^{-1} (≈ 50 to 75 µg C L^{-1}).

Table 2. Comparison of literature data on maximum ingestion rates (I_{max}) and maximum clearance rates (F_{max}) among different stages of *Oithona davisae* feeding on the same type of prey (*Oxyrrhis marina*) in the laboratory.

Stages	I_{max} ± SE (cells $female^{-1}day^{-1}$)	F_{max} ± SE (mL $female^{-1}day^{-1}$)	T (ºC)	Reference
NI-NII	118 ± 6	0.38 ± 0.02	20.5	Almeda et al. 2010
NV-NVI	296 ± 19	0.57 ± 0.04	20.5	Almeda et al. 2010
C2-C3	517 ± 25	1.11 ± 0.05	20.5	Almeda et al. 2010
Males	640	2.6 ± 0.3	22	Kiørboe 2008
Adult females	-	6	21	Saiz et al. 2003
Adult females	737	7.3	18	this study

The lack of feeding threshold in adults of *O.davisae* could be explained as the result of differences in prey detection capabilities between adult and juveniles. Both juveniles and adults of *Oithona* are strict ambush predators that detect motile prey from hydromechanical signals (Paffenhöfer 1993; Svensen and Kiørboe 2000). It could be that different number and length of mechanoreceptors between developmental stages of copepods could explain differences in their feeding performances (Paffenhöfer 1998). In this regard, it has been described how copepod size is positively related to the sensitivity to fluid deformation in the detection of potential predators (Kiørboe et al. 1999: *Acartia tonsa*). Although those observations were focused on the stimuli that a potential predator may originate, we think it could also be applied to hydromechanical perception of prey. That would imply that later stages might have a better perceptive performance to detect prey than earlier stages.

It is also important to notice that the satiating food concentrations (K_s) reported in this study for adult females of *Oithona davisae* (249 cells $mL^{-1} \approx 56$ µg C L^{-1}) are much lower than those reported for the early stages of the same species by Almeda et al. (2010) (from 700 cells $mL^{-1} \approx 200$ µg C L^{-1} for NI-NII to 1130 cells $mL^{-1} \approx 320$ µg C L^{-1} for CII-III stages). This fact is unexpected, because it is typically considered that adult stages have higher satiating food concentrations than earlier stages. However, low values of satiating food concentrations for adult females of *Oithona davisae* have been previously found in the same species (100 µg C L^{-1}, Saiz et al. 2003) and on *Oithona nana* (50-100 µg C L^{-1}, Lampitt and Gamble 1982). The lack of type III Holling functional response (i.e., the lack of a feeding threshold concentration) of *Oithona davisae* adult females, and the comparatively low critical food concentration seem to reflect a particular strategy in the adult stage to perform well at low food availability. In fact, it has been observed how *Oithona similis* can fed at very low concentrations (< 1 μg C L^{-1}, S. Zamora-Terol et al. 2013), even below our lowest food level, therefore indicating the possibility of a lack of feeding threshold for adults of *Oithona* spp.

Maximum specific ingestion rates found in this study (ca. 80% body carbon day^{-1}) for adult females of *Oithona davisae* are very likely within the maximum possible rates for similar-sized *Oithona* species (80-125% day^{-1}, Saiz et al. 2003); and certainly much higher than most previously reported maximum rates in the field for other *Oithona* species: e.g., 17% (Castellani et al. 2005*b*), 27% (Nakamura and Turner 1997) and 35% (Drits and Semenova 1984) found for *O.similis*, or 34% for *Oithona* spp. (Atkinson 1996). Despite of that, the maximum daily rations reported here are still lower than those typically reported for similar sized free-spawning copepods (which can be up to two times higher, Paffenhöfer 1988) and egg-carrying calanoids (148% body weight, Paffenhöfer and Harris 1976: *Pseudocalanus elongatus*). Such difference on daily rations (DR, % body carbon ingested per day) between *Oithona* and calanoid copepods has been attributed to the ambush feeding behavior, low motility and low respiration rates of *Oithona* (1.4 – 31.4% in a range of 4-30ºC, Lampitt and Gamble 1982; Hiromi et al. 1988; Castellani et al. 2005*a*).

Egg production

Oithona davisae egg production rates and clutch sizes found in this study are in good agreement with the in situ values reported for the same species by Uye and Sano (1995) in Fukuyama Harbour (i.e., 0.6 and 5.6 eggs female^{-1} day^{-1} in winter and in summer, respectively). Overall the values reported here for *O.davisae* fall within the range of values reported in situ for other *Oithona* species, and in particular the maximum specific-egg production rates we observed are very similar to the ones reported in the literature (Table 3). It is worth noting that in our calculations of egg production rates we followed the approach by Uye and Sano (1995) using the interclutch time instead of the embryonic time, more widely used in the literature. We assumed that the interclutch time was only dependent on the temperature, although we cannot discard that very likely food availability may also affect it.

In our experiments, food concentration had an effect on egg production of *Oithona davisae* by influencing the clutch size and also the percentage of ovigerous females in the population (Fig. 2). Even at the lowest food concentrations we observed the production of eggs, but with smaller clutch sizes (Fig. 4A). Larger clutch sizes corresponded to larger eggs, contrarily to what is observed in other *Oithona* species (Castellani et al. 2007; Dvoretsky and Dvoretsky 2009). We also found a positive relationship between clutch size and female body weight, as has been found in other cyclopoid and calanoid copepods (Runge 1984; Castellani et al. 2007). In our particular case, however, food concentration determined the final size and carbon content of the females, therefore confusing the effects of any direct relationship between female body weight and clutch size. In the case of in situ studies the largest clutches for certain *Oithona* species typically occur in the most favorable conditions within the year i.e., when high availability of food is present in the water column (Uye and Sano 1995; Castellani et al. 2005*b*; but *see* also Temperoni et al. 2011 for the lack of seasonal variation). In our study, however, the largest clutch sizes corresponded to the largest females (Fig. 7B) not necessarily by direct relationship but simply because those females were grown at the highest food levels, making difficult to ascertain causal relationships.

Regarding the reproductive status of the population, the number of reproductive females in the population was affected by food availability and increased through time during the experimental period, reaching maximum values of 80% of females in ovigerous state (i.e., carrying egg sacs; Fig. 2). In agreement with our results, Sabatini and Kiørboe (1994) found similar percentages of ovigerous females (80%) on *Oithona similis* at the highest food levels tested (300 µg C L^{-1}). The females kept at low food concentrations started to evidence the ovigerous state 5-8 days later than those at higher food levels, lagging behind. This trend suggests a limitation in the gonad development (Niehoff 2004).

We found that even at the lowest food concentrations (10 µg C L^{-1}) *Oithona davisae* showed relatively high weight-specific ingestion and egg production rates (approximately 30% and 4%, respectively). The capability of *O.davisae* to reach maximum egg production rates at low food concentrations may explain the year-round egg production observed for this species and likely for other congeneric species. A similar strategy was described by Jonasdottir (1989) for the egg-carrying calanoid *Pseudocalanus newmani*, whose capability to achieve maximum egg production rates at low food concentrations could explain the observed year-round production of eggs of *P.newmani* in Puget Sound. It is worth noticing, however, that our results contrasts with the observations for *Oithona similis* in the laboratory study carried out by Sabatini and Kiørboe (1994), in which no egg production was observed at flagellates concentrations below 20 µg C L^{-1}. Although differences in life strategies or body size between both *Oithona* species could explain that discrepancy in food threshold, an alternative explanation could be the use of a non-optimal diet, since the maximum clutch sizes reported for *O.similis* by those authors (13.7 ± 0.4 eggs $clutch^{-1}$) are lower than the values found in field studies for the same species (up to 20-30 eggs $clutch^{-1}$, Nielsen and Sabatini 1996; Castellani et al. 2007; Dvoretsky and Dvoretsky 2009) (*see* Table 3).

The maximum weight-specific egg production rate found in this study (0.12 day^{-1} at 18ºC) for adult females of *Oithona davisae* was lower than the maximum specific growth rate reported for nauplii (0.33 day^{-1} 20.5ºC) but similar to copepodites (0.12 day^{-1} 20.5ºC) of the same species (Almeda et al. 2010). This is in agreement with the observations of Uye and Sano (1998) following the demography of the natural population of *O.davisae* in Fukuyama Harbour, in which the maximum weight-specific rates found for the adult females (0.14 day^{-1}) were lower than those for nauplii (0.35 day^{-1}), although they also reported high values for copepodites (0.44 day^{-1}). Sabatini and Kiørboe (1994) also found a similar pattern for *Oithona similis* (0.1 and 0.2 day^{-1} for respectively adult females and juveniles) in the laboratory. In the case of *Oithona davisae*, the discrepancy between growth rates found for adults (in the present study) and nauplii (Almeda et al. 2010), under presumably optimal and not limiting food conditions, could be explained by the negative relationship between specific-growth rates and body weight under satiating food conditions described by Almeda et al. (2010). In any case, such differences between adult and juvenile weight-specific growth rates appear not to be only particular on *Oithona*, but also extended to calanoid egg-carrying species (Paffenhöfer and Harris 1976: *Pseudocalanus elongatus*). However, under optimal food conditions in the laboratory, small broadcasting calanoids exhibit maximum weight-specific egg production rates closer to the maximum specific juvenile growth rates (Berggren et al. 1988: *Acartia tonsa*). Our results, therefore, question the equivalence between weight-specific egg production and juvenile somatic growth for *Oithona*, which application in field studies could result on erroneous estimations of total copepod production.

Table 3. Comparison of egg production rates and clutch sizes among different species of *Oithona* from field and lab studies.

Species	Location	Prosome length (μm)	Temperature (ºC)	Type
O. aruensis	Cape York rivers	280	22.2-30.6	Field
O. attenuata	Exmouth Gulf	340	21.3-23.2	Field
O. colcarva	Chesapeake Bay	650-770[a]	15	Field
	"	"	15	Lab
	"	"	20	Field
	"	"	20	Lab
	"	"	25	Field
	"	"	25	Lab
O. davisae	Tokio Bay	-	20	Lab
	Fukuyama Harbour	276-331	10-28	Field
	Barcelona Harbour	326-330	18	Lab
O. nana	Argentinian Sea	609 ± 36[a]	10-21	Field
	Jamaican waters	325	28	Field
O. plumifera	South east continental shelf	≈ 600	20	Field
	Jamaican waters	540	28	Field
O. setigera	Indian Ocean	1140-1900[a]	25-28	Field
O. similis	Halifax Harbour	-	10	Lab
	Øresund	≈ 487	15	Lab (diet 1)[e]
	Øresund	450-487	15	Lab (diet 2)[f]
	North Atlantic	498-510	≈ 3-11	Field
	Scotia Sea	-	-1.5-3.3	Field
	Barents Sea	508-517	-1.6-10	Field
	Northern North Sea	438-575	6.3-14.7	Field
	North Sea	493-585	7-12	Field
O. simplex	Exmouth Gulf	270	21.3-23.2	Field
	Jamaican waters	≈ 270	28	Field
Oithona spp.	North Atlantic	479-501	3-11.1	Field
Oithona sp.	Cape York rivers	320	22.2-30.6	Field

[a]Total length, [b]Eggs per sac, [c]Clutch size estimated from hatched nauplii,
[d]Maximum specific-egg production rate,

Mean clutch size (± SE) (eggs female^{-1})	Range clutch size (eggs female^{-1})	Egg production rate (eggs female^{-1} day^{-1})	Specific-egg production rate (day^{-1})	Reference
-	3-9 [b]	0.8-11.3	0.01-0.12	McKinnon and Klumpp 1998
11.4 ± 0.2	7-18	3.2	0.023	McKinnon and Ayukai 1996
15.0 ± 2.4	-	2	0.045[d]	Lonsdale 1981 a,b
8.2 ± 9.9[c]	-	-	-	Lonsdale 1981 a,b
20.0 ± 4.2	-	3.6	-	Lonsdale 1981 a,b
6.1 ± 4.2[c]	-	-	-	Lonsdale 1981 a,b
16.8 ± 3.7	-	11.6	-	Lonsdale 1981 a,b
10.5 ± 9.0[c]	-	-	-	Lonsdale 1981 a,b
9.9 ± 4.6	3-20	3.7	0.14[d]	Uchima 1985
-	10-30	0.6-5.6	0.08-0.39	Uye and Sano 1995
15 ± 3	8-21	1.8-6.3	0.04-0.12	This study
12 ± 2[b]	9-15[b]	1-6	0.02-0.1	Temperoni et al. 2011
17.0 ± 4.6	9-26	-	-	Hopcroft and Roff 1996
11.6	7-16	3.8	0.08[d]	Paffenhöfer 1993
12.9 ± 3.7	5-21	-	-	Hopcroft and Roff 1996
11	-	-	-	Sazhina 1985
9.3 ± 4.7	4-19	1.6	0.03[d]	Eaton 1971
13.7 ± 0.4	-	4.48	0.1[d]	Sabatini and Kiørboe 1994
≈ 8.2	6-9	1.5-1.9	0.04	Sabatini and Kiørboe 1994
-	-	≈1.6- 2.1	0.025-0.06	Castellani et al 2005*b*
15.8[b]	6-31	0.03-1.12	0-0.02	Ward and Hirst 2007
21.5 ± 0.5	14-32	0.2-1.8	0.005-0.04	Dvorestsky and Dvoretsky 2009
14	5-22	0-4	0-0.12	Drif et al. 2010
-	10-15[b]	1.01-5.21	0.014-0.10	Nielsen and Sabatini 1996
5.3 ± 0.2	4-8	2.4	0.069	McKinnon and Ayukai 1996
7.5 ± 1.6	4-10	-	-	Hopcroft and Roff 1996
10.5 ± 1.5[b]	5-20[b]	1-6	0.017-0.13	Castellani et al. 2007
-	3-9 [b]	2.3–15.3	0.02-0.13	McKinnon and Klumpp 1998

Egg production efficiency (GGE)

The estimated egg production efficiency for *Oithona davisae* in the present study (16%) did not reach the typical 30% gross-growth efficiency assumed for egg production in calanoid copepods (Ikeda and Motoda 1978). It is indeed slightly lower than the value for juvenile stages of *Oithona davisae* (21%) reported by Almeda et al. (2010). The low growth efficiencies found during our study for *Oithona davisae* suggest therefore, either higher metabolic costs or lower assimilation efficiencies than calanoid copepods. As it has been typically attributed to *Oithona* low metabolic rates (Paffenhöfer 1993; Sabatini and Kiørboe 1994; Nakamura and Turner 1997) a lower assimilation efficiency seems more likely. However, this contrasts with the study by Almeda et al. (2011) on early stages of *O.davisae*, who reported assimilation efficiencies no different from the range of values reported in the literature for calanoids.

We do not really know why assimilation efficiency would be so different between juveniles and adults. It could be argued that *Oxyrrhis marina* is not a suitable diet for egg production. However, *Oxyrrhis marina* has been shown to be nutritionally a good food item for calanoid copepods (Klein Breteler et al. 1982), furthermore, in our stock cultures *Oithona davisae* has been easily maintained for years, indicating it is a suitable prey. An alternative and more likely explanation could be that, maximum food intake of adult females of *O.davisae* (and probably also of late copepodites), constrained by the strict ambush feeding mode, is relatively low in comparison to metabolic requirements, which for this species at our experimental temperature would be in the order of 21% (Hiromi et al. 1988). We cannot dismiss either, when comparing with free-spawning calanoids, that in cyclopoid species spawning frequency is limited by the fact that a new clutch can only be released when the previous one has hatched, whereas for nauplii the somatic growth might not be too different. The lack of other studies with *Oithona*, however, does not allow for strong conclusions about our hypothesis. In this regard, it is also worth noticing that Castellani et al. (2005*b*) found higher GGE for *Oithona similis* (47% on average), which was interpreted as a consequence of a preponderantly carnivorous diet (ciliates); differences in calculation methods could also help to explain the differences with our data.

From an ecological point of view, the comparatively low productive rates described for *Oithona* are probably counterbalanced by the expected lower encounter rate with predators due to their low motility. In addition, the low critical food concentrations exhibited by adult females in our study explain their capability to grow at maximum rates in very diluted environments. Therefore, we suggest that high abundances of *Oithona*, especially in situations of unfavorable trophic conditions for calanoid copepods, and their presence in a wide range of ecosystems, can be explained by their feeding and reproductive strategies.

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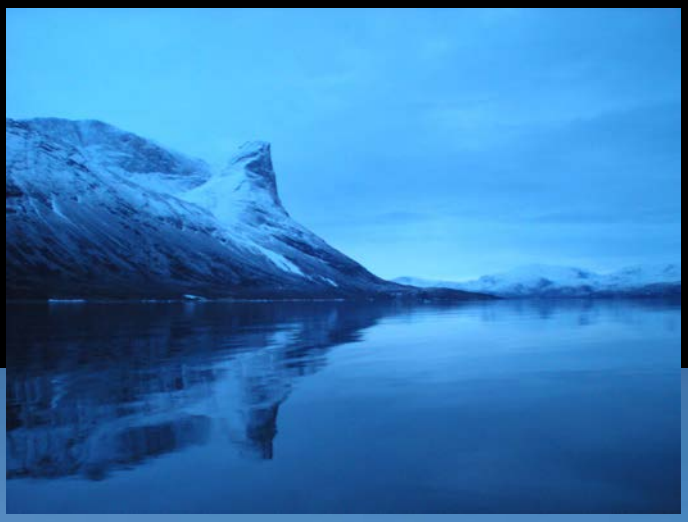


Chapter 2

Population dynamics and production of *Oithona similis* in the Kapisigdlit fjord, West Greenland

Based on the manuscript: Zamora-Terol, S., Sanne Kjellerup, Rasmus Swalethorp, Enric Saiz, and Torkel Gissel Nielsen. Population dynamics and production of the small copepod *Oithona similis* in a subarctic West Greenlandic fjord.

Abstract

Oithona similis is a small, abundant, and worldwide-distributed copepod with an important role in Arctic and subarctic marine pelagic ecosystems. However, aspects related to their population biology, production, and patterns of distribution have received little attention limiting our ecological understanding of these small copepods. The abundance, vertical distribution, and diel vertical migration of the developmental stages of *O. similis* in a fjord of West Greenland was described from winter to late summer. *O. similis* showed a marked seasonal variation in abundance, which was very low during winter-early spring, when late copepodites and adults were most abundant, and peaked in summer, when earlier stages dominated in the water column. In general, all the developmental stages of *O. similis* remained in the upper 100 m, although nauplii stages were found shallower. Although our results suggested non-seasonal migration of *Oithona* spp., we found evidences of a possible deeper distribution of the adult females in winter. The fecundity of *O. similis*, in terms of clutch size, percentage of ovigerous females, and egg production was also investigated. Egg production rates showed a significantly positive relationship with both temperature and protozooplankton concentration, and no correlation with Chl a. *Oithona* has been described to be more independent from phytoplankton blooms than larger calanoids, and therefore capable to maintain stable populations throughout the year; however, strict environmental conditions, such as very low temperature, might greatly affect the population dynamics of *Oithona* spp. in high latitude environments.

INTRODUCTION

Understanding the factors controlling the distribution and productivity of copepods is essential knowledge required to predict how future changes in the marine environment will affect plankton communities. In strongly-seasonal ecosystems like the Arctic and subarctic, current paradigm states that copepods optimize their survival success by a winter diapause, and maximize recruitment by synchronizing their reproduction with the phytoplankton spring bloom (Varpe et al. 2007). These strategies are well documented for large calanoids, such as Calanus, due to their key role in high latitude plankton communities in the productive season. However, the small cyclopoid copepods are in general less sensitive to seasonal variations in phytoplankton availability, do not undergo diapause, and remain active from late summer throughout the winter (Møller et al. 2006, Madsen et al. 2008, Svensen et al 2011). Thus, in comparison to the large calanoid copepods, the phenology of cyclopoid copepods in high latitudes might be less affected by climate change, which stresses their potential relevance in Arctic and subarctic plankton food webs in a warmer future.

The egg-carrying copepod *Oithona similis* is the most abundant cyclopoid copepod worldwide, and dominates numerically in Arctic and subarctic waters (Hopcroft et al. 2005, Madsen et al. 2008) when larger copepods are not present in the surface layer (Møller et al. 2006; Zamora-Terol et al. 2013). *Oithona* spp. populations show relatively high abundances throughout the year compared to calanoid copepods (Sabatini & Kiørboe 1994, Nielsen & Sabatini 1996), present low mortality rates (Eiane and Ohman 2004; Thor et al. 2008), and feed on the microbial web (Atkinson 1995; Castellani et al. 2005a, Zamora-Terol et al. 2013). These characteristics have very likely made *Oithona* so successful globally (Paffenhöfer 1993). Despite the importance of *O. similis* in Greenlandic waters (Pedersen et al. 2005, Madsen et al. 2008, Arendt et al. 2013), there is surprisingly limited information about their phenology and ecology. Seasonal and diel migration patterns of Calanus species in Arctic ecosystems are well described (Dawson 1978, Grainger 1989, Hirche 1997); comparatively, migration patterns of small size copepods are poorly studied, and in particular, it is unclear whether *Oithona* spp. performs any vertical migration (Turner and Dagg 1983, Shaw and Robinson 1998).

The present study was conducted in the fjord branch Kapisigdlit, located in the Godthåbsfjord system in Western Greenland, through the winter-summer transition. We investigated the population dynamics and fecundity of *Oithona similis*, as well as their vertical distribution and the presence of diel vertical migration patterns. In particular, we aimed to improve our knowledge on the phenology of *O. similis* to further our understanding of how plankton communities might change in a high latitude ecosystem affected by climate warming.

MATERIALS AND METHODS

Study site

The Godthåbsfjord fjord (Fig. 1) is the biggest fjord system on the West Greenlandic coast. The fjord is influenced by melted freshwater from the Greenland ice sheet, and saline water from the West Greenland Current (Mortensen et al. 2011), supporting a very productive ecosystem within the area that is of high importance to the local communities.

Sampling was conducted in the Kapisigdlit fjord (Fig. 1), from March 22nd to August 5th of 2010. Except for the period 16-18th June, where sampling was conducted from R/V Dana, samples were collected from vessel Lille Masik. Four stations were sampled along the 25 km length of the Kapisigdlit fjord (Fig. 1; Table 1): St. 2, located close to the entrance of the fjord; St. 4, situated in the middle, and defined as the main station of the study; St. 5, located on a slope in the inner part leading up to the shallow St. 6, situated close to Kapisigdlit River (Fig. 1). A total of 15 cruises were conducted during the period of study, during which all stations were sampled. During the period of sampling there was no presence of sea ice at the sampling stations, but the river was frozen until June 20th.

Sampling

Sampling on St. 4 was conducted for 24 hours (at 06:00, 12:00, 18:00, and 00:00 h local time) on every third cruise, to assess the presence of diel changes in vertical distribution; the rest of samplings for that station were conducted at 18:00 h. The time of sampling for the rest of the additional stations varied between cruises (1:00-14:00 at St. 2, 10:00-23:00 at St. 5 and 5:00-17:00 at St. 6). Vertical profiles of salinity and temperature were measured down to 10 m above the bottom by using a Seabird CTD (SBE 19 plus), and water samples for chlorophyll a analysis (only at St. 4) were taken from 8 depths (1, 10, 20, 50, 75, 100, 150, and 250 m) using a Niskin bottle.

Chlorophyll a (chl a) samples were split into 2 subsamples and filtered onto GF/F and 10

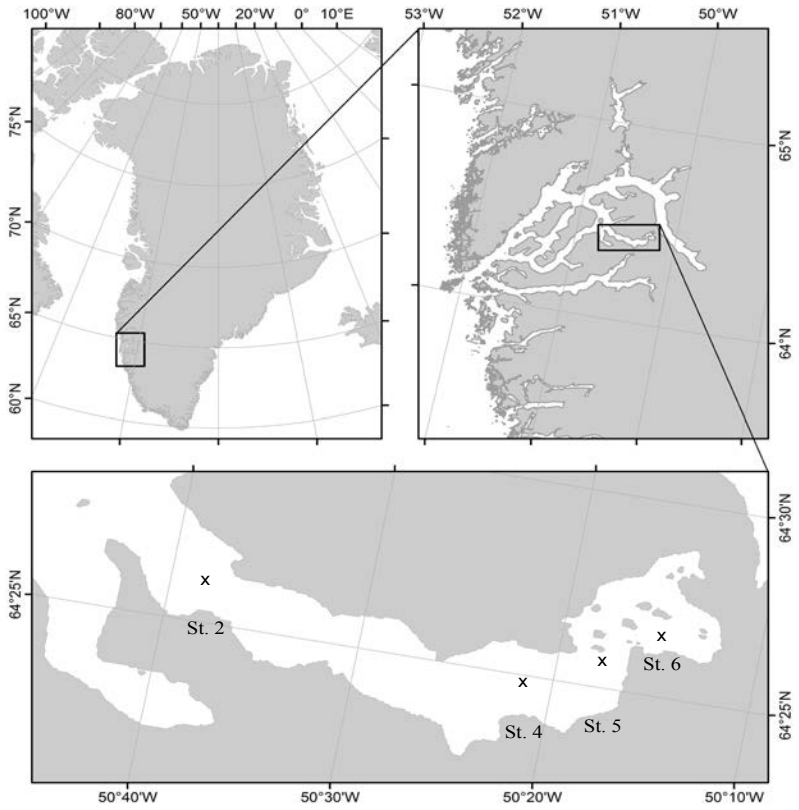


Fig. 1. Map showing the Godthåbsfjord system on the western coast of Greenland, and the sampling stations in the Kapisigdlit fjord.

μm filters by triplicate; filters were extracted for 12-24 hours in 96% ethanol, and finally chl a concentration was measured using a Turner TD-700 fluorometer. At station 4, additional samples for protozooplankton identification (250-300 mL) were collected at 5 depths (1, 10, 20, 50-60, and 100 m) and preserved in acid Lugol's solution (2% final concentration); samples were kept cool and dark until inspection under an inverted microscope (see Riisgaard et al. 2013).

Table 1. Location and depth of the stations along the fjord, and sampling depth of the vertical tows during the study.

Station	Coordinates	Station depth (m)	Sampling depth (m)
2	64° 26 N, 50° 39 W	194	100-150
4	64° 25 N, 50° 22 W	251	235
5	64° 25 N, 50° 18 W	125	75-100
6	64° 26 N, 50° 15 W	85	50

Zooplankton was collected from several depth strata (Table 1) by vertical hauls (0.2-0.3 m s^{-1} speed) using a 50 μm Hydrobios Multinet (type Mini, opening 0.25 m^2) or a 50-μm WP-2 net (opening 0.28 m^2) equipped with a non-filtering cod; both plankton nets were equipped with flow-meters. When long samplings were conducted at St. 4 (i.e. 24 h samplings), the Multinet was used on all stations, except at St. 6 where the WP-2 net was used instead. In the rest of samplings, the Multinet was only used at St. 4, whereas St. 2, 5, and 6 were sampled using the WP-2 net. Detailed information of sampling depth and plankton nets used in each date are provided in Table 3. Samples collected from the nets were immediately preserved in borax-buffered formaldehyde-seawater solution (4% final concentration).

Sample analysis and calculations

Zooplankton samples preserved in buffered formaldehyde were split in subsamples, and all organisms present were counted and identified in the laboratory. From each sample, nauplii and copepodite development stages of all copepods were identified under microscope down to species or genus level, and length was measured of up to 10 individuals of each development stage. Zooplankton abundance was calculated assuming that all water passed by the net was filtered, and no clogging of the nets occurred; integrated abundances (ind. m^{-2}) and depth-weighted abundance was calculated. The carbon content of developmental stages of each group of copepods was calculated using length-weight regressions from literature.

We did not distinguish among *Oithona* congeners in the counts, but occasional checking indicated that most adults belonged to *Oithona similis*, so hereafter we will only refer to this species throughout the article. For *O. similis* the number of females carrying egg sacs, the total number of egg sacs (attached to females or free), and the average number of eggs per sac (n = 10) were quantified.

The weighted mean depth (WMD) of the developmental stages of *Oithona similis* was calculated as follows:

$$WMD = \sum n_s d_s / \sum n_s$$

where d_s is the sampling depth (in our case calculated as the mean depth of the water strata) and n_s is the abundance (ind. m^{-3}) of the developmental stage (s).

Egg production estimation

At each sampling, reproductive parameters (i.e. number of ovigerous females, number of eggs, clutch size) were calculated for each depth strata, and then depth-weighted averages were estimated. The abundance of ovigerous females in the population was estimated for each depth strata by the sum of number of females with egg sacs and the number of detached egg sacs divided by 2 (assuming that all ovigerous females had 2 eggs sacs). The abundance of eggs was calculated by multiplying the number of egg sacs by the average number of eggs per sac.

Egg production rates (EPR, eggs female^{-1} day^{-1}) was calculated using the egg-ratio method based on egg and female abundance, and on the egg hatching time (HT, day) according to Edmondson (1971) as follows:

$$EPR = (eggs/females) \times 1/HT$$

where HT was calculated according to Nielsen et al. (2002):

$$HT = (0.064+0.0145\ T)^{-1}$$

where T is water temperature (ºC). In order to compute the weight-specific egg production, the egg and female carbon contents were estimated from size, using the conversion factor 0.14 pg C μm^{-3} (Kiørboe et al. 1985) for eggs, and the length-weight relationship provided by Sabatini and Kiørboe (1994) for adult females of *Oithona similis*.

Linear regression analyses were used to describe relationships between fecundity rates and physical (average temperature in the upper 100 m) and biological variables (average chl a and protozooplankton concentration in the upper 50-100 m). Those analyses were only conducted on St. 4 data as chl a and protozooplankton concentrations were only measured at that station.

RESULTS

Oceanography

The physical conditions along the fjord were in general similar among stations, and developed similarly throughout the study. The water column was well mixed from March to April, with temperatures around 1ºC, low chl a concentration (0.5-1 μg L^{-1}), and salinity around 33 that slightly decreased at the end of April resulting in the formation of a weak halocline (Fig. 2). From late May, surface temperature (i.e. upper 50 m) increased from 1 to 6 ºC, and a flow of melted water was established in the surface culminating with the break-up of ice in the Kapisigdlit River around June 20. Hereafter the surface salinity rapidly decreased

from 31 to 16 by the beginning of August and the surface warmed up rapidly (Fig. 2). When stratification was strong in the water column, a subsurface phytoplankton bloom developed with a peak of 12 µg chlorophyll a L^{-1} (Fig. 2).

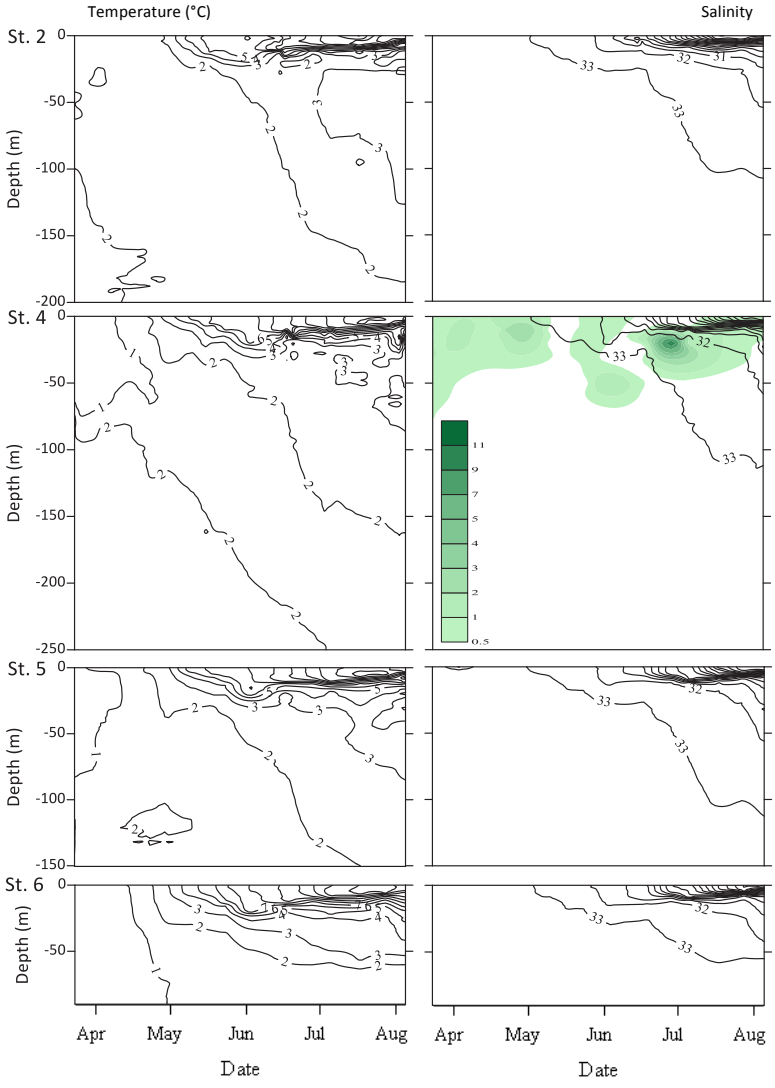


Fig. 2. Temporal variation of temperature (left panel) and salinity (right panel) of the stations along the Kapisigdlit fjord between March 24th and August 5th 2010. The temporal variation of chlorophyll *a* is shown only for station 4 (right panel). Note different scales on y-axis.

Seasonality in copepod community structure and stage seasonal abundance of *Oithona similis*

The small copepods present in the fjord dominated by far the copepod community in terms of abundance, mainly due to the extraordinary high abundance of Microsetella spp. The relative abundance of *Oithona similis* increased during the period of our study, becoming one of the dominant copepods in summer (Fig. 3). The high relative abundance of Microsetella spp. masked the relevance of the rest of copepods present in the water column. Thus, in terms of biomass, Metridia spp. was the most important copepod in winter-spring, and the smaller-sized copepod species (Microsetella spp., Oncaea spp., *Oithona* spp., Pseudocalanus spp.) became more important in summer, accounting for > 50% of the copepod biomass (Fig. 3). The total biomass of copepods was up to 24 g C m^{-2} in March, when large-sized copepods were present, and decreased to 1.5 g C m^{-2} in June, when smaller-sized species dominated (Fig. 3).

