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SCHOOL OF OCEAN AND EARTH SCIENCE

Reproduction and larval biology of North Atlantic asteroids related to the invasion of the deep sea

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ABSTRACT
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<u>Doctor of Philosophy</u>

REPRODUCTION AND LARVAL BIOLOGY OF NORTH ATLANTIC ASTEROIDS
RELATED TO THE INVASION OF THE DEEP SEA

by Francisco Benitez Villalobos

A very important objective of ecological research is to explain the evolution of life histories, more specifically how natural selection modifies reproduction and development in order to generate the patterns that are observed in nature. With few exceptions, the reproductive mechanisms and patterns found in deep-water echinoderms are entirely similar to those found in shallow-water species. The aims of this study were 1) to examine the reproductive biology of the many deep-sea asteroids found on the continental slope to the west of Europe in order to determine if the reproductive adaptations are a function of depth, distribution or are phylogenetically controlled, and 2) to conduct experiments on the effects of pressure and temperature on larval development of Atlantic asteroids, to investigate the physiological potential for deep sea invasion by shallow-water species. Eggs of the shallow-water asteroids *Asterias rubens* Linnaeus and *Marthasterias glacialis* (Linnaeus) were fertilized *in vitro* and incubated through the early embryonic cleavages until the larval stage. They were subjected to different temperature/pressure regimes. Early embryos were able to tolerate pressures up to 150 atm at 15°C and 100 atm at 10°C. Survivorship of *A. rubens* swimming bipinnaria remained high (> 70%) after incubation at all the pressure/temperature combinations. In *M. glacialis* the highest survival of swimming larvae was 100% at 1 atm/5, 15 and 20°C and 50 atm/15 and 20°C. Data for the temperature and pressure effects on the later stages of development suggest that all the larval stages are more temperature/pressure tolerant than the early embryos and survivorship becomes greater with larval age. Therefore, the larvae of these two species could survive transport to deeper waters and these species may be capable of sending colonists to the deep sea. In the deep NE Atlantic the habitat has selected for species with specific reproductive traits, which provide them with successful and advantageous life history strategies. This can be clearly observed in the upper bathyal zone between 700 and 1100 m, where the environmental conditions have selected for small species with low fecundity and large eggs, plus habits related directly or indirectly with suspension feeding. These species exhibit reproductive features with trends to the opportunistic strategy and are distinctive of unpredictable environments, although their large egg size probably follows the general trend observed in species from cold waters in order to provide the larvae with energy sufficient for a high survival possibility. Conversely, phylogenetic and evolutionary factors are also important and seem to be decisive at the deepest waters where basically mainly species belonging to the strict deep-sea family Porcellanasteridae are found. All these species possess a mixture of features typical of classical *K* strategists and equilibrium strategists, which enable them to persist in a relatively stable environment with low energy availability. A comprehensive knowledge of the reproductive processes of the deep-sea fauna is essential in order to evaluate the level of variability caused in the environment principally by human activity and the possible effects on life-history of the species.

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To my family for always looking after me like if I was the youngest member.

To Falcon, a lightning that came and went too fast and left the scar of his light in everyone who knew him.

Prière de l'étoile de mer

Seigneur
L'abîme est fermé sur moi
Ne suis-je pas
quelque Lucifer
tombé du ciel
et délaissé
aux torments des vagues?
Voyez,
j'ai l'air d'une étoile de sang.
J'essaie de me souvenir
de ma royauté lontaine
mais en vain.
Rampant sur le sable,
j'écarte mes branches
et je rêve, je rêve, je rêve…
Seigneur,
un ange
ne m'arrachera-t-il pas
du fond des mers
pour m'incruster de nouveau
dans Votre ciel?
Ah! qu'un jour,
       Ainsi sois-il!
Carmen Bernos de Basztold,
Choral de Bêtes, Editions du Cloitre,
Paris.

Prayer of the starfish

Lord,
The depths have closed over me.
Am I not
like Lucifer
fallen from heaven
and abandoned
to the torment of the weaves?
See,
I look like a blood star.
I try to remember
my distant royalty
but in vain.
Crawling on the sand,
I open my arms
and I dream, I dream, I dream…
Lord,
could not an angel
pull me up
from the bottom of the sea
to place me again
in Your heaven?
Ah! one day,
      So be it!
Translated by John Lawrence
(A functional biology of
echinoderms,
Croom & Helm, London &
Sidney)

Every time a single larva is released, the entire ocean lights up with possibilities.
Francisco Benitez Villalobos

CHAPTER ONE – GENERAL INTRODUCTION

1.1- The invasion of the deep sea

The factors that control the diversity of communities and the bathymetric distribution of individual species probably involve several mechanisms that act during the larval stage. These mechanisms may include larval physiological tolerances to temperature and pressure, larval orientation and swimming behaviours as well as availability of food (Tyler & Tyler, 1966). Factors that limit depth of occurrence may place limits on the invasion of the deeper water by shallow water species, but in most deep-sea animals, such limiting factors for individual species are unknown (Tyler & Young, 1998). In addition, there are only a few physiological experiments on early life-story stages of deep-sea benthic animals. (Dayton *et al*., 1982; Young & Tyler, 1993; Young *et al*., 1996ab, 1997; Tyler & Young, 1998).

There are two current hypotheses that may explain the origin of the deep-sea fauna. The first of these proposes that species colonized the deep sea fauna in cold, near-isothermal waters from shallow seas at high latitudes (Kussakin, 1973; Menzies *et al*., 1973) and that diversity subsequently increased through specialization and adaptive radiation at depth. The second hypothesis suggests that the deep-sea fauna moved from warm shallow seas at low latitudes during the late Mesozoic or early Cenozoic, when the oceanic water column was warm and vertically homogeneous (Menzies *et al*., 1973; Hessler & Wilson, 1983; Young *et al*., 1997). In the first hypothesis, no adaptation to different thermal conditions would be required. In the second one, nevertheless, species would have to adapt to increasingly colder water over geological and evolutionary time. Both hypotheses assume that thermal tolerances are narrow and conservative. Campbell & Rowe (1997) described the new

species of asteroid *Patiriella paradoxa*, which being a temperate taxon inhabits southern Arabian waters. This fact could only be explained in terms of this species being a relict who evolved to survive continual conditions in this tropical location, supported by the influence of local seasonal upwelling. The authors propose that the present-days areas of upwelling, including those within the Indian Ocean were established around 25-15 mya when the Drake's passage became completely open establishing the circum-Antarctic current generating an important drop in surface and bottom sea water temperatures (Van Andel, 1981). The major area of upwelling off the coast of Dhofar produces turbulent surface waters, rich in nutrient and almost temperate conditions (Savidge *et al.* 1986), which have been used by *P. paradoxa* over evolutionary time to remain in this location isolated from other congeneric species (Campbell & Rowe, 1997).

Pressure is a potential limiting factor in the hypotheses that propose deep-sea invasion by shallow water animals. Pressure forms a long and absolutely continuous environmental gradient everywhere in the oceans of the World. Temperature often decreases with depth discontinuously because of seasonal or permanent thermoclines. Young *et al*. (1997) found that larvae of shallow water Mediterranean echinoids tolerate relative high pressures at temperatures that prevail in the modern Mediterranean Sea, those findings have given impetus to the hypothesis that the Mesozoic and early Cenozoic periods were the principal times for the invasion of the deep sea, because during these periods, the water column was warm and isothermal as is the modern Mediterranean (Tyler, 2003).