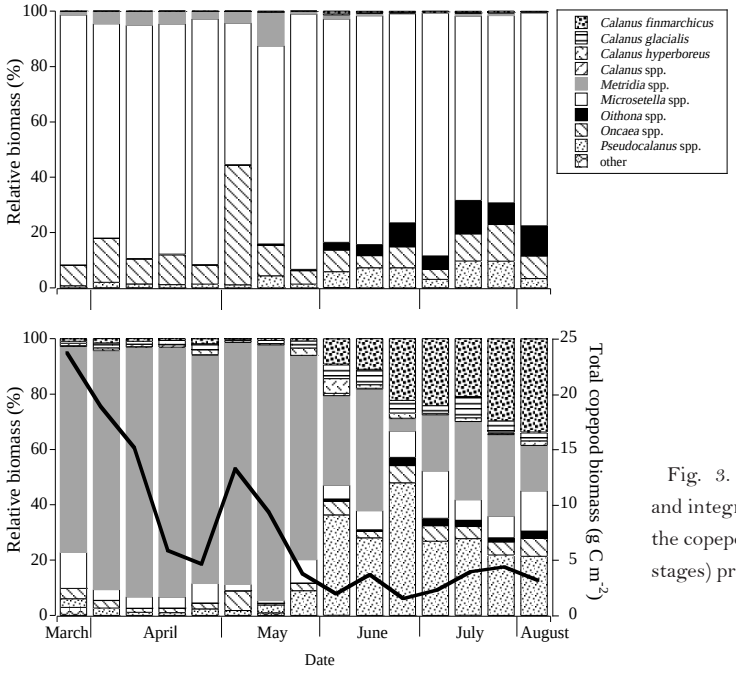


Fig. 3. Relative abundance (%) and integrated biomass (mg C m^{-2}) of the copepod community (including all stages) present at station 4 during the period of study.

Maximum abundance of *Oithona similis* occurred in mid-late summer (St. 2 in July > $1.1 \cdot 10^6$ ind. m^{-2}, left panels of Fig. 4), whereas minimum abundance was found at the end of April (1,800 ind. m^{-2}) at St. 5 and St. 6 (Fig. 4). Overall the abundance of *O. similis* was very low during early spring, when the population was mainly composed of late copepodites and adults. Nauplii and early copepodites of *O. similis* were almost absent until May (Fig. 4).

In terms of relative abundance (Fig. 4, right panel), adult females of *Oithona similis* accounted for 20-83% of the population from April to June, and decreased in summer (< 25%) when younger stages developed. The males were present most of the time, and reached the highest abundance in April-May (10-22%). Late copepodites stages (C4-C5) showed two peaks, in April (15-58%) and in July (13-40%), whereas the younger copepodites stages dominated in May-June (10-17 %). Nauplii represented 0-5% of the population in March-April, increased up to 20-30% in early May, and peaked in late May-June (> 50%) (Fig. 4; St. 4, right panel). Overall, the phenological patterns of *O. similis* observed along the fjord were similar (Fig. 4), and this was further confirmed by the fact that seasonal variability in *O. similis* abundance seemed to account for larger differences that the variability among fjord stations (Table 2).

Table 2. Coefficient of variation based on weighted average abundances (individuals m^{-3}) of all developmental stages of *Oithona similis*.

	Coefficient of variation (%)						
Station	March*	April	May	June	July	August*	All months
2	-	83	54	88	96	-	195
4	-	44	86	47	24	-	121
5	-	138	74	45	48	-	149
6	-	25	54	61	45	-	140
All stations	51	151	66	59	91	34	

* In March and August only one sampling was conducted

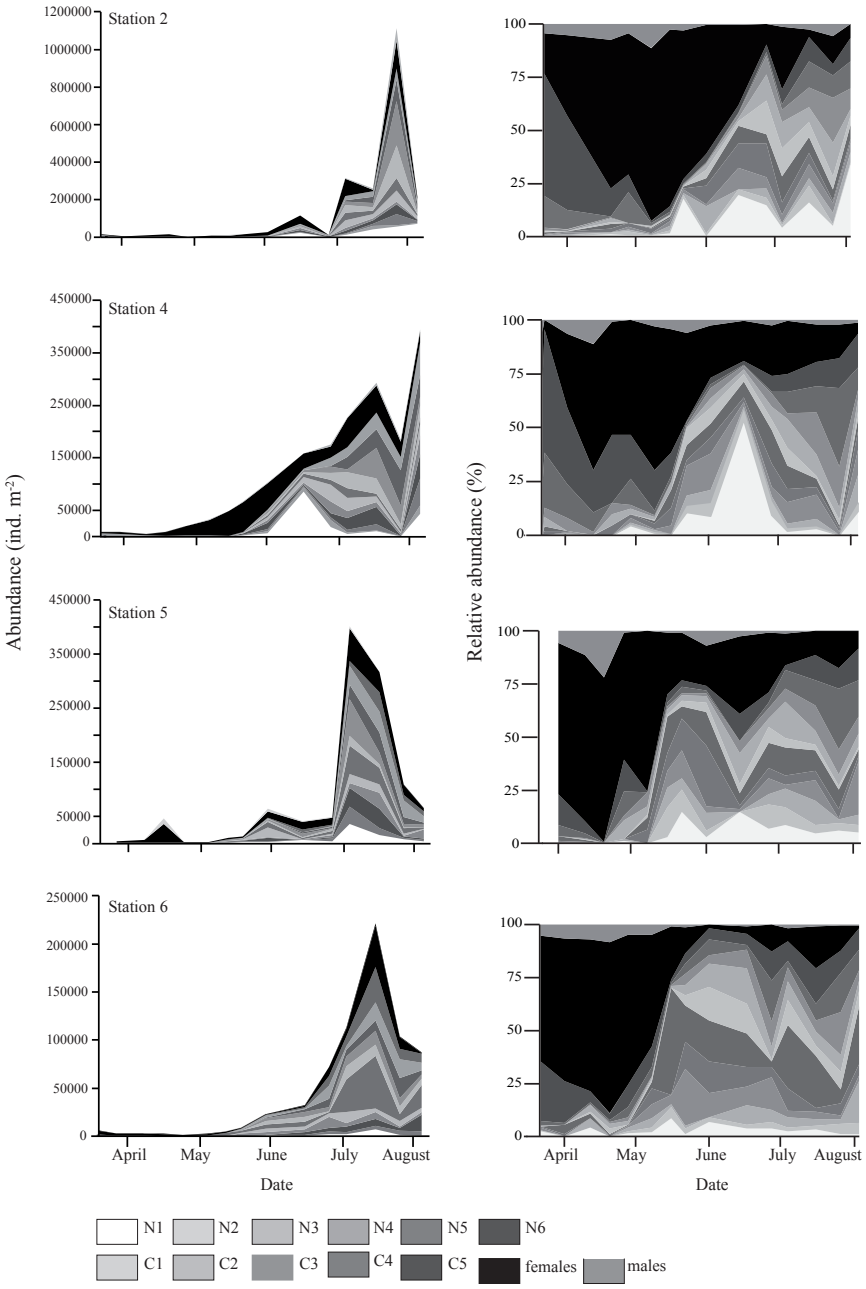


Fig. 4. Temporal variation of integrated abundances (left panel) and relative abundance (right panel) of developmental stages of *Oithona similis* at the stations along the fjord. Notice different scales on the y-axis in the integrated abundance.

Ontogenetic and seasonal vertical distribution of *Oithona similis*

Throughout most of the study *Oithona similis* inhabited the upper 100 m, except in March-April when 30-50% of the population (mainly adult females and C5) was found in the deepest layers (150-230m) (data not shown). Earliest stages (mainly nauplii) were mostly located in the upper 50 m, except for the two first samplings. Copepodites were distributed throughout the entire water column in March-April with a non-defined vertical pattern; from May on they were located shallower (i.e. upper 50 m), and became the most abundant stages both in 0-50 m and 50-100 m depth strata at the end of July (28th, Fig. 5). The maximum concentrations of adult females of *O. similis* were found at the 50-100 m depth strata, being the most abundant stage in the deepest depth strata (Fig. 5). Although female distribution in the water column did not show a strong seasonal pattern, a higher percentage was found in deeper layers in winter-spring (two first samplings), while being more homogenously distributed in surface layers in summer (Fig. 5). However, male maximum abundances were found in the 0-50 m depth strata, overall shallower in the water column than the females.

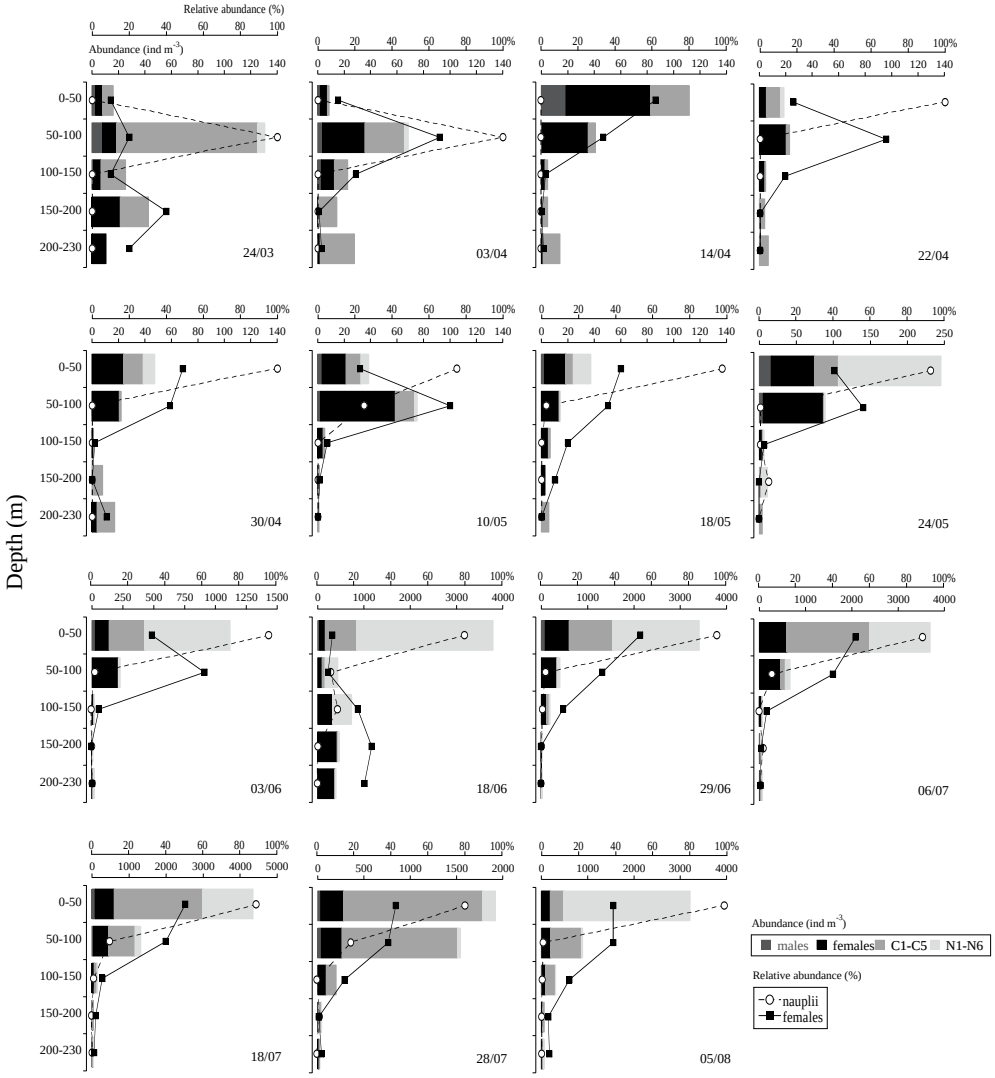
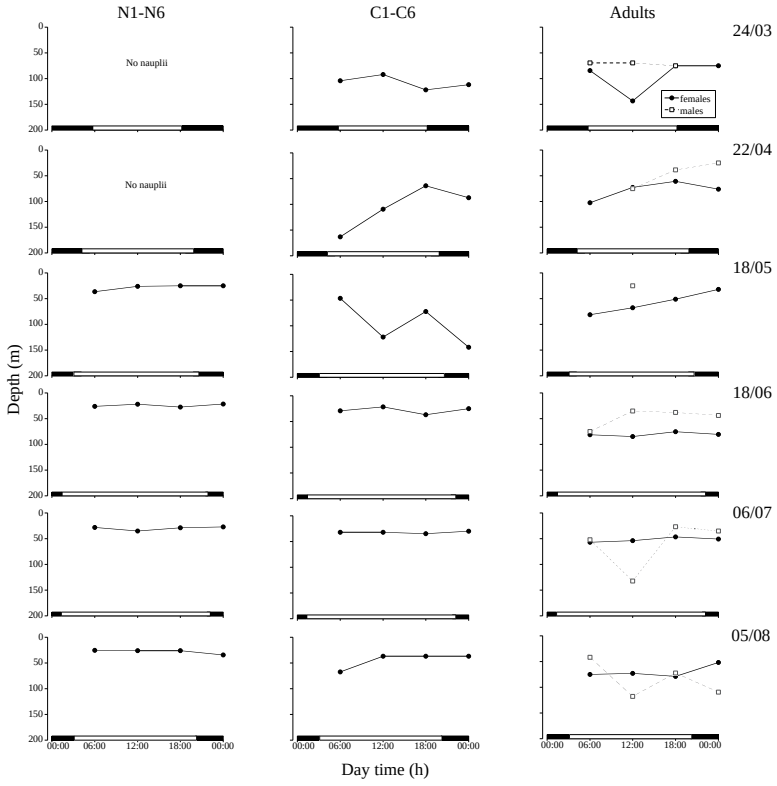


Fig. 5. Vertical distribution of nauplii, copepodites, adult females, and males in terms of abundance (ind. m^{-3}). from samplings conducted at 18:00 in St. 4. Circle and square symbols indicate the relative abundance (%) of respectively nauplii and adult females in the water column. Notice different scale on the x-axis of abundance.

Diel vertical migration of *Oithona similis*

Nauplii appeared in May (very low abundances were found before) located in the upper 50 m (mean depth of 28 ± 4 SD m), independent of time of the day or light levels. Copepodites showed similar distribution patterns as nauplii from June to August (30 ± 5 SD m), but in winter-early spring, copepodites exhibited more varied patterns, which were likely due to the low number of individuals found in those samples (see Discussion). Females showed a distribution with no clear evidence of diel vertical migration pattern along the period of study; however, in the first 3 samplings there was a slight tendency towards upward migration at night (Fig. 6, right panel). Adult females were found in a mean depth of 72 ± 22 SD m in the water column (Fig. 6, right panel). Males showed a similar pattern to the females in winter-spring, but they were located a bit closer to the surface; however, in summer (July-August) they showed a migration from 40-50 m down to 120-130 m between dawn and midday (Fig. 6, right panel), and went up again before dusk.



Seasonal variation of fecundity and egg production

Through the study there was a clear increase in mean temperature, ranging from a minimum below 0ºC in March, to a maximum of approximately 6ºC in August (Fig. 7A), with the highest temperatures recorded at the innermost station close to the river (i.e. inner stations 5 and 6). The size of adult females of *Oithona similis* ranged from 430 to 520 μm (Fig. 7B). Smaller females were present in late spring, whereas slightly larger females were found in winter, early spring and summer (Fig. 7B); this difference in size was more evident at stations 5 and 6. Nevertheless, no correlation between body size and temperature was found ($p = 0.45$). The relative abundance of ovigerous females in the population was quite variable through the period of study, although in March-April the number of ovigerous females was generally low, whereas in May-June tended to be higher (Fig. 7C).

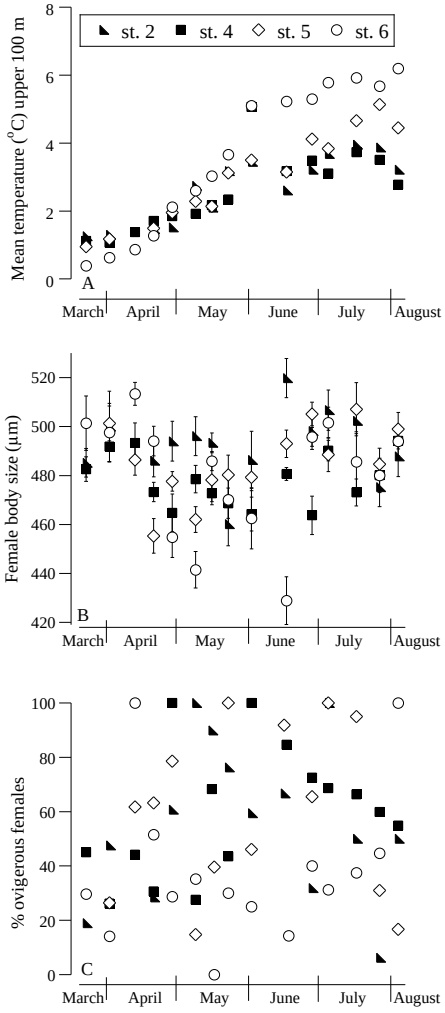


Fig. 7. Temporal variation of (A) temperature, (B) mean (± SE) prosome length of adult females of *Oithona similis*, and (C) percentage of ovigerous females of *O. similis* along the fjord. Symbols in the legend indicate the stations along the fjord.

Fig. 6 (left page). Diel variation in the weighted mean depth of nauplii, copepodites, and adults of *Oithona similis* collected at St. 4 during the 24 h intensive samplings. Black and white bars indicate the dark/light pattern present in the water column during the season.

The mean clutch size varied from 16 to 30 eggs per female, corresponding to March and June respectively (Fig. 8A). Smaller clutch sizes were found between March and May, while larger clutch sizes were found between June and August (Fig. 8A). The egg production rates were low (< 0.5 eggs female^{-1} day^{-1}) in March and in the beginning of April at all stations, and reached maximum values (> 1.4 eggs female^{-1} day^{-1}) in June-July for all the stations (Fig. 8B). The weight-specific egg production rates followed the same pattern, with mean values ranging between 0.2 and 3 % day^{-1} in March and July, respectively (Fig. 8C).

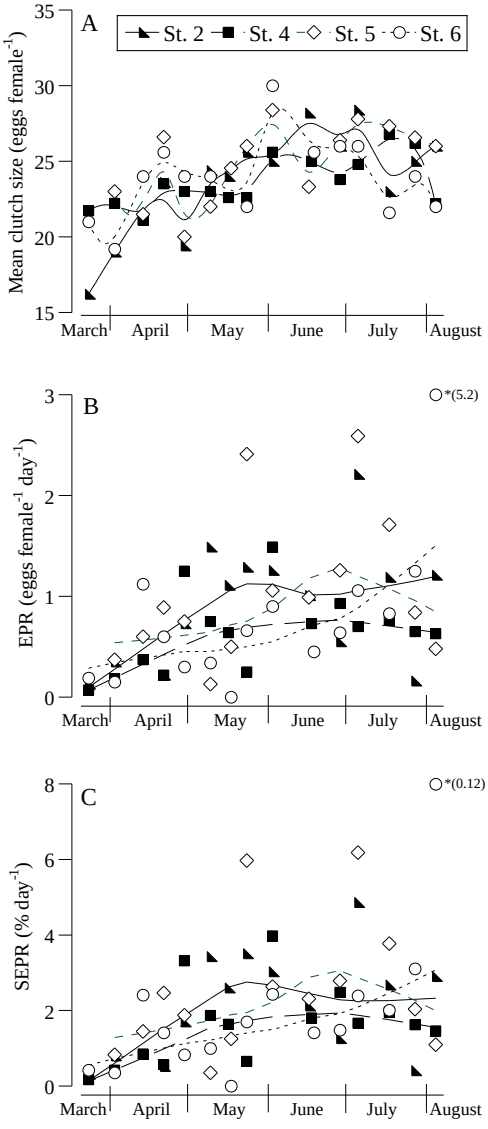


Fig. 8. Fecundity of *Oithona similis* in terms of (A) mean clutch size, (eggs per female), (B) egg production rate (EPR, eggs female^{-1} day^{-1}), and (C) weight-specific egg production rate (SEPR, % day^{-1}). The corresponding stations are indicated by different symbols; and weighted fits are shown. (*) Indicates outlier values.

The relationship between egg production rate (at St. 4) and the environmental variables (i.e. temperature, chlorophyll a and protozooplankton concentration) are shown in Fig. 9. The egg production was linearly related to temperature ($r^2 = 0.46$, $F_{1,13} = 11.13$, $p < 0.01$; Fig. 9A). No significant linear relationship was found between egg production and chlorophyll a concentration ($r^2 = 0.12$, $F_{1,13} = 1.70$, $p = 0.2$; Fig. 9B). However, a positive and significant ($r^2 = 0.77$, $F_{1,7} = 10.43$, $p < 0.01$) linear relationship was found between the egg production and the protozooplankton concentration (Fig. 9C).

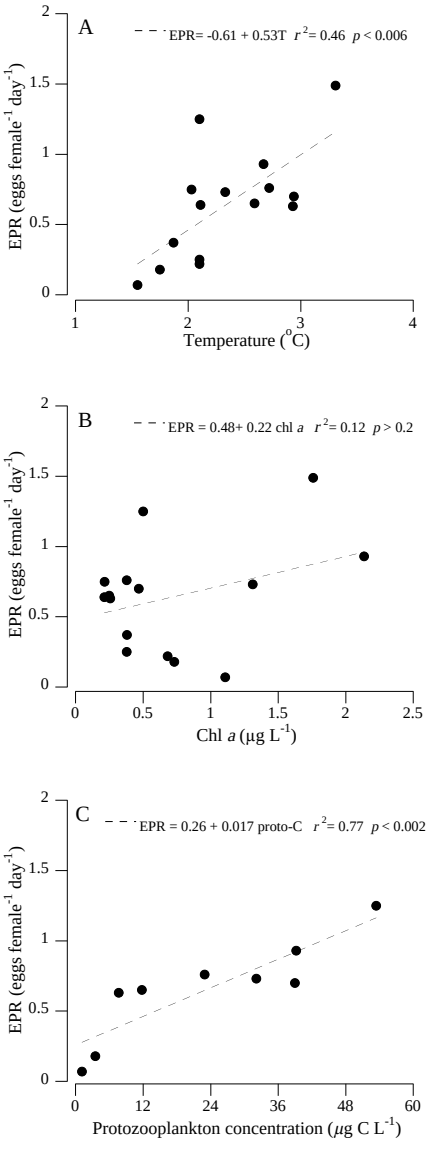


Fig. 9. Relationship between *Oithona similis* egg production rate (EPR, eggs female^{-1} day^{-1}) at St. 4 and, (A) temperature (°C), (B) chlorophyll *a* concentration (µg L^{-1}), and (C) protozooplankton concentration as weighted average (µg C L^{-1}). Linear regressions are shown. Proto-C: protozooplankton concentration (µg C L^{-1}).

DISCUSSION

Seasonal population dynamics

Seasonal succession of *Oithona similis* showed a pronounced cycle, from low abundance of all developmental stages in late winter, to peak abundance in the summer. During winter-spring the late stages of *O. similis* dominated, whereas in summer all developmental stages were present.

Oithona similis is a dominant species in polar environments (Atkinson 1998, Madsen et al. 2008), although its contribution to biomass has been generally considered low (Ashjian et al. 2003). In our study, the shift from large to small copepod species was responsible for the decrease of the total copepod biomass in summer, when small copepods numerically dominated. However, Møller et al. (2006) did not find a decreased in the total copepod biomass when small copepods dominated the zooplankton community in the Greenland Sea. It is worth mentioning that in Møller et al. (2006), the significant contribution of *Oithona* spp. to the copepod biomass was observed from late summer to winter, a period that we did not sample. In the same regard, Svensen et al. (2011) also found a significant contribution of *Oithona* spp. to the zooplankton biomass of the Fram Strait in autumn.

The abundance of *Oithona similis* found in summer is in accordance with previous seasonal studies in temperate (Baltic Sea: Hansen et al. 2004; Irminger Sea: Castellani et al. 2007), tropical (Sea of Japan: Takahashi and Uchiyama 2008) and polar seas (Madsen et al. 2008, Dvoretsky and Dvoretsky 2009). Maximum abundance found in our study ($1.2 \cdot 10^6$ ind. $m^{-2} \approx$ 11,000 ind. m^{-3}) is slightly above the maximum abundances ever reported for *Oithona similis* ($\approx 7 \cdot 10^5$ ind. m^{-2}: Lischka and Hagen 2005; $\approx$ 9,000 ind. m^{-3}: Dvoretsky and Dvoretsky 2009). Differences in maximum abundances found for *Oithona* spp. in similar ecosystems could be due to an underestimation because of using coarse mesh sizes to capture small copepods (Calbet et al. 2001, Turner 2004), thus high abundances of *Oithona* spp. closer to our maximum values might likely be found in other areas.

Adults and late copepodites were the most abundant stages during winter and early spring, but a change in the relative abundance of stages occurred in May-June, when contribution of

younger stages increased. The fact that the hatching time for *Oithona* spp. eggs is estimated to be 14-12 days at 2-3ºC (Nielsen et al. 2002) suggests that the most productive spawning event during our study (i.e. increase of nauplii during June-July, Fig. 4) was due to the ovigerous females present in high abundances in May-June (Fig. 7C). The increase of the abundance of younger stages of *Oithona* spp. in summer might be linked to an increase of temperature and food availability in the water. Besides that, a lower predation pressure, due to the decline in the relative abundance of larger-sized copepods after June (Fig. 3), could also explain the high abundance of the youngest stages of *Oithona similis* during summer (Metz and Schnack-Schiel 1995). High abundance of *O. similis* nauplii in early summer, when the water was strongly stratified, have previously been reported for the Greenlandic Arctic (Hansen et al. 1999, Thor et al. 2005) and central Arctic Ocean (Auel and Hagen 2002).

We had expected to identify several cohorts of *Oithona similis* in our study. However, the continuous reproduction throughout the year, as well as the long hatching and development times due to low temperature, resulted in the overlapping generations (egg hatching time: from 18 to 10 days at 1-4ºC respectively, Nielsen et al. 2002; estimated developmental times from the Bêlehràdek equation: from 143 to 78 days at 1-4ºC) making difficult to identify cohorts clearly. In other studies conducted in Antarctica, nauplii and copepodites of *Oithona* spp. were also present throughout the year, and no clear cohorts could be identified during any season (Fransz and Gonzalez 1995, Metz 1995).

Ontogenetic vertical distribution and migration

Oithona similis is typically ascribed to be epipelagic all year-round both in Antarctic and Arctic waters (Metz 1995, Atkinson 1998, Ashjian 2003, Madsen et al. 2008). Interestingly in our study, we found evidences that suggest that in winter the adult females might reside at relatively deeper waters than in summer. This observation is in agreement with the observations of deeper populations of *Oithona* spp. (mainly *O. similis*) in Icelandic waters in winter (Gislason 2008). Although our seasonal coverage did not include the whole winter period, it seems likely that winter populations of *O. similis* might reside deeper in the water

column and maintain physiologically and reproductively active, either feeding on the available protozooplanktont or using lipid reserves (Narcy et al 2009, Zamora-Terol et al. 2013). Although *O. similis* has been described as a non-seasonal migrant in Antarctica (Metz 1995, Atkinson and Sinclair 2000), Lischka and Hagen (2005) described this species as seasonally migrant in Svalbard. In any case, this presumably winter deepening in the vertical distribution of *O. similis* is not comparable in extent to the deep seasonal migration of large calanoids that go into diapause (Conover 1988, Hirche 1997), because adult females of *O. similis* are active in winter (Dvoretsky and Dvoretsky 2009, Zamora-Terol et al. 2013). In our study, nauplii and early copepodites of *O. similis*, generally inhabited shallower waters than the adults and older copepodites, in agreement with general patterns described not only for cyclopoid copepods (Titelman and Fiksen 2004), but also for the calanoid (Williams et al. 1987, Hirche 1997).

No clear evidence of diel vertical migration was found in this study for any developmental stage of *Oithona similis*, although as mentioned before, there was a tendency of females to swim upwards at night during winter-early spring. It is generally accepted that the day-night light cycle is an important factor regulating the vertical migrations of zooplankton populations (Davis 1984), however we found no evident relationship between the time of the day and the mean depth where all developmental stages of *O. similis* were located. Other investigations conducted in the Baltic (Hansen et al. 2004) and the North Sea (Eiane and Ohman 2004) on the same species could not document diel vertical migration either. However, Tanimura et al. (2008) found diel changes in the vertical distribution of *O. similis* in Antarctica.

Differences in the seasonal and diel migratory behavior of *Oithona similis*, reported in different studies, could be explain by the presence of different predators in the plankton community, as it has been reported for calanoid copepods (Bollens and Frost 1989, Ohman 1990). However, changes in the physical structure of the water column throughout the year could also result in migration patterns of the copepods. Advection processes in the fjord (Mortensen et al. 2011) might also determine the vertical distribution and migration of *O. similis*, in the same way that cod eggs are retained in the shallow innermost area of Kapisigdlit fjord with less risk of upwelling and advection (Swalethorp et al. unpubl.), as described for

Kimmerer et al. (2001) in estuaries. The adaptive value of staying in the physically stable layer in the water column might minimize the likelihood of *O. similis* to be advected out of the fjord (Eiane et al. 1998, Gorsky et al. 2000).

Fecundity and egg production

Ovigerous females were present throughout the study, suggesting continuous reproduction in this population of *Oithona similis* from at least March to August. Other long-term studies have also described year-round reproduction for the same species not only in Arctic (Madsen et al. 2008, Dvoretsky and Dvoretsky 2009) and Antarctic seas (Atkinson 1998), but also in temperate areas (Nielsen and Sabatini 1996).

The variations in the percentage of ovigerous *O. similis* females observed in our study, with a non-clear seasonal pattern, could be explained by the simultaneous presence of "old" (i.e. from autumn-winter population) and "new" (i.e. developed from eggs present in winter) females in the population. The very high percentages occasionally found, reaching 100% of the female population, are much higher than the values reported for Barents Sea (41%, Dvoretsky and Dvoretsky 2009), the Southern Ocean (67%, Ward and Hirst 2007) or the North Sea (82%, Castellani et al. 2007) for the same species. The higher proportion of ovigerous females of *O. similis* reported here might be due to the use of coarser mesh sizes in other studies (63 μm, Castellani et al. 2007; 100 μm Ward and Hirst 2007; 168 μm, Dvoretsky and Dvoretsky 2009), allowing for the loss of detached egg sacs when the nets were towed.

The average clutch size of *Oithona similis* showed a slight seasonal pattern in which the number of eggs per female increased from June on, and kept more or less steady during summer (Fig. 8A). The values found (16-30 eggs per female) were in accordance with clutch size reported for the same species in high latitude environments (6-31 Ward and Hirst 2007; 18-29 Dvoretsky and Dvoretsky 2009; 14-38 Zamora-Terol et al. 2013). No clear relationship between clutch size and any of the studied environmental factors was found. The lack of correlation with chl a was not surprising, since most investigations carried out on the same (Ward and Hirst 2007, Drif et al. 2010) and other *Oithona* species (Temperoni et al. 2011),

have also found no relationship. *O. similis* is considered an omnivorous species, and it is more likely that protozooplankton concentration could have a stronger influence on their fecundity. However, a non-significant correlation was found between clutch size and protozooplankton concentration, suggesting that clutch size of *O. similis* in our study is little affected by food concentration. The size of the adult females did not explain the variation in clutch size either (analysis not shown), confirming the lack of importance of body size of *Oithona* on the egg production also found in other studies (Drif et al. 2010, Zamora-Terol et al. 2013). In contrast, Castellani et al. (2007) and Dvoretsky and Dvoretsky (2009) found that body weight of adult females of *O. similis* was correlated with clutch size. In our case we think it is more likely that the mixing of females from the "old" and "new" generations (see above) masked any trend with size.

Egg production rates found for *Oithona similis* in the present study are within the range of previous reported values for the same species in polar (Ward and Hirst 2007, Dvoretsky and Dvoretsky 2009) and temperate seas (Nielsen and Sabatini 1996, Castellani et al. 2007, Drif et al. 2010). Similarly, maximum egg production rates found here (≈ 2.6 eggs female^{-1} day^{-1}) were also in accordance with maximum values found for the same species in areas with a range of temperatures similar to our study (2.1 and 1.1 eggs female^{-1} day^{-1} in respectively, Castellani et al. 2007, and Ward and Hirst 2007), but lower than those reported in warmer areas for *Oithona* spp. (5.2 and 5.6 eggs female^{-1} day^{-1} in Nielsen and Sabatini 1996, and Uye and Sano 1995, respectively).

Our results showed how egg production of *Oithona similis* did not show a pronounced seasonal cycle as often happens with co-occurring calanoid copepods. However, a slight increase in the egg production occurred when temperature increased (Fig. 8). Seasonal studies on the egg production of *Oithona* spp. in high latitude systems suggest the importance of temperature as a main factor controlling their fecundity (Metz 1995, Ward and Hirst 2007, Zamora-Terol et al. 2013). The long hatching times for egg carrying copepods at low temperatures limit their capability to reach maximum egg production, since they are not able to produce new egg sacs before the previous ones has hatched. In contrast, in other studies

Oithona spp. did not follow a seasonal pattern in the egg production (Castellani et al. 2007, Temperoni et al. 2011). However, in those investigations the range of temperature was never below 5ºC, temperature below which egg production rates rapidly decline, as suggested by Ward and Hirst (2007).

The positive relationship between temperature and fecundity of *Oithona similis* found has been reported in most of the seasonal studies conducted on *Oithona* spp. (Uye and Sano 1995, Ward and Hirst 2007, Dvoretsky and Dvoretsky 2009). Nonetheless, in other studies food condition instead of temperature was correlated with fecundity of *O. similis* (Sabatini and Kiørboe 1994, Nielsen and Sabatini 1996, Castellani et al. 2005). We found a positive correlation between egg production of *O. similis* and protozooplankton biomass, but not with the chl a concentration (Fig. 9). This observation is in agreement with previous studies that found a positive correlation between egg production and protozooplankton concentration, but not with phytoplankton abundance (North Sea, Nielsen and Sabatini 1996; West Greenland, Zamora-Terol et al2013). This fact confirms the advantage of the life strategy of *Oithona* spp. to maintain active populations year-around, since they are not strictly linked to seasonal phytoplankton blooms as in the case of most calanoid copepods (Nielsen and Sabatini 1996). In conclusion, both temperature and food availability are influencing the egg production of *Oithona* spp., as well as the indirect influence that both factors have on the size of the females, also considered to influence the fecundity of *Oithona* spp. (Castellani et al. 2007, Dvoretsky and Dvoretsky 2009). However, we should consider that although *Oithona* spp. is linked to microbial food webs, and thus their production patterns are more stable than those of calanoid copepods, strict environmental conditions, such as very low temperature, might greatly affect the population dynamics of *Oithona* spp.

This study has reported on the population dynamics and seasonality of *Oithona similis* in a subarctic environment from winter through summer transition. In contrast to the large calanoid copepods, characteristic for the Arctic and subarctic, which typically go into deep waters and diapause, no ontogenetic or seasonal distribution patterns were evident for *O. similis*. This species seems to be active throughout winter, although at low reproductive

rates (Zamora-Terol et al. 2013). Temperature appeared to be a major factor controlling the fecundity and recruitment of *O. similis* in this study, suggesting that this species may be successful in a future warmer climate. In conclusion, the year-round presence of active *O. similis*, and their high abundance in some periods of the year, stresses the importance of these small copepods in plankton communities within high latitude ecosystems.