Tyler & Young (1998) were the first to examine temperature and pressure tolerances of the dispersal stages of congeneric species of echinoids. The species had different bathymetric distributions, and it was inferred that physiological tolerances of

the larvae controlled the adult distribution. They proposed that larval tolerances affected the rate of colonization and speciation. The authors also proposed that the genus *Echinus* invaded the deep sea during isothermal glacial periods and that speciation ocurred either by geographical (depth) isolation or by reproductive (temporal) isolation. Reproductive isolation could occur as a result of differences in the timing of spawning in relation to seasonal changes in the vertical flux of organic matter to the seabed (Tyler & Young, 1998).

Young *et al*. (1996b) experimented with early embryos of 7 littoral species of tropical echinoids from Hawaii and the Bahamas and 3 bathyal species from the Bahamas. The embryos were incubated at pressures as great as 300 atm. In every case, embryos tolerated pressures greater than those of their adult normal distributional ranges, but at temperatures found in shallow water. This suggests that pressure does not set actual depth limits for most species and would not prevent recruitment or invasion of depths as great as 2000 m.

In research carried out by Sewell & Young (1999) it was concluded that the geographic distribution of the echinoid *Echinometra lucunter* (Blainville) does not appear to be limited by the temperatures at which normal embryonic/larval development occurs, but by 1) adult temperature tolerances, 2) temperatures needed for growth or spawning, or 3) hydrographic features that limit larval settlement and juvenile survival.

Only one experiment has been carried out onto pressure tolerances of a bathyal asteroid species, *Plutonaster bifrons* (Wyville Thomson) (Young *et al*. 1996a). The greatest percentage of embryos developing normally occurred at pressures equivalent to 2000 m depth, the depth at which the species is most common. No normal development occurred at a pressure corresponding to 3000 m depth. Therefore,

embryonic tolerances could determine the bathymetric limits of distribution for *Plutonaster bifrons*.

1.2- Larval type and its role in dispersal

Dispersal plays a significant part in the establishment and maintenance of biological diversity and is thought to have important consequences at ecological, biogeographic, genetic and evolutionary levels. In ecological time, dispersal potential can influence biological interactions, such as competition or reduction of predation. In addition, the capability of larvae to explore a variety of prospective environments will allow a species to persist in heterogeneous environments (Strathmann, 1974; Palmer & Strathmann, 1981). In evolutionary time, dispersal can increase geographic range of the species allowing 1) exploitation of new habitats, 2) the reduction of inbreeding and 3) the maintenance of genetic continuity between metapopulations (Scheltema, 1986; Wilson & Hessler, 1987).

It is generally recognized that, in a given habitat, the distribution and abundance of sessile marine benthic species are controlled by larval recruitment, asexual reproduction and mortality whereas for mobile benthic organisms, immigration and emigration are also important agents of control (Chia, 1989). Demographic features have important implications in community dynamics, particularly considering larval and/or juvenile mortality in relation to successful recruitment events. Community structure regarding larval recruitment is controlled by larval supply, success at settlement, and the mechanisms acting in larval transport at a particular site. These components are very important in order to explain species fluctuation in space and time (Giangrande *et al*., 1994) and the long term,

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evolutionary stability of common cohesiveness among extensive populations (Hedgecock, 1986).

Restricted dispersal may indirectly cause decreased local genetic variability and increased differentiation among populations by encouraging the effects of local selection, inbreeding, and drift (Slatkin 1985). Extensive dispersal on the other hand promotes gene flow and this may confers a short-term, adaptive advantage by damping genetic responses of local populations to temporary conditions.

The distance dispersed is partially correlated with time spent in the plankton (Crisp, 1976). The time interval over which dispersal occurs may be 1) a few minutes or a tidal cycle, 2) many months, or even 3) a year or more (Scheltema, 1986). Dispersal range will strongly influence the geographical range of species and the genetic structure of populations (Crisp 1978; Jackson 1986; Scheltema 1989), with potentially important consequences for phylogeny (Taylor, 1988)

Pelagic larvae occur in many different taxa and are the principal means of dispersal (Strathmann, 1985). For most deep-sea benthic marine invertebrates dispersal occurs during larval stages although some taxa, such as peracarid shrimps, elasipod holothurians and some molluscs can disperse as juveniles or adults either in the plankton or at the seabed (Gebruk *et al*., 1993). For example, the shelf gastropod *Buccinum polaris*; which lacks a planktonic larva and seems to have "walked" around the Arctic shelf since the Miocene (Scheltema, 1989b). The deep-sea holothurian *Psychropotes longicauda* Théel like other psychropotid species produces large yolky eggs, which are subsequently provided with both buoyancy and an energy store for prolonged development. These large eggs develop directly into juveniles in the water column and the juveniles may reach at least 35 mm before they settle on the sea bed (Tyler and Billett, 1987). This strategy allows the juvenile *P. longicauda* to be

dispersed over great distances and is important for the wide geographic distributions of the psychropotids (Hansen, 1975).

Larval variability in many organisms is linked with patterns of dispersal. A complex relationship occurs between 1) seasonal signals that stimulate larval development, 2) physiological features inherent of larvae such as positive or negative geo- and phototaxis (Hall & Wake, 1999) and 3) interactions between larvae and environment that establish both competence for the selection of a site and settlement (Chia & Rice, 1978; Morse, 1991; Fell, 1997). For example, in shallow waters the release of planktotrophic larvae is coordinated in females in response to phenolic compounds released by seasonal blooms of phytoplankton (Starr *et al.*, 1990).

Raimondi & Keough (1990) noted significant intraspecific variation in the settlement behaviour of most larvae and suggested that this variation may be important and adaptive, predominantly as a way of dealing with variation in the environment occupied by adults (Krug, 2001; Toonen & Pawlik, 2001). Differentiation of populations in marine invertebrates with pelagic larvae can occur from either physical or biological barriers to larval dispersal or from disparity of survival or fecundity in immigrants (Hedgecock, 1986). Larval settlement behaviour in addition, has the potential to affect larval dispersal strongly. Larvae that settle relatively indiscriminately are expected to settle shortly after they acquire the ability to do it (*i.e.* become competent), while those using specific settlement signals are expected to spend more time in the plankton before they discover the appropriate signal (Toonen & Pawlik, 1994; Krug, 2001).

Young *et al.* (1997) suggested on the basis of data in Emlet *et al.* (1987), that echinoderm eggs with a diameter 300 μm or larger produced lecithotrophic larvae and smaller eggs produced planktotrophic larvae. In addition they suggested it should be

assumed that lecithotrophic eggs developed into pelagic larvae unless brooding had actually been observed.

Scheltema (1986) reviewed developmental mode in relation to zoogeographic range in shallow water organisms. In shallow water it is expected that species with planktotrophic development will have more extensive distributions than species with "abbreviated" lecithotrophic development. A similar pattern was expected for the deep-sea fauna (Wilson & Hessler, 1987). However, there have been some studies that contradict this view, with examples of species with an ample bathymetric and geographical distribution and non planktotrophic mode of development:

Jackson (1986) found that there was no clear correlation between geographic range and length of larval life among sessile organisms, whether reproduction was aclonal, clonal or both. Young & Cameron (1987) studied floatation rates of eggs from the bathyal echinoid *Phormosoma placenta* both *in vivo* and *in situ*, and noted that this species, even though it produced lecithotrophic larvae was widely distributed throughout the North Atlantic. Laegdsgaard *et al*. (1991) compared the reproduction of sympatric populations of two echinoid species, *Heliocidaris erythrogramma* (Valenciennes) and *H. tuberculata* (Lamarck) in the Sydney region in Australia. They found differences in oogenesis and egg size between the two species that were related to different patterns of larval development. *H. tuberculata*, which produces small eggs and a planktotrophic larva, had a limited distribution compared with *H. erythrogramma*, which produced large eggs typical of lecithotrophic larval development.