Acknowledgments

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Chapter 3

Plankton community structure and role of *Oithona similis* on the western coast of Grenland during the winter-spring transition

Based on the manuscrit: Zamora-Terol, S., Torkel Gissel, Nielsen, and Enric Saiz (2013) Plankton community structure and role of *Oithona similis* on the western coast of Greenland during the winter-spring transition. Marine Ecology Progress Series, 483, 85-102.

Abstract

The cyclopoid copepod *Oithona similis* is one of the most abundant copepods in the world's oceans, and has a potentially important role in pelagic food webs. However, there is a lack of knowledge on aspects of Oithona's biology and function in plankton communities. In the present study, we aimed to assess and compare its trophic role in Greenlandic coastal waters during the winter-spring transition, with a focus on their winter behaviour, when large calanoids are not present in the surface layer. Two locations were studied: waters offshore from Godthåbsfjord (Nuuk) in winter, and Qeqertarsuaq (Disko Bay) in spring (bloom and post-bloom period). The potential prey of adult females of *O. similis* was quantified, and grazing experiments were conducted to determine feeding rates of adult females on phytoplankton and protozooplankton >10μm. The abundance, stage composition, and egg production of *O. similis* was also investigated. We found that ciliates were the preferred prey for *O. similis*, what confirms its importance linking the microbial food web to higher trophic levels. We observed high egg production rates and efficiencies of *O. similis* in winter, confirming that they are active and successfully reproductive in food limiting winter conditions. Our results stress that *O. similis* is a key component in Arctic and subarctic waters throughout the year linking the microbial part of the food web to higher trophic levels.

INTRODUCTION

Most studies on copepods carried out in Arctic and subarctic seas have focused on large calanoid species, such as *Calanus*. This is mainly due to their high contribution to the copepod biomass relative to smaller species, but also conditioned by the historical undersampling of small copepods due to use of coarse (> 200 μm) plankton nets (Gallienne and Robins 2001). Recent investigations, however, have shown the relevance of small copepods in the Arctic (Auel and Hagen 2002, Hopcroft et al. 2005, Svensen et al. 2011). Although smaller in size, these copepods are important in terms of abundance, biomass and production, especially in coastal waters and fjord systems of the northern hemisphere (Nielsen and Andersen 2002; Lischka and Hagen 2005; Arendt et al. 2010). From an abundance point of view, small copepods often outnumber larger species (Møller et al. 2006, Madsen et al. 2008); and in contrast to large calanoids, which spend winter in diapause (Conover et al. 1988, Conover and Siferd 1993), small copepods can be present all year-round in polar seas (Ward and Hirst 2007, Dvoretsky and Dvoretsky 2009a).

Among small copepods *Oithona* is the most abundant pelagic zooplankton genus on the western coast of Greenland (Head et al. 2003, Thor et al. 2005, Madsen et al. 2008), and within the genus, *Oithona similis* is a cosmopolitan species. *Oithona* spp. have been described to have year-round presence (Hansen et al. 2004) and reproductive activity (Nielsen and Sabatini 1996, Ward and Hirst 2007), and it has been argued that these copepods can exploit microbial food webs more efficiently than calanoid copepods (Nielsen and Sabatini 1996), characteristics that make *Oithona* spp. populations more stable in time and space than those of calanoid copepods (Paffenhöfer 1993). Consequently, *Oithona* spp. could be important food sources for fish larvae and larger zooplankton, especially in those periods of the year when other potential prey are not available.

In Arctic marine environments only few studies on the biology of *Oithona* spp. are available (Nielsen et al. 2002, Madsen et al. 2008, Ward and Hirst 2007, Narcy et al. 2009) and even less is known about their feeding ecology. It has been reported that *Oithona similis* prefer motile prey, especially ciliates (Nakamura and Turner 1997; Lonsdale et al. 2000; Castellani et al. 2005a), although many aspects of their natural diet are still unknown. Thus, our knowledge on their feeding activity in winter in high latitude environments is almost non-existent, since most of the studies have focused on more productive periods of the year (Atkinson 1996, Møller et al. 2006), and lower latitudes (Castellani et al. 2005a).

The present study represents the first attempt to investigate the role of *Oithona similis* on the western coast of Greenland, mainly focusing on aspects of its feeding ecology. For that purpose we carried out different samplings and feeding experiments from winter to late spring to describe seasonal differences in the role of *Oithona similis* in the plankton community, with a strong interest on their feeding behaviour in winter. Additionally, other aspects of *O. similis* biology and ecology (egg production and population structure) were also investigated during the period; and the microplankton and copepod community were described.

METHODS

Study sites and sampling

Sampling for the present study took place in two areas located at different latitudes on the west coast of Greenland (Fig. 1), waters offshore from Godthåbsfjord (Nuuk) and Qeqertarsuaq (Disko Bay).

1. Waters offshore from Godthåbsfjord

In Godthåbsfjord, the study was conducted in a 350 m deep station located at the entrance of the fjord (64 °07'N, 51 °53'W) (Fig. 1), a dynamic area (Mortensen et al. 2011) that represents the transition zone between the fjord and the marine system at Fyllas Banke, which is never covered by sea ice. Samples were collected from the small boat 'Erisaalik' (Greenland Institute of Natural Resources) in the daytime (09.00 to 15.00) from 16th February to 29th March.

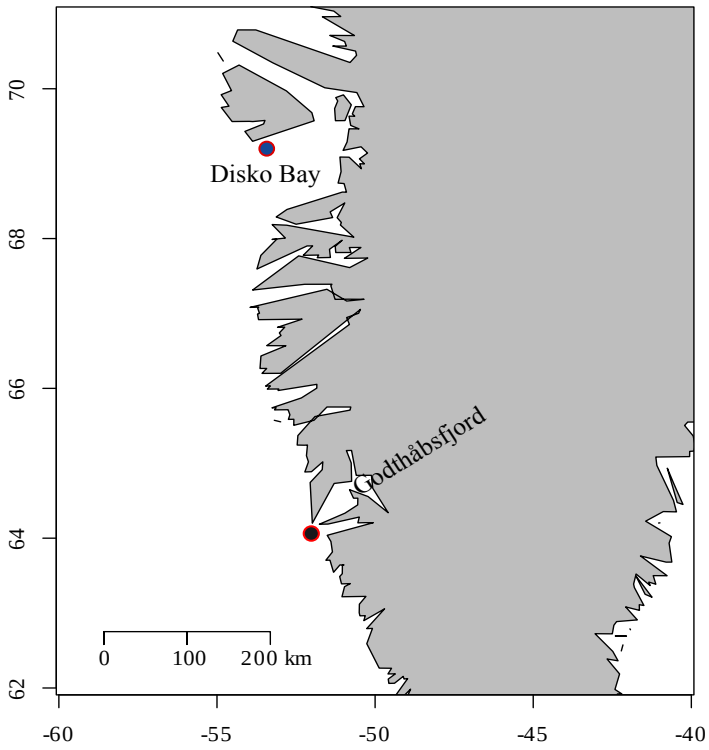

Fig.1. Map showing the sampling stations on the west coast of Greenland. One located at the entrance of the Godthåbsfjord, in Nuuk; and the other one situated approximately 2 km off Qeqertarsuaq, in Disko Bay.

Vertical profiles of water temperature, fluorescence, salinity, and density were measured down to 340 m using a CTD (SBE19plus, SeaCat) equipped with a Seapoint chlorophyll a fluorometer and a Biospherical/Licor sensor. Water samples for chlorophyll a analyses and for estimation of vertical profile of microplankton were taken from 4 depths (1, 15, 50 and 100 m depth) using a 10 litre Niskin water sampler.

2. Disko Bay

The sampling in Disko Bay took place in a 250 m deep station (69º 15'N, 53º33'W) (Fig. 1) from 16th April to 24th May, once the sea ice was broken. The samples were collected from RV 'Porsild' (Arctic Station, University of Copenhagen) from 09.00 to 14.00. Vertical profiles of salinity, temperature, and fluorescence were taken at each sampling occasion using a Seabird SBE25-01 CTD probe, equipped with a fluorometer. Fluorescence was calibrated against chlorophyll a concentrations determined spectrophotometrically in water samples taken at 8 depths (1, 20, 50, 75, 100, 150, 200 and 250 m) with a 10 l Niskin water sampler. In addition, samples for determining the vertical profile of microplankton were taken from 4 depths (1, 15, 50 and 100 m).

Phyto- and protozooplankton

Samples taken for chlorophyll a determination (1000 ml in Godthåbsfjord and 250 ml in Disko) were filtered onto GF/F filters and kept frozen (-18ºC). Later, they were extracted in 96% ethanol for 18 hours, and fluorescence was then measured using a fluorometer (TD-700, Turner Designs) calibrated against a pure chlorophyll a standard.

Microplankton samples were fixed with acid Lugol's solution (2% final concentration), settled (from 50 to 1500 ml depending on the in situ concentration), and counted under an inverted microscope. Diatoms, dinoflagellates, and ciliates (>10μm) were identified and classified into 10-μm size classes.

Microplankton biovolumes were determined from their linear dimensions and volume equations for appropriate geometric shapes (Hillebrand et al. 1999, Olenina et al. 2006), and finally converted into carbon biomass according to the equations provided by Menden-Deuer and Lessard (2000).

Zooplankton

Zooplankton samples for taxonomic and quantitative purposes were collected by vertical hauls using a 45-μm WP-2 closing net (56 cm diameter) equipped with a flowmeter, in Godthåbsfjord; and with a 50-μm Hydrobios type midi (net opening 50 cm x 50 cm) equipped with a flowmeter, in Disko. In Godthåbsfjord the samples were collected above 300 m (0-50, 50-100, 100-200, 200-300 m), whereas sampling was slightly shallower in Disko (0-50, 50-150, 150-200, 200-250 m). The samples collected for zooplankton analysis were preserved in 4% buffered formaldehyde (final concentration).

All copepods were identified, with a special focus on *Oithona* spp. naupliar and copepodite composition. The abundance of females with and without eggs sacs was quantified for *Oithona similis*, and the average number of eggs per sac was determined (n = 10). The carbon content of *Oithona* spp. nauplii and copepodites was calculated by using the length-weight regression given by Sabatini and Kiørboe (1994).

Feeding experiments

Copepods for feeding experiments were collected from the upper 50-100 m using a 50-μm WP-2 net fitted with a large non-filtering cod end. Water for incubation was collected from the depth of maximum fluorescence using a Niskin bottle. A nutrient mixture (15μM NH_4Cl and 1μM Na_2HPO_4) was added to the seawater used in the experiments to compensate for nutrient enrichment due to copepods excretion (Saiz et al. 2013). Adult females of *Oithona similis* were sorted in ice-chilled petri dishes under a stereomicroscope, washed out in filtered seawater, and transferred to polycarbonate bottles previously filled up with the collected seawater (Godthåbsfjord: 2000 ml bottles, 28-35 females per bottle; Disko: 600 ml bottles, 9-15

females per bottle). The bottles were completely filled, plastic film was put over the mouth, and bottles were then placed on a plankton wheel and incubated in a temperature-controlled cold room at light and temperature conditions close to in situ. At least 3 experimental (with copepods) and 3 control (without copepods) bottles were prepared in each experiment and incubated; in addition, 2 initial bottles (control at time 0) were immediately preserved with acid Lugol's (2% final concentration) at the beginning of the experiment to obtain initial prey concentrations.

Experiments were run for approximately 24 hours, and the survival of the females was checked under a stereomicroscope at the end of the incubation. At least 15 females were preserved in 4%-formaldehyde (final concentration) for later sizing and estimation of carbon content. Control and experimental bottles were fixed with acid Lugol's as the initial ones, and kept in a dark room at 4°C until analysis. The identification and counting of the Lugol's samples (initial, control and experimental) were conducted in the same way as for the vertical profile (*see* above).

Clearance and ingestion rates of *Oithona similis* were calculated according to Frost's equations (Frost 1972) after verification that prey growth rates in experimental bottles were significantly different than those in the control ones (t-test, $p < 0.05$). Weight-specific rates were estimated using our measurements of the size of the potential prey (i.e. diatoms, dinoflagellates, and ciliates) and the adult females of *O. similis*, and then converted into carbon using carbon content-size relationships from the literature (see above). To determine possible size preferences in the diet of *O. similis*, the different groups of protozooplankton were pooled into size classes of 10 μm intervals (10-20, 20-30, 30-40, and > 40 μm), and clearance rates were then calculated for each size class of ciliates and dinoflagellates.

Egg production measurements

The abundance of ovigerous females in the population was estimated for each depth strata by the sum of attached and detached egg sacs divided by 2 (assuming that all ovigerous females had 2 eggs sacs). The percentage of ovigerous females at each sampling day was then calculated as the quotient of the estimated abundance of ovigerous females with respect to the total number of females. The abundance of eggs was calculated by multiplying the number of egg sacs by the average number of eggs per sac.

Egg production rates (EPR, eggs female^{-1} day^{-1}) were calculated using the egg-ratio method (Edmondson 1971) based on egg and female abundance data from the 4%-formaldehyde samples collected by vertical tows, and on the egg hatching time (HT, d) as follows:

$$EPR = (eggs/females) \times 1/HT$$

The egg hatching time (HT) was calculated by using the equation given by Nielsen et al. (2002):

$$HT = (0.064+0.0145\ T)^{-1}$$

where T is water temperature (ºC). Weight-specific egg production was calculated using the egg carbon content that was estimated by using the egg volume conversion factor of 0.14 pg C μm^{-3} (Kiørboe et al. 1985), and the female carbon content estimated by the length-weight relationship provided by Sabatini and Kiørboe (1994). The egg production efficiency (i.e., GGE, %) of adult females of *Oithona similis*, i.e., the fraction of ingested food converted into egg production, was calculated as the quotient of weight-specific egg production and ingestion rate, expressed as percentage.

All parameters used for estimating reproductive parameters were calculated for each depth strata of each sampling day, and then depth-weighted averages were calculated to use in the final estimates of egg production measurements (i.e. number of ovigerous females, number of eggs, carbon content of the females, clutch sizes).

RESULTS

Hydrography

The monitoring station at the entrance of the Godthåbsfjord system (Fig. 1), sampled during winter conditions, is located in a turbulent area, with strong vertical mixing, also influenced by the presence of the intense tidal forces in the region (Mortensen et al. 2011). This is corroborated by the CTD profiles which showed a well-mixed water column (Fig. 2), with salinities between 33-33.5, and temperatures below 1ºC. The very low chlorophyll a concentrations found (Table 1), with a uniform distribution in the upper 100 metres, indicated winter conditions, a situation also confirmed by the low diatom concentrations (Fig. 3).

In Disko Bay the structure of the water column was completely different, during the spring bloom and post-bloom. That area is characterized by the presence of sea ice cover during winter, followed by the ice breakup together with the influence of glacier melt water (from Jakobshavn glacier), which results in strong stratification of the water column in spring (Hansen et al. 2012) (see Fig. 2). Once the sea ice breaks up, light enters the water column and triggers the phytoplankton growth (Table 1, Fig. 3).

Table 1. Mean (± SE) chlorophyll *a* concentrations ($\mu g\ l^{-1}$) in the upper 100 metres from each sampling date.

Water depth	Winter			Spring bloom				Post-bloom	
(m)	23 Feb	5 March	29 March	19 April	26 April	3 May	10 May	17 May	24 May
1	0.07	0.07	0.13	8.3 ± 0.09	20.4 ± 2.23	12.8 ± 1.86	10.0 ± 1.13	5.2 ± 0.51	0.9 ± 0.09
15	0.07	0.07	0.14	6.6 ± 0.27	17.9 ± 2.66	14.7 ± 2.78	9.0 ± 1.52	8.2 ± 1.07	1.5 ± 0.21
50	0.08	0.08	0.14	0.9 ± 0.02	5.0 ± 1.02	1.8 ± 0.01	4.3 ± 0.59	8.3 ± 1.08	2.4 ± 0.32
75	0.07	0.07	0.14	0.3 ± 0.09	1.5 ± 0.28	0.8 ± 0.24	1.4 ± 0.22	1.5 ± 0.21	1.4 ± 0.03
100	0.07	0.07	0.14	0.1 ± 0.06	0.8 ± 0.03	0.5 ± 0.04	0.9 ± 0.23	0.9 ± 0.16	1.4 ± 0.20

The stratification in the water column was characterized by warmer and more saline water in the bottom during the bloom period (Fig. 2). The temperature of the surface increased by ca. 2ºC, due to the increase of solar heating, creating a more-defined surface thermocline situated at 20-30 metres depth during the decaying bloom phase (Fig. 2). Notice that vertical profiles corresponding to 24 April and 17 May are missing in Figure 2 due to technical problems with the equipment in those sampling dates; for the sake of comparison, a profile from 22 April was added to the figure. The concentration of chlorophyll a observed in Disko Bay illustrated the transition from the beginning of a bloom to a post-bloom situation (Table 1, Fig. 2).

Potential prey: phyto- and protozooplankton

The winter phytoplankton community in the upper 100 metres was dominated by the chain-forming diatoms *Chaetoceros* spp. and *Thalassiosira* spp. (on average 92% of phytoplankton biomass), with lower densities of *Pseudo-nitzschia* spp., *Navicula* spp., *Skeletonema* spp., and undetermined centric diatoms, as well as the silicoflagellate *Dictyocha speculum* (data not shown). The integrated average biomass of phytoplankton (98% diatoms) was very low with values that ranged from 0.03 to 0.65 μg C L^{-1} corresponding to February and late March, respectively (Table 2). The protozooplankton community was dominated by small (10 to 40 μm) naked oligotrich ciliates such as *Strombidium* spp., *Strobilidium* spp., and *Lohmaniella* spp., with the presence in lower abundances of *Mesodinium* spp. Tintinnids were either present in low abundance or absent in the samplings. Heterotrophic dinoflagellates were also present, dominated by the athecate genera *Gymnodinium* and *Gyrodinium*, and with the presence in lower concentrations of thecate dynophisoid and peridinoid dinoflagellates. The integrated average biomass of ciliates ranged from 0.06 to 0.43 μg C L^{-1}, and the corresponding biomass of dinoflagellates from 0.02 to 0.11 μg C L^{-1} (Table 2). The vertical distribution of protozooplankton in general mirrored the water column structure (Fig. 3).

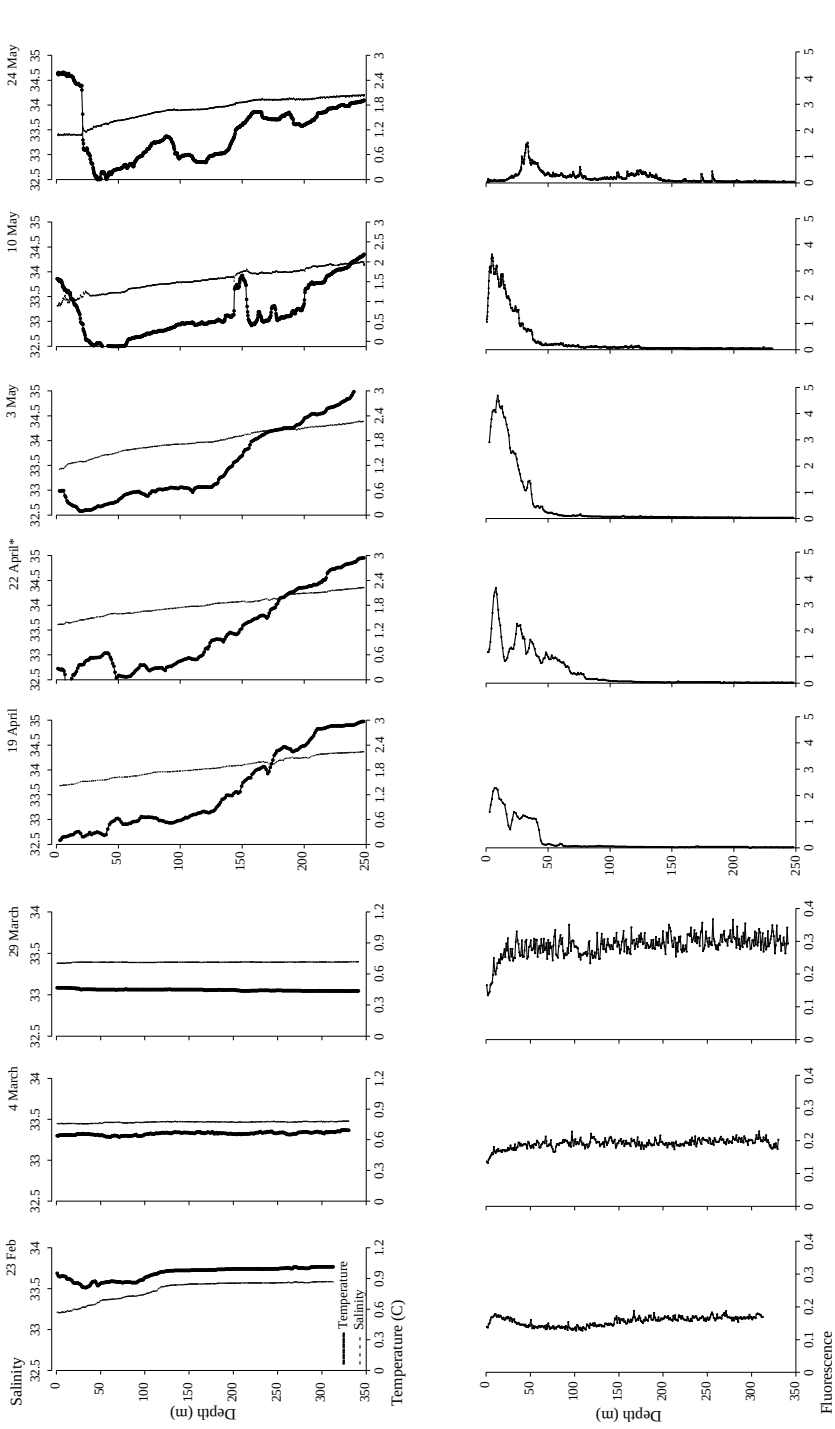

Fig. 2. Vertical profiles of temperature and salinity (top panel), and fluorescence (bottom panel). The asterisk (*) indicates a sampling date replacing the sampling of 26 April. Notice a different scale and bottom depth in the winter samplings.

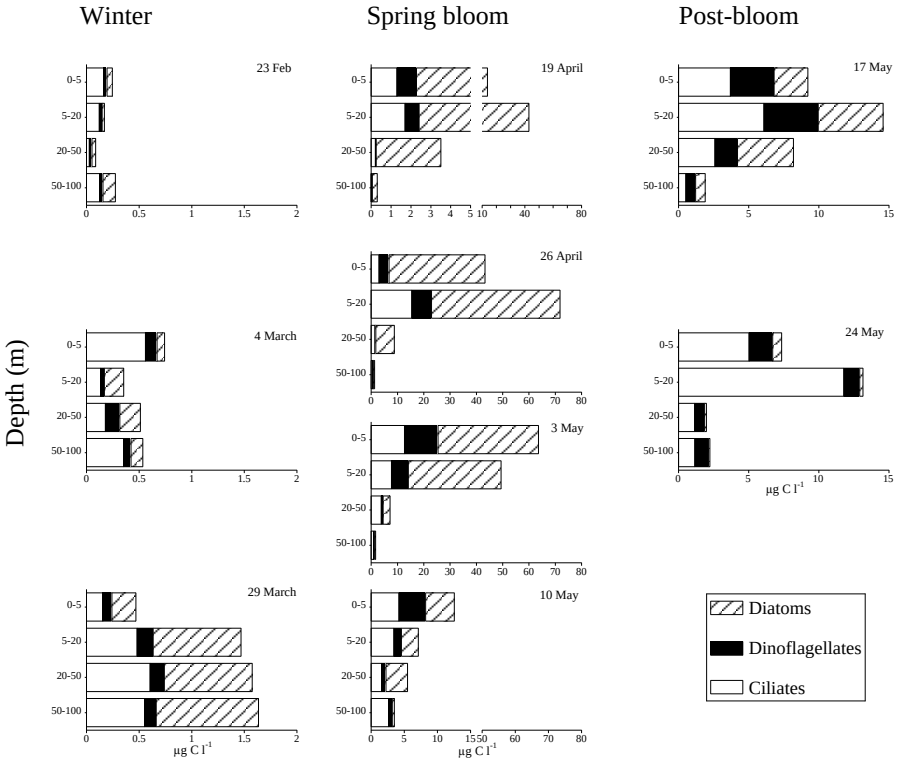

Fig. 3. Vertical distribution of diatom, dinoflagellate, and ciliates biomass (μg C l^{-1}) in the upper 100 m. Note different scales for each period.

During spring, the composition of the phytoplankton community was similar to that found in winter, but the abundances were higher. During the spring bloom the dominating diatom was *Thalassiosira* spp., and high abundances of the small flagellate *Phaeocystis* spp. were present. The integrated average biomass of phytoplankton (excluding *Phaeocystis* spp.) ranged from 2.4 to 17.9 μg C L^{-1} in the bloom phase, and from 0.2 to 3.1 μg C L^{-1} in the post-bloom (Table 2).

Ciliates, again, dominated the protozooplankton community during the spring bloom and post-bloom (Fig. 3; Table 2); and the most abundant ciliates and heterotrophic dinoflagellates were similar to those found in winter. The integrated average biomass of ciliates ranged from 0.6 to 4.7 μg C L^{-1} during the bloom period, and from 2.9 to 3.9 μg C L^{-1} during the post-

bloom; whereas for dinoflagellates the biomass ranged from 0.3 to 2.9 μg C L^{-1} during the bloom, and from 0.8 to 1.9 μg C L^{-1} in the post-bloom phase (Table 2).

The peak abundance and biomass of phytoplankton, dinoflagellates and ciliates were found in the upper 20 m, with a dominance of diatoms in that depth strata during the early phase of the bloom, and a dominance of ciliates later in the decay phase of the bloom (Fig. 3).

Table 2. Mean depth integrated abundance (cells ml^{-1}) and biomass (μg C L^{-1}) in the upper 100 m of each potential prey category (diatoms,dinoflagellates, and ciliates) by sampling location and date.

Location	Date	Period	Diatoms		Dinoflagellates		Ciliates	
			Biomass μg C L^{-1}	Abundance cells mL^{-1}	Biomass μg C L^{-1}	Abundance cells mL^{-1}	Biomass μg C L^{-1}	Abundance cells mL^{-1}
Godthåbsfjord	23 Feb	Winter	0.03	0.14	0.02	0.07	0.06	0.06
	4 March		0.14	0.51	0.08	0.13	0.18	0.15
	29 March		0.65	1.63	0.11	0.17	0.43	0.38
Disko	19 April	Spring bloom	12.25	17.73	0.27	0.52	0.61	0.47
	26 April		17.93	39.96	2.28	2.82	4.68	3.41
	3 May		12.83	22.18	2.91	3.70	4.62	3.31
	10 May		2.38	6.15	0.91	1.60	2.06	1.54
Disko	17 May	Post-bloom	3.07	5.20	1.93	4.19	2.93	3.98
	24 May		0.17	0.39	0.78	2.30	3.86	9.42

The copepod community

Copepods dominated the mesozooplankton, constituting 71%, 94%, and 88% of the total abundance in winter, spring-bloom, and post-bloom periods respectively. The abundance in the different depth strata fluctuated, from a few tens (winter) to 14,000 ind. m^{-3} (post-bloom period) (Fig. 4); and between a few hundreds to 4000 ind. m^{-3} (weighted average) for the whole water column.

During winter the small copepods *Microcalanus* spp., *Microsetella* spp., *Oithona* spp., *Oncaea* spp., and *Pseudocalanus* spp. dominated, whereas the larger copepods *Calanus* spp. and *Metridia* spp.were less abundant (Fig. 4). Average abundances across depths and dates during winter were 105 ind. m^{-3} for *MicroCalanus* spp., 120 ind. m^{-3} for *Microsetella* spp., 67 ind. m^{-3} for *Oithona* spp., and 74 ind. m^{-3} for *Oncaea* spp. The group "others" was composed of much-less-abundant copepod species and other mesozooplankton components (e.g. foraminifera, thecostomata and tintinnidae), which accounted for 25-35 % of the total mesozooplankton community. In winter, *Oithona* spp. contributed 10-15% to the total mesozooplankton abundance, and 15-20% to the copepod abundance. The vertical distribution of the copepod community did not follow any specific pattern, probably due to the strong mixing in that dynamic sampling area (Fig. 4).

During the spring bloom at Disko Bay the diversity of copepods was similar to that observed in winter at the Godthåbsfjord, but in contrast, larger calanoids dominated the copepod community both in abundance (Fig. 4) and biomass (data not shown). However, during the initial phase of the bloom small copepods were still very abundant, and Oncaea spp. and *Oithona* spp. together contributed > 50% of the total copepod abundance (Fig. 4, 19 April). At the peak of the spring bloom there was a decrease in the relative abundance of small copepods due to a large increase in the abundance of *Calanus* spp. (especially nauplii). *Calanus* species reached the maximum abundances in the post-bloom period (Fig. 4). The mean abundance of copepods fluctuated between 1817 ind. m^{-3} during the bloom period, to 2982 ind. m^{-3} during the post-bloom phase (Fig. 4).

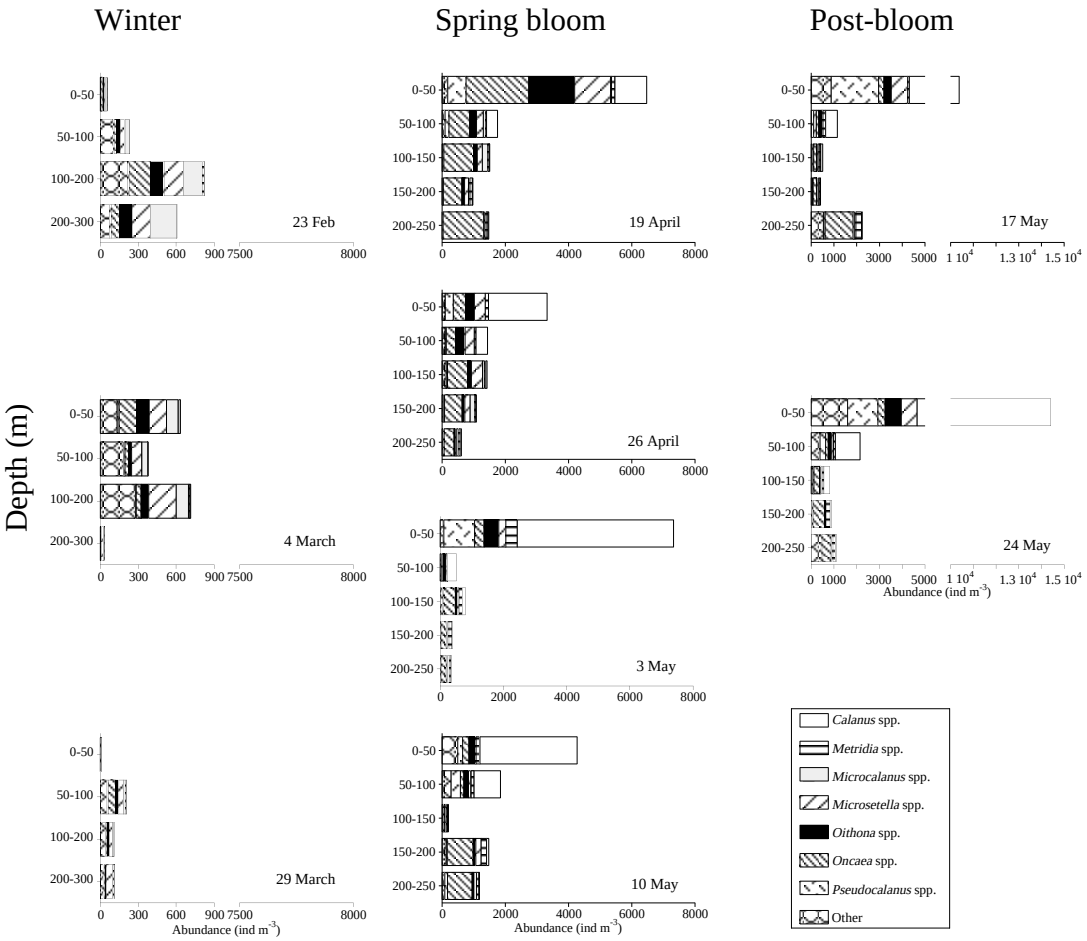

Fig. 4. Vertical distribution of copepod abundance (ind. m^{-3}) in winter (0-50, 50-100, 100-200 and 200-300 m depth strata) and in spring (0-50, 50-100, 100-150, 150-200, 200-250 m depth strata). Each date of sampling is indicated. Note that the scale for 17 and 24 May goes up to $1.5 \cdot 10^4$ ind. m^{-3}.

The maximum abundance of copepods were always located in the upper 50 m, in association with the highest microplankton concentrations (located in the upper 20 m, Fig. 4). *Oithona* spp. contributed 4-15% of the total mesozooplankton abundance during the spring-bloom and post-bloom periods. The other mesozooplankton groups represented less than 10% in all samplings.

It should be mentioned that although some *Oithona* atlantica were observed in the vertical tows, their abundance was negligible and therefore *O. similis* was the target species in this study. From now on we will refer to *Oithona* spp. as *Oithona similis*, considering the presence of other *Oithona* species in the tows as negligible.

Stage vertical distribution, abundance and composition

During winter, the vertical distribution of nauplii and copepodites of *Oithona similis* showed no clear pattern, although maximum abundances for both stages were found at the same depth strata at each sampling date (Fig. 5). From the beginning to the end of the winter, the abundance (weighted average) of naupliar and copepodite stages of *O. similis* varied from 41 to 7 nauplii m^{-3}, and from 33 to 4 copepodites m^{-3} (data not shown). During the winter period the most abundant stage (weighted average over dates) was C5 (~ 8 ind. m^{-3}), followed by N3 (~5 ind. m^{-3}), N5 (~ ind. 5 m^{-3}) and adult females (~ 4 ind. m^{-3}) (data not shown). The relative stage composition was diverse but constant throughout the winter period, both nauplii and copepodites accounted approximately for half of the population at each sampling date, with a slight increase in the contribution of nauplii in the last sampling (Fig. 6). The proportion of adult females was quite stable representing approximately 10% of the population (Fig. 6).

In contrast to the winter in Godthåbsfjord, nauplii and copepodites did not show the same vertical pattern during spring in Disko Bay. Nauplii were mainly located in the upper 50 m, whereas copepodites, although also abundant in the upper layers, were comparatively much more abundant in deeper waters (Fig. 5). The abundance of nauplii stages varied between sampling dates with a non-defined trend, although the latest stages (N4-N6) were most abundant (Fig. 5).