Young *et al*. (1997) tested the hypothesis that for bathyal and abyssal echinoderms and ascidians of the Atlantic Ocean, species with planktotrophic larval development had broader biogeographic and bathymetric ranges than species with

lecithotrophic development. The prediction that species with planktotrophic larvae should have more extensive geographic ranges than species with lecithotrophic larvae did not hold for echinoderms. Asteroid and echinoid species with planktotrophic development had more constrained distributions compared with lecithotrophic species. Some of the most widespread species in the deep sea reproduce without the hypothetical advantage of planktotrophy, although some studies have identified a few species, which seem to have developed a way to use the advantage of both modes of development, for example the larvae of the echinoid *Aspidodiadema jacobyi* develop from a small egg (90 µm) like typical planktotrophs, but unlike other species, development of a mouth is delayed for the first 21 days, as the blastocoel becomes filled with yolky cells from the vegetal plate. These yolky cells permit dispersal and perhaps vertical migration for up to two months before planktonic food is required (Young et al., 1989).

There is some further evidence that lecithotrophic larvae have sufficient energy and nutrients store in order to remain in the water column for long periods of time. Birkeland *et al*. (1971) noted that larvae of the temperate starfish *Mediaster aequalis* Stimpson survived in culture for more than a year before settlement. Young & Cameron (1989) carried out experiments about the developmental rate as a function of depth with the bathyal echinoid species *Linopneustes longispinus*, showing that the development of embryos maintained at 5° C was delayed at the gastrula stage for over four months and the embryos never became plutei at this temperature. Nevertheless, gastrulae relocated to temperatures at 10 and 15° C rapidly developed into plutei. These results highlighted that at low temperatures the potential for dispersal without feeding is even greater because of low metabolic demand. Shilling & Manahan (1994) demonstrated experimentally that the lecithotrophic larvae of some Antarctic

echinoderms have such low mass-specific respiration rates that may enable them to disperse for months, or even years (ranging from 10 months to 5 years) relying exclusively on the reserves of the egg. Hoegh-Guldberg & Emlet (1997) investigated energy use during development of the lecithotrophic echinoid species *Heliocidaris erythrogramma* and the planktotrophic *H. tuberculata*. They observed that the energy required to generate a juvenile on a per mass basis in the species with planktotrophic larvae is essentially the same as that in the species with pelagic, lecithotrophic larvae, when the body size of juveniles is taken into account. Marsh *et al*. (1999) established that the lack of particulate food resource does not appear to cause a "starvation" stress on the early larvae of the Antarctic echinoid *Sterechinus neumayeri* (Müller). This indicated that feeding larval stages were not dependent on the availability of phytoplankton to complete early development. In addition, there is great potential for embryos and larvae to uptake dissolved organic material, which might also reduce the demand for resources of particulate food.

It is likely that the same phenomenon occurs in the cold waters of the deep sea as in Antarctica. Species with planktotrophic development may not necessarily spend any longer time in the plankton than pelagic lecithotrophs. Evidence that lecithotrophy does not limit deep-sea dispersal is provided by studies on the genetics of hydrothermal vent organisms (Black *et al*. 1997; Craddock *et al.* 1997; Vrijenhoek, 1997). Analysis of allozymes in four species of archaeogastropod limpets with nonplanktotrophic modes of development provided results contrary to the prediction that dispersal should occur in a "stepping-stone" mode. Among those four species two of them did not exhibit the expected decline in rates of gene flow with increasing geographic distances between sampled localities, suggesting that the lecithotrophic larvae of these species are capable of dispersal over very long distances (Craddock *et*

al., 1997). In a review (Vrijenhoek, 1997) citing hydrothermal vent-endemic molluscs, two species of bivalves and two species of limpets showed evidence of high rates of gene flow that were not restricted by the topology of the ridge system or by geographical distance.

In the deep sea, species with planktotrophic larval development may be restricted to regions where there is sufficiently high surface production in order to produce an important amount of fine detrital food at bathypelagic and abyssopelagic depths. Therefore planktotrophic larvae could be limited by food in oligotrophic areas, whereas lecithotrophic larvae may survive even if advected to areas of low productivity using energy stored internally (Young *et al.*, 1997). A very important case to mention regarding planktotrophy and widespread distribution is the deep-sea synaptid holothurian *Protankyra brychia* Verrill, which essentially has a cosmopolitan distribution. The oocyte diameters of *P. brychia* indicate planktotrophic larval development or lecithotrophic larval development followed by planktotrophy (Billet, 1991; Pearse, 1994). Pawson *et al.* (2003) presented evidence that this species has a near surface planktotrophic larval stage which had been reported in the literature as the Giant Auricularia larva *Auricularia nudibranchiata* Chun (Chun, 1896; Oshima, 1911; MacBride, 1920; Inaba, 1934; Garstang, 1939).

1.3- Deep-water formation and species dispersal

Developmental mode is not the only factor that might determine dispersal distance. Scheltema (1986b) established that "the maximum potential distance that a larva can be dispersed and the likelihood that it survives to settlement is related to (a) the length of its planktonic life and (b) to the rate and direction of the currents that transport it".

Larval dispersal patterns depend also on physical forcing mechanisms and such mechanisms may be different in distinct regions. Therefore, larval dispersal patterns vary between populations according to the relative importance of tidal advection and eddy diffusion in local waters, so that local larval retention is increased where tidal advection is weak (Ellien *et al*., 2000). Small-scale eddies caused by local features of the seabed may retain eggs and larvae near the site of spawning in some regions, whereas larvae that spend equivalent times in the plankton may disperse greater distances where the level of retention produced by eddies is low. Hydrographical conditions and irregular distribution of suitable substrata consequently are considered to restrain gene flow, creating opportunities for local genetic differentiation even if pelagic larvae possess a high dispersal potential (Goldson *et al*., 2001).

Fenaux *et al.* (1994) showed that In the Eastern Alborean Sea, where surface currents form a complex frontal zone, with associated eddies and gyres, the distributions of larvae and postlarvae of echinoderms vary according to hydro dynamical structures. Larvae are numerous in the Atlantic Geostrophic Jet, which passes along the African coast and they are accumulated in an anti-cyclonic gyre to the west of the jet. In the anti-cyclonic eddy of Mediterranean water, north of the frontal zone, the larvae in contrast were scarce.

Wind forcing increases the effect of advection on larval transport and modifies significantly the level of larval retention. Wind-induced currents however, may produce larval transport from one population to another and might be involved in restoration of depleted populations (Ellien *et al*., 2000). Larval dispersal from eight populations of the ophiuroid *Ophiothrix fragilis* Abildgaard in the English Channel was examined by Lefebvre *et al*., (2003) using an advection/diffusion model.

Although larval dispersal and settlement of this species are apparently hydrodynamic constrained in almost all populations larval retention appeared to be sufficient to ensure local recruitment, in spite of short larval life span and/or meteorological conditions. Whereas some populations were mainly self–seeding, larval supply from neighbouring populations could exceed local retention for other populations depending on wind forcing.

The flow of water in the deep sea is still not fully understood compared to the surface circulation. Therefore, in most cases, it is not an easy task to predict where larvae released at a particular location will go and how long the journey will take. Marsh *et al.* (2001) studied the larval dispersal potential of the tube worm *Riftia pachyptila* at deep sea hydrothermal vents on the East Pacific Rise. They showed the prevailing importance of current flow in determining dispersal potential and suggested that populations at different vent sites may have different dispersal limits depending on local current conditions. In this region at least, it is apparent that the dispersal distance of *R. pachyptila* was not limited by the physiological performance of the larvae, but by 1) temporal oscillations in the long axis currents and 2) larval loss in cross-axis flows.

Global termohaline circulation occurs by a variety of pathways that transport warm waters to high latitudes where they become denser, sink, and finally spread throughout the oceanic floor (Smethie *et al.*, 2000). One of the most important pathways by which this process occurs is the formation and spreading of North Atlantic Deep Water (NADW). The extension of its influence on the deep-ocean circulation can be measured as the properties of this water mass have been observed throughout much of the deep ocean and it may extend as far as the North Pacific (Reid & Lynn, 1971).

There is increasing evidence for accelerated deep-water formation at the end of glacial periods (Knorr & Lohmann, 2003). During deglaciation, the Atlantic thermohaline circulation becomes more vigorous. It changes from a weak glacial mode into a strong interglacial mode, triggered either by 1) southern warming and increased northwards transport of denser, salty waters from the tropics into the "sinking regions" at high latitudes, where they ultimately initiate thermohaline circulation, or 2) by injection of fresh water from the Antarctic ice-sheet. In consequence, the density of deep and intermediate waters from Antarctica, which compete in the Atlantic with North Atlantic Deep Water (NADW), is reduced, eventually accelerating the termohaline circulation (Knorr & Lohmann, 2003).

There is also recent evidence that there may be a variety of sites of deep-water formation in the N. Atlantic (Pickart *et al*., 2003). If this more vigorous formation of deep water occurs at transitional periods similar to the end of glacial periods it may be that the warming of surface waters stimulates accelerated reproduction, both through temperature and primary production and that the resultant larvae using the thermohaline 'conveyer belt', are able to penetrate the deep sea.

Tyler *et al.* (1995) hypothesized on the basis of biogeographic distributions in the genus *Echinus*, that the western boundary undercurrent, which transports North Atlantic Deep Water (NADW), formed in the Norwegian Sea, south along the slope of the eastern margin of North America, was capable of transporting larvae from the centre of speciation in the North Atlantic to the South Atlantic and Pacific Oceans. Reduced flow of NADW during glacial periods has probably resulted in allopatric speciation of isolated populations of *Echinus* at these sites (Young *et al*., 1997).

Experimental work on the pressure and temperature tolerances of embryos and

larvae of shallow water echinoids from the northern Mediterranean (Young *et al.,* 1997b) show that larvae are sufficiently tolerant of high pressures to follow an isothermal layer of warm water into the deep Mediterranean Sea. Embryos and larvae of the shallow water echinoids *Paracentrotus lividus* Lamarck, *Arbacia lixula* , and *Sphaerechinus granularis* tolerated pressures as high as 150 atm at 15°C. Lower temperatures (<11°C) exacerbated the effects of pressure. Although the distributions of those species belong to very shallow waters, living larvae have been collected from depths as great as 400 m (Pedrotti, 1990) indicating that invasion of deeper waters could in fact take place in a single generation.

Tyler & Young (1998) have examined the temperature and pressure tolerances of embryos and larvae of the shallow water species *Echinus esculentus* Linnaeus shallow and bathyal populations of *E. acutus* Linnaeus and lower bathyal populations of *E. affinis* (Linnaeus). Embryos and larvae of both *E. esculentus,* and *E. acutus* were able to tolerate pressures more than 250 atm, which is far outside the adult range, although, unlike the Mediterranean species, developmental arrests and abnormalities did not increase with lower temperatures. Of particular significance was that embryos of *E. acutus* var. *norvegicus* from the bathyal zone tolerated a significantly broader range of pressure and temperature than did embryos of *E. acutus* from shallow subtidal habitats. *E. acutus* var. *norvegicus* also developed more rapidly at lower temperatures, suggesting that *E. acutus* is a very plastic species and may be currently in the process of invading the deep sea by slowly adapting to increased pressure.

CHAPTER TWO- IMPORTANCE OF THE LARVAL STAGE IN THE LIFE HISTORY OF MARINE INVERTEBRATES, SPECIFICALLY ASTEROIDS: A REVIEW

2.1. Introduction

An animal starts its development as a zygote. The amount of development that takes place before and after hatching varies considerably from group to group, and frequently from species to species within the same group (Thorson, 1950). There are phyla, such as the Entoprocta in which all known species have larvae. In other phyla, such as the Chaetognatha all individuals hatch in a form resembling minuscule adults. They have no larvae, and development is assumed to be direct. However, in most phyla there is a mixture of developmental modes, some species with larval stages, and some without. In the words of Young (2002) "Indeed, larval development is the most common developmental pathway in the animal kingdom. Representing the future generations of most marine animals, larvae are, in a very real sense, the lifeblood of the sea".

Species that never had a larval phase throughout their evolutionary history or phylogeny show primary direct development. Those without larvae, but that at some point during evolution have evolved from species with larvae, show secondary direct development (Strathmann, 1993; Nielsen, 1998). Jägersten (1972) affirms that there are no factual cases of primary direct development, but Williamson (1992) identifies this category for some phyla such as the Chaetognatha, that show no trace of a larval form at any time during their evolutionary history. With respect to time spent in larval stage, some organisms like the polychaete genus *Spirorbis*, have a larval life of only a

few hours, whereas other species such as the European eel *Anguilla anguilla* Linnaeus, takes three years to complete its larval development (Williamson, 1992).