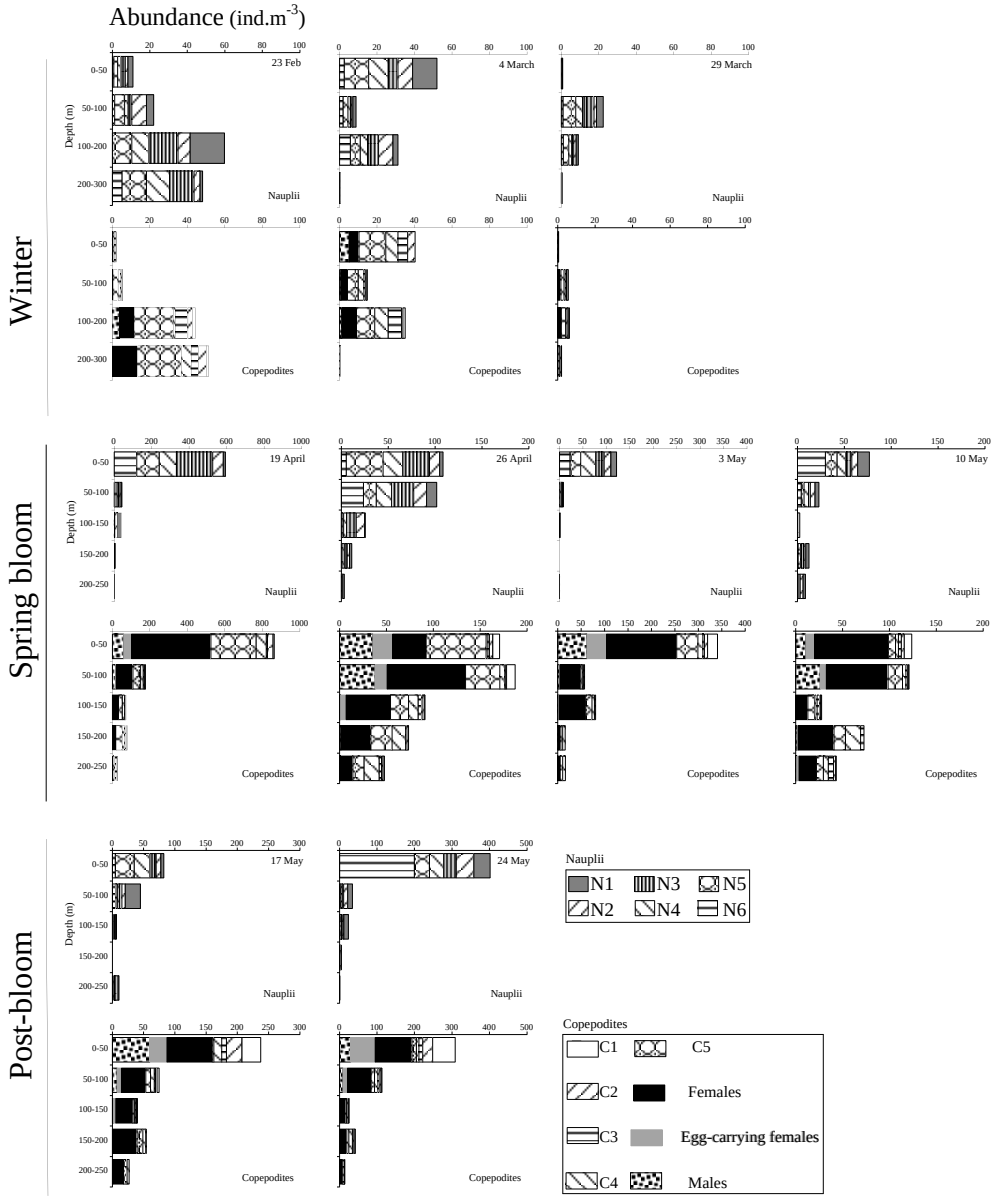

Fig. 5. Vertical distribution of nauplii and copepodites of *Oithona similis* (ind. m^{-3}) during winter, spring bloom and post-bloom. Depth strata as in Fig. 4.

The abundance of copepodites was clearly dominated by adult females, with abundances (weighted average) between 46 and 122 ind. m^{-3} (data not shown) during the spring. Overall, the stage composition of *Oithona similis* during the spring-bloom and post-bloom periods was dominated by later copepodite stages, with the exception of the last sampling of the post-bloom period, in which the number of nauplii increased reaching 50% of the total stage abundance (Fig. 6). During the bloom, the population of *O. similis* became increasingly dominated by adult females, which were by far the most abundant stage, and contributed 30 to 46% of the total abundance of developmental stages (Fig. 6).

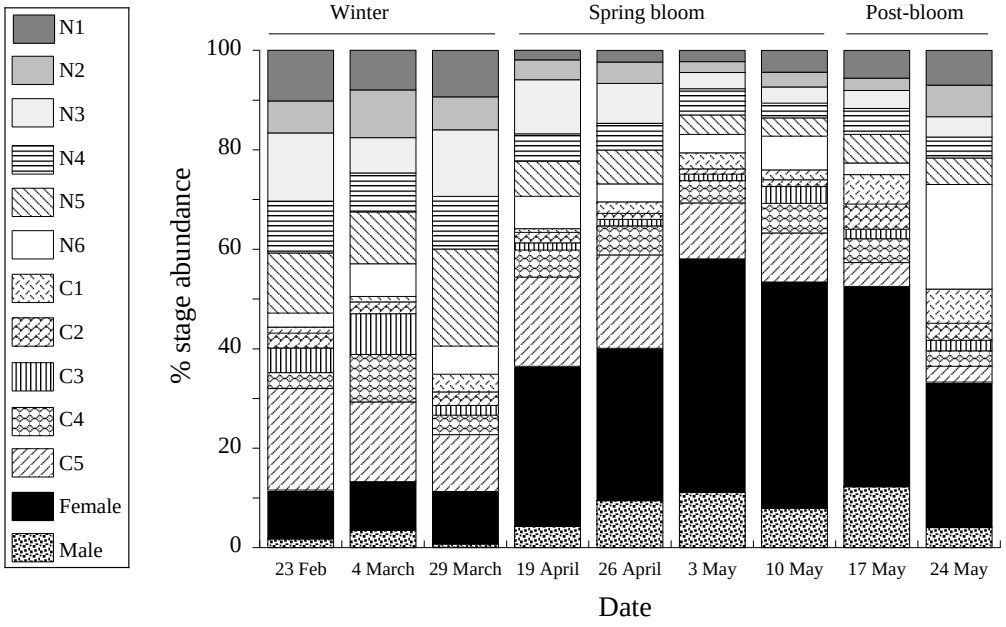

Fig. 6. Relative stage composition of *Oithona similis* (%) for each sampling date.

Feeding rates and prey selection

Ciliates were the most abundant components of the protozooplankton community, and the preferred prey for adult females of *Oithona similis*. There was a significant relationship between ingestion and protozooplankton abundance (n = 9, p = 0.03; data not shown). However, the relation was mainly due to the abundance of ciliates, as evidenced when ciliates alone were considered in the feeding rate calculations (Fig. 7, Table 3). Clearance rates on diatoms were always negative (data not shown), and therefore no feeding rates could be computed.

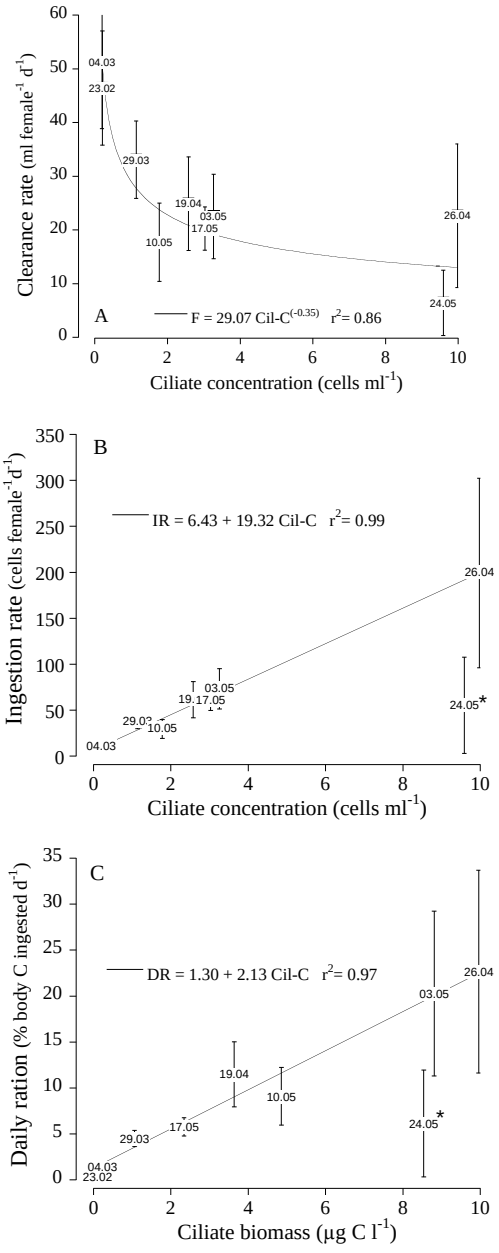

Fig. 7. Feeding rates of *Oithona similis* adult females on ciliates. (A) Clearance rate (*F*, ml female^{-1} d^{-1}), (B) ingestion rate (IR, cells female^{-1} d^{-1}), and (C) daily ration (DR, % body carbon ingested female^{-1} d^{-1}). Each point represents the mean value (± SE) of 3-4 replicates. Cil-C: ciliate concentration, either in cells or carbon. Asterisk (*) indicates a date not included in the fitted equations, because the presence of negative values.

Clearance rates (average ± SE) on ciliates were inversely correlated to ciliate concentration and ranged from 6 ± 6.1 to 51 ± 12.3 ml female^{-1} d^{-1} (Fig. 7A, Table 3). Ingestion rates on ciliates (average ± SE) ranged from maximum values of 199 ± 103 ciliates female^{-1} d^{-1} during the spring bloom, to minimum values of 9 ± 1.4 ciliates female^{-1} d^{-1} during winter (Fig. 7B, Table 3).

Ingestion rates, in terms of daily rations (% body carbon ingested per day), were low in winter (1.4%), and reached maximum values of approximately 20-23% during the spring bloom (Fig. 7C; Table 3). Feeding rates were positively correlated with ciliate abundance and biomass (Fig. 7B and 7C).

Table 3. Experimental conditions of the feeding experiments. For each experiment the number of replicates (*n*), concentration of adult females of *Oithona similis* in the bottles, and female body weight are indicated. Initial concentration of dinoflagellates (dino.) and ciliates in cell and biomass is shown. Mean (± SE) clearance and ingestion rates of dinoflagellates and ciliates are shown, as well as the percentage of body carbon ingested per female and day (daily ration).

Location	Date	Period		Female		Initial concentration			
						Dino.	Ciliates	Dino.	Ciliates
			n	ind. L^{-1}	µg C ind.$^{-1}$	cells mL^{-1}		µg C L^{-1}	
Nuuk	23 Feb	Winter	3	15	0.71	0.16	0.21	0.09	0.22
	4 March	Winter	3	15	0.81	0.15	0.2	0.08	0.23
	29 March	Winter	3	15	0.77	0.27	1.14	0.14	1.06
Disko	19 April	Spring bloom	3	18	0.75	6.45	2.58	2.77	3.64
	26 April	Spring bloom	3	18	0.86	14.34	9.98	11.81	9.96
	3 May	Spring bloom	4	18	0.89	7.33	3.26	11.68	8.81
	10 May	Spring bloom	4	18	0.81	4.25	1.78	4.15	4.86
Disko	17 May	Post-bloom	4	18	0.81	4.81	3.03	2.42	2.34
	24 May	Post-bloom	3	18	0.81	4.41	9.59	1.98	8.54

* Signifiicant rates (*t*-test $p < 0.05$)
n.v.: negative value

The highest clearance rates on ciliates were measured when adult females of *Oithona similis* fed on cells between 20 and 40 μm size (equivalent spherical diameter), and reached maximum values (≈113 ml female^{-1} d^{-1}) when feeding on the size range 30-40 μm (Fig. 8A). Ciliates in the size range 20-40 μm were mainly comprised of *Strombidium* spp. and *Lohmaniella* spp., and lower numbers of *Strobilidium* spp.

Overall, results of feeding rates on dinoflagellates were inconclusive, with negative values in some replicates, and statistically non-significant values in most of the experiments. The results of feeding rates on dinoflagellates are shown in Table 3 and will be discussed later. Clearance rates on dinoflagellates by sizes tended to be higher on the 10-30 μm prey size range (Fig. 8B). In general, for similar-sized prey, ciliates were cleared more efficiently than dinoflagellates (Fig. 8).

Clearance rate		Ingestion rate		Daily ration	
Dino.	Ciliates	Dino.	Ciliates	Dino	Ciliates
mL cop d^{-1}		cells cop d^{-1}		% body C ingested d^{-1}	
19.0 ± 7.8 *	46.4 ± 10.6 *	2.8 ± 1.0*	9.4 ± 1.4*	0.21 ± 0.08	1.4 ± 0.2
8.8 ± 4.7	51.2 ± 12.3 *	1.2 ± 0.6	9.9 ± 1.9*	0.08 ± 0.04	1.4 ± 0.3
21.3 ± 11.3	33.1 ± 7.2 *	5.5 ± 2.7	37.3 ± 7.2*	0.38 ± 0.19	4.5 ± 0.9
-1.3 ± 15.7	24.9 ± 8.7 *	n.v.	61.4 ± 19.8*	n.v.	11.5 ± 3.54
7.7 ± 23.0	22.6 ± 4.9	7.7 ± 301.9	199.2 ± 103	n.v.	22.7 ± 11.0
8.3 ± 4.5	22.5 ± 6.7*	60.5 ± 32.5	73.2 ± 21.8*	15.0 ± 5.8	20.3 ± 8.9
4.6 ± 3.2	17.7 ± 7.3*	18.4 ± 13.1	29.6 ± 10.2*	2.1 ± 1.5	9.1 ± 3.1
2.4 ± 3.1	20.3 ± 4*	10.3 ± 14.3	60 ± 10.2*	0.7 ± 0.9	5.8 ± 1.0
2.2 ± 5.0	6.4 ± 6.1	7.9 ± 22.6	55.3 ± 52.3	0.4 ± 1.3	6.1 ± 5.8

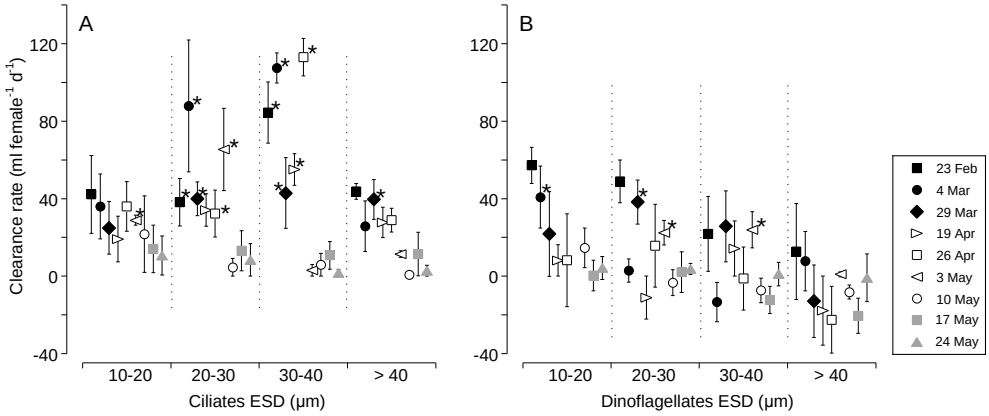

Fig. 8. *Oithona similis*. Size-dependent clearance rates (mL female^{-1} d^{-1}) of adult females on (A) ciliates and (B) dinoflagellates.Symbols in black, white and grey represent winter, spring bloom and post-bloom, respectively. Each point represents the mean value (± SE). Asterisks (*) indicate statistically significant grazing rates (*t*-test, $p < 0.05$)

Egg production and egg production efficiency

Egg-carrying females of *Oithona similis* were present both in winter and spring, with values surprisingly high in winter when compared with spring, and most abundant in the post-bloom period (Table 4). The relative abundance of ovigerous females varied from a minimum of 8% (winter) to a maximum of 56% (post-bloom) (Table 4). Clutch sizes (eggs female^{-1}) ranged from 16 to 26 eggs in winter; from 14 to 36 eggs in the spring bloom; from 14 to 38 eggs in the post-bloom period (Table 4). Mean clutch sizes did not show important differences between the bloom phases, and varied on average from 18 (spring bloom) to 22 (post-bloom) eggs per female (Table 4). Variability between average clutch sizes on different sampling dates is reported in Table 4. Egg size did not vary much during the period of the study, averaging 57 ± 3 μm (n= 20) in diameter.

Table 4. Fecundity of *Oithona similis*. Proportion of ovigerous females, clutch size, egg production rates (EPR), and weight-specific egg production rates (SEPR) are based in weighted average values from WP-2 net and multinet samples. Egg production efficiencies (GGE) are estimated from weight-specific ingestion rates only on ciliates.

Location	Date	Period	Temperature (°C)	Ovigerous females	Clutch size		EPR	SEPR	GGE
				%	eggs female^{-1}	average and range	eggs female^{-1} d^{-1}	% d^{-1}	%
Godthåbsfjord	23 Feb	Winter	1.0	8	16.3 ± 0.33**	20 (16-23)	0.09	0.2	16
	4 March	Winter	0.7	24	19.7 ± 0.93**		0.30	0.7	47
	29 March	Winter	0.4	14	23.9 ± 1.22**		0.17	0.4	9
Disko	19 April	Spring bloom	0.3	15	15.9 ± 1.53	18 (14-36)	0.17	0.4	3
	26 April	Spring bloom	0.4 *	29	17.7 ± 0.39		0.19	0.4	2
	3 May	Spring bloom	0.2	12	18.7 ± 0.10		0.09	0.2	1
	10 May	Spring bloom	1.0	31	20.5 ± 0.56		0.30	0.7	7
Disko	17 May	Post-bloom	1.5 *	36	20.1 ± 1.18	22 (14-38)	0.51	1.1	20
	24 May	Post-bloom	2.3	56	23.2 ± 0.94		0.91	1.9	31

* Temperature not estimated from CTD profiles

** Clutch sizes estimated from n < 10 egg sacs dissected

Mean egg production rates varied from 0.09 (winter and spring bloom) to 0.91 (post-bloom) eggs female^{-1} d^{-1}; and weight-specific egg production rates varied approximately from 0.2 to 2 % d^{-1} (Table 4). *Oithona similis* egg production rates (either per capita or weight-specific) were not very variable from winter to the spring bloom, although females found in the tows were slightly smaller in winter (average 0.60 μg C female) than those ones found in spring (average 0.68 μg C). During the post-bloom period, however, egg production rates increased on average 3.8 times the rates found in the earlier periods. On the other hand, egg production efficiencies were relatively high in winter (on average 23%), decreased during the spring bloom (3%), and increased again during the post-bloom (25%) (Table 4).

DISCUSSION

The environmental settings

The CTD profiles revealed a mixed water column during winter, with homogenous and very low chlorophyll a concentrations in Godthåbsfjord, in contrast to the development of a stratified water column with increasing chlorophyll a during the spring-bloom and post-bloom phases in Disko Bay (Fig. 2). The two localities chosen for the study represented the typical events in the seasonal succession in the oceanography and plankton along western Greenland (Mortensen et al. 2011, Hansen et al. 2012), and can therefore be merged and used to illustrate the winter-spring transition in general. In the present study, the microplankton community of southwestern Greenland was for the first time investigated in winter, showing a clear dominance of ciliates and dinoflagellates. The similar winter importance of protozooplankton in Arctic plankton communities documented further north (Levinsen et al. 2000), enable us to hypothesize that protozooplankton constitute a main food source in the winter feeding of copepods in those latitudes.

The winter copepod community described in this study in the southwest of Greenland was dominated by small copepods, as previously documented in the same area for the late summer (Tang et al. 2011), and for the periods of the year when *Calanus* spp. are not present in the surface layer in Disko Bay (Madsen et al. 2008). Our study documents the abundance of small copepods in winter, suggesting they comprise an important food web component, often unconsidered, outside the period of the main bloom. The even vertical distribution of different species of copepods found in winter could be explained by the turbulent regime in the area (Mortensen et al. 2011), which could blur the "natural" distribution patterns of species separated vertically in less-energetic systems (Haury et al. 1990). However, during the spring bloom and post-bloom, when *Calanus* spp. took over the surface water, the smaller copepods were more abundant in deeper waters.

Population dynamics of *Oithona similis*

Even though it is one of the most abundant copepods in Arctic and subarctic regions (Auel and Hagen 2002, Hopcroft et al. 2005, Møller et al. 2006, Madsen et al. 2008, Svensen et al. 2011), the population dynamics of *Oithona similis* have not been investigated thoroughly in Greenland. In this study, we found all developmental stages of *O. similis* present during the period of sampling. The presence of egg-carrying females during winter confirmed the year-round reproduction strategy described for *O. similis* from lower latitudes (Kiørboe and Nielsen 1994, Sabatini and Kiørboe 1994). Moreover, the presence of the earliest nauplii stages in winter evidenced a successful recruitment despite unfavourable food conditions. In spring, *O. similis* females were present in the entire water column but the younger stages were mostly found near the surface.

We observed an increase in nauplii abundance by the end of May (post-bloom), parallel to an increase in ovigerous females abundance indicating that the peak production of *O. similis* must occur later in the season. The eggs produced by females late in the period will form the basis of the high abundance of *O. similis* normally observed in summer-autumn (Hansen et al. 1999, Madsen et al. 2008, Zamora-Terol et al. unpubl.).

However, 2010 was a historically warm year in Greenland (Jensen and Rasch 2011), which could result in an early initiation of the high productive period. Periods of low productivity are rarely included in studies of copepod life history, in that sense our study is unique because included the pre-bloom population dynamics of *O. similis*, thereby improving our knowledge on the ecology of this copepod in the period of the year when it dominates the plankton community.

Feeding rates and prey selection

In all the feeding experiments carried out in the present study we found that *Oithona similis* had a clear preference for medium-sized ciliates (20-40 μm). Preference for ciliates has been reported from ecosystems at lower latitudes, not only for *O. similis* (Nakamura and Turner 1997, Castellani et al. 2005a) but also for other *Oithona* species (Atienza et al. 2006: O.nana, Zamora-Terol et al. unpubl.: O.attenuata). Experiments with *O. similis* feeding on natural plankton populations found that when dinoflagellates were as abundant as ciliates, *O. similis* still preferred ciliates (Nakamura and Turner 1997, Castellani et al. 2005a), confirming the pattern observed in this study and suggesting the active selection for ciliates by *Oithona* spp. In this context, our grazing rates of *O. similis* on dinoflagellates were controversial throughout the different experiments. We observed no significant ingestion (t-test > 0.05) in most of the experiments conducted, occasionally resulting in negative clearance values in some replicates, probably as a consequence of trophic cascade effects. Therefore, we cannot fully discard that *O. similis* grazed on dinoflagellates, but in any case predation on ciliates seemed to be more important.

As expected, we found no ingestion of diatom, in agreement with most previous studies (Uchima 1988, Lischka and Hagen 2007, Nishibe et al. 2010). Although occasionally in the literature reports on diatom consumption by *Oithona* spp. can be found (Lampitt and Gamble 1982, Atkinson 1996, Atienza et al. 2006, Pond and Ward 2011), it is not clear what mechanisms are involved.

In this regard, the work by Svensen and Kiørboe (2000) suggests that ambush raptorial copepods as *Oithona similis* should rely on hydromechanical signals for detecting individual prey; and Kiørboe and Visser (1999) further showed that the hydromechanical signals from non-motile and slow-sinking particles are not in the detection threshold of *O. similis*.

In the case of size preference, there are also different opinions on the capability of *Oithona* spp. to feed on very small or large prey. We did not check the ingestion of small flagellates (<5μm), but we observed that small (<5-10 μm) single diatoms were not ingested in any of the experiments. In the present study, *Oithona similis* captured particles mainly within the 10-40 μm size range, what is in agreement with previous investigations on the same species (Drits and Semenova 1984, Castellani et al. 2005a, Nishibe et al. 2010); however, wider range of sizes has also been reported (4-300 μm for O.nana, Lampitt and Gamble 1982). It is very likely that in natural environments, size preferences will be influenced by the abundance and type of prey comprised in that range, in particular Strombidium-like and Strobilidium-like ciliates.

Daily rations (% body C ingested d^{-1}) of *Oithona similis* found in this study were positively correlated with the abundance of ciliates, and were in average 1.4% in winter, 16% during the spring bloom, and 6% in the post-bloom. Assuming that metabolic costs would account for 1.4% d^{-1} (at 4ºC, Castellani et al. 2005b), the ingestion rates measured in spring were more than enough to cover respiration costs at mean temperature encountered in Disko Bay. For the winter period, however, ingestion rates were rather low and only occasionally could be sufficient to cover metabolic requirements, suggesting the need to use lipid reserves to ensure the winter survival strategy of *O. similis* (Narcy et al. 2009). In contrast to large calanoid copepods that go into diapause, during the winter *O. similis* appears to be actively feeding and reproducing (see below), even if at low rates, despite the low temperatures and nutritionally diluted environment.

Egg production rates and efficiency

Overall, the fecundity of *Oithona similis* in the present study did not respond strongly to the development of the spring bloom and associated microbial community (Fig. 9A and 9B), and it was only during the post-bloom that a substantial increase in egg production occurred.

The proportion of ovigerous females found here (8-56%) was in the range of reported values in other high latitude environments (0-67% Ward and Hirst 2007, 22-52% Dvoretsky and Dvoretsky 2009b). The maximum percentage of ovigerous females (56%) was observed at the end of May (post-bloom), and was close to the maximum values reported for *O. similis* in the Southern Ocean (67%, Ward and Hirst 2007). The presence of ovigerous females in winter also suggests they are reproductively active throughout the year, as previously reported in other polar environments (Fransz and Gonzalez 1995, Metz 1995, Lischka and Hagen 2005).

Clutch sizes found for *Oithona similis* were within the range reported in the literature (18-26 eggs per female, Castellani et al. 2007, Dvoretsky and Dvoretsky 2009a). Despite the changes in food availability throughout the study period, clutch size did not vary much (16-24 eggs female^{-1}), in agreement with previous studies that also reported little variation throughout the year (Danish coastal waters: Kiørboe and Nielsen 1994; Barents Sea: Dvoretsky and Dvoretsky 2009a; Southwest Atlantic: Temperoni et al. 2011). We could not find a correlation between clutch size and food availability (Fig. 9A) or any other factors, suggesting that clutch size is weakly dependent on environmental factors. Egg production rates in the present study (0.1-0.9 eggs female^{-1} day^{-1}) are within the range of the lowest values reported in earlier studies on the same species from lower (Castellani et al. 2005a, Ward and Hirst 2007), and similar (Dvoretsky and Dvoretsky 2009b) latitudes.

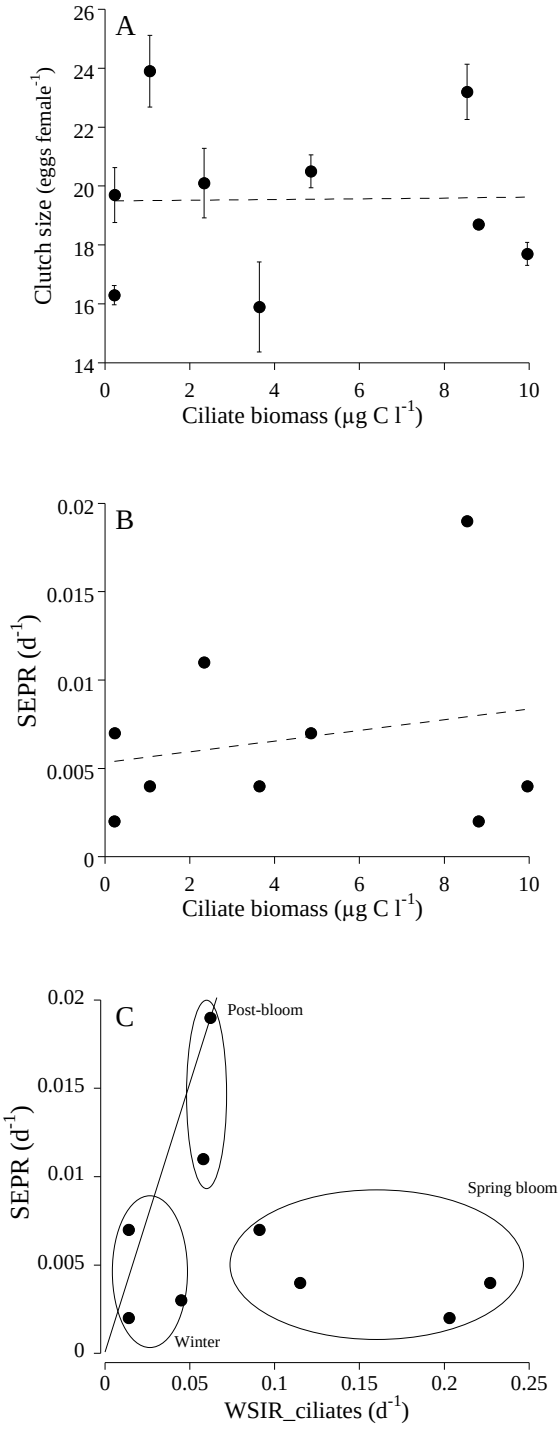

Fig. 9. *Oithona similis* adult females. (A) Clutch size (eggs female^{-1}) and (B) weight-specific egg production rates (%, d^{-1}) plotted against the average biomass of ciliates (µg C l^{-1}); dash lines show linear regressions. (C) Egg production efficiency (i.e. GGE); continuous line indicates 30% GGE.

Egg production rates are influenced by different factors, and food availability has been considered one of the most important factors driving feeding and egg production rates for calanoid copepods (Kiørboe and Nielsen 1994, Saiz and Calbet 2011). We did not find any clear relationship between food availability and any reproductive parameter of *Oithona similis*. In fact, average egg production rates were the same in winter and during the spring bloom, 0.2 eggs female^{-1} day^{-1}. However, an increase of the egg production (0.5-0.9 eggs female^{-1} day^{-1}) was observed during the post-bloom phase, very likely due to a delay between coupling of food availability (which increased during the spring bloom) and fecundity. These observations are in agreement with studies that covered a longer part of the reproductive season for *O. similis*, and that reported reproductive peaks in summer or early autumn (Lischka and Hagen 2007, Madsen et al. 2008, Dvoretsky and Dvoretsky 2009a). In this regard, we should not ignore the temperature as an important factor controlling the egg production of *Oithona* spp. Maximum egg production rates in egg-carrying copepods are greatly determined by hatching times, which are at the same time dictated by temperature (McLaren et al. 1965; Nielsen et al. 2002). Studies on the egg production of *Oithona* spp. have described the strong effect of water temperature on hatching times and egg production rates (Sabatini and Kiørboe 1994, Uye and Sano 1995, Drif et al. 2010), as has also been documented in calanoid copepods (see Mauchline 1998).

Weight-specific egg-production rates reported in this study for *Oithona similis* (0.2-2% d^{-1}) are in agreement with previous investigations on the same species (0-2% d^{-1} Ward and Hirst 2007, 0.5-4% d^{-1} Dvoretsky and Dvoretsky 2009b), and slightly higher than values found for *Oithona* spp. in the same area during the same time of the year (0.004-1.5 % d^{-1}, Madsen et al. 2008). In general, our maximum weight-specific egg-production rates are in good agreement with previous studies in high latitude environments at temperatures similar to ours (Ward and Hirst 2007, Dvoretsky and Dvoretsky 2009b).

Surprisingly we found that the egg-production efficiency (i.e. gross growth efficiency, GGE) was high during winter and very low during the spring bloom (Table 4). As mentioned above, it is very likely that females of *Oithona similis* were using lipid reserves in winter to compensate for the low food concentration, and these reserves contributed to fuel egg production. In this regard, it has been observed how *O. similis* exhibit low content of lipids in spring in the Arctic (Lischka and Hagen 2007). This fact would explain that the high feeding rates in spring do not translate into high egg production, but are very likely allocated to refuel the lipid reserves. Although some studies have investigated reproduction of *O. similis*, very few have compared feeding with egg production and calculated their growth efficiencies (Castellani et al. 2005a). The egg production efficiency (i.e. GGE) found in this study was in average 15%, whereas Castellani et al. (2005a) reported 47% in the North Atlantic. Difference in estimations, however, could be due to the use of egg-production rates calculated from the population, instead of individual females measurements. The GGE found in this study was in good agreement with the value estimated in the laboratory for O.davisae (16%) based on individual female incubations (Zamora-Terol and Saiz 2013).

In this study we have documented how *Oithona similis* is actively feeding and successfully reproducing during winter. We have also confirmed the preference of *Oithona* spp. for ciliates as prey items, highlighting their key role in pelagic food webs as a link between the microbial food web and higher trophic levels. This role is especially relevant in Arctic and subarctic plankton webs when larger *Calanus* spp, are not active in the water column. In conclusion, the capability of *Oithona* spp. to survive and succeed when unfavourable conditions are present in the water, might explain the success of the genus, not only in polar environments but also in marine environments worldwide.