After a series of cleavages (Fig. 2.1) all metazoan embryos pass through the blastula stage before hatching. At this stage the dividing cells form a more or less hollow sphere, with a fluid filled space within called a blastocoel (Fig. 2.2). In most phyla embryonic development continues further although in many echinoderms the cells of the blastula are ciliated and the animal hatches in this stage (Williamson, 1992). In the following stage, the gastrula, a tube-like inner layer of cells called the archenteron, or primitive gut, is formed. The internal end of the archenteron is closed, and the opening at the vegetal pole is called the blastopore (Figs. 2.2, 2.3).
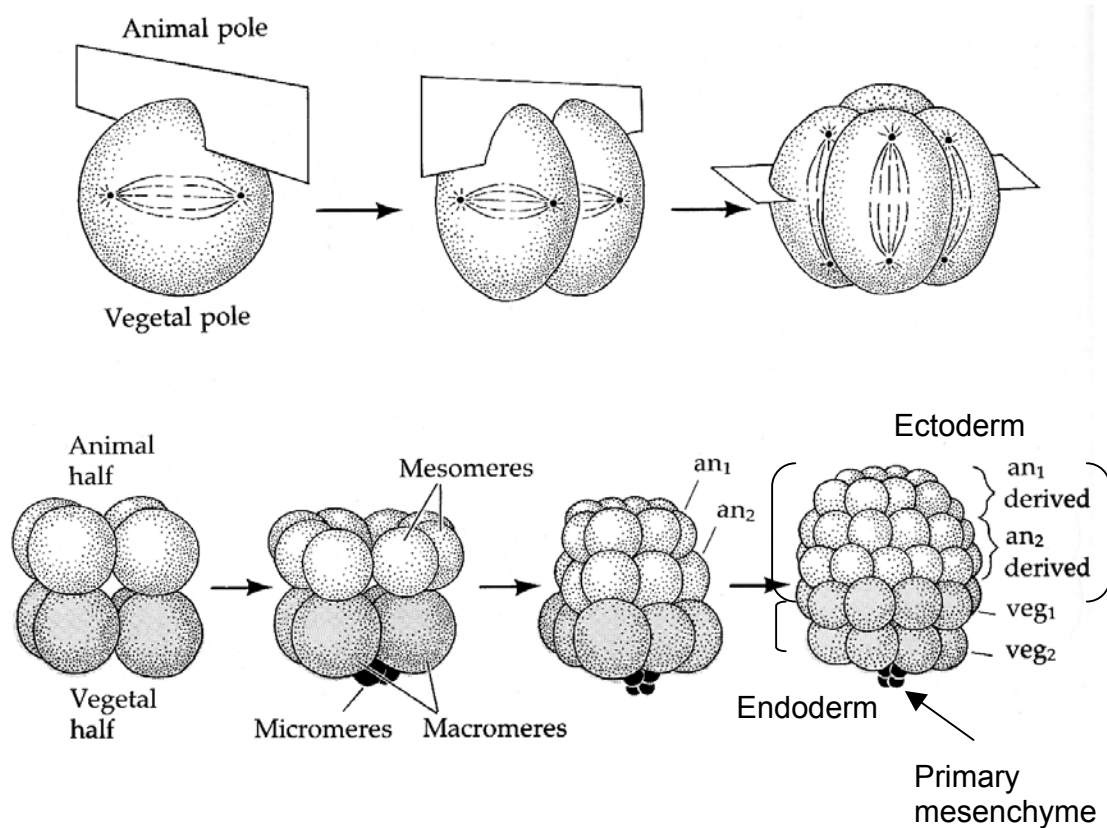
Fig.2.1. Cleavage in Deuterostomes showing the cell divisions from the original zygote to the late divisions prior the blastula stage. Abbreviations: an_1, an_2 refer to cells derived from the animal half; veg_1, veg_2 refer to cells derived from the vegetal half (Modified from Barnes *et al.* 2001).

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In the larval development of echinoderms, the coelom starts to take its form from outgrowths of the archenteron, which soon form separate sacs. The blastopore then becomes the anus, and subsequently the mouth develops as a new opening. This type of coelom, derived from the archenteron, is named an enterocoel and this type of mouth, which develops independently of the blastopore, is called a deuterostome. The terms enterocoel and deuterostome are also applied to all other animal groups in which embryonic development shows similar features (Fig. 2.2).
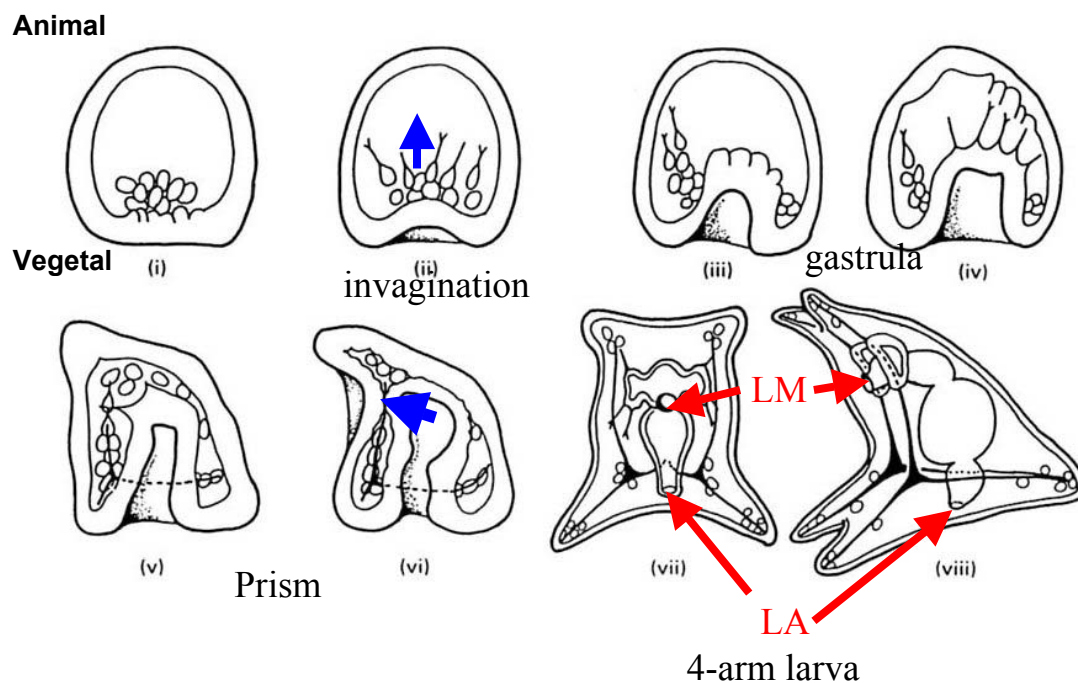
Fig. 2.2. Gastrulation and prism formation in echinoids showing the development from blastula stage to the 4-arm larval stage. Abbreviations: *LA* Larval anus; *LM* Larval mouth (Modified from Hyman, 1955).

The diversity of larval forms and developmental patterns have become progressively better known during the past 20 years. Nevertheless the classification of development patterns has been ambiguous because developmental patterns have not

been defined as a unique sequence of well defined character states (McEdward & Janies, 1993).
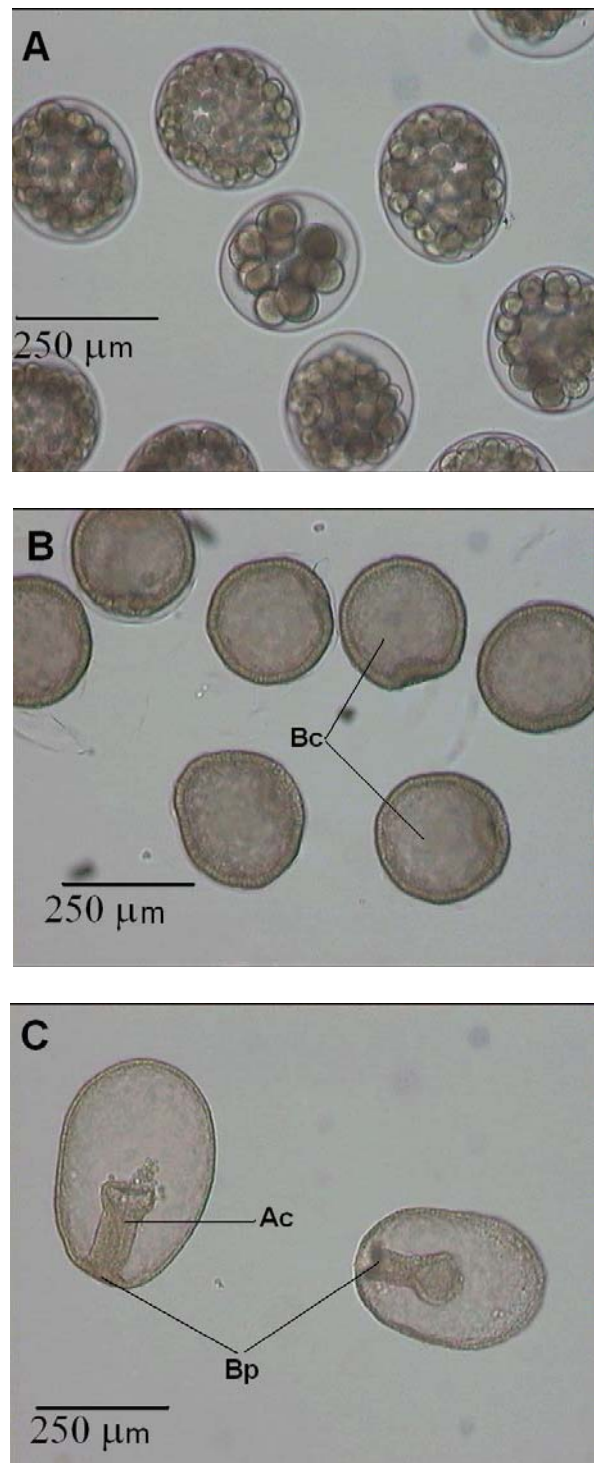
Fig.2.3. Developmental stages of the starfish *Marthasterias glacialis* (Linnaeus). **A** Cleavege at approximate 64-cel stage after 6 h of fecundation. **B** Late blastula stage after 24 h of fecundation. **C** Late gastrula stage after 48 h of fecundation. Abbreviations: *Ac* Archenteron; *Bc* Blastocoel; *Bp* Blastopore. (Micrographs by Francisco Benitez)

2.2. A revision of the definitions of the early-life story stages

McEdward & Janies (1993) reviewed the definitions of some important features that have been described inconsistently in the literature. Their re-definitions took into consideration the continuous nature of development and the evolutionary diversification of ontogenetic sequences.

2.2.1. Embryo

In general, embryos can be defined morphologically as cleavage stages; blastulae, and gastrulae. These are particular stages in the development of all invertebrates. The blastula and gastrula are stages inherent of the Metazoa (Buss, 1987; Booner, 1988; Margulis, 1990). It is the uncertainty concerning the exact state of transition involving the start of morphogenesis and the completion of a definitive form that makes the distinction between late embryo and early larva, impossible to identify with precision. For this reason the expression "embryo" is best restricted to the stages of development which are of universal incidence (cleavage, blastula, and gastrula) and it should not be used to refer to the subsequent period of transition (McEdward & Janies, 1993).

2.2.2. Larva

In spite of numerous attempts, no author has provided a specific and generally accepted definition of the term "larva". Such a definition is very difficult to obtain especially because some authors on one hand consider the distinction between a larva and an embryo, and a larva and a juvenile on the other to be at some point arbitrary (Strathmann, 1985). The first problem when trying to define larva is in relation to recognizing boundaries between developmental stages. The second problem is that structural, ecological, and morphogenetic definitions generally do not coincide;

therefore a developing form may well be a larva by one definition, but not by the others.

In the structural definition, a larva is a structural state or series of states that occur between the start of morphogenesis, which follows embryonic development (cleavage, blastula, and gastrula), and metamorphosis to the adult. The larva is a combination of temporary features and adaptations inherent to larval life that 1) are lost at metamorphosis, 2) are modified to generate adult functions at metamorphosis, and 3) are features of the adult that begin their development before metamorphosis occurs. In the structural definition, as long as morphogenesis continues to produce larval structures, and as long as those structures are lost (literally lost or absorbed by the rudiment) during metamorphic transformation, there will be a larva.

The structural definition is in conflict with the standard ecological definition of a larva, especially when regarding brooded development in which young stages can be larval by morphological and developmental criteria, but do not live in the plankton and therefore do not provide larval dispersal (McEdward & Janies, 1993)

From the ecological point of view, the developing organism is a larva only if it hatches and passes through a pelagic phase. The ecological definition considers the larva as a dispersive phase of the life history. Thus, an organism is still an embryo if it develops in a benthic egg mass or capsule, even if it possesses all the structural features of a larva. As an example, Chia (1974) defines that "Larva is a developmental stage, occupying the period from post embryonic stage to metamorphosis, and it differs from the adult in morphology, nutrition, or habitat. Post embryonic stage designates the time after emerging (hatching) from the primary egg membrane; prior to that it is considered as embryonic stage". The difficulty with this definition from an evolutionary point of view, however, is the definition of post-embryonic stages as the

"time after emerging (hatching) from the primary egg membrane". This limits the term significantly. On the other hand, there are pelagic propagules that undergo direct development and consequently qualify as larvae only in the sense that they are dispersed by water movements (McEdward, 1992).

The third definition of a larva is basically morphogenetic. For this definition, larva is the premetamorphic consequence of type 1 embryogenesis, a type of specification of cell fates that is fundamentally different in invertebrate larvae, until metamorphosis (Davidson, 1991; Davidson *et al.,* 1995; Hall and Wake, 1999). The problem with this definition if that it applies most specifically to larvae that are strictly indirect-developing, such as those of echinoderms, in which there is a considerable degeneration (reabsortion) of larval tissues at metamorphosis, but it is not similarly applicable to all invertebrate larvae.

Finally, McEdward & Janies (1993) suggest it is important to recognize that there cannot be an absolute distinction between larvae and mesogens (see pag. 21), because the evolution from indirect to direct development usually involves the successive reduction and elimination of larval structures. Thus, in order to answer the questions about "what is a larva?" and "what is not?" McEdward & Janies (1993) propose that a larva will only be a larva when the strictest morphological and developmental features are taken into account. Everything else-including a mesogen-is not.

2.2.3. Juvenile

The expression "juvenile" has been used in a very broad sense. At one extreme, it makes reference to "post-larval" stages, either directly following rapid metamorphosis from a definite larval stage or at some point, not adequately defined, during a prolonged, gradual metamorphosis. At the other extreme, juveniles are "pre-

adults" that have the definitive adult morphology, but are small and/or not sexually mature. According to the definitions of McEdward and Janies, (1993), a juvenile 1) has attained the adult body plan (symmetry, general body shape), 2) is a form in which the major systems are functional (especially locomotion and feeding), with the exception of reproduction, and 3) excludes the transitional period during metamorphosis.

2.2.4. Metamorphosis

Definitions of metamorphosis usually affirm it involves a change in form and function that is accomplished over a relatively short period of time and defines the transition from larva to adult (Burke, 1989).These definitions have been often restricted to cases where there is a drastic and rapid change in morphology. However, it would be more useful to consider metamorphosis as the transition from the larval body plan to the juvenile body plan, regardless of the rate or magnitude of the change (McEdward & Janies, 1993).

2.2.5. Indirect development

This refers to the pattern of development in which the embryo is followed by intermediary stages with structural features that are not directly implicated in the morphogenesis of a juvenile. These intermediary stages are larvae, and the construction of their specialized structure and transitory body plan, followed by metamorphosis, defines the indirect nature of a developmental succession.

2.2.6. Direct development

This refers to the pattern of development in which the embryonic stages are followed directly by the morphogenesis of the juvenile, without an intervening larval stage. This development possibly will involve short, simple ontogenetic sequences or long, complex series. In contrast to indirect development, the juvenile develops

directly from the embryo, through a series of intermediate stages, all of which are transitional and without morphogenesis of any larval structures. McEdward & Janies, (1993) introduce the term "mesogen" (middle stage) to nominate the developmental stages that occur between the embryo and the juvenile in the direct pattern of development given that the term larva only applies to the specialized stage in the indirect mode of development.