Acknowledgements

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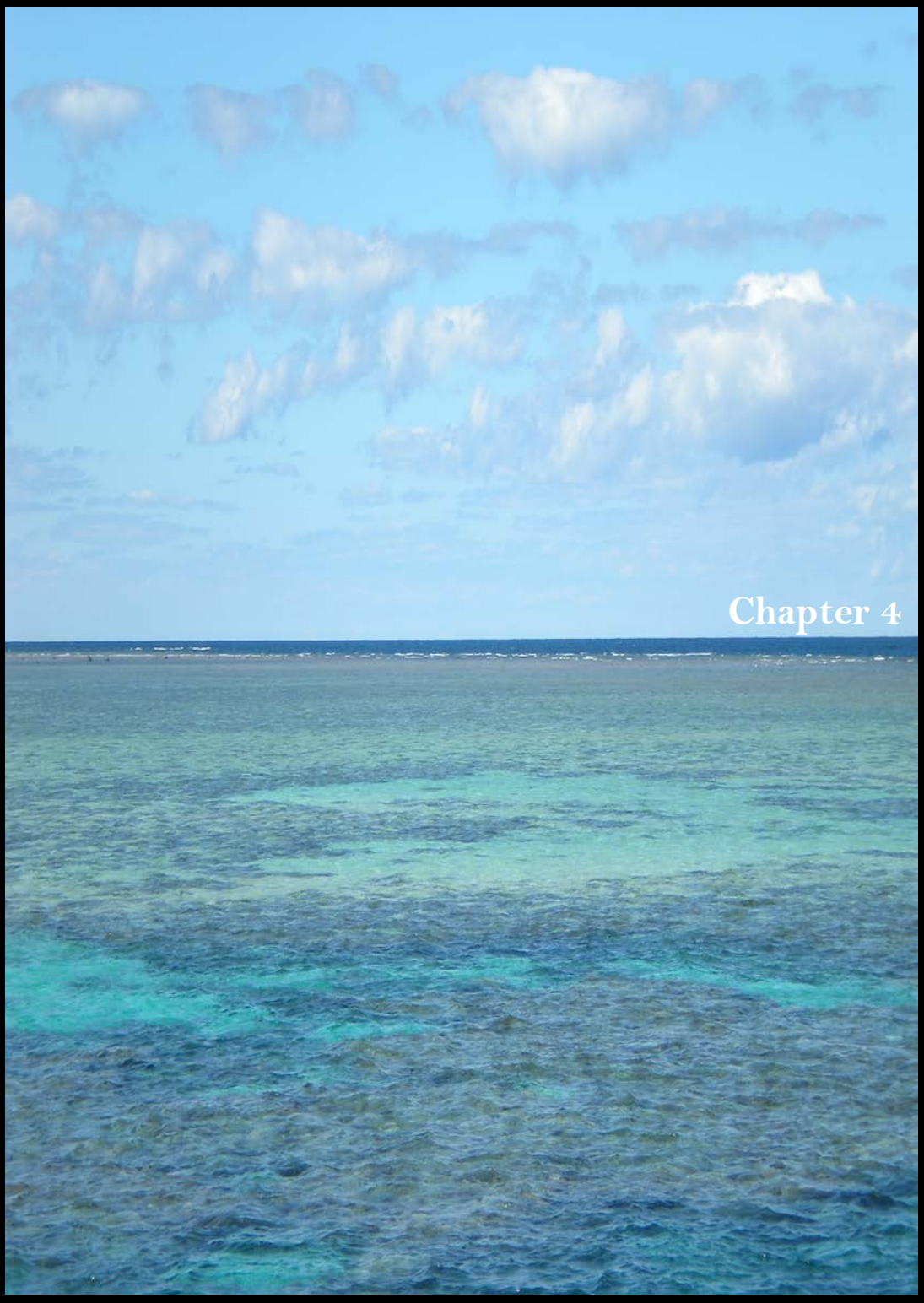

Chapter 4

Feeding and egg production of *Oithona* spp. in tropical waters of North Queensland

Based on the manuscript: Zamora-Terol, S., A.D. McKinnon, and Enric Saiz. Feeding and egg production of *Oithona* spp. in tropical waters of North Queensland, Australia

Abstract

Despite the acknowledged importance of small copepods of the genus *Oithona* in marine pelagic ecosystems, there is still little information about their ecological role, potential food resources, and egg production in tropical environments. In the present study, feeding and egg production rates of adult females of two species of *Oithona* were determined in two different tropical food webs in Northeastern Australia. *Oithona attenuata* was studied in waters of the Great Barrier Reef, and *Oithona cf dissimilis* was studied in a mangrove system. Both species ingested both dinoflagellates and ciliates preferentially to other prey items of the nano- and microplankton community. Clearance rates ranged from 3.7 to 10.4, and from 4.3 to 18.1 mL female^{-1} day^{-1} for dinoflagellates and ciliates respectively. The percentage of body carbon ingested per female and day was less than 1% feeding on dinoflagellates, and varied from 1 to 10% feeding on ciliates. Our results suggested that *Oithona* spp. fed on small flagellates (5-20 μm), although the contribution of carbon to the diet was low (2% body carbon). Egg production and weight-specific egg production rates ranged from 0.22 to 3.34 eggs female^{-1} day^{-1}, and 0.2 to 4.5 % day^{-1} respectively. The ingestion rates measured in all the feeding experiments were too low to sustain both metabolic and egg production costs, indicating that other food resources contribute to the diet of *Oithona* spp. in tropical environments.

INTRODUCTION

Zooplankton have a key function as a link between primary producers and higher trophic levels in pelagic food webs, as well as an important role both as grazers and in regenerating nutrients in plankton communities. Copepods are the most abundant and ubiquitous group of zooplankton in the oceans (Humes 1994), and small copepods, in particular, are very important components of plankton communities as intermediates between the microbial and classical food web (Wickham 1995). This role is especially important in oligotrophic tropical ecosystems such as coral reefs, where small organisms such as nano- and microplankton comprise an important fraction of the total biomass (Furnas et al. 1990). In contrast, mangrove habitats are highly productive tropical systems that could act as a nutrient reservoir of zooplankton production, and where small copepods are often the most abundant zooplankton with an important role in regenerating and exporting nutrients (McKinnon and Ayukai 1996). Although nowadays the importance of small species of copepods in marine ecosystems is widely accepted (Turner, 2004), their function in plankton communities is not completely understood, and their trophic role in tropical waters has been poorly studied.

The cyclopoid copepod genus *Oithona* is one of the most abundant in temperate and polar seas all around the world (Gallienne and Robins 2001, Castellani et al. 2007; Møller et al. 2006; Svensen et al. 2011.), and tropical waters are not an exception (Hopcroft et al. 1998; Satapoomin et al. 2004; McKinnon et al. 2008). In Australia, *Oithona* spp. is one of the most abundant copepods in coastal waters of the Great Barrier Reef (McKinnon and Thorrold 1993; McKinnon, Duggan, & De'ath, 2005), and a dominant copepod in mangrove habitats (Robertson et al. 1988; McKinnon and Klumpp 1998). Many studies have reported the importance of *Oithona* in terms of abundance in different marine ecosystems (Gallienne and Robins 2001), but their role in food webs is poorly known since only a few studies have addressed this topic (Calbet et al. 2000; Atienza et al. 2006; Zamora-Terol et al. 2013). *Oithona* is considered an omnivorous copepod with preference for motile prey (Nakamura and Turner 1997), preferring ciliates over other components of the microplankton (Lonsdale et al. 2000; Castellani et al. 2005; Zamora-Terol et al. 2013). However, there is still a controversy on the

contribution of other potential prey items, such as diatoms, small flagellates, or dinoflagellates (Atkinson 1996; Calbet et al. 2000; Atienza et al. 2006).

This study was conducted in two different ecosystems on the northeastern coast of Australia: 1) waters of the Great Barrier Reef lagoon (GBR), where we found a transition between the oceanic oligotrophic clear waters of the Coral Sea and turbid waters near the coast; 2) and a mangrove system situated in Townsville. The main food resources and egg production of adult females of *Oithona* spp. were investigated with the goal of understanding their ecological role in the plankton community in these contrasting tropical ecosystems. Keeping in mind that in tropical waters we expect high metabolic costs associated with high water temperatures as well as generally low stocks of food resources that limit the growth of copepods (McKinnon and Duggan 2003), we also assessed the gross growth efficiency of *Oithona* spp.

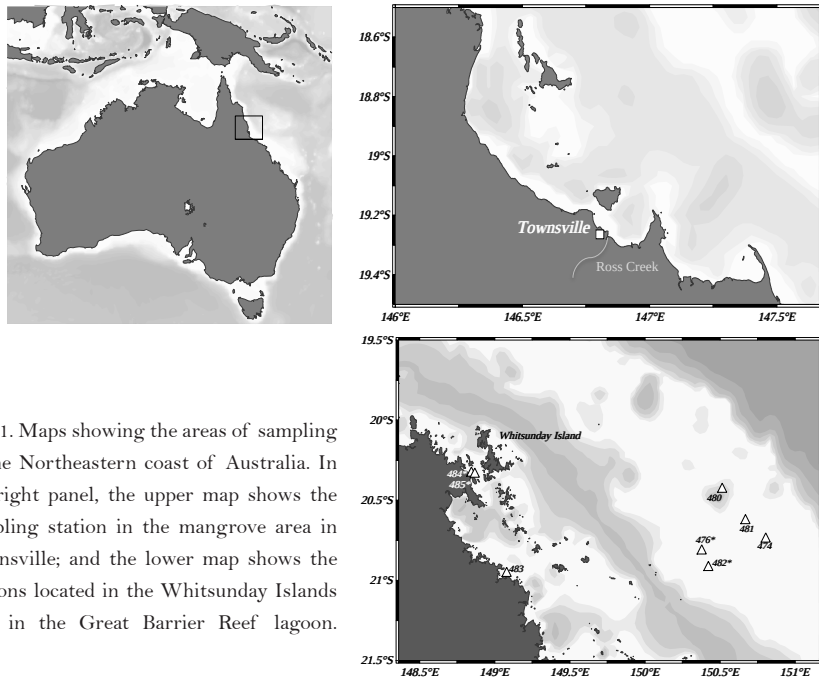

Fig. 1. Maps showing the areas of sampling in the Northeastern coast of Australia. In the right panel, the upper map shows the sampling station in the mangrove area in Townsville; and the lower map shows the stations located in the Whitsunday Islands area in the Great Barrier Reef lagoon.

METHODS

Study site and sampling

The fieldwork in the GBR lagoon took place on board R/V Cape Ferguson in the Whitsunday Islands during the period 24 March-6 April 2011; whereas the fieldwork in the mangrove took place in Ross Creek, an estuarine inlet in the city of Townsville, during April-June 2011 (Fig. 1). In the GBR lagoon 11 stations were sampled during the cruise, but feeding experiments were only conducted in 4 of them due to bad weather conditions (Table 1); in the mangroves a single station was sampled twice and two experiments carried out (Table 1) In the GBR lagoon the potential copepod prey was collected by sampling subsurface water using a 10L Niskin bottle, and the copepods were collected by gentle oblique tows using a 50 µm plankton net at different stations not deeper than 20 metres (Table 1). In the mangrove, seawater containing the microplankton was collected with a bucket and gently transferred to a carboy, whereas copepods were collected in short subsurface tows using a 100-µm plankton net. A coarser net was used in the mangrove to avoid high abundance of early stages of *Oithona spp.* in the samples collected. The copepods were collected in the morning, when the tide was the maximum in the area, to ensure the presence of *Oithona* species in the samples.

Feeding experiments at GBR lagoon

Feeding experiments were conducted with natural particle assemblages. The water collected in the Niskin bottle was gently transferred to a carboy and amended with a nutrient mixture (15 μM NH_4Cl and 1 μM Na_2HPO_4) to compensate for nutrient enrichment due to copepod excretion. This incubation water was then used to fill 2 replicates for time 0 bottles, and 3-4 replicates for control (without copepods) and experimental (with copepods) bottles.

After the catch, the copepods were immediately transferred into a bucket and transported to the lab on board where between 30-35 adult females were sorted, washed in filtered seawater, and placed in 500 mL polycarbonate bottles (experimental bottles). All bottles were completely filled and plastic film was placed in the mouth to avoid the formation of bubbles during incubation.

Table 1. Sampling stations. Location, sampling date, station depth, temperature, salinity and average chlorophyll *a* (Chl *a*) in the water column.

Location	Station	Coordenates	Date	Max depth (m)	Temperature (ºC)	Salinity	Chl *a*
Great Barrier Reef	POM476*	20º 48.73' S 150º 22.67' E	29-Mar	13	27.2	34.52	2.48
	POM480	20º 44.28' S 150º 48.32' E	30-Mar	51	27.3	34.90	1.89
	POM481	20º 37.38' S 150º 40.22' E	31-Mar	53	27.3	34.85	2.04
	POM482*	20º 54.92' S 150º 25.4' E	01-Apr	17	26.9	33.34	0.98
	POM483	20º 57.13' S 149º 4.81' E	02-Apr	6	25.7	27.44	4.65
	POM484*	20º19.61 S 148º 50.64' E	03-Apr	13	26.3	30.95	2.28
	POM485*	20º 19.54' S 148º 50.64' E	04-Apr	13	26.2	30.84	2.43
Ross Creek	Stanley St. bridge*	19º 26.04 S 146º 81.82' E	12-May	3	24.0	≈ 35**	-
	Stanley St. bridge*	19º 26.04 S 146º 81.82' E	08-Jun	3	24.0	≈ 35**	-

*Feeding experiment conducted
**Aprox. value from McKinnon and Klumpp 1998

Oithona attenuata was the species chosen for the experiments in the GBR lagoon because it was more often found in the samples than other *Oithona* species. After placing the copepods in the experimental bottles, both experimental and control bottles were incubated on deck for 24 hours, and mixed by repeatedly turning upside down every 2 hours (daytime) and 4-6 hours (night) to avoid sedimentation of cells.

From the time 0 bottles, 20 mL were removed for nanoplankton identification using DAPI staining, and 300 mL fixed with 2% acid Lugol's solution (Kiørboe and Nielsen 2002) for microplankton identification. After the incubation time, control and experimental bottles were processed in the same way as the 0-times. The females were removed from the experimental bottles and examined under a microscope to check their viability during the experiment, and then preserved in formaldehyde (4% final concentration) for later measuring of the prosome length ($n = 20$) and estimation of the mean clutch size on those females carrying egg sacs (see below egg production measurements).

Samples for estimation of grazing on the nanoplankton community (i.e. flagellates < 10 µm) were fixed with 1% glutaraldehyde and kept at 4ºC for at least 6 hours; later the samples were filtered onto a 2 µm black polycarbonate membrane filter and stained with the nucleic acid stain DAPI (5 µg mL^{-1}). The filters were mounted on slides with immersion oil and frozen for a later analysis with a Zeiss epifluorescence microscope. For the estimation of grazing on the microplankton community, 100-ml aliquots of the samples preserved in acid Lugol's solution were settled (Utermöhl 1958) and analysed under the inverted microscope. Diatoms, dinoflagellates and ciliates were identified, counted, and classified by sizes (10-µm size classes). Microplankton biovolumes were determined from their linear dimensions and volume equations for appropriate geometric shapes (Hillebrand et al. 1999; Olenina et al. 2006), and finally converted into carbon biomass according to the equations provided by Mender-Deuer and Lessard (2000).

Feeding rates were calculated according to Frost´s equations (Frost 1972) after checking that prey growth rates in experimental bottles were significantly different than in the controls (*t-test* $p < 0.05$). Weight-specific ingestion rates were calculated by using the length-weight regressions given by McKinnon and Klumpp (1998) for adult females of *Oithona attenuata*.

Feeding experiments in the mangrove

The feeding experiments in Ross Creek were carried out at the Australian Institute of Marine Science in Townsville. Natural particle assemblages were also used in these experiments, and the water, in this case, was collected *in situ* using a bucket and gently transferred to a carboy. The copepods collected were transferred into a bucket filled up with surface water, and immediately transported to the laboratory. Once in the laboratory, the nutrient mixture (see above) was added and the incubation water was gently siphoned through a 132-µm mesh to remove juveniles of *Oithona* spp. (very abundant in the samples) and other potential grazers. The water was then transferred into 250 mL polycarbonate bottles (2 replicates for time 0, and 4 replicates for both control and experimental treatments). Although different species of *Oithona* were found in the samples (e.g. *O.nishidai*, *O.aruensis*, *O.attenuata*, *O. cf dissimilis*),

we chose *Oithona cf dissimilis* because that was the most abundant one. Between 15-19 adult females were sorted and placed in the experimental bottles. The time 0 treatment bottles were fixed with 2% acid Lugol's solution, and the control and experimental bottles were placed in a plankton wheel rotating at 0.5 r.p.m in a constant temperature room for 24 hours, with a natural light cycle. The bottles from time 0 were fixed with 2% acid Lugol's solution for later counting and identification of microplankton. After the incubation time, the samples were fixed, settled and identified in the same way as in the GBR; feeding rates were also calculated in the same way (*see* above).

Egg production measurements

Zooplankton samples for the estimation of egg production rates were taken using a 73-µm plankton net in the GBR lagoon, and a 50-µm plankton net in the mangrove, and preserved in 4% (final concentration) formalin for later analysis. The samples were divided into subsamples using a Folsom plankton splitter in the lab. An aliquot (5 mL) was taken using a Stempel pippete (Hensen 1887), and *Oithona* spp. females were counted in a mini-Bogorov chamber, and classified into those carrying eggs and those not carrying egg sacs. The egg sacs found were dissected (10-40 eggs sacs dissected depending on the abundance) to estimate the mean clutch size, which was used for the estimation of the egg production rate.

The average population egg production rates (EPR, eggs female^{-1} d^{-1}), computed for the ensemble of ovigerous and non-ovigerous females, was calculated using the egg-ratio method according to the following equation, modified from Uye and Sano (1995),

$$EPR = CS \times OF / TF \times D$$

where CS is clutch size (eggs female^{-1}), OF is the number of ovigerous females present in the sample, TF is the total number of females, and D is the embryonic time of the eggs.

The egg development time (D) was calculated by using the equation from McKinnon and Klumpp (1998) for *Oithona nishidai* (as *Oithona* sp. 1) from nearby mangrove creeks in North Queensland as follows:

$$D = 1.68\ (T\text{-}21.56)^{-0.26}$$

Weight-specific egg production rates were calculated by using the length-weight regressions given by McKinnon and Klumpp (1998) for adult females of *Oithona attenuata*, and the egg carbon content estimated from the equation given by Uye and Sano (1995). The egg production efficiency (i.e. gross growth efficiency) of *Oithona* spp. was estimated by dividing the weight-specific egg production rate by the weight-specific ingestion rate.

In addition, we investigated the clutch size, hatching success, and hatching time of adult females of *Oithona aruensis* from the mangrove. We placed 48 individual adult females of *O. aruensis* in multi-well plates and monitored them during 4 days. The plates were placed in a dark room at 25ºC and checked twice a day.

RESULTS

Environment

During the cruise conducted in the GBR lagoon the temperature was approximately 26-27ºC, the salinity varied between 28 and 35, and chlor *a* concentration ranged from 0.98 to 4.65 μg L^{-1} (Table 1). The lowest salinities and highest chlorophyll *a* concentration were found in those stations located closer to the coast (i.e. POM483, 484 and 485), and the highest chlor a concentration recorded (4.65 μg L^{-1}) occurred in the station located closest to Mackay, in the proximity of the mouth of the Pioneer River. On the other hand, in the sampling conducted in the Ross Creek, the temperature was 24ºC.

Plankton community

The microplankton community of the GBR lagoon was mainly composed of flagellates, dinoflagellates, and ciliates. In the copepod community, different species of *Oithona* were identified, such as *Oithona attenuata*, *Oithona nana*, *Oithona oculata*, *Oithona setigera*, and *Oithona simplex*. *Oncaea* spp. and unidentified nauplii were also present in high abundances in the samples, together with *Microsetella* spp. and unidentified calanoid copepods. On the other hand, in the mangrove ecosystem the plankton community was totally dominated by *Oithona* spp. (*Oithona aruensis*, *O. attenuata*, *O. cf dissimilis*, and *O. nishidai*), with very high abundances of juveniles. Although present in both ecosystems, *Oithona* was one of the dominant copepods in the plankton community of the mangrove estuary, whereas it was less abundant in the GBR lagoon.

Potential prey for *Oithona* spp. and feeding rates

In the GBR lagoon organisms between 2-10 μm numerically dominated the micro- and nanoplankton community (> 500 cells mL^{-1}), whereas dinoflagellates and ciliates were found in similar low abundances, never reaching more than 1 cell mL^{-1} (Table 2). On the other hand, the mangrove microplankton community was dominated by ciliates, which reached a maximum of 17 cells mL^{-1}. Diatoms were present in both ecosystems, with concentrations of 2-8 cells mL^{-1} in the GBR lagoon, and 11-20 cells mL^{-1} in the mangrove (data not shown).

In the GBR lagoon significant ingestion (*t-test* < 0.05) of ciliates and dinoflagellates was found (Table 2). Clearance rates found for *Oithona attenuata* in the GBR lagoon ranged approximately from 8 to 18 mL $female^{-1}$ day^{-1} on ciliates, and from 7 to 10 mL $female^{-1}$ day^{-1} on dinoflagellates, (Fig. 2; Table 2). Ingestion rates in terms of cells ranged from 10 to 105 ciliates $female^{-1}$ day^{-1}, and from 5 to 36 dinoflagellates $female^{-1}$ day^{-1} for (Table 2), whereas when expressed as percentage of body carbon ingested per female per day, daily rations were in general low, ranging from 1 to 1.7% for ciliates, and from 0.3 to 0.7% for dinoflagellates (Table 2).

In the feeding experiments conducted in the mangroves, only ciliates were significantly ingested (Fig. 2; Table 2). Clearance rates of *Oithona cf dissimilis* in the mangroves ranged from 4.3 to 6.8 mL female^{-1} day^{-1} for ciliates, and from 3.7 to 6.3 for dinoflagellates. Ingestion rates varied between 22.6 to 104.6 cells female^{-1} day^{-1} for ciliates, and between 5 to 35.8 cells female^{-1} day^{-1} for dinoflagellates. The percentage of body carbon ingested from ciliates was 3.7-10.1%, and from dinoflagellates was 0.2-1.2% (Table 2).

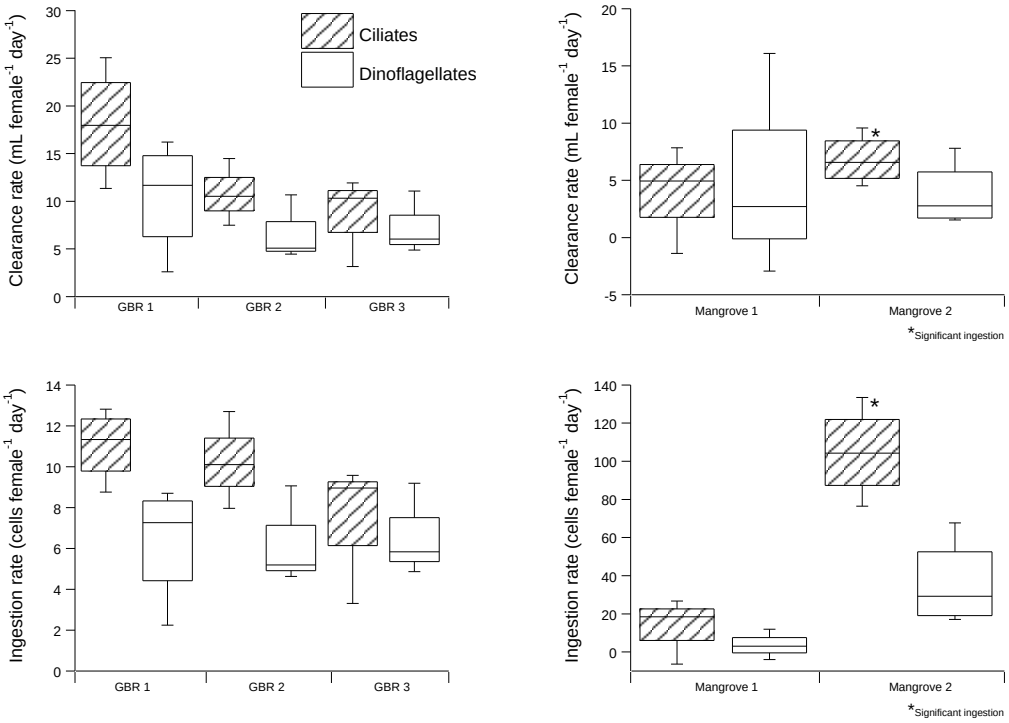

Fig. 2. Feeding rates of *Oithona* spp. Clearance (upper figures) and ingestion (lower figures) rates in the Great Barrier Reef lagoon (left panel) and mangrove (right panel). All feeding rates were significant in the GBR, and an asterisk (*) indicates significant ingestion rates in the mangrove

Table 2. Feeding experiments in the Great Barrier Reef lagoon (GBR lagoon) and mangrove. The *Oithona* species, number of replicates, and date of the experiments are indicated. The initial prey concentration (in cells and biomass), clearance rates (mL female^{-1} day^{-1}), and ingestion rates (in cells and % of body carbon ingested) are shown. Asterisks (*) indicate significant feeding rates.

Location	Date	Species	Replicates	Prey	Initial biomass (µg C L^{-1})	Initial concentration (cells mL^{-1})	Clearance rate (mL female^{-1} day^{-1})	Ingestion rate (cells female^{-1} day^{-1})	% body C ingested (day^{-1}
Great Barrier Reef	01-Apr	*Oithona attenuata*	4	Dinoflagellates	0.30	0.7 ± 0.07	10.4 ± 2.93**	6.3 ± 1.45	0.7 ± 0.13
			4	Ciliates	0.66	0.6 ± 0.06	18.1 ± 2.90***	11.1 ± 0.87	1.7 ± 0.13
	03-Apr	*Oithona attenuata*	3	Dinoflagellates	0.18	1.0 ± 0.06	6.7 ± 1.98**	6.3 ± 1.40	0.3 ± 0.06
			3	Ciliates	0.58	1.0 ± 0.04	10.8 ± 1.25***	10.3 ± 0.87	1.4 ± 0.12
	04-Apr	*Oithona attenuata*	3	Dinoflagellates	0.29	0.9 ± 0.05	7.3 ± 1.90**	6.6 ± 1.31	0.3 ± 0.06
			3	Ciliates	1.20	0.9 ± 0.07	8.5 ± 2.70**	7.3 ± 2.00	1.0 ± 0.29
Mangrove	12-May	*Oithona cf dissimilis*	3	Dinoflagellates	0.18	1.0 ± 0.18	6.3 ± 4.97	5.0 ± 3.61	0.2 ± 0.12
			3	Ciliates	2.69	3.9 ± 0.35	4.3 ± 2.29	22.6 ± 4.14	3.7 ± 0.68
	08-Jun	*Oithona cf dissimilis*	4	Dinoflagellates	0.72	10.3 ± 0.27	3.7 ± 1.4	35.8 ± 11.51	1.2 ± 0.37
			4	Ciliates	2.83	15.7 ± 0.73	6.8 ± 1.09***	105 ± 11.9	10.1 ± 1.14

** $p < 0.05$
*** $p < 0.01$

Egg production

Egg production of *Oithona attenuata* in the GBR lagoon ranged between 0.22 to 1.44 eggs female^{-1} day^{-1}, corresponding to weight-specific egg production rates of 0.2 to 1.6% day^{-1} (Table 3). Ovigerous females were not present in the tows, so the percentage of ovigerous in the population estimated from detached egg sacs was 1.5-10.3% (Table 3). Mean clutch sizes ranged between 14-15 eggs per female, reaching maximum values of 30 eggs per female. The mean prosome length of the females varied from 423 to 435 μm, with corresponding carbon contents of 0.45 to 0.49 μg (Table 3). *Oithona cf dissimilis* egg production rates were higher than those of *O. attenuata*, with a range of 1.69-3.34 eggs female^{-1} day^{-1}, and weight-specific egg production rates in the range 2.2-4.5% day^{-1}. The mean clutch size varied from 10 to 18 eggs per female, and maximum clutch sizes were up to 24 eggs per female. Ovigerous females were present in the tows, and the percentage in the population varied between 35.7 to 39%. The mean prosome length of the females was 389-394 μm, with female body carbon of 0.37-0.38 μg (Table 3).

The egg production efficiency (i.e. gross growth efficiency, GGE) varied among experiments between 13 and 67%. An average of 40% was estimated from the slope of the linear regression between SEPR and WSIR (Fig. 3).

Clutch size of *Oithona aruensis* ranged from 6 to 15 eggs per female. During the monitoring we found that 5 of the 48 females produced only one egg sac (3-6 eggs). Hatching success was always 100%, and hatching time occurred in approximately less than 24 hours.

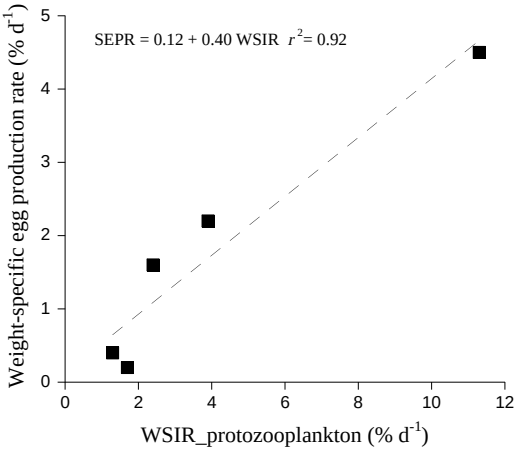

Fig. 3. Relationship between weigh-specific egg production (SEPR, % day^{-1}) and weigh-specific ingestion (WSI, % day^{-1}) on protozooplankton. Linear regression fit to the data is shown.

Table 3. Fecundity of *Oithona* spp. in the Great Barrier Reef lagoon (GBR lagoon) and mangrove. Prosome length (PL, µm) and female body weight (µg C) for each species are indicated. The percentage of ovigerous females, average clutch size (eggs per female ± SE), egg production rates (EPR, eggs per female and day), and weight-specific egg production rates (SEPR, % day^{-1}) are shown. The egg production efficiency (i.e. GGE, %) is also shown.

Location	Date	*Oithona* species	Prosome length (µm)	Female weight (µg C)	Ovigerous females (%)	Avergage clutch size (eggs female^{-1} ± SE)	Maximum clutch size (eggs female^{-1})	EPR (eggs female^{-1} day^{-1})	SEPR (% d^{-1})	GGE (%)
Great Barrier Reef	01-Apr	*Oithona attenuata*	423	0.45	10.3*	14 ± 0.9	30	1.44	1.6	67
	03-Apr	*Oithona attenuata*	435	0.49	1.5*	15± 2.2	24	0.22	0.2	13
	04-Apr	*Oithona attenuata*	426	0.46	2.5*	14 ± 1.2	20	0.34	0.4	29
Mangrove	12-May	*Oithona cf dissimilis*	394	0.38	39	10 ± 0.6	16	1.69	2.2	57
	08-Jun	*Oithona cf dissimilis*	389	0.37	36	18 ± 0.5	24	3.34	4.5	40

*Ovigerous females estimated only from dettached eggs

DISCUSSION

Importance of *Oithona* spp. in tropical environments

During this investigation we dealt with two contrasted tropical ecosystems, oligotrophic waters in the GBR lagoon (mesotrophic in the stations closest to the river), and eutrophic waters in a mangrove system. It is well described the importance in terms of abundance of *Oithona* spp. in different habitats of Australia (Robertson et al. 1988, McKinnon and Klumpp 1998), as well as its dominance in tropical estuarine areas (Duggan et al. 2008, Chew and Chong 2011). Although in this study we did not attempt to describe the copepod community, we observed that the relative abundance of *Oithona* spp. was higher in the mangrove, in great part due to the high abundance of juveniles. This pattern is in agreement with the study conducted by McKinnon and Klumpp (1998) in different rivers of North Queensland, where *Oithona* spp. was by far the dominant copepod. On the other hand, different species of *Oithona* have been found in the Great Barrier Reef (McKinnon et al. 2005), and *Oithona attenuata* has been described as one of the most abundant copepods (McKinnon and Thorrold 1993).

Feeding rates of *Oithona* spp.

With the feeding experiments carried out in this study we expected to clarify the natural diet of *Oithona* spp. in tropical areas. We confirmed the preference of *Oithona* spp. for protozooplankton as prey, although we found low ingestion rates not sufficient to cover metabolic cost and egg production.

The importance of protozooplankton in the diet of *Oithona* spp., and its preference for ciliates, has been reported in previous studies carried out in other environments (Nielsen and Sabatini 1996; Nakamura and Turner 1997; Lonsdale et al. 2000; Castellani et al. 2005; Zamora-Terol et al. 2013). We found that dinoflagellates contributed to *Oithona* spp. female's diet, especially when protozooplankton concentration was low, however the contribution of ciliates to the diet was always more relevant. When diluted conditions were present, *Oithona attenuata* ingested dinoflagellates at similar rates than ciliates in the GBR lagoon (i.e. 35-45%

of the protozooplankton diet was composed of dinoflagellates). On the other hand, ciliates were most abundant in the mangrove, and then the contribution of dinoflagellates to the diet of *Oithona* spp. was lower than in the reef (i.e. 18-26% of the protozooplankton diet were dinoflagellates). Higher feeding rates of *Oithona* spp. on ciliates found in earlier studies (Nakamura and Turner 1997, Castellani et al. 2005, Zamora-Terol et al. 2013) have came to speculate about an active selection on ciliates. However, *O.similis* in subantarctic sea cleared heterotrophic dinoflagellates at rates 1.3 times higher than ciliates (Atkinson 1996). Thus, *Oithona* spp. might not be necessarily selecting ciliates over dinoflagellates, or the selection by ciliates might be determined by the environmental food conditions.

As mentioned above, feeding rates measured for adult females of *Oithona* spp. in the present study were rather low when compared with previous reported values for other *Oithona* species (Castellani et al. 2005, Atienza et al. 2006, Zamora-Terol et al. 2013). The feeding environment was very diluted in the GBR lagoon ($\leq$ 1 protozooplankton cell mL^{-1}), and if we compare with feeding rates reported for *Oithona similis* in Greenland in similar feeding conditions, feeding rates are quite similar. However, we should consider that vital rates increase at higher temperatures, so we might expect higher feeding rates in a tropical than in a polar environment, when similar food conditions are present in the water column.

In the mangrove feeding rates were higher than in the reef, as it was the availability of food, but even so, feeding rates did not reach expected values to cover energetic expenses (Table 2). Thus, it is very likely that we have missed other potential prey in the diet of *Oithona* spp.

Alternative food resources

Diatoms. Feeding on diatoms by *Oithona* spp. was also assessed in this study, but no evidence of ingestion was found. Phytoplankton > 10 μm (mainly diatoms) was identified and counted, and no ingestion was observed, supporting the theory that *Oithona* spp. might not be feeding on diatoms (Uchima and Hirano 1986; Uchima 1988; Lischka and Hagen 2005; Nishibe et al. 2010), even in diluted environments (Zamora-Terol et al. 2013).

Few studies have reported little contribution of diatoms to the diet of *Oithona* spp. (Marshall and Orr 1966; Atienza et al. 2006). However, other studies have reported significant ingestion of diatoms by *Oithona* spp. (Turner 1986; Hopkins and Torres 1989; Atkinson 1996; Pond and Ward 2011). It is worth mentioning than only in the study conducted by Atkinson (1996) direct ingestion of diatoms was observed, whereas the other studies used indirect methods (i.e. gut and faecal pellet content).

Flagellates. In this study we expected to find positive ingestion rates of *Oithona* spp. on small cells (<10 μm), such as nanoflagellates, as it has been found in a subtropical area (Calbet et al. 2000), especially in very diluted conditions of food. However, the analysis of DAPI filters only showed a significant ingestion of cells > 5 μm, whereas smaller cells were not significantly ingested (Table 4). Nevertheless, it is worth mentioning that the samples from the reef were very "dirty", a lot of sediments were present in the filter, what could have interfere in the counting. So we do not completely discard the possibility that *Oithona* spp. found in the reef is feeding on small cells (< 5 μm), although it might be necessary to corroborate the results with more experiments.

Table 4. *Oithona attenuata* feeding experiment on the nanoplankton community in the Great Barrier Reef lagoon. The initial prey concentration (in cells and biomass), clearance rates (mL female^{-1} day^{-1}), and ingestion rates (in cells and % of body carbon ingested) are shown. Asterisks (*) indicate significant feeding rates.

Location	Date	Species	n	Prey	Initial biomass (μg C L^{-1})	Initial concentration (cells mL^{-1})	Clearance rate (mL female^{-1} day^{-1})	Ingestion rate (cells female^{-1} day^{-1})	% body C ingested (day^{-1})
Great Barrier Reef	01-Apr	*Oithona attenuata*	4	< 5 μm Autot.	0.25	84.42	3.9 ± 2.60	263.5 ± 209.83	0.18
			4	< 5 μm Heter.	0.80	267.70	5.9 ± 3.98	1546 ± 783.50	1.00
			4	> 5 μm Autot.	0.97	44.20	13.9 ± 1.67**	407.2 ± 26.15	2.00
			4	> 5 μm Heter.	1.10	48.30	3.2 ± 3.14	129.9 ± 135.24	0.64

In agreement with our findings about the contribution of flagellates to the natural diet of *Oithona* spp., Calbet et al. (2000) found that both *Oithona nana* and *Oithona simplex* ingested (nanoflagellates) small cells (< 5μm) in subtropical waters of Hawaii. The values of ingestion reported by Calbet et al. (2000) seem extremely high ($1{,}2 \cdot 10^5$ cells cop^{-1} d^{-1}) for *Oithona nana* in some of the experiments, and maybe caused by cascade effects in the bottles. In any case, they found a significant ingestion on nanoflagellates by two species of *Oithona.*

Aggregates and faecal pellets. Feeding on detritus could be an alternative food resource, but we think it is unlikely for *Oithona* spp. Signals from sinking aggregates are too weak to provide remote cues to an ambush feeder copepod as *Oithona* (Kiørboe and Thygesen 2001). However, the often turbulence found in mangrove areas, could enhance prey encounter rates for an ambush feeding predator (Kiørboe and Saiz 1995), thus enhance the feeding on non-usual prey. Gonzáles and Smetacek suggested that *O.similis* could feed on faecal pellets by using chemoreception (Gonzáles and Smetacek, 1994), however Svensen and Kiørboe (2000) described how prey capture in ambush feeders requires mechanosensory detection or direct prey interception. Recent investigations have confirmed the unlikely coprophagous feeding of *Oithona* spp. when no observation of faecal pellet capture or ingestion was observed by *Oithona similis* (Reigstad et al. 2005; Iversen and Poulsen 2007).

Nauplii. It has been widely reported the ingestion of nauplii by *Oithona* spp. (*Calanus* nauplii: Marshall and Orr 1966, Lampitt 1978, Lampitt and Gamble 1982, Drits and Semenova 1984, Uchima and Hirano 1986, Nakamura and Turner 1997).

McKinnon and Klumpp (1998) recorded how *Oithona* spp. from different rivers of North Queensland fed on paracalanid nauplii; when 3-5 nauplii were present, 1-2 nauplii were consumed per day and female. Due to the high abundance of copepod nauplii in the mangrove, and to confirm this cannibalism in a mangrove system, we checked the potential feeding of 2 species of *Oithona* spp. (*O. aruensis* and *O. nishidai*) on nauplii.

Non-ovigerous adult females of *Oithona* spp. (O. *aruensis* and O. *nishidai*) were placed individually in a 24 multi-well plate, and 2 small (< 100 µm) and 1 large (> 100 µm) unidentified nauplii were added in each well. The females were monitored for a period of 3 days, during which a fresh supply of filtered (0.2 µm) seawater was added daily to each well, and new-hatched nauplii were removed. The disappearance of the nauplii placed in each well was reported, but we did not make quantitative estimations of the ingestions. Although we did not check clearance or ingestion rates of nauplii from natural abundances, we could estimate the contribution to carbon budget from previous studies (*see* below).

Lipid storage

It could be argued that low ingestion rates reported here for *Oithona* spp. are compensated by the use of storage reserves. The proximity to coastal areas, maybe more productive areas, could explain the possibility to storage lipids when more favourable conditions are present in the water column. In the same way, seasonal phenomena in tropical and subtropical areas, like changes in the wind fields (i.e. monsoons or trade winds) could enhance primary production by upwelling processes (Postel 1990, Peterson 1998) in some periods the year. Furthermore, it has been reported how protein may function as a metabolic reserve in *Oithona similis* in tropical coastal waters of South India (Perumal et al. 2009). Thus, in conclusion we cannot discard the use of storage reserves as an explanation for the low daily rations estimated here from direct ingestion of *Oithona* spp. on natural particle assemblage.

Metabolic requirements

Metabolic requirements for *Oithona* spp., at temperatures similar to the ones found in this study, have been described to be 20.9-22.50% of the body carbon (at 20-30ºC, Hiromi et al. 1988). However, for *Oithona similis* daily rations up to 40% body carbon, has been described at 20ºC (Nakamura and Turner 1997). Castellani et al. (2005) estimated an energy demand of 32% for *Oithona similis* at 25ºC. Thus, considering those values, daily rations found in this study are far from being enough to sustain metabolic costs.

The daily ration estimated assuming either an egg production efficiency (i.e., GGE) of either 16% (*Oithona davisae*: Zamora-Terol and Saiz 2013), or 30% (Straile 1997), or 41% (estimated from our own data), are higher than our daily rations estimated from direct ingestion, but still low (Table 4).

In the GBR lagoon we could include an ingestion of flagellates < 5 μm, what would increase in approximately 1.2% the carbon ingested (Table 4). We can also consider the potential predation on nauplii, as previously reported by Nakamura and Turner (1997) and McKinnon and Klumpp (1998). If we assume that 1 adult female of *Oithona* spp. can capture 1.2 nauplii per day (McKinnon and Klumpp 1998), and that the average carbon content of one nauplii can varied from 32 to 92 ng C (from NI to NVI) (*O.davisae* nauplii; Almeda et al. 2010), then we would increase between 7-21% and 8-24% of the body carbon ingested, in females from the reef and the mangrove respectively. Considering these other potential prey, we would have daily rations of approximately 12-27% in the GBR lagoon (considering extra feeding on flagellates and nauplii), and 12-35% in the mangrove (considering extra feeding only on nauplii). We should consider that in the GBR lagoon, maybe the density of nauplii was not high enough to make up this "estimated carbon budget".

In conclusion those "recalculated" daily rations might be sufficient to sustain basic metabolism at our temperatures, considering the above-mentioned studies. However, the respiration rates estimated by Atienza et al. (2006) for *Oithona nana* (0.33 μg C ind.$^{-1}$) were 0.18-0.26 μg C day^{-1} at 25.5-27ºC, which could not be covered by our daily rations, even including alternative food sources. This fact suggests that there are still other alternative food sources for *Oithona* spp. in tropical waters, or a possible underestimation of the feeding rates.

Egg production

The percentage of ovigerous females found in the GBR was low, whereas in the mangrove was within values previously reported for other *Oithona* species (Ward and Hirst 2007; Castellani et al. 2007; Dvoretsky and Dvoretsky 2009). It could be argued that the reason of the low percentage of ovigerous females in the reef could be due to damage during the capture of copepods, and the loss of egg sacs through the net. However, we use a fine mesh (50 μm) in the plankton net, so we think it is unlikely that most of the egg sacs slip out the net. In tropical waters we often find high metabolic costs associated with high water temperatures, and in those conditions low stocks of food resources could limit the growth of copepods (McKinnon and Duggan 2003). Thus we should consider the possibility that in such conditions in the coral reef, the females were food limited (*see* below), and maybe the extremely bad weather could explain that females lost the egg sacs before being captured by the net.

Clutch sizes found for *Oithona* spp. in the present study (≈ 10-30 eggs per female) are in agreement to those reported for similar-sized *Oithona* spp. in tropical regions (≈ 17 eggs *O.nana*, Hopcroft and Roff 1996; 10-20 eggs *O.davisae*, Uye and Sano 1995; 14-36 eggs *O.attenuata*, McKinnon and Ayukai 1996; 6-18 eggs *O.aruensis*, McKinnon and Klumpp 1998).

Egg production rates (EPR) found in this study for *Oithona attenuata* (0.22-1.44 eggs female^{-1} day^{-1}) are lower than reported value for the same species in Northwestern Australia (3.2 eggs female^{-1} day^{-1}, McKinnon and Ayukai 1996). The egg production rate found here for *Oithona cf dissimilis* (1.69-3.34 eggs female^{-1} day^{-1}) is also lower than previous reported values for different *Oithona* species from river of Northeastern Australia (0.8-15.3 eggs female^{-1} day^{-1}, McKinnon and Klumpp 1998). Considering that the proportion of ovigerous females in the population is one of the factors determining the egg production rates, one feasible reason for those differences in the EPR might be the difference in the percentage of ovigerous females found in the studies. Our percentage of ovigerous females was very low (1.5-39%), whereas in the study conducted by McKinnon and Klumpp (1998) in most samplings at least 40% of the female population was ovigerous.

Values of clutch size and egg production observed in the present study for *Oithona* spp. (*see* Table 3) are in agreement with reported values for *Oithona davisae* feeding on very low food concentrations in the lab. In food conditions of 33 cells mL^{-1} (≈ 10 μg C L^{-1}), the corresponding clutch size and egg production were ≈ 8-10 eggs $female^{-1}$ and ≈ 1.8-2.4 eggs per $female^{-1}$ day^{-1}, respectively (Zamora-Terol and Saiz 2013).

Reported values of clutch size and egg production in the field for *Oithona* spp. of tropical and subtropical areas are also in agreement with our results (Hopcroft and Roff 1996; McKinnon and Klumpp 1998; Satapoomin et al. 2004; Etilé et al. 2012) (*see* Table 5). However, maximum egg production rates in this study are rather low in comparison with most of other field studies (Table 5). Egg production rates in our study seemed to be clearly food limited, as it has been reported before in McKinnon and Klumpp (1998) for congeneric species.

Table 5. Comparison of fecundity parameters of differents species of *Oithona* from tropical and subtropical areas. PL: prosome legth; EPR: egg production rate; SEPR: weight-specific egg production rate.

Species	Location	Area	Mesh plankton net	Temperature	Chl *a*	PL
			µm	ºC	µg L^{-1}	µm
O. aruensis	Haughton River	tropical	Niskin bottle	-	-	280
Oithona sp 1*	"	"	"	-	-	320
O. aruensis	Cape York Rivers	tropical	Niskin bottle	22.2-29.5	-	280
Oithona sp 1*	"	"	"	22.2-30.6	-	320
Oithona sp 2	"	"	"	24.7-28.9	-	280
O. attenuata	Exmouth Gulf,	tropical	150	21.3-23.2	0.15-0.35	340
O. simplex		"	"	21.3-23.2	0.15-0.35	270
O. davisae	Fukyama Harbour	subtropical	62	10-28	1.00-3.23	276-331
O. brevicornis	Grand-Lahou Lagoon	tropical	64	20-30	-	-
O. plumifera	Andaman Sea	tropical	50	28-30**	0.19	559 ± 1.7
O. plumifera	Jamaica	tropical	64	28 ± 1.5	0.11-2.6	-
O. nana	"	"	"	28 ± 1.5	0.11-2.6	-
O. simplex	"	"	"	28 ± 1.5	0.11-2.6	-
O. nana	Jamaica	tropical	64	28	-	-
O. plumifera	"	"	"	"	-	-
O. simplex	"	"	"	"	-	-

**Oithona* sp 1, described later as *O. nishidai* (McKinnon 2000)

***Surface temperature

Weight	Range clutch size (mean value)		EPR	SEPR	Reference
µg C	eggs per sac	eggs per female	eggs female^{-1} d^{-1}	d^{-1}	
0.30	3.1-9.3	-	0.8-12.3	0.01-0.12	McKinnon and Klumpp 1998
0.61	3.2-9.5	-	2.3-15.3	0.02-0.13	"
0.30	3-6.7	-	0.87-5.25	0.015-0.088	"
0.61	3.9-6	-	2.28-5.17	0.019-0.042	"
0.30	3.3-7.1	-	0.08-11.85	0.001-0.22	"
0.55	7-18	-	3.2	0.023	McKinnon and Ayukai 1996
0.31	4-8	-	2.4	0.069	"
0.20-0.25	-	11-28.5	2.6-11.6		Uye and Sano 1995
-	-	10-16	0.11-3.33	0.001-0.046	Etilé et al. 2012
0.81	-	-	0.2-11.2	0.05 ± 0.01	Satapoomin et al. 2004
-	-	5-21 (12.9 ± 3.7)	-		Hopcroft and Roff 1996
-	-	9-26 (17-20)	-		"
-	-	4-10 (7)	-		"
0.53 (0.21)	-	-	-	0.31-0.37	Hopcroft and Roff 1998
1.9 (0.76)	-	-	-	0.56	"
0.55 (0.22)	-	-	-	0.21	"

CONCLUSION

We have confirmed the preference of *Oithona* spp. for protozooplankton as part of its diet, and point out about the potential importance of smaller prey in oligotrophic tropical environments. The carnivorous feeding of *Oithona* in tropical environments might be playing an important contribution to the diet, since although taking into account the contribution of flagellates to the daily ration, the increase of carbon in the diet is not extremely high, so it is more likely were are missing larger prey that might have a higher contribution to increase the daily carbon ingested by adult females of *Oithona* spp. Egg production seemed to be food limited in the GBR lagoon, and very likely dependent on how often nutritious prey are part of the diet.

Many studies have described the dominance of *Oithona* spp. in nearshore tropical areas (Robertson et al. 1988; McKinnon et al. 2005), and their contribution as food source for fish larvae (Sampey et al. 2007, Chew et al. 2007). Thus, *Oithona* spp. might have a relevant trophic role in coastal habitats of tropical seas, especially in those areas where clearly dominate the plankton community.

Tropical areas have received little attention in comparison with higher latitudes in terms of investigations on small copepods, despite that the historical underestimation of their contribution to the secondary production is even more severe in tropical areas. For this reason, more effort on investigating the ecological role of small copepods, such as *Oithona*, is required in tropical areas for a complete understanding of the functioning of marine ecosystems.

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Synthesis of results and general discusssion

This thesis started with the aim of getting deeper insights into the biology and ecology of the cyclopoid copepod genus ***Oithona***. We have combined laboratory (**Chapter 1**) and field investigations (**Chapter 2, 3, and 4**), as well as observations at the individual (**Chapter 1, 3, and 4**) and population levels (**Chapter 2 and 3**) in order to improve our understanding of their role in the plankton community and their success in the marine environment.

With this discussion I aim to summarize and discuss the results of the chapters of this thesis in relation with the previous knowledge of the subject. The structure of this general discussion will be divided into 3 main sections: *Feeding*, *Reproduction*, and *Population dynamics, distribution and life strategy*. The two first sections will focus on aspects of the feeding and reproductive biology of *Oithona*, whereas the third section will discuss some aspects of the relationship with the environment, dealing with aspects related to distribution, life cycle and population dynamics of *Oithona* in contrasted environments.

FEEDING

The purpose of feeding is to obtain the energy to survive, grow and reproduce. The sensory-motor capabilities of the copepods have evolved to optimize the net nutritional gain, but at the same time constrain their feeding strategies and capabilities. In this section I will discuss physiological aspects of feeding such as the functional response of *Oithona* spp. to food concentration, the relationship between ingestion rates and metabolic requirements, aspects of the ambush feeding behaviour, and the diet of *Oithona* spp. Finally, some methodological aspects of the feeding experiments will be discussed.

With the results of our feeding experiments both in the lab (**Chapter 1**) and in the field (**Chapter 3 and 4**) we have been able to (partially) answer these questions:

1. How much is *Oithona* eating? (**Chapter 1, 3 and 4**)
2. What is *Oithona* eating? Is the diet of *Oithona* limited by its ambush feeding behaviour? (**Chapter 3 and 4**)
3. Is *Oithona* eating enough to survive and reproduce? (**Chapter 3 and 4**)

How much is Oithona eating?

Food demand of a copepod is a function of the growth and metabolism, and there are different aspects that will affect the ingestion rates, i.e. its reaction to the food offered in a feeding experiment: its life history, the current nutritional state (i.e. starving, replete), the light, and the temperature, food type, size and motility, palatability and nutritional quality, among others (Mauchline 1998).

In the laboratory, under controlled conditions of light, temperature and food concentration we found a feeding response type II for adult females of *Oithona davisae* feeding on a culture of the heterotrophic dinoflagellate *Oxyrrhis marina* (**Chapter 1**). That is, **we did not find a critical food concentration below which adult females were not able to feed** (**Chapter 1**).

There are several studies that have investigated the effect of food concentration on the feeding rates of *Oithona* spp. in laboratory-controlled conditions (Lampitt and Gamble 1982; Drits and Semenova 1984; Uchima and Hirano 1986; Saiz et al. 2003). The same kind of functional response (Type II) was found in all of them, with the exception of some of the experiments conducted by Lampitt and Gamble (1982) in which a linear response (Type I) was found when *Oithona nana* fed on *Thalassiosira* and *Dunaliella*. The type II functional response, derived by Holling (1959*b*), describes the average feeding rate of a predator when the predator spends some time searching for prey and some time processing each captured prey item (i.e., handling time) (Skalskii and Gilliam 2001).

In contrast to the type II functional response found for adult females of *Oithona davisae* (**Chapter 1**), juveniles (nauplii and early copepodites) of the same species shown a type III functional response under similar controlled conditions (Almeda et al. 2010). The type III functional response differs from type II in the presence of a 'feeding threshold', i.e., a prey concentration below which the copepod stops feeding (Wlodarczyk 1988) or reduces its clearance rates (Kiørboe et al. 1985). This kind of functional response in juveniles implies the existence of a feeding threshold below which they are not able to feed (900 cells mL^{-1}, Uchima and Hirano 1986; 153-235 cells mL^{-1}, Almeda et al. 2010).

Although nauplii of *Oithona davisae* have the ability to feed right after hatching (Eaton 1971; Uchima and Hirano 1986), their low ability to feed at very low food concentrations (Uchima and Hirano 1986; Almeda et al. 2010) might be explained by a likely less effective mechanoreceptor system (Paffenhöfer 1998).

The lack of feeding threshold and low concentration of food to reach maximum ingestion and egg production rates (**Chapter 1**) might be an advantage for the females of *Oithona* spp. to **survive and successfully reproduce in very diluted environments, as it will be further discussed in** *Reproduction*).

What is Oithona eating?

Diatoms, flagellates, dinoflagellates, ciliates, faecal pellets, nauplii, detritus, and aggregates have been considered and studied as potential contributors to the natural diet of different species of *Oithona*. There are two aspects of the natural diet of *Oithona* spp. we have investigated in this thesis: the type and size of prey (**Chapter 3 and 4**). Regarding the type of prey, we have reported how **ciliates and at lesser extent dinoflagellates are the main food items in the diet of *Oithona* spp.** feeding in natural conditions. These results have been previously reported in investigations that have used natural assemblages, and the bottle incubation method (Atkinson 1995; Nakamura and Turner 1997; Lonsdale et al. 2000; Castellani et al. 2005a; Atienza et al. 2006; Nishibe et al. 2010).

In early investigations about feeding of *Oithona*, a controversy on the type and size of the preferred prey of *Oithona* spp. already existed. Lebour (1922) found diatoms in the stomach of *Oithona* sp.; Zaika et al. (1975) found fragments of plants and crustacean in the gut of *Oithona setigera*: Kozhevnikov (1979) reported feeding of *O. similis* on phytoflagellates, protists, and bacterial aggregates; and Petipa (1981) using a radiocarbon method, documented the ability of *Oithona* spp. to feed on algae and small crustacean. Lampitt and Gamble (1982), and Drits and Semenova (1984) reported feeding on algae by *Oithona nana* and *Oithona similis*, respectively.

On the other, Marshall and Orr (1966) found that *O. similis* did not consume algae, and fed on *Calanus* nauplii, in agreement with Lampitt (1978), who also reported the carnivorous feeding of *O. nana*.

Because the diet is the product of a feeding strategy, and of the available prey, feeding experiments contribute to gain insight into the ways in which copepods respond to their food environments. In this thesis we have investigated aspects related with the natural diet of *Oithona* spp. in **Chapter 3 and 4**, in two very contrasted areas.

Laboratory and field investigations *Oithona* spp. have considered *Oithona* spp. to be omnivorous (e.g. Castellani et al. 2005), carnivorous (e.g. Marshall and Orr 1966; Lampitt 1978; Nakamura and Turner), herbivorous (e.g. Gauld 1966; Eaton 1971), and coprophagous (González and Smetacek 1994), and suggested to feed on detritus (Lampitt and Gamble 1982; Ambler et al. 1994; McKinnon and Klumpp 1998). These assumption are the result of investigations conducted on different species, different environments, and using different techniques.

In the old investigations we found controversy or lack of agreement of the potential prey of *Oithona* spp. However, in recent investigations there is a tendency to define *Oithona* spp. as a carnivorous copepod, because the main contribution of heterotrophic dinoflagellates and ciliates to the diet (e.g. Lonsdale et al. 2000; Castellani et al. 2005a; Atienza et al. 2006). In agreement with these works, **our results would define *Oithona* spp. more as a carnivorous copepod** (**Chapter 3 and 4**).

In this section our results of the natural diet of *Oithona*, together information in the literature regarding the potential food items of the natural diet of this ambush feeding copepod will be discussed, as well as the relevance of other potential prey (i.e. phytoplankton, protozooplankton, nauplii, faecal pellets).

Protozooplankton: the main diet?

The contribution of heterotrophic dinoflagellates and ciliates to the diet of *Oithona* spp. has been reported in most of the field investigations (Atkinson 1996; Nakamura and Turner 1997; Lonsdale et al. 2000; Castellani et al. 2005a; Atienza et al. 2006; Nishibe et al. 2010) including our feeding experiments conducted in Greenland (**Chapter 3**) and Australia (**Chapter 4**) on different species of *Oithona*.

The **major contribution of oligotrich ciliates to the diet of *Oithona* spp.** when feeding on natural assemblages observed in our results (**Chapter 3 and 4**), is in agreement with previous studies (Nakamura and Turner 1997; Lonsdale et al. 2000; Castellani et al. 2005a; Atienza et al. 2006)). In general, in our results, **when dinoflagellates and ciliates were present at similar concentrations, ciliates were cleared at higher rates**. It could be speculated that higher clearance rates for ciliates might indicate an active selection for ciliates (Castellani et al. 2005a); however, higher clearance rates for dinoflagellates reported for *Oithona* spp. in other investigations (e.g. Atkinson 1995) could indicate that they do not necessarily select ciliates over dinoflagellates.

The contribution of dinoflagellates to the diet of *Oithona* spp. is more variable than that of ciliates. We have reported ingestion of dinoflagellates by *Oithona similis* in Greenland, especially when food was very scarce in the water (**Chapter 3**), and also by *Oithona attenuata* in the Great Barrier Reef, and *Oithona cf dissimilis* in a mangrove system (**Chapter 4**). However, in many occasions under the doubt that many of the feeding rates were not significant. It should be considered that dinoflagellates could be more affected by trophic cascade effects in the bottles, than ciliates, resulting in controversial feeding rates on dinoflagellates.

Despite the agreement of the important contribution of protozooplankton in the diet of *Oithona* spp., in most of the investigations, the consequent calculation of daily ration, only based on protozooplankton ingestion, have occasionally evidenced too low carbon feeding rates to sustain basal metabolism. In our results, the daily rations reported in winter (≈ 1.5-4.5%) in Greenland (**Chapter 3**), and in the Great Barrier Reef (≈ 1.3-2.4%) (**Chapter 4**) were too low to sustain the temperature-equivalent respiration rates.

These results, in our case and as well as in other investigations (Drits and Semenova 1984; McKinnon and Klumpp 1998; Castellani et al. 2005a), have lead to hypothesize about the potential contribution of other prey to the diet of *Oithona* spp. to cover the metabolic expenses and growth.

Diatoms

Diatoms are one of the food items we have considered as a potential prey in diet of *Oithona* spp. (**Chapter 3 and 4**). We did not found ingestion of diatoms in any of the experiments (including all replicates separately) we have conducted. In agreement, other investigations that have used the same method to estimate feeding rates (i.e. bottle incubation) did not observe direct diatom ingestion either (Uchima and Hurano 1986; Lishcka and Hagen 2007; Nishibe et al. 2010). In contrast, several studies have reported the presence of diatoms in the guts of *Oithona* spp. (Hopkins and Torres 1989; Atkinson 1996), and detected them in biochemical compounds (Pond and Ward 2011); and diatoms have been successful used as a diet in laboratory investigations (Lampitt and Gamble 1982; Li and Zhang 2009; Santhaman and Perumal 2012). But only sporadic observations of feeding on diatoms have been observed in the field, although in most of the cases at non-significant rates (Castellani et al. 2005a; Atienza et al. 2006).

It has been hypothesized how the sinking-swimming behaviour of diatoms, and the signal they emit, are not in the hyrdromechanical threshold detection of *Oithona* to be considered a potential prey (Kiørboe and Visser 1999; Svensen and Kiørboe 2000). In the same regard, the investigation conducted by Henriksen et al. (2007) reported how *Oithona davisae* nauplii (which are also ambush feeders) were unable to feed on non-motile prey (i.e. diatom *Thalassiosira weissflogii*). Thus, the mechanosensory capabilities reported for *Oithona* spp. together with the results reported in our feeding experiment, allow us to hypothesize about **diatoms as a non-feasible prey in the natural diet of *Oithona* spp. in the environments investigated in this thesis**.

However, we did not discard the potential contribution of diatoms to the diet of *Oithona* spp. in exceptional conditions and/or particular environments, and a possible ingestion not detected due to trophic cascade effects in the bottles; in any case, it seems unlikely that diatoms are an important component of the natural diet of *Oithona* spp.

Although cautiously, our findings regarding the natural diet of *Oithona* spp. might confirm that **diatoms are not part of the diet of the species of *Oithona* investigated in this thesis, and that they apparently prefer ciliates as prey in most occasions**. However, there are some aspects of the diet of *Oithona* spp. that we either did not study or we could not confirm or discard due to lack of evidences.

Nanoplankton (flagellates <10 µm)

The capability of *Oithona* to capture and ingest cells smaller than 5 µm has also been discussed, with no conclusive results until today (Calbet et al. 2001; Vargas and González 2004; Atienza et al. 2006). The controversy on the contribution of small flagellates to the natural diet of *Oithona* spp. is more a question of size, rather than type of prey.

Although there are several studies that have reported the capability of *Oithona* spp. to feed on small flagellates in the lab (e.g. Eaton 1971; Lampitt and Gamble 1982); the *in situ* ingestion on nanoflagellates is not clear. Nakamura and Turner (1997) reported that *Oithona similis* did not fed on cells 2-8 µm; however Calbet et al. (2000) and Vargas and González (2004) have reported nanoflagellates as the main prey ingested by different species of *Oithona* from subtropical environments. **It is very likely that nanoflagellates have a more important contribution to the diet of *Oithona* in oligotrophic environments** (tropical and subtropical areas), and that is one of the reasons why we investigated the contribution of small flagellates to *Oithona attenuata* in waters of the Great Barrier Reef (**Chapter 4**).

We found evidences that *Oithona* spp. ingested flagellates > 5 µm in tropical waters, and sporadically flagellates <5µm (**Chapter 4**). However, the data obtained in our experiments was not enough to confirm that small flagellates are important prey items of the natural diet of *Oithona* spp.

The lack of investigations including nanoflagellates in the feeding experiments, gives fragmented information on the contribution of nanoflagellates to the natural diet of *Oithona* spp., especially in high latitude environments. Castellani et al. (2005a) reported little contribution of small flagellates to the diet of *Oithona similis* in the Irminger Sea. However, cells were fixed with Lugol's solution, which is not the most adequate fixative for nanoflagellates, and could have resulted in erroneous estimations.

In any case, if we consider the potential contribution of nanoflagellates in terms of daily ration, as estimated in **Chapter 4** (max. 3.8% carbon ingested), nanoflagellates seems not to be main contributors to the diet of *Oithona* spp Thus, in high latitude environments where nanoflagellates are often less abundant than in warmer areas, they might contribute even less to the total carbon ingested by *Oithona* spp.

Carnivorous feeding on nauplii

It is accepted that *Oithona* spp. can ingest nauplii as part of its diet (Marshall and Orr 1966, Lampitt 1978, Lampitt and Gamble 1982, Drits and Semenova 1984, Uchima and Hirano 1986, Nakamura and Turner 1997). Clearance and ingestion rates on nauplii have been reported in the laboratory (Lampittt and Gamble 1982; Nakamura and Turner 1997; McKinnon and Klumpp 1998) to check the potential relevance of nauplii as part of the natural diet of *Oithona* spp. In **Chapter 4** we made an estimation of the potential contribution of nauplii to the diet of *Oithona* spp. in a mangrove system (increase of 7-24% of carbon ingested including one nauplii in the daily diet), which was reported high enough to cover the metabolic expenses not covered when only protozooplankton ingestion were considered.

The contribution of nauplii to the diet of *Oithona* spp. might be important in environments with high density of juveniles (i.e. mangrove and other nearshore habitats); however, if we consider common densities of nauplii in the sea, this contribution might not be so relevant. As hypothesized by Lampitt (1978), at natural prey densities, feeding rates based on carnivorous feeding will unlikely provide >25% of the metabolic requirements of adult femals of *Oithona* spp.

Coprophagous feeding

The few studies conducted to investigate the potential feeding of *Oithona* spp. on faecal pellets, have presented contradictory results. González and Smetacek (1994) reported feeding of *Oithona* spp. on faecal pellets, suggesting that they could retard the vertical flux of particle matter in the oceans, and enhance nutrient regeneration in the photic layer. Svensen and Nejstgaard 2003 found in mesocosms experiments a negative correlation between faecal pellets and *Oithona* biomass. However, further investigations rejected the coprophagous feeding of *Oithona* spp. when no observation of faecal pellet capture or ingestion was observed by *Oithona similis* (Reigstad et al. 2005; Iversen and Poulsen 2007). Reigstaad et al. (2005) concluded that *Oithona* spp. might be an indicator species for a 'retention ecosystem', but that they are probably not the single factor explaining faecal pellet retention in the system. Thus, it is probably more likely that the presence of *Oithona* spp. in high retention ecosystems is more a coincidence than a direct implication of the copepods on the ingestion of faecal pellets.

On the size spectrum of prey

Conover (1968) suggested that a raptorial feeding might confine the food size to a narrower range than suspension feeding. However, the range of prey size reported for *Oithona* spp. in the literature is wide (e,g. *Oithona nana*: Lampitt and Gamble 1982, Atienza et al. 2006; *Oithona similis*: Drits and Semenova 1984, Nakamura and Turner 1997).

Gauld (1966) estimated that adult *Oithona* would not feed on prey smaller than 15 μm (Eaton 1971); in contrast, Eaton (1971) reported effective feeding on particles down to 8-10 μm, and demonstrated that naupliar stages were able to ingest smaller algal species (2-5 μm) than the adults (> 8-10 μm). Conover found that preferred food range of *Oithona similis* ranged from 8 to 10 μm in all seasons (cited in Eaton 1971). Lampitt and Gamble (1982) reported the highest clearance rates on nauplii (NI) of *Acartia clausi*, but algae in the size range 10-67 μm were the main carbon contribution to the diet. Drits and Semenova (1984) reported the feeding rates of *Oithona similis* on cultured algae of 10-40 μm, but they also reported its capability to feed on nauplii of 500 μm (*Artemia salina*).

In more recent investigations, Castellani et al. (2005a) reported the highest clearance rates (23 mL female^{-1} day^{-1}) of *Oithona similis* feeding on *Strombidium* spp. of 20-30 µm, what is in agreement with our results on the same species in west Greenland, feeding on oligotrich ciliates (**Chapter 3**).

We found that *Oithona* ***similis*** **seemed to select ciliates of 20-40 µm in Greenlandic waters**, size range in which we found the highest clearance rates (70-110 mL female^{-1} day^{-1}). And this preference was also confirmed in *Oithona* species from tropical waters (**Chapter 4**).

Thus, **our results indicate an optimal food size at which** *Oithona* **spp. seems to feed more effectively** (Chapter 3), possibly related with the size of the females (Berggreen et al. 1988). However, it is unlikely that the raptorial feeding confine the food size to a narrower range than suspension feeding (Conover 1968), and it is more likely that the motility of the cells could limit the feeding behaviour of *Oithona* spp.

Is the diet of Oithona limited by its ambush feeding behaviour?

I have mentioned in the introduction that *Oithona* spp. prefer motile prey (Uchima and Hirano 1986; Turner and Granéli 1992), and this behaviour and preference is related to the ambush (and raptorial) feeding mode of the copepod. In fact, **we have confirmed this preference for motile prey in the feeding experiments conducted *in situ* in polar, subpolar and tropical waters** (**Chapter 3 and 4**). We have not reported ingestion of any non-motile prey (diatoms).

In *Oithona*, as in all copepods, the first antennae presents numerous setae (mechanorreceptors), which are used to perceive the precise location of moving prey at a distance (Paffenhöfer 1998). This could seem a less effective mechanism for feeding if we compare with suspended-feeder copepods, which scan more water volume per unit time. However, it has been shown how potent mechanosensory capabilities can lead to extraordinary feeding rates (*Acartia tonsa*: Saiz and Kiorboe 1995).

There are many advantages of ambush feeding over other prey encounter strategies. Ambush-feeders have reduced encounters with predators, and they spend less energy for swimming (Kiørboe 2009). These characteristics can explain why obligatory ambush-feeders, such as *Oithona* spp., have lower metabolic rates (Castellani et al. 2005b) and experience less mortality (Eaine and Ohman 2004) than similar-sized and co-occurring copepods with more active feeding strategies (e.g. suspended-feeders). However, it is not clear if this feeding mode will restrict the number of taxa on which *Oithona* spp. are able to feed on.

If we consider our results of *in situ* feeding rates, it might be hypothesized that *Oithona* spp. is feeding only on a few taxonomic groups, which include motile cells (i.e. dinoflagellates and ciliates). However, when looking at the wide range of prey type reported in the literature, we might think about a highly varied diet of *Oithona* spp. As mentioned before, the kind of experiments, methodologies, environments, and species studied make it difficult to deny or accept the hypothesis that have been raised up in the feeding investigations on *Oithona* spp.