2.3. Types of larvae recognized in scientific literature

Balfour (1880) summarized the evidence for two kinds of larvae: (1) primary larvae, as modified ancestral forms, which have existed as free larvae "from the time when they constituted the adult form of the species" and (2) secondary larvae, established secondarily into the life history of a species that previously had a pattern of direct development. He sets the planula (the ancestral form of coelenterates) as a primary larva, and apart from all the other larval forms. Secondary larval adaptations were thought to arise from 1) changes in larval life, 2) changes in the order of emergence of structures or 3) to be eventually associated to the effort implicated for existence when settling.

Currently zoologists recognize two fundamental types of invertebrate larvae corresponding to protostome and deuterostome modes of development:

The trochophore larva is found in animals with a protostome mode of embryonic development and spiral cleavage. Further cladistic analyses performed on the distribution of larvae are consistent, demonstrating the trochophore as the type of larva possessed by the last common ancestor of arthropods and chordates (Peterson *et al*., 1997).

The dipleurula is the hypothetical type of ancestral larva that is found in animals with a deuterostome mode of embryonic development. The also ancestral tornaria larva found in hemichordates and the auricularia larvae of echinoderms are possibly the least modified dipleurula larvae (Willmer, 1990). The divergence of the deuterostomes may well have started with the transformation of the protostome trochophore larva to the dipleurula larva (Peterson *et al.*, 1997)

2.4. Larvae as a mechanism for dispersal

Garstang (1922) considered larvae as a mechanism for dispersal similar to the seeds of a plant. He considered the secondary decline of the free swimming larval stage to be the consequence of the implementation of an incubatory mode of development. As an example, cephalopods do not have larvae, avoiding the trochophore and veliger larvae and omitting metamorphosis, because the adult is locomotory, thus a dispersive larval stage is not necessary. The relationship between larval and adult stages is also sufficiently illustrated in organisms from groups that normally include a larval stage as the primitive condition, but which have either modified or lost the larva. Those direct-developing species (e.g. many echinoids and some ascidians) hatch as minuscule adults, demonstrating the plasticity of early development processes and ontogenic stages (Hall & Wake, 1999).

2.4.1. Classification of larvae regarding dispersal potential

In recent years, the importance of early life-history stages in population dynamics has been recognized and there has been increased interest in the movement and fate of invertebrate larvae (Scheltema, 1986; Levin, 1990; Young, 1990). In 1993 Kelman and Emlet measured the vertical velocity of eggs and larvae on the asteroid *Pteraster tesselatus* Ives, which produces large floating eggs (1100 to 1500 µm

diameter) that develop into lecithotrophic larvae and may spend several weeks in the plankton before settlement. The results showed that the patterns of buoyancy of eggs and swimming velocity of larvae should initially facilitate dispersion in *P. tesselatus.*

Scheltema (1971) introduced into the scientific literature several expressions that explicitly address larval dispersal abilities, recognizing the need for terms that describe dispersal potential.

Teleplanic (tel= far, planos= wanderer) are those larvae with planktonic periods exceeding two months and possibly enduring a year or more. Teleplanic larvae are common amongst architectonid and cymatiid gastropods (Scheltema, 1966, 1988), bivalve veligers (Scheltema & Scheltema, 1984), spionid and chaetopterid polychaetes (Scheltema, 1986b, 1988, 1992), sipunculan pelagosphera (Scheltema & Hall, 1975), and lobster phylosoma (Phillips & Sastry, 1980). Teleplanic dispersal is also known to occur in enteropneust tornaria (Scheltema, 1987), phoronid actinotrochs, brachyuran zoea and megalopae, and echinoderm bipinnaria, auricularia (Scheltema, 1986b), and ophioplutei (Hendler, 1991). Distributions of teleplanic larvae suggest that they are capable of transoceanic dispersal in both the Atlantic (Scheltema, 1968, 1971) and the Pacific Oceans (Scheltema, 1986b, 1988).

The term *actaeplanic* (actaeos= coastal, planos= wanderer) was introduced in 1989 to refer to shoreline larvae with planktonic periods from one week to less than 2 months. About 70 % of temperate sublittoral species are included in this category; most of them have planktonic development times of 4 to 6 weeks (Levin & Bridges, 1995).

Anchiplanic (Anchi= near, planos= wanderer) refers to "nearby wanderers" larvae, which remain in the plankton from a few hours to a few days. This abbreviated dispersal ability can be found in almost every phylum, and is particularly common

among tunicate tadpoles, sponge and bryozoan larvae, and some coral planulae, as well as certain polychaete families.

The non-planktonic larvae are at the end of the spectrum. This kind of larva is considered A*planic* (a= without). Examples of aplanic larvae include forms that emerge from the adult with the capability to settle, but also include some lecithotrophic larvae in which development occurs completely during encapsulation or while crawling on the seabed, as happens in some corals (Gerrodette, 1981; Fadlallah & Pearse, 1982).

2.5. Larval types of Asteroids

The class Asteroidea includes seven orders and 34 families of echinoderms incorporating approximately 1800 species (Clark & Downey, 1992; Hendler *et al*., 1995). Asteroids have basically two characteristic larval types: bipinnaria and brachiolaria (Fell, 1967). In addition, there are two more larval forms that are derived from the planktotrophic larvae: yolky brachiolariae and barrel-shaped larvae (Young, 2002). Ultimately some species do not have a larval stage and undergo direct development via a mesogen (Fell, 1967; Oguro, 1989; Chia & Walker, 1991; Chia *et al*., 1993; McEdward & Janies, 1993; McEdward & Miner, 2003).

2.5.1. The Bipinnaria

The bipinnaria is a pelagic, feeding larva with a complex body form characterized by the bilateral arrangement of the pre- and post- oral ciliated, swimming and feeding bands that are originated on the arms (McBride, 1914; Kume & Dan, 1968) (Fig. 2.4.AB). The ovoid body has two ventral folds (oral and anal hoods) and multiple lateral lobes. Hollow extensions of the body wall form elongate bipinnarian arms that contain blastocoelic space and are not supported by calcareous

skeletal rods (Hyman, 1955). Although, the number and size of bipinnarian arms fluctuates between species, the arms can be identified by their anatomical location using the nomenclature designated by Mortensen (1898). A complete and functional gut is present. The bipinnaria larva occurs in the life cycles of all asteroids with planktotrophic larval development (McEdward & Janies, 1993) and has been documented in four of the asteroid orders: Forcipulatida, Notomyotida, Paxillosida, and Valvatida (Young, 2002)

2.5.2. The Brachiolaria

The brachiolaria is also a complex, feeding larva, and is characterized by the presence of two specialized structures for the attachment on the pre-oral lobe: the brachiolar arms and attachment disk. The brachiolar arms are distinguished from bipinnarian arms because they contain extensions of the larval coelom (Gemill, 1914; Barker, 1977). Brachiolar arms are utilized by larvae to explore the substratum and provide initial, temporary adhesion during settlement. The adhesive disk secretes cement and provides more permanent attachment for metamorphosis (Fig. 2.5.A). The only difference in larval morphology that distinguishes the bipinnaria from the brachiolaria stage are the presence of the brachiolar arms and attachment disk. Although these larvae are given different names, they have not evolved independently, but are sequential developmental stages. Planktotrophic brachiolaria occur in the orders Forcipulatida and Valvatida (Young, 2002).