Is Oithona eating enough to survive and reproduce?

Daily rations of *Oithona* spp. in the field have been reported in a range of 1-40% (Lampitt and Gamble 1982; Nakamura and Turner 1997; Lonsdale et al. 2000; Castellani et al. 2005; Atienza et al. 2006; **Chapter 3 and 4**), although some investigations have reported extreme values, sometimes over 100% of body carbon ingested (e.g. Calbet et al. 2000; Vargas and González 2004). Factors like experimental temperature, diet, body weight, make difficult to compare metabolic demand of *Oithona* spp. with weight-specific ingestion (i.e. daily ration).

Oithona spp. maximum weight-specific ingestion rates (% body carbon ingested, based on protozooplankton diet) reported *in situ* in Greenland (≈22%) and Australia (≈10%) (**Chapter 3 and 4**), are in good agreement with other *in situ* investigations in which *Oithona* spp. mainly fed on protozooplankton (17-35%, Atkinson 1996; Nakamura and Turner 1997; Castellani et al. 2005a). In contrast, in laboratory experiments, maximum daily rations of *Oithona* spp. in general have been reported higher than those in the field (35-80%, Lampitt and Gamble 1982,

Drits and Semenova 1984, **Chapter 1**). However, it should be mentioned that in laboratory experiments, single-prey diet was often offered as food, instead of natural prey assemblages, normally used in field investigations, what could determine different maximum daily rations reported for *Oithona* spp. in the field and in the lab.

In general, maximum daily rations of *Oithona* spp. are lower than those of similar-sized egg-carrying (148% *Pseudocalanus elongatus*, Paffenhöfer and Harris 1976) and free-spawners (Paffenhöfer 1988 and references therein) calanoids. Lower ingestion rates of cylopoids in comparison with calanoids have been hypothesized (Paffenhöfer 1993), and although our results confirm this assumption, in field investigations, we often find low daily rations for large calanoid copepods (e.g. Pasternak et al. 2008).

With the information of the carbon ingested by *Oithona* spp. and information on the metabolic requirements of *Oithona* spp. (Marshall and Orr 1966; Klekowski et al. 1977; Lampitt and Gamble 1982; Hiromi et al. 1988; Nakamura and Turner 1997; Castellani et at. 2005b; Atienza et al. 2006) we can estimate if *Oithona* is eating enough to maintain basal metabolism, and to reproduce.

Oithona similis in Arctic and subarctic environments, where low temperatures are present, seems to feed enough to sustain basic metabolism (≈1% at 4ºC, from Castellani et al. 2005*b*), and furthermore to successful reproduce, as we have reported in **Chapter 3**. However, when maximum daily rations were reported, egg production efficiency was low.

Metabolic demands at higher temperatures (31.4% at 30ºC, Hiromi et al. 1988; 26% at 25ºC, Atienza et al. 2006) did not fit well with our reported daily rations of *Oithona* spp. in tropical environments (max. 10% body carbon, **Chapter 4**). Thus, the **carbon ingested from protozooplankton by *Oithona* spp. in tropical ecosystems was too low to sustain basic metabolic demands** estimated for similar-sized *Oithona* at similar experimental temperatures (≈24-28ºC).

To elucidate how basic metabolism could be covered, and reproductive activity present, when ingestions are too low, we propose several explanations. On one hand, the possible use of storage lipids (especially relevant in Arctic environments, **Chapter 3**) or structural proteins in *Oithona* under adverse food conditions (**Chapter 4**); and on the other hand, a possible underestimation of feeding rates in our experiments, which might not have accurately contemplated the full prey size spectrum of *Oithona*.

Lipid biochemistry. The seasonal dynamics on lipid composition and quantity has been investigated on *Oithona similis* from the Arctic (Lischka and Hagen 2007; Narcy et al. 2009). Living under arctic conditions requires adaptations to cope with food paucity during the unfavourable season. The accumulation of lipid reserves, especially of large herbivorous calanoids, is very well known in polar marine areas (Lee et al. 2006).

There are only a few investigations on the lipid biochemistry of *Oithona* spp., all conducted on the same species, *Oithona similis*. Two studies were focused on the fatty acid and alcohol composition, one from the Antarctic (Kattner et al. 2003) and the other one from the Arctic (Lischka and Hagen 2007), one study on the amount of lipid reserve using an optical method, also conducted in the Arctic (Narcy et al. 2009); in tropical waters of India, Perumal et al. (2009) reported the biochemical composition of *Oithona similis*. Lischka and Hagen (2007) suggested that high percentages of 18:1(*n*-9) fatty acids in all stages of *Oithona similis* and during all seasons point to a generally omnivorous diet (Dalsgaard et al. 2003 and references therein). In the same regard, Kattner et al. (2003), based on its lipid composition, described *O. similis* to feed opportunistically on a great variety of organisms. In disagreement, our results maintained that the diet of *Oithona* is mainly based in a few taxa, and mostly fed carnivorously on protozooplankton.

Lipid sacs made of wax esters, which are known as the major storage lipids in high latitude species (Lee et al. 2006). Accumulation of wax esters is usually linked to seasonal feeding patterns (i.e. herbivorous copepods) (Falk-Petersen et al. 1987), and generally, high wax ester levels are indicated by clearly visible large lipid droplets (in *Oithona similis*, Narcy

et al. 2009).

Deposits of wax esters in *Oithona similis* have been reported, with maximum in summer, a decrease in winter and a minimum in spring (Kattner et al. 2003; Lischka and Hagen 2007), what might indicate that *O. similis* is using lipid reserves to overwinter. The maximum deposit of lipid reserves in summer indicates that *O. similis* is building up the reserves in spring, and this is in agreement with our founding in the Arctic, where **maximum ingestion rates of *O. similis* in spring were not translate into egg production, very likely because they were allocated to build up the lipid reserves** (Chapter 3). This assumption also means that **part of the egg production reported in winter is very likely fuelled by storage lipids**, and that would explain the low daily rations reported (**Chapter 3**). In contrast, Narcy et al. (2009) reported a different seasonal pattern, in which females of *O. similis* presented the highest accumulation of lipids before the phytoplankton bloom. Since Lischka and Hagen (2007) and Narcy et al. (2009) conducted the study in the same fjord, it is unlikely to consider different life strategies of *O. similis*, thus the methodology used could be the reason of different seasonal pattern in the lipid content of adult females.

Protein. In tropical waters, Perumal et al. (2009) found that protein was the major biochemical component of *Oithona similis*, whereas the lipid content was rather low. If the protein is, as hypothesized by Perumal et al. (2009), a main metabolic compound for *Oithona* spp. in tropical waters, then we could explain the low ingestion of carbon in our results from Australia by the use of protein. Furthermore, egg production rates were very low, in accordance with the low ingestion rates reported, especially in the Great Barrier Reef Lagoon. The hypothetical use of protein might constrain egg production, in the way that energy obtained by protein is very likely used for respiration. Thus, ***Oithona* spp. in tropical waters were very likely food limited, what might explain the low egg production rates found** (Chapter 4).

CONCLUDING REMARKS OF THE FEEDING

- Since the acknowledge of the microbial food web concept, our vision of the pelagic food web has changed from a simple linear food chain to complex group of interactions, what at the same time has revealed the diversity in copepods diets. *Oithona* spp. is one of those copepods, link of the complex interactions between processes in the microbial food web and higher trophic levels, as confirmed by our results in the *in situ* feeding experiments (**Chapter 3 and 4**).
- Although ability to eat different kind of prey (i.e. omnivory diet) may enhance the probability to cover metabolic requirements in diluted food environments, our results might indicate a narrow prey spectrum (i.e. dinoflagellates and ciliates) in the diet of *Oithona* spp. (**Chapter 3 and 4**). This would contradict the hypothesis that cyclopoids are less specialized than calanoid copepods in terms of feeding.
- Low food concentrations at which maximum production rates are reached might contribute to successful exploit diluted environments (**Chapter 1**).
- The deep lack of experimental studies on specific aspects of the biology of *Oithona* spp. in the laboratory limits our capability to understand their ecological role in the seas.

REPRODUCTION

The females have a major role in the production of the population, and throughout this thesis we have investigated different aspects of the fecundity of adult females of *Oithona* spp., and their egg production efficiency, both in the lab (**Chapter 1**) and in the field (**Chapter 2, 3, and 4**). In this section some aspects of the reproductive biology of *Oithona* will be discussed, as well as the effect of environmental factors on their egg production, hatching success, and the relationship between feeding and egg production (i.e. egg production efficiency).

Female body size

Body size (weight) is a key parameter determining vital rates of copepods (feeding: Saiz and Calbet 2012; egg production: Hopcroft and Roff 1998, Bunker and Hirst 2004; respiration: Ikeda et al. 2001), and for this reason when studying reproduction, the size of the adult females is often considered to influence egg production rates. Food concentration and temperature are typically considered the major factors affecting body size of copepods; in general patterns body size is inversely correlated with temperature and positively with food concentration (Klein Breteler and González 1988).

In this thesis **we did not find a correlation between food availability and temperature with prosome length of adult females of *Oithona* spp. in the field**. However, in the field, the range of variation of environmental factors can sometimes be too low to observe a significant correlation. The variability of the size of adult females of *Oithona* spp. has been studied in relation with reproductive characteristics and environmental factors in several studies (e.g. Castellani et al. 2007; Dvoretsky and Dvoretsky 2009; Drif et al. 2010). In agreement with our results, Drif et al. (2010) pointed out the lack of importance of body size in reproductive characteristics of *Oithona;* in contrast, Castellani et al. (2007) and Dvoretsky and Dvoretsky (2009) argued that the effect of temperature on the fecundity of *Oithona similis*, was indirectly mediated by body size . In calanoid copepods, Halsband and Hirche (2001) found strong relationships between body size of the females and clutch size and egg production rates of calanoid copepods, with the exception of the egg-carrying *Pseudocalanus* spp.

If the effect of body size on the fecundity of copepods is related to spawing strategies, however, is not clear.

Typically, in free-living copepods, adult body size is inversely related to temperature during development (McLaren 1965; Miller et al. 1977; Burns 1979; Runge and Myers 1986); when forced through a fixed number of moults quickly by high temperature, copepods can not grow to large sizes. In agreement with this, the influence of temperature on the body size of *Oithona* spp. has been found in several studies, where an inverse relationship between prosome length and temperature has been reported (Uye and Sano 1995; Castellani et al. 2007; Dvoretsky and Dvoretsky 2009). Although we did not find a relationship between temperature and body size, it should be considered that long generation times of *Oithona* spp. at low temperatures, might not allow to see any relationship between the final body size and environmental variables (Klein Breteler and González 1988).

A way to untangle the different variables involved in the body size and weight of copepods is the conduction of experiments under controlled conditions. **In the lab the adult females of *Oithona davisae* grown under the highest food concentrations were larger, both in length and width, than those under lower food conditions** (Chapter 1). In most of the investigations, only the length of the females is considered when checking correlation of the size and environmental factors, what could result in wrong results (Geller and Müller 1985; Klein Breteler and González 1988). In this regard, Bonet and Carlotti (2001) found that *Centropages typicus* of similar prosome length were often different in body dry weight. Thus, an alternative explanation for the lack of relation between body size of adult females of *Oithona* spp. and food concentration in the field, could be the use of length measurements alone and not consider the effect of food in the width or the actual biomass. One solution is to estimate the dry weight, but the small size of *Oithona* spp. implies a high number of animals necessary to conduct analysis (and replicates) of dry weight, what is not always possible when working in the field. Uye (1982) used more than 1000 organisms to estimate the dry weight of *Oithona* spp.

In conclusion, we found a positive relationship between body size of adult females of *Oithona* spp. and egg production rate in the lab, very likely because of the indirect effect of food concentration. However, in the field the relationship between temperature and food with females body size was not clear, very likely influenced by the inverse relationship between temperature and food concentration often found in the field (Deevey, 1960).

Clutch and egg size

The effect of body size on the fecundity has been largely reported in copepod investigations (e.g. Corkett and Mclaren 1969; Kiørboe and Sabatini 1995; Halsband and Hirche 2001; Bunker and Hirst 2004), and generally a positive relationship between body size and clutch size is described for calanoids (Runge 1984).

The relationship between female body size and clutch size of *Oithona* spp. was investigated in all the chapters of this thesis. **In the lab**, although the results showed **larger clutch sizes in larger females**, the effect of food concentration masked a possible direct relationship between body and clutch size (**Chapter 1**). In the field studies, in contrast, **we did not find any clear intraspecific relationship between the size of the females of *Oithona* and their clutch size** (Chapter 2, 3 and 4). Some studies have reported a positive correlation between body and clutch size of *Oithona* spp. (Castellani et al. 2007; Dvoretsky and Dvoretsky 2009), however, other studies found no clear pattern (Drif et al. 2010; Temperoni et al. 2011).

A clear trend in our field studies was that clutch size of *Oithona* spp varied little in relation with seasonal changes and/or environmental factors (**Chapter 2 and 3**). Other studies have also reported little variation in the clutch size of *Oithona* spp. throughout the year (Dvoretsky and Dvoretsky 2009; Temperoni et al. 2011). In contrast, Uye and Sano 1995) found a bicyclal variation of clutch size of *Oithona davisae*, with peaks in early summer and autumn; unfortunately no information of the food environment in that study was provided which could help to explain such pattern.

It is worth mentioning that the investigations that have reported little seasonal changes of the clutch size of *Oithona* spp. have mainly been conducted in high latitude environments, what could give strength to the hypothesis that egg production is less variable in egg-carrying copepods than in free-spawners, at least in these environments, if the effect of temperature is not considered.

We found that the mean clutch size of the small-sized species of *Oithona* spp. inhabiting tropical waters (**Chapter 4**) was smaller than that of the larger congeneric species *Oithona similis* inhabiting high latitude environments (**Chapter 3**). However, **maximum clutch sizes were quite similar among species of *Oithona* spp.** (ca. 30 eggs female^{-1} in *Oithona similis* and *Oithona attenuata*, and 24 in *Oithona cf dissimilis* with mean prosome length of 500, 430, and 390 μm respectively) (**Chapter 3 and 4**).

It is believed that organisms must divide their reproductive effort between a few large and many small offspring (Smith and Fretwell 1974; Lloyd 1987; Duarte and Alcaraz 1999). In the experiments conducted in the **lab** (**Chapter 1**) we found a **positive correlation between the clutch and egg size**, at the same time **related with the highest food concentration and the largest females**. In the field, we observed variability in the egg size among egg sacs and even within the same egg sac (pers. obs.). Castellani et al. (2007) observed in *Oithona similis* that the largest egg sacs contained the highest number of eggs, but not the largest eggs.

Egg size varies among species of *Oithona* spp.; generally the largest eggs belong to the largest species, in agreement with data from the literature (e.g. McKinnon and Klumpp 1998; Nielsen et al. 2002). The difference in egg sizes among species of *Oithona* spp. might explain why maximum clutch sizes are not so different, which means that **the largest species of *Oithona* spp. might produce largest eggs instead of more eggs in comparison with smaller species,** when non-limited food conditions are present in the environment.

Egg size affects the proportion of eggs hatching and the naupliar survival; and the food quantity and quality can determine the organic content of the eggs, which could affect the hatching success (Guisande and Harris 1995). It has been reported for calanoid copepods that producing larger eggs benefits the hatching success and naupliar survival, as well as a positive

relationship between availability of food and egg size (*Calanus helgolandicus*, Guisande and Harris 1995). However, the effect of egg size on the hatching success of *Oithona* spp. has not been investigated.

Effect of food on egg production

Among the principal factors regulating egg production in copepods are both the quality and quantity of food (reviewed by Mauchline 1998). It is well established that egg production increases when food availability increases (e.g. Marshall and Orr 1955; Runge 1985; Niehoff and Hirche 2000).

Our laboratory experiments showed how **food concentration affected the egg production of *Oithona davisae* in terms of time of appearance of ovigerous females in the population and clutch size** (**Chapter 1**). In the same regard, Niehoff (2000) reported the effect of food availability in the gonad maturation of *Calanus finmarchicus*, which will cause a delay in the appearance of ovigerous females in the population.

In the field, however, the effect of the ambient food concentration on the fecundity of the females might not be so obvious, because of the influence of other factors, like the reproductive status of the females (Ianora 1990), can also be important. Previous feeding history (i.e. periods of high or low food availability) can affect the timing of fecundity as well as the number of eggs produced, if the energetic reserves of the copepods (if present) are not enough to sustain the egg production at normal rates. In tropical waters, the egg production of *Oithona* spp. was greatly affected by food limitation (**Chapter 4**), however, in the high latitude environments studied, although food limitation was also evident, the egg production was comparable to that in non-limiting food conditions (**Chapter 3**). As mentioned earlier in this discussion, *Oithona similis*, at high latitudes, might be relying on lipid reserves for fueling egg production under unfavourable conditions. Thus, **the presence of reserves could explain the different effect of food limitation on the egg production of *Oithona* spp. from tropical and polar environments**.

In our results we found two puzzling situations for *Oithona similis* in our high latitude investigations. In **Chapter 2** we reported a positive correlation between egg production rate of *Oithona similis* and protozooplankton biomass in the environment, whereas in **Chapter 3** no significant correlation was found (very likely related to an allocation of food intake into reserves instead of egg production). In general, we might consider a positive correlation between egg production rate of *Oithona* spp. and protozooplankton concentration (if we do not consider the effect of temperature), as previously reported in other studies (Nielsen and Sabatini 1996; Castellani et al. 2005a; Castellani et al. 2007). In agreement, in free-spawner calanoid species, such as *Calanus*, bloom conditions in the water can accelerate the egg production (Frost 1985).

The effect of food quality on the egg production of *Oithona* spp. has received little attention and almost no information is available in the literature. Protozoans are recognized as important and nutritious food source for copepods (Stoecker and Capuzzo 1990). The effect of food quality on reproductive characteristics of calanoid copepods has been reported in several studies (e.g. Jónasdóttir 1994; Pond et al. 1996; Shin et al. 2003; Jónasdóttir et al. 2009). However, the effect of food quality on cyclopoid copepods is almost non-existent. This is an interesting topic to be studied under controlled conditions in laboratory experiments. With this information we might be able to explain why similar egg production rates are derived from different ingestion rates, if we know the nutritive value of the prey ingested and their effect on the egg production of *Oithona* spp.

Effect of temperature on egg production

Together with food concentration, temperature affects the physiology of species in terms of egg production, growth and development (Uye 1980). The contribution of each of these factors in driving egg production of a copepod population, however, depends very much on the environmental conditions and seasonal variations. For instance, although food concentration is a major factor limiting the fecundity of calanoids, in some species the temperature affects the egg production rather than the food (Uye 1981; Ambler 1986; Halsband and Hirche 2001).

Generally, *in situ* rates of fecundity in epipelagic copepods are positively correlated with temperature (Bunker and Hirst 2004). **The fecundity of *Oithona similis* was not very affected by temperature in terms of clutch size in the field** (Chapter 2 and 3), and this is in agreement with other studies conducted on *Oithona* spp. (Ward and Hirst 2007), and also on calanoid copepods (*Calanus finmarchicus*, Hirche et al. 1997). In our studies, **the limiting effect of temperature on egg production rates was mainly consequence of the long hatching times**, and not related with the number of eggs per female (**Chapter 2 and 3**), in agreement with other seasonal studies on reproductive characteristics of *Oithona* spp. in high latitude environments (Metz 1995; Ward and Hirst 2007; Dvoretsky and Dvoretsky 2009).

Thus, clutch size does not seem to be the main fecundity parameter affected by temperature, and it is more likely that temperature limits the spawn frequency of the clutches rather than their size in *Oithona*. Uye and Sano (1995) reported a positive correlation between weight-specific egg production of *Oithona davisae* and temperatures below 22ºC.

However, Castellani et al. (2007) reported that *Oithona similis* egg production rates increased with both ciliates and dinoflagellates concentrations, rather than with temperature in the Irminger Sea. This is in agreement with Nielsen and Sabatini (1996) who found a correlation between the egg production of *Oithona* spp. and protozooplankton in the North Sea. Thus, it is very likely that a combination of food availability and temperature have an effect on the fecundity of *Oithona*, although in some cold areas, temperature seems to have a major role.

Egg production efficiency

The lower fecundity of *Oithona*, in comparison with free-spawning calanoids, has been attributed to its inefficient feeding (Kiørboe and Sabatini 1994), however, data on egg production efficiency (i.e. GGE) of *Oithona* spp. are very scarce in the literature. We have addressed this topic under controlled conditions in the lab (**Chapter 1**) and in contrasted environments in the field (**Chapter 3 and 4**).

In our field investigations, the egg production efficiencies (i.e. gross growth efficiency, GGE) ranged from 1% (during the spring-bloom in Greenland, **Chapter 3**) to 67% (in the GBR lagoon in Australia, **Chapter 4**) when considering experiments separately. In tropical waters, **the high values of egg production efficiency reported are very likely due to the miss of some of the potential prey of *Oithona* spp.** (i.e. underestimation of ingestion) (Chapter 4). Whereas, **in the Arctic, the low GGEs reported for *Oithona similis* during the spring bloom are probably the result of the allocation of ingestion into lipid reserves instead of egg production** (Chapter 3). In addition, estimation of egg production from the females of the population (i.e. samples from the tows) instead of from individual incubations can lead to under or overestimation of egg production rates, because from individual observations we obtain the actual egg production of the female based on its own fecundity characteristics (clutch size, body weight, egg size) and not a derived estimation. In the lab (**Chapter 1**), the estimation of egg production efficiency of *Oithona davisae* was directly derived from individual observations of feeding and egg production, what provides a more reliable value (16%).

The diet can also have an important role in the egg production efficiency, in the way that a nutritive diet will allow for a more efficient egg production, but as I have mentioned before, this topic has not been assessed on *Oithona* spp. From our results in the field, it is difficult to discern between the influence of the composition of diet, the ingestion rate, and other factors on the GGE of *Oithona* spp.

Hatching success

The recruitment success determines the success of a species, thus the success of *Oithona* spp. in terms of high abundances and survival in unfavourable conditions is conditioned by their successful reproductive strategy. The egg-carrying strategy did not allow for high egg production rates when temperature is limiting the egg development, hatching and inter-clutch times. However, this reproductive strategy has some advantages.

Related with this, the few studies that have addressed the hatching success of *Oithona* spp. have reported values of 100% in most of the observations (see Annex B). This means that although the reproduction in cyclopoid copepods is limited by the fact of carrying egg sacs, the recruitment success is high, what could be very likely linked to a nutritive diet. We found high percentages of hatching success of *Oithona similis* in the Arctic and subarctic, as well as of *Oithona aruensis* in Australia (Annex B and pers. obs. not publ.). We observed **hatching success of 100%** in approximately 99% of the observations, when *Oithona* spp. was **feeding on a diet based mainly on ciliates and dinoflagellates**.

Weight-specific egg production as estimate of growth

The assumption that the weight-specific egg production rate of the females is equal to the growth rate of the younger stages (Sekiguchi et al. 1980; Berggreen et al. 1988) is not verified for cyclopoid copepods, and it has been argued for calanoid copepods (Hirst and Sheader 1997; Hopcroft and Roff 1998; Hirst and McKinnon 2001). When food availability/suitability is not limiting, this equivalence of juvenile growth and weight-specific egg production has been demonstrated for calanoid copepods (e.g. Berggreen et al. 1988; Fryd et al. 1991).

However, our estimations of weight-specific egg production rates of *Oithona davisae* under presumably optimal and non-limiting food conditions (**Chapter 1)** were lower than growth rates for juveniles of the same species under similar food conditions (Almeda et al. 2010). In the same regard, it has been reported in a tropical area how growth of nauplii seem to be not food limited, and higher than that from adult stages, very likely because of the ability of nauplii to feed on smaller cells (Roff et al. 1995). Thus, in some of oligotrophic environments, the use of SEP might result in wrong estimation of production. Thus the equivalence between growth of juveniles and SEPR of adult females might not be valid for cyclopoid copepods.

Hatching time vs inter-clutch time

Most investigations on reproductive characteristics of *Oithona* spp. have used hatching time (the embryonic development time) to estimate egg production rates, not considering that once the eggs have hatched there might be a time lag before a new clutch is released (i.e. period of time before another sac can be laid). There are very few estimates of this time between hatching and the production of a new clutch, which indicated that the time from hatching to the production of a new clutch might be independent of temperature (Uye and Sano 1995; Ward and Hirst 2007). However, Saiz et al. (1997) found that egg sacs of *Oithona* appeared faster when food availability was higher, suggesting a dependence on food concentration.

Sabatini and Kiørboe (1994) estimated the time between clutches of *Oithona similis* to be approximately 3.4 days and the egg hatching to be 3 days at 15ºC (then the interval of clutch was 0.4 days). Uye and Sano (1995) estimated a mean period of interval of clutch of 0.75 days from observations of adult females of *Oithona davisae* under a range of temperatures from 10 to 30ºC. This estimation of **time from hatching of egg sacs to the development of new ones in *Oithona similis* at 4ºC** was less than 1 day in 60% of our observations (Annex B), what indicates that this time can be longer. Thus, if the inter-clutch time is approximately one day longer than the hatching time, the egg production rates using the hatching time might be overestimated in approximately 17% of that using the inter-clutch time. If the time from hatching to the production of a new clutch increases at temperatures below 4ºC, this overestimation could be even more severe. There is no data available on the effect of such low temperature on the inter-clutch time of *Oithona* spp., for this reason we should be cautious in the estimation of secondary production of *Oithona* spp. in terms of egg production at low temperatures. And it should also be considered that intrinsic characteristics of the females, such as the age, can also affect these times, as suggested by the variability observed in *Oithona similis* (Annex B).

CONCLUDING REMARKS OF REPRODUCTION

- Little variability of clutch sizes of *Oithona* might indicate that reproductive effort is put on producing larger eggs than larger clutch sizes, as indicated by the results of *Oithona* fecundity in the field (Chapter 2, 3, and 4).
- *Oithona davisae* did not reach the typical 30% of gross growth efficiency (**Chapter 1**) described for copepods (Ikeda and Motoda 1978; Straile 1997), very likely due to low assimilation efficiency of the adult females. High metabolic costs to explain low GGE are unlikely because the low metabolic requirements of *Oithona*, related with its ambush feeding behaviour, are well known.
- **Temperature, food (Bunker and Hirst 2004) and female size are important factors controlling copepod egg production rates.** The importance of one factor over the other seems more evident in high latitude environments where low temperatures are present, and in which temperature is greatly limiting the egg production of *Oithona* spp. In environments where we do not find extreme conditions, a combination of the factors might be controlling the egg production of *Oithona* spp.
- The food concentration affects *Oithona* egg production in terms of number of ovigerous females in the population and the interval at which clutches are produced rather than the clutch size itself.

POPULATION DYNAMICS, DISTRIBUTION, AND LIFE STRATEGY

The environmental conditions determine the relationship between the standing stock and the production of a population, and the seasonal variation will at the same time affect the food supply and climatic conditions that determine the environment (Lenz et al. 2000). Production and feeding strategy are related, thus a complete and nutritive diet will contribute to enhance the success for an individual, what will ultimately enhance the success of the population within the environment.

In earlier sections, the effect of food supply in individual biological aspects has been discussed, and in this section I will discuss the consequences in the population, as well as the effect of temperature, a decisive factor controlling the growth rate. Population abundance is the result of the balance between the birth and the mortality rate, and although we have not estimated mortality rates, they will also be considered in this discussion.

POPULATION DYNAMICS

Seasonal changes in the population density of marine copepods can be, in great part ascribed to variability in egg production, which in turn is affected by temperature and food quantity and quality (Guisande and Harris 1995). In addition to the breeding intensity *per se*, the hatching success of the eggs and the survival of the early larval stages may also affect recruitment to populations of marine copepods (Mullin 1991).

To understand the seasonal population dynamics of *Oithona* spp. and their response to environmental changes we need information on how seasonality will affect the life cycle of the organisms (**Chapter 2**). Studies on the seasonal changes in numbers of each developmental stage can give information of the number of generations occurring in a year, however it is not always successful due to the life history of the species and the overlap of generations. Although we were not successful to identify cohorts in the seasonal study conducted in the Kapisigdlit fjord, we described seasonal changes in the population composition (**Chapter 2 and 3**).

Copepods have developed life strategies to adjust their growth and reproduction periods to those times of the year when optimal conditions are present in the water. Overwintering or a resting stage in order to overcome adverse environmental conditions, can be found in different forms: diapause eggs, resting or dormant eggs, dormant copepodites, and dormant and diapausing adults (Norrbin 1993; Marcus 1996; Williams-Howze 1997 and references therein). Overwintering strategy plays a key role in the life strategy of the co-existing *Calanus* spp. with *Oithona* spp. in high latitude marine ecosystems. *Calanus* species, from temperate and high latitudes, have developed adaptations to survive long unfavourable-food periods (e.g. ontogenetic vertical migration, reduce of metabolism in late stages), including physiological aspects to cope reproduction with optimal food environmental conditions (Hirche 1996, and references therein). Diapause for cylcopoid copepods has only been described in freshwater species. Santer and Lampert (1995) reported summer diapause of *Cyclops* species as a strategy to avoid mortality caused by either predation or competition.

Diapause has not been reported for marine cyclopoids, on the contrary *Oithona* has been found in surface waters in unfavourable conditions in many studies (e.g. Castellai et al. 2007; Dvoretsky and Dvoretsky 2009), often at high abundaces (Dvoretsky and Dvoretsky 2009). Related to this activity in unfavourable conditions, one important conclusion of this thesis is the reported **winter activity of the population of** *Oithona similis* (**Chapter 2 and 3**). The difference in winter activity between large calanoids and cyclopoids stresses the importance of small copepods in high latitude environments (**Chapter 2 and 3**).

The year-round presence of *Oithona* spp. in the plankton community have been reported in many studies, and although this is probably the case of the cosmopolitan species *Oithona similis*, in certain environments, the succession of species in other areas has received little attention (Mazzocchi and Alcalà 1995). In the Arctic, where biological processes occur at lower pace than in lower latitudes, *Oithona* spp. present active populations throughout the year, with low densities in winter, but able to maintain a residual population which we have reported is feeding and reproducing at low rates (**Chapter 2 and 3**).

Several authors (Marshall, 1949; Frolander, 1962; Heinrich, 1962; EI-Maghraby, 1965; Faber, 1966) have reported on year-round reproduction of *Oithona*, as well as the maintenance of a high population density in winter, which suggests that metabolic needs are met during this season of low primary production (Lampitt and Gamble 1982). Although we found evidences of the year-round reproduction, winter densities were lower than in spring and summer (**Chapter 2 and 3**). **Temperature affected the development of the population of *Oithona similis*, together with the availability of food, and high densities were not reached in winter** (Chapter 2 and 3). Although *Oithona* is capable to maintain a residual population in unfavourable conditions, peaks of productivity were present when more favourable conditions were present in the water (**Chapter 2**). However, we very likely did not observe the period of maximum production of *Oithona* in polar areas, which has been reported to occur in late summer-autumn, when temperatures are generally higher, and protozooplankton abundant (e.g. Madsen et al. 2008; Dvoretsky and Dvoretsky 2009).

Based on several studies of the abundance of *Oithona* spp. in marine environments from different latitudes, we can conclude that they can be as abundant in the Arctic as in the tropics. However, the importance in terms of abundance and biomass might vary depending on the co-existing copepods in a determine environment. In the case of the Arctic and subarctic we have observed the importance of *Oithona similis* in periods when larger copepods go into diapause and small copepods dominate the zooplankton community (Chapter 3). In tropical environments there is not a marked succession in the copepod composition in the plankton community, however, *Oithona* spp. is an extremely abundant copepod in neritic areas.

DISTRIBUTION

Studying the distribution and seasonal cycle of marine zooplankton has to face the problem that the sea is a highly dynamic environment, with a steady motion and mixing of water masses. The currents and advection of new water masses makes it almost impossible to follow the same population of organism (Huntley and Niiler 1995). And although these processes exist in fjord systems, it somehow can provide the certainty that we are dealing with the same population. For this reason, aspects of the population dynamics and life strategy of *Oithona* spp. were investigated in a fjord system (**Chapter 2**).

The ontogenetic changes in vertical distribution of calanoid copepods are relatively well known in cold waters (REF). The daily migration, rising at dusk and descending at dawn, has the main function of avoiding predation, and often extends over several hundreds metres. On the other hand, the seasonal vertical migration is very typical of members of the genus *Calanus* in high latitudes, and can reach depths of hundreds up to a few thousand metres.

Patterns of vertical distribution have been poorly studied on cyclopoid copepods, and only a few studies have addressed this topic on *Oithona* (Kosobokova 1980; Gislason 2008; Tanimura et al. 2008; Takahashi and Uchiyama 2008; Saiz et al. 2013). We have reported that **all developmental stages of *Oithona similis* were mainly located in the upper 100 m** although a deeper distribution of adult females of *Oithona similis* could be expected in winter (**Chapter 2 and 3**). This pattern of ontogenetic change has been observed for *Oithona* (Ueda 1987; Takahashi and Uchiyama 2008). However, Takahashi and Uchiyama (2008) reported different vertical pattern distribution of nauplii of different-sized *Oithona* (*Oithona atlantica* and *Oithona similis*), what might indicate different life strategies. The ontogenetic changes in vertical distribution, in which the earliest stages are located in shallower waters and moved to deeper waters when they became larger, however, is not particular of *Oithona*, since it is widespread among neritic planktonic copepods.

The presence of *Oithona* spp. has been observed in deep waters in the Arctic in winter (Gislason 2008); although some of our samplings were conducted in winter, the dynamism of the area did not allow to see any clear pattern of distribution, which very likely was influenced by the hydrography. It should also be considered that reported presence of *Oithona* in deep waters (Saiz et al. 2013) could be due to the presence of different species (e.g. *Oithona atlantica* or *Oithona plumifera*) in the water column, with different patterns of vertical distribution.

In the investigation conducted in the Kapisigdlit fjord, **we did not find patterns of diel migration of *Oithona similis*, and the seasonal migration was not clear** (**Chapter 2**). In contrast, Tanimura et al. (2008) reported small-scale diel vertical migration of *Oithona similis* in Antarctica, with an unexpected pattern in which copepods remained in the upper layer during the day, and moved into deeper waters at night. Although the authors attributed this behaviour to changes in food availability in the water column, an alternative explanation could be to have an opposite diel vertical migration to that of larger species what might allow *Oithona* to avoid mortality by predation. Although a seasonal migration has been reported for *Oithona* in a high latitude environment, the animals were located shallower than other copepods in winter; furthermore, the larger coexisting *Calanus* showed a greater seasonal migration (Gislason 2008), what indicates different life strategies between *Oithona* and *Calanus* species of high latitude environments.

LIFE STRATEGY

The life strategy of *Oithona* spp. is considered the clue of their successful and wide distribution in marine environments. If we put together that they are well adapted to unfavourable food conditions, capable to survive at low metabolic rates, and that they have low mortality rates (related with their capability to avoid predation), it might actually be a successful life strategy that *Oithona* has.

Although little is known about the role of cyclopoid copepods in the plankton community, their life strategy could follow a different spatio-temporal pattern from calanoid copepods, especially in high latitude environments. Other small copepods, also abundant in the high latitude environments studied were *Oncaea* spp., *Microsetella* spp., and *Pseudocalanus* spp. (**Chapter 2 and 3**). Among them, only *Oncaea* spp. have a comparable wide and ubiquitous distribution to *Oithona* spp.; however, the feeding behaviour of *Oncaea* spp. is different from *Oithona* spp. and probably limit their capability to exploit some environments where *Oithona* is present. For this reason, among cyclopoid copepods *Oithona* is considered the most successful; however, information on *Oncaea* is even more limited than for *Oithona*. In the same regard, many aspects of the ecology of small copepods, which are co-existing species with *Oithona* spp. in the plankton community, have been little studied.

Cyclopoid species seem to show less specialization than calanoids (Paffenhöfer 1993), and they are able to survive over an extended range of environmental conditions. However, it has been speculated, and in this thesis observed, how ***Oithona similis* fitness in a high latitude environment can be severely limited by temperature** (Ward and Hirst 2007; **Chapter 2 and 3**).

Reproductive strategy

The reproductive strategy is one of the main aspects very likely involved in the different life strategy of *Oithona* and calanoids. In polar areas, an increase in food availability is rapidly translated into egg production in *Calanus* species, whereas for *Oithona* this increase is constrained by the frequency of hatching. In addition, the feeding strategy of *Oithona* limits ingestion, metabolism, and egg production. The feeding strategy allows for lesser metabolic expenditure and predation risk than calanoids with a suspension-feeding strategy. However, the lower ingestion rates of *Oithona*, implies lower fecundity in comparison with calanoids with high daily rations. Furthermore, the small size of *Oithona* contributes to a lesser risk of predation than larger copepods, but it has the disadvantage that limits clutch size and the capability to storage big lipid reserves, in contrast with the large calanoids in the Arctic.

A free-spawner female can produce and release eggs almost continuously (Runge 1987; Niehoff and Hirche 1996; Hirche et al. 1997), whereas egg-carrying copepods cannot produce new eggs until the sacs they are carrying hatch. This fact is very likely the clue of such differences in maximum egg production for similar-sized calanoids and *Oithona* spp. in areas where temperature has a limiting role in the development of copepods. For calanoid copepods we find a great variation between minimum and maximum numbers of egg production (e.g. *Calanus finmarchicus* from 3 to 96.5 eggs per female and day, Niehoff and Hirche 2000) in comparison with *Oithona* spp. (*see* **Chapter 1**). Large copepods present a proportional much bigger volume than smaller ones, in a theoretical relation of L^3, (given a *Calanus finmarchicus* of 3.5 mm and O. similis of 0.5 mm prosome lenght, the 7 times decrease in size corresponds in the order of almost 400 times decrease in body volume; Saiz et al. 2013). This fact might indicate a constraint in the maximum clutch sizes and egg production rates due to the biovolume of the copepod.

In addition, in egg-carrying copepods, however, exist a limitation in the maximum clutch size, due to the reproductive strategy. **The size of the females of *Oithona* spp. seems to limit the maximum number of eggs per clutch they can produce**, very likely limited by the size of the ovaries. However, detailed information on the structure of female gonads of

cyclopoids is very scarce, and mainly focused on freshwater species (Wyngaard and Chinnapa 1982). Besides this limitation, egg sacs that a female can carry should not be too large, because they could limit the swimming capability of the copepod; furthermore, there might exist a limitation related to the resistance of the membrane of the egg sac.

The maximum clutch size reported for *Oithona similis* represents around 10-12% of their body weight (considering a clutch size of 30 eggs per female, and female and egg weight of 0.80 and 0.05 μg respectively). In the co-existing calanoid, *Calanus finmarchicus*, maximum clutch size can represent >100% of their body weight (Hirche et al. 1997). Thus, it is very likely that two main aspects limit the maximum egg production of *Oithona*, one is the egg-carrying strategy, and the other, the small size.

Mortality rates

Verity and Smetacek (1996) argued that dominant species in the marine pelagic systems attain their status as much by reducing mortality due to predation as by increasing growth or reproductive rates. It has been hypothesized that low mortality rates of *Oithona* spp. could be one of the clues of their success in marine plankton communities. However, studies on the mortality rates of cyclopoids are very scarce, as well as the mortality of juveniles and adults of *Oithona* spp. in the field (Eiane and Ohman 2004; Thorr et al. 2008; Hirst and Ward 2008).

Although information on the mortality rates of *Oithona* spp. is limited, their capability to avoid predation based on its swimming behaviour is well known. The small size and transparency, and low motility of *Oithona* spp. consequence of their feeding behaviour, combined with its mechanoreceptor system, are together characteristics that might contribute to avoid predation. In addition, the egg-carrying strategy has some advantages to reduce the mortality of the eggs and ensure the survival of the nauplii.

Uye and Sano (1995) reported that *Oithona davisae* population was reduced in mid-summer in Fukuyama Harbour due to predation by a ctenophore rather than the reduction of its own reproductive rate. Furthermore, they pointed out that this species barely overwinter, and that its survival was mainly dependent upon the survival of the adult females. This is a clear example that predation is regulating the population of *Oithona* spp., and that the capability to avoid predation might be crucial for the successful development and survival of the population.

The motility of the ambush feeding of females of *O. davisae* is very low; in contrast, the males swim rapidly in order to search for females what often sacrifice their feeding and lead to higher predator encounter rates (Kiørboe 2007). The elevated mortality rates in adult males to females of *Oithona* spp., is predominantly due to higher predation on males (Hirst et al. 2010).

Ovigerous females are more susceptible to visual predation than those free-spawners, what might imply a higher mortality of egg-carrying females (Sandstrom 1980; Bollens and Frost 1991). In some species there has been found a migratory behaviour of females to avoid predation, in which ovigerous females would remain in deeper waters than the non-ovigerous ones (Bollens and Frost 1991). However, the migratory ability of small cyclopoid copepods is limited (Boxshall 1977; Ueda 1987).

Free-spawning calanoid have shorter hatching times than most cyclopoids. This is a strategy to compensate for the higher mortality of free eggs in the water (which can be up to 100%, Tang et al. 1998) than those attached in sacs to the body of the female. The lower developmental time of eggs in free-spawning calanoids implies that first nauplii have not developed a complete gut, thus they are not able to feed until NII-NIII stage (Landry 1983; Berggren et al. 1988). However, nauplii from the cyclopoid species *Oithona* are capable to feed immediately after hatching (Eaton 1971; Uchima and Hirano 1986). High mortality in the nauplii of *Oithona davisae* at low food levels may be attribute to their poor feeding ability in the first feeding and to their vulnerability to starvation (Uchima and Hirano 1986). It has been described that when newly hatched nauplii starve they do not develop past the first stage

(Eaton 1971; Uchima and Hirano 1986). Thus, the feeding environment in which eggs hatch will be the major factor to determine the survival of nauplii of *Oithona* spp.

In summary, laboratory studies are a good tool to experiment with the effect of parameters on the biology of the organisms, but unfortunately the information of *Oithona* based on experimental data is very limited. Both the lack of success in maintaining in culture wild animals, and the difficult of handling such small organisms, have contributed to limit the number of experiments conducted under controlled conditions in the laboratory. The scarce experimental studies conducted on cyclopoid copepods, limit our comprehension of their ecological success.

We still need to develop better tools to investigate in detail the feeding ecology of *Oithona* spp. in marine ecosystems. This information will contribute to understand how these small copepods will respond to variability in the environment. We also need efforts to model copepod feeding, information that will be useful for predictions of changes in marine ecosystems. Laboratory experiments under controlled conditions would give useful information for that purpose, without forgetting that *in situ* investigations are also needed to validate data obtained in models.

List of conclusions

The results obtained in this thesis lead for the following conclusions:

1. The low critical food concentrations exhibited by adult females of *Oithona davisae* in the laboratory indicates their capability to exploit oligotrophic environments successfully (**Chapter 1**).

2. Different functional responses of adult females of *Oithona davisae* (Type II) and their juveniles (Type III, Almeda et al. 2010), suggests different sensory capability to detect and capture prey at low food concentration (**Chapter 1**).

3. High fecundity rates reported for *Oithona* at low food concentrations are in agreement with records of year-round reproduction even in periods and/or environments with low food availability (**Chapter 1 and 3**). Hypothesis 4 is not valid.

4. Food concentration affects the fecundity of *Oithona* in terms of number of ovigerous females in the population and the interval at which clutches are produced rather than the clutch size itself (**Chapter 1, 3, and 4**).

5. A diet based mainly on components of the protozooplankton, confirms the role of *Oithona* as a link between the microbial food web and upper trophic food levels (**Chapter 3 and 4**). Hypothesis 7 is valid.

6. In polar areas, temperature limits the development of *Oithona* populations, and the capability of the females to achieve maximum egg production rates (**Chapter 2 and 3**). Hypothesis 5 and 8 are valid.

7. The life strategy of *Oithona* in polar areas is different from that of large-sized calanoid species inhabiting those waters, and it benefits from the independence of phytoplankton blooms to maintain active populations throughout the year (**Chapter 2 and 3**).

8. The winter activity of *Oithona similis* highlights the importance of small-sized copepods in high latitude environments, in particular when large-sized copepods disappear from the upper productive layers (**Chapter 3**).

9. In tropical areas, the production of *Oithona* is food limited and daily rations appear too low to sustain basic metabolism. Including a wider variety of prey (nanoflagellates and nauplii) in the diet, might allow reaching the carbon requirements needed to cover basic metabolism (**Chapter 4**). Hypothesis 8 is valid.

10. The ontogenetic vertical distribution indicated the presence of juveniles (mainly nauplii) in shallower waters than the adults (**Chapter 2 and 3**).

11. Seasonal patterns in vertical distribution were not evident, but results suggested a likely deeper distribution of the adult females in winter (**Chapter 2**).

12. High recruitment success together with a presumably lower mortality due to the particular ambush behaviour of this genus, might explain the ecological success of the populations of *Oithona* spp. in contrasted environments (**Chapter 3, 4 and Annex B**).

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Annex A

COPEPOD FEEDING RATES, CONVERSION FACTORS AND OTHER CALCULATIONS

In this thesis we have expressed the copepod feeding as clearance rate, ingestion rate, and daily ration. There are different ways to calculate these different expressions of feeding rates, sometimes making the comparison between results of different investigations difficult. Here I provide a little discussion of methodological aspects of feeding experiments, details on the way we have calculated feeding rates, and which conversions factors between length and carbon we have applied. In addition, other calculation methods used in this thesis are provided.

METHODOLOGICAL ASPECTS OF FEEDING EXPERIMENTS

One of the relevant questions in copepod biology is the kind of prey eaten and the amount of food needed to survive and reproduce. Several methods have been developed in order to estimate feeding rates and describe the composition of diet. We can roughly divide them into those that are direct measurements of ingestion (e.g. prey removal experiments in bottle incubations), and those that give qualitative information of the diet of the copepod by indirect measurements, which do not require incubation and can be conducted *in situ* (e.g. gut and faecal pellet content).

In the laboratory we can supply copepods with different kind and quantity of prey under controlled conditions, and expect that their behaviour in the laboratory will be similar to that at the sea in natural conditions. Laboratory estimations are a useful tool to predict how environmental changes could affect the biology of the organisms, and we can extract useful information than we can later apply to understand the results of experimental investigations conducted *in situ*. Under controlled conditions we can reduce the experimental factors to few variables of interest.

The natural diet of copepods can be investigated in situ, using food removal methods in bottle incubation, with which qualitative and quantitative estimations can be obtained (Chapter 3 and 4). However, qualitative approaches might give better results, because the trophic cascade effect, what could result in under or overestimations of ingestion. Possible solutions have been presented to minimize this trophic cascade effect when calculating feeding rates, but none of them with totally satisfactory results (e.g. size fractionation methods, and the use of equations to correct estimates, see below). In addition, the methods to quantify the cells ingested by copepods (e.g. counts under the microscope, Coulter counters) often give different results, what can make difficult comparisons between investigations.

It could be argued the existence of several limitations of the bottle incubation method, such as the crowding effect, unnatural turbulence, and the stress of capture, collectively known as "bottle effects" (Roman and Rublee 1980); besides that, the often time-consuming analysis (i.e. identification and count under the microscope). However, the bottle incubation method is the simplest direct method to estimate feeding rates, and is one of the longest in use (Gauld 1951; Paffenhöfer 1988). It is, as well, the only method that allows direct quantification of feeding rates on non-phytoplankton data, therefore a wider prey spectrum to be investigated (Båmstedt et al. 2000).

Another quantitative estimation is the measurement of gut fluorescence. However, this method to estimate feeding rates only consider phytoplankton ignoring other potential prey, and have the added problem that chlorophyll is destroyed within the gut (Mauchline 1998 and references therein). Atkinson (1996) reported diatoms ingestion of Oithona spp. by measuring the gut fluorescence, although he reported higher clearance rates of ciliates than of similar-sized diatoms from the bottle incubation method. In fact, the ingestion of diatoms reported by Atkinson (1996) under the microscope was much lower than clearance of total chlorophyll a reported by fluorescence, demonstrating how different methodologies can end up in controversial results.

Among other methods to investigate the feeding and diet of copepods that can also provide qualitative information, we find the measurement of gut content, faecal pellet content,

and lipid and protein content and composition.

The use of estimations of gut contents might be not good enough to determine the natural diet of *Oithona* spp. because on the one hand, certain prey ingested by *Oithona* spp. could not be detected in gut contents because their degradation (i.e. oligotric ciliates, which have no theca). On the other hand, the presence in the gut of some kind of prey (e.g. diatoms) could be consequence of their presence in the gut of larger prey ingested by Oithona spp. For instance, Pond and Ward (2011) suggested that diatom markers found in biochemical analysis in *Oithona* spp. could be derived indirectly from the ingestion of diatom-feeder prey. In the same way, the use of faecal pellet content would have the same problems when dealing with the diet composition. Approaches to quantify gut contents of copepods have been conducted in the base of genetic and molecular analyses (Troedsson et al. 2009). One of the problems of these techniques is the fast degradation of the DNA after ingestion. In any case, the use of molecular techniques is not yet optimize (Nejstgaard et al. 2008; Durbin et al. 2008; Simonelli et al. 2009) and has not been applied to small copepods, in great part to the extreme difficulty of the methodology (e.g. obtaining genomic libraries, design of primers, specificity of the test).

Lipid and protein content (i.e. biochemical methods) give insights into the diet of copepods, physiological state of individuals, and population fitness (in *Oithona* spp., Narcy et al. 200; Perumal et al. 2009). The lipid composition, with some exceptions, reflects the different modes of plankton species (Lee et al. 2006). Changes in the fatty acid composition can be used to determine the contribution of microalgae in herbivorous zooplankton, and how these phytoplankton fatty acid patterns are modified in omnivorous and carnivorous zooplankton (Dalsgaard et al. 2003).

Feeding rate calculations

Nejstgaard et al. (1997, 2000) pointed out the possible underestimation of copepod feeding rates on phytoplankton due to trophic cascade effects. If copepods present high clearance rates on microplankton, there is a release of grazing pressure on phytoplankton in the experimental bottles (with copepods) in comparison with control bottles (without copepods). Nejstgaard et al. (1997, 2001) proposed some equations to correct this cascade effect considering estimates of microzooplankton grazing from dilution experiments (Landry and Hassett 1982).

However, there is still controversy about how precise it is to apply those equations to correct, or reduce, errors of underestimation when conducting feeding experiments with the incubation method (Saiz and Calbet 2011). One of the main problems of applying corrections in the calculation of feeding rates is the use of an estimate of microzooplankton grazing in the equations (Saiz and Calbet 2011). The method proposed by Nejstgaard et al. (1997, 2001) imply the use of dilution experiments (Laundry and Hasset 1982) in parallel to the feeding experiments using copepods, but most studies use an estimation of microzooplankton grazing from the literature instead of conducting a parallel experiment. We have used the classical equations provided by Frost (1972) (*see* Annex a), since the logistic of our experiments did not allow for conduction of parallel dilution experiments to make corrections of trophic cascade effects.

FEEDING RATE CALCULATIONS

For the estimation of the feeding rates we have used the equations provided in Frost (1972).

Clearance rate

The clearance is a filtration rate, defined as the volume of water cleared of food by a consumer (i.e. copepod), expressed per unit time and per copepod (or body mass). The result of volume of water filtered assumes a 100% of capture efficiency.

The changes in prey concentration during the feeding experiments can be expressed as follows:

The growth in the bottles with only prey (control bottles), μ, was calculated from,

$$C_2 = C_1 e^{\mu(t_2 - t_1)}$$

where C_1 and C_2 are the prey concentration in the control bottles at time t_1 and t_2.

In the bottles with added copepods (experimental bottles) the grazing coefficient, *g*, was calculated from,

$$C_2^* = C_1^* e^{(k-g)(t_2 - t_1)}$$

where C_1* and C_2* are the prey concentration in the experimental bottles at time t_1 and t_2.

Using intrinsic growth rate (μ) and the grazing coefficient (g), the average cell concentration (C) for each experimental bottle during the incubation (time interval of t_2-t_1) is,

$$C = \frac{C_1^* \left[e^{(\mu - g)(t_2 - t_1)} - 1 \right]}{(t_2 - t_1)(\mu - g)}$$

The clearance rate (or volume swept clear, F), defined as the volume of ambient medium from which prey are removed by copepods, is given by,

$$F = Vg/N \ \ (\text{mL copepod}^{-1}\ \text{day}^{-1})$$

where V is the volume (mL) of the bottle and N is the number of copepods in the bottle.

Ingestion rate

Ingestion rate (I) is the number (or mass) of prey ingested per unit of time and copepod.

Using the average cell concentration (C) and clearance rate (F), then the ingestion rate is defined as,

$$I = C \times F \text{ (cells eaten copepod}^{-1} \text{ day}^{-1}\text{)}.$$

Although we have expressed the clearance and ingestion rates by days, other authors express those equations by hours.

Daily ration

The ingestion rate can be expressed as mass of prey ingested per mass of copepod and day, the weight-specific ingestion rate (WSI, in **Chapter 1**)

And the daily ration (DR) is the mass of food ingested, expressed as a percentage of copepod body mass,

$$DR = WSIR \times 100$$

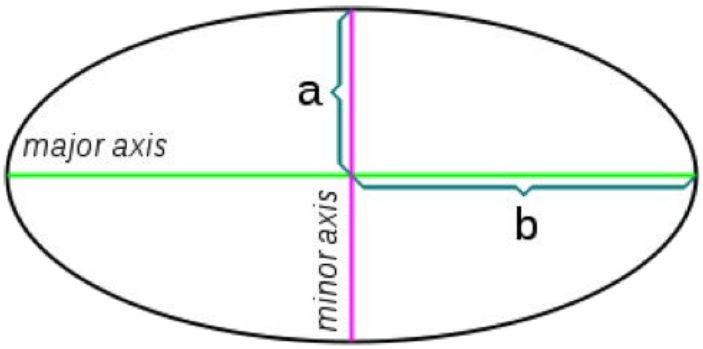
Carbon-to- biovolume factor for *Oithona davisae*

The carbon content or weight of a copepod is estimated using dry weights, however in Chapter 1 we were not able to measure the dry weight of the females. One of the problems was the small size of *Oithona davisae*, what made necessary a high number of copepods on the filters to be able to detect a difference of weight between the filter before and after the addition of the copepods. Another problem was that *Oithona* is an egg-carrying copepod, and we had mixed population of ovigerous and non-ovigerous females in each cylinder. Thus, to estimate the carbon content of a female we should either use non-carrying females, or deduct the weight of the eggs, and for that we needed to know the clutch sizes of the females on the filters.

We made an attempt to weight samples with mixed female, and with non-ovigerous females, but the amount of animals on the filters was not enough to obtain a good estimation. For this reason, we decided to try an indirect estimation of the carbon content using the shape of the prosome of the females, expecting that females under higher concentration of food were probably somehow wider. And from the geometrical shape of the females we estimated the volume.

First we calculated the biovolume of the females at each food level. We measured their prosome (major axis) and width (minor axis) using digital pictures (as explained in Chapter 1), and we considered the body as a prolate spheroid to calculate the corresponding volume using the next equation:

$$V = 4/3\ \pi\ a^2 b$$



Then using only the major axis (prosome length) we estimated the carbon content (C, µg C) of the females at each food level using the equation by Uye (1982),

$$C_{female} = 1.26 \times 10^{-4} \times PL^{1.31}$$

Finally, using an average of the biovolume and carbon content of the females from each food condition, we computed a carbon : biovolume factor. The final factor used to estimate the female biomass from biovolume measurements, was obtained by the average of factors for each food condition.

As an example, some of the values estimated for the females feeding in the food level of 800 cells *of Oxyrrhis marina* per

Female	Food level	Axis (µm)		Biovolume	Body weight (Uye 1982)	Body weight from biovolume
		Major	Minor	µm³	µg C	µg C
1	800	329.95	163.27	4605317.12	0.25	0.31
2	800	333.00	133.90	3126108.23	0.25	0.21
3	800	318.70	160.64	4306139.32	0.24	0.29
4	800	329.58	151.36	3953499.70	0.25	0.27
5	800	326.28	153.37	4018554.95	0.25	0.27
6	800	330.70	147.49	3766673.43	0.25	0.26
7	800	329.27	159.86	4405857.06	0.25	0.30
8	800	324.98	159.98	4354984.78	0.25	0.29
9	800	336.67	157.04	4347340.07	0.26	0.29
10	800	328.17	154.54	4103735.06	0.25	0.28
11	800	326.54	150.85	3890681.14	0.25	0.26
12	800	323.01	139.18	3276183.77	0.24	0.22

For females at [800] cells mL^{-1}

Average biovolume	4012922.89 µm^3
Average body weight (Uye 1982)	0.25 µg C

Conversion factor (body weight : biovolume) 6.20646E-08 µg C µm^{-3}

Then we estimated an average conversion factor to apply. This average carbon content : biovolume factor **6.74 × 10^{-8} µg C µm^{-3}** was then used to estimate the female biomass from the biovolume measurements.

Food level	Conversion factor
[50]	7.60616E-08
[100]	7.09791E-08
[200]	6.65174E-08
[400]	6.49893E-08
[800]	6.20646E-08
[1200]	6.3812E-08
	6.7404E-08

Carbon content of *Oithona similis*

To calculate the carbon content of both nauplii and copepodites of *Oithona similis* we used the equations given by Sabatini and Kiørboe (1994),

For nauplii: $W = 5.545 \times 10^{-8} L^{2.71}$
For copepodites: $W = 9.4676 \times 10^{-7} L^{2.16}$

Where *W*, is the body weight in µg C, and L is the total length (µm) for nauplii, and the cephalothorax length (µm) for copepodites.

The carbon content of the eggs (µg C) was calculated from the volume of the eggs by using the equation (Checkley1980; Uye 1981; Kiørboe et al. 1985):

$$0.14 \times 10^{-6} \text{ µg C µm}^{-3}$$

The volume of the egg was calculated considering the egg as a sphere: $4/3\ \pi\ r^3$

Calculation of sunrise and sunset times in Kapsigdlit (Greenland)

With the maximum number of sun hours (N), it is possible to calculate the dawn (sunrise) and dusk time. Considering that midday (12:00 h) is the time of the day when the Sun is at the highest position, the dawn and dusk time can be estimated by deducting the maximum number of sun hours divided by 2:

$$12 - (N/2)$$
$$12 + (N/2)$$

The photoperiod in the latitude of our samplings in Kapisigdlit was calculated using the statistical computing software R. The function "Daylength" (based on Forsythe et al. 1995) of the package Geosphere was used in R, with the required information of latitude and date (i.e. day, month and year).

Our data set was:

64° 25 N, 50° 22 W						
Julian day	date	max number solar hours	orto time	sunset time	orto real time	sunset real time
82	24/03/2010	12.45	5.77	18.23	05:46:22	18:13:38
93	03/04/2010	13.67	5.17	18.83	05:09:58	18:50:02
104	14/04/2010	14.89	4.56	19.44	04:33:19	19:26:41
112	22/04/2010	15.79	4.11	19.89	04:06:26	19:53:34
120	30/04/2010	16.69	3.66	20.34	03:39:22	20:20:38
130	10/05/2010	17.82	3.09	20.91	03:05:26	20:54:34
137	18/05/2010	18.60	2.70	21.30	02:41:53	21:18:07
144	24/05/2010	19.37	2.32	21.68	02:18:58	21:41:02
154	03/06/2010	20.37	1.81	22.19	01:48:52	22:11:08
168	18/06/2010	21.31	1.34	22.66	01:20:39	22:39:21
180	29/06/2010	21.23	1.38	22.62	01:23:00	22:37:00
187	06/07/2010	20.80	1.60	22.40	01:36:04	22:23:56
199	18/07/2010	19.69	2.15	21.85	02:09:15	21:50:45
209	28/07/2010	18.62	2.69	21.31	02:41:22	21:18:38
217	05/08/2010	17.73	3.13	20.87	03:08:03	20:51:57

Microplankton biovolume. Fitted shapes and volume equations used to estimate the biomass of the most frequent microplankton cells in Godthåbsfjord and Qeqertarsuaq (Greenland).

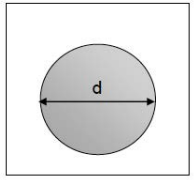
DIATOMS	Fitted shape	Equation
Chaetoceros spp.	Oval cylinder	$V=\pi/4 \cdot d_1 \cdot d_2 \cdot h$
Thalassiosira spp.	Cylinder	$V=\pi/4 \cdot d^2 \cdot h$
Pseudonitzschia spp.	Parallelepiped -20%	$V=l \cdot w \cdot h$
Navicula spp.	Parallelepiped -40%	$V=l \cdot w \cdot h$
Skeletonema spp.	Cylinder	$V=\pi/4 \cdot d^2 \cdot h$
Pseudo-nitzschia seriata	Parallelepiped -20%	$V=l \cdot w \cdot h$
Nitzschia longisima	2 cones	$V=\pi/12 \cdot d^2 \cdot h$
Thalassionema nitzschoides	Parallelepiped	$V=l \cdot w \cdot h$
Coscinodiscus spp.	Cylinder	$V=\pi/4 \cdot d^2 \cdot h$
Pseudonitzschia delicatissima	Parallelepiped -20%	$V=l \cdot w \cdot h$
Rizhosolenia 300 µm??	Cylinder	
Dictyocha speculum	Half-sphere	$V=\pi/12 \cdot d^3$

DINOFLAGELLATES	Fitted shape	Equation
Gyrodinium-like	2 cones	$V=\pi/12 \cdot d^2 \cdot h$
Gymnodinium-like	Rotational ellipsoid round cross section	$V=\pi/6 \cdot d^2 \cdot h$
	Flattened ellipsoid elliptic cross section	$V=\pi/6 \cdot a \cdot b \cdot h$
Protoperidinium (bipes?)	Half cone	$V=\pi/24 \cdot d^2 \cdot h$
Gonyaulax (runde thecate)	Sphere-25%	$V=\pi/6 \cdot d^3 \cdot 0.75$
Protoperidinium (depressum?)	Cone+half sphere - 20%	$V=\pi/12 \cdot d^2 \cdot (h+d/2) \cdot 0.8$
Dinophysis spp.	Flattened ellipsoide	$V=\pi/6 \cdot a \cdot b \cdot h$
CILIATES		
Round ciliates	"Sun", "ball"	$V=\pi/6 \cdot d^3$
Mesodinium	2 spheres*5/8	$V=\pi/6 \cdot d^3 \cdot 2 \cdot 5/8$
Strombidium-like	Half rotational ellipsoide circular cross section	$V=(\pi/6 \cdot h \cdot d^2)/2$
	"Cone"	$V=\pi/12 \cdot d^2 \cdot h$
Tintinnids	Ovale	$V=\pi/6 \cdot a \cdot b \cdot h$

Sphere

Volume: $V = \pi/6 * d^3$

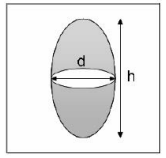
V: volume
d: diameter



Rotational Ellipsoid

Volume: $V = \pi/6 * d^2 * h$

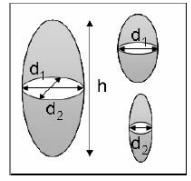
V: volume
d: diameter
h: height



Flattened Ellipsoid

Volume: $V = \pi/6 * d_1 * d_2 * h$

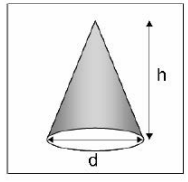
V: volume
d_1: large diameter
d_2: small diameter
h: height



Cone

Volume: $V = \pi/12 * d^2 * h$

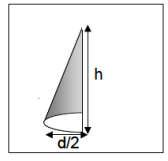
V: volume
d: diameter
h: height



Half Cone

Volume: $V = \pi/24 * d^2 * h$

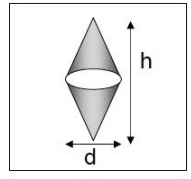
V: volume
d: diameter
h: height



Double Cone

Volume: $V = \pi/12 * d^2 * h$

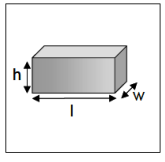
V: volume
d: diameter
h: height



Parallelepiped

Volume: $V = l * w * h$

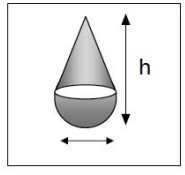
V: volume
l: length
h: height
w: width



Cone with half Sphere

Volume: $V = \pi /12 * d^2 * h$

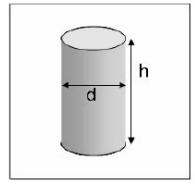
V: volume
d: diameter
h: height



Cylinder

Volume: $V = \pi/4 * d^2 * h$

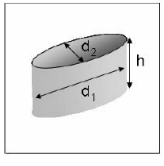
V: volume
d: diameter
h: height



Oval Cylinder
(ellipsoid or prism on elliptic base)

Volume: $V = \pi/4 * d_1 * d_2 * h$

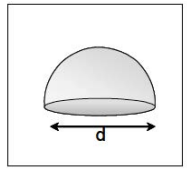
V: volume
d_1: large diameter
d_2: small diameter
h: height



Half Sphere

Volume: $V = \pi/12 * d^3$

V: volume
d: diameter



Olenina et al. 2006

Volume to carbon conversion factors used for microplankton in Greenland

From Mender-Deuer and Lessard (2000):

Diatoms. The volume (V, μm^3) conversion factor used for diatoms was:

$$Pg\ C\ cell^{-1} = 0.288 \times V^{0.811}$$

Dinoflagellates. The volume conversion factor used for all dinoflagellates was:

$$pg\ C\ cell^{-1} = 0.760 \times V^{0.819}$$

Ciliates. The volume conversion factor used for ciliates was:

$$pg\ C\ cell^{-1} = 0.216 \times V^{0.939}$$

Annex B

OBSERVATIONS ON FECUNDITY AND RECRUITMENT SUCCESS OF *OITHONA SIMILIS* AT A FIXED TEMPERATURE

The fecundity and recruitment success determine the success of a species. In a period of the year of maximum production (June) we monitored individual adult females of *Oithona similis* in the Godthåbsfjord in Nuuk (Greenland).

Method

Adult females of *Oithona similis* were collected in Godthåbsjord during the BoFYGo cruise in late spring of 2010. A 50-µm plankton net with a non-filtering cod end was used.

Egg-carrying females of *Oithona similis* were placed individually in 24-multi well plates containing 3 mL of filtered surface water and incubated at constant temperature of 4°C. Seawater in each well was renewed every day by *in situ* seawater from max chlor *a*, to ensure a food supply for the females, and test only the temperature as a factor controlling fecundity.

Every 12 h the wells were inspected under a stereomicroscope, and the number of females from the eggs had hatched was noted. The hatching success was also recorded by recording the number of eggs the females were carrying and the number of viable nauplii hatched.

Results

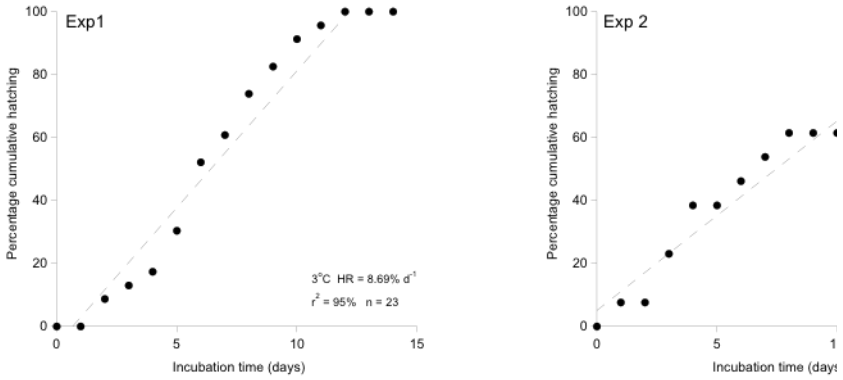


Fig. 1. Percentage of cumulative hatching (%) of *Oithona similis* at 4ºC in relationship with the incubation time.

- The eggs in early development are difficult to distinguish and count in the sac, only when they are more developed (more transparent) is possible to count them. In general, this makes underestimation of the number of eggs that the female is carrying.
- In some occasions, the second clutch observed presented "empty" eggs, maybe not viable.
- The process of hatching could take several hours, not all the eggs hatched at the same time. In several observations a few eggs remained attached to the body of the female from some hours.
- The second clutch is normally smaller.
- In most of the females the time for hatching to the appearance of a new clutch is less than 24 hours.
- In 62% of the observations the time from hatching to the development of a new clutch was less than 24 hours, in 17%this process took between 24-48 hours; in 5% 48-72 h; and in 14% more than 72 h.
- Hatching success: 71% females presented 100% hatching success; 9% presented > 90% hatching success; 20% females presented < 90% hatching success.

Discussion

In the egg ratio method used to estimate in situ egg production rates (Edmondson 1971) it is commonly used the hatching time instead of the intercluth time. The results found here show on the one hand, how time between hatching and the production of a new clutch can be up to several days, and on the other hand, that the egg development time showed an individual variability. These observations imply that the common use of hatching times in the calculation of *in situ* egg production rates will result in a considerable overestimation in the production of *Oithona* spp., at least at low temperatures.

In the first chapter we used the interclutch time for the estimation of egg production rates of *Oithona davisae*; however, in the egg production calculations of *Oithona similis* we did not consider to change the hatching time for the interclutch time.