2.5.3. The yolky brachiolaria: a derived larval form

Some asteroid species develop by means of non-feeding, pelagic brachiolarian larvae, in which the feeding structures, bipinnarian arms, and a functional gut are absent. The brachiolar arms and attachment disk are the only conspicuous external larval structures.
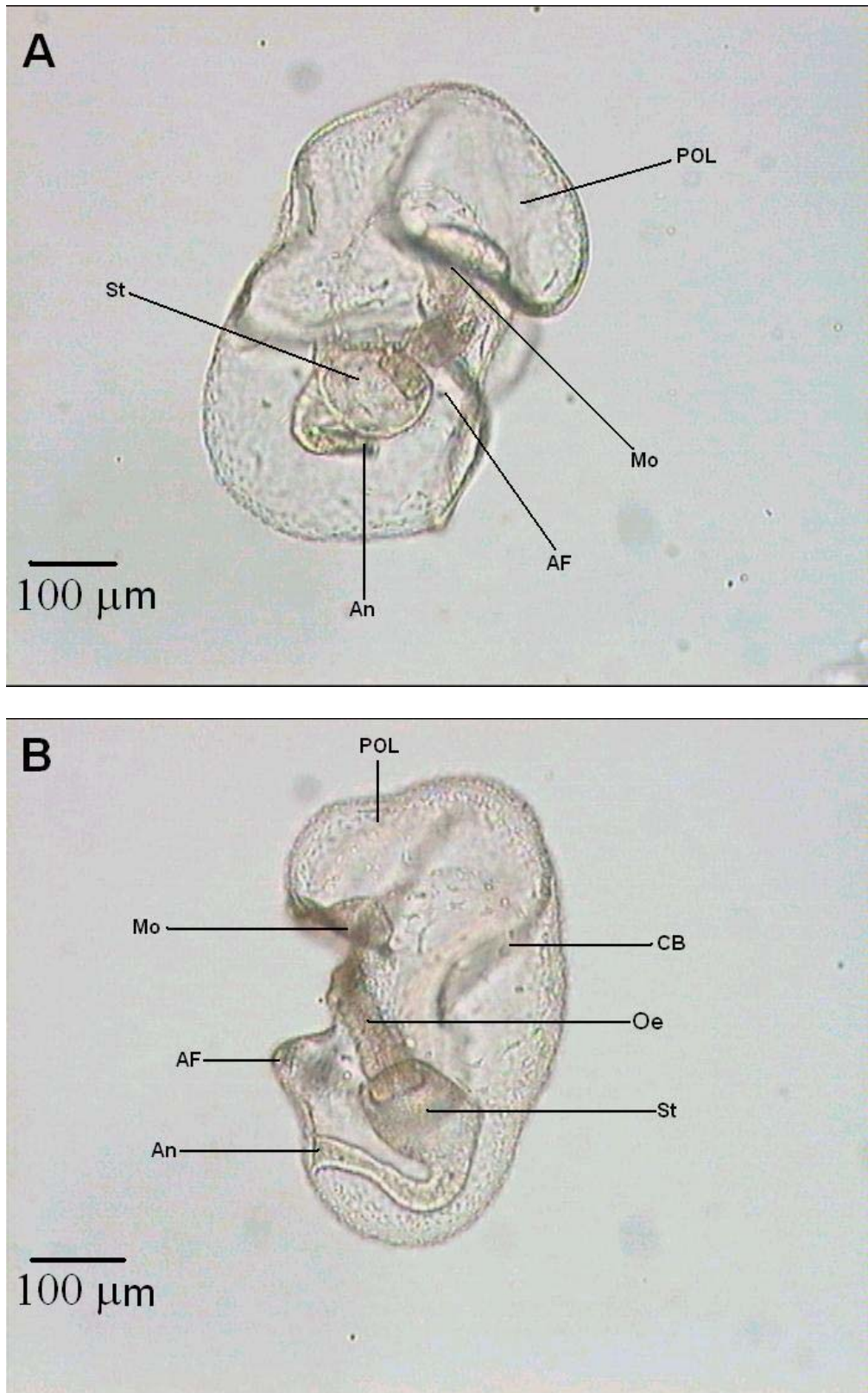
Fig.2.4. Bipinnaria larvae of the asteroid species *Marthasterias glacialis* (Linnaeus). **A** Front view. **B** Lateral view. Abbreviations: *AF* Anal Field; *An* Anus; *CB* Ciliary Band; *Oe* Oesophagus; *Mo* Mouth; *POL*; Pre-Oral Lobe; *St* Stomach. (Micrographs by Francisco Benitez)

In general, brooded brachiolariae retain well-developed and functional brachiolar arms and adhesive disks, although some asteroid species develop via a benthic brachiolaria that has reduced brachiolar structures. For example, in the intragonadal brooders, *Patiriella vivipara* Verrill and *P. parvivipara* Verrill, the brachiolar arms are present, but they are reduced and non-functional (Byrne & Cerra, 1996). Yolky brachiolariae occur in the orders Forcipulatida, Spinulosida, Valvatida, and Velatida.

2.5.4. The barrel-shaped larva: a derived larval form

Barrel-shaped larvae are also non-feeding larvae with abbreviated development and simplified morphology. These larvae are characterized by a large transparent, non-yolky pre-oral lobe, and by the absence of larval arms, ciliated bands, mouth, and anus. The larval coelom does not extend anteriorly into the conspicuous pre-oral lobe. Barrel-shaped larvae occur in some species of paxillosids (e.g., *Astropecten latespinosus* Meissner, Komatsu, 1975; Komatsu *et al*., 1988; *Ctenopleura fisheri* (Perrier), Komatsu, 1982; *Astropecten gisselbretchti* Meissner, Komatsu & Nojima, 1985).

2.6. Patterns of development in asteroids

Worldwide, slightly more than one half of the asteroid species for which development type is known are free spawners. The number of species with planktotrophic and lecithotrophic larvae is approximately the same. Brooding has been reported in 40% of the species (Emlet *et al*., 1987). There are more than five times as many free spawning species from the northern hemisphere as from the southern hemisphere and brooding appears more common in the southern oceans, especially at high latitudes (Emlet *et al*., 1987).

In marine invertebrates there is a trade-off between fecundity and the amount of energy that can be invested in each egg. Thus as a rule, a species produces either many small eggs with planktotrophic development or fewer, large eggs with lecithotrophic development (Thorson, 1950). Regarding asteroids, the presence of small (less than 200 μm diameter) freely spawned eggs is considered indicative of planktotrophic larval development, whereas large (greater than 700 μm) freely spawned eggs indicate pelagic lecithotrophy.

The distribution of egg sizes in many asteroids is conspicuously bimodal. Sewell & Young (1999) performed a re-examination of asteroids and echinoids egg sizes and tested the prediction of bimodality in holothuroids and ophiuroids. Eggs diameters in asteroid species range from 100 to 3500 μm, and the two modes are found in the ranges of 100 to 150 μm and 700 to 1000 μm. All species known to have planktotrophic larvae have small eggs, ranging from near 100 μm in several species up to 230 μm in *Astropecten scoparius* Verrill (Emlet *et al*., 1987). Species with pelagic lecithotrophic larvae have eggs from 300 μm in *Astropecten latespinosus* to 1460 μm in *Pteraster tesselatus* Sladen. Brooding species have eggs that range from 300 μm in *Asterina scobinata* Verrill to 3500 μm in *Rhopiella Koehleri* Fisher. The ranges in egg diameter for planktotrophic and lecithotrophic species do not overlap, but egg sizes overlap considerably between lecithotrophic and brooding species (Emlet *et al*., 1987).

McEdward & Janies (1993) proposed a multifactor classification scheme for patterns of development in asteroids. Developmental patterns were classified using three completely independent characters, 1) the morphological nature of development can be clearly described using the distinction between indirect and direct development. In their scheme all indirect types have larvae and all direct types do not,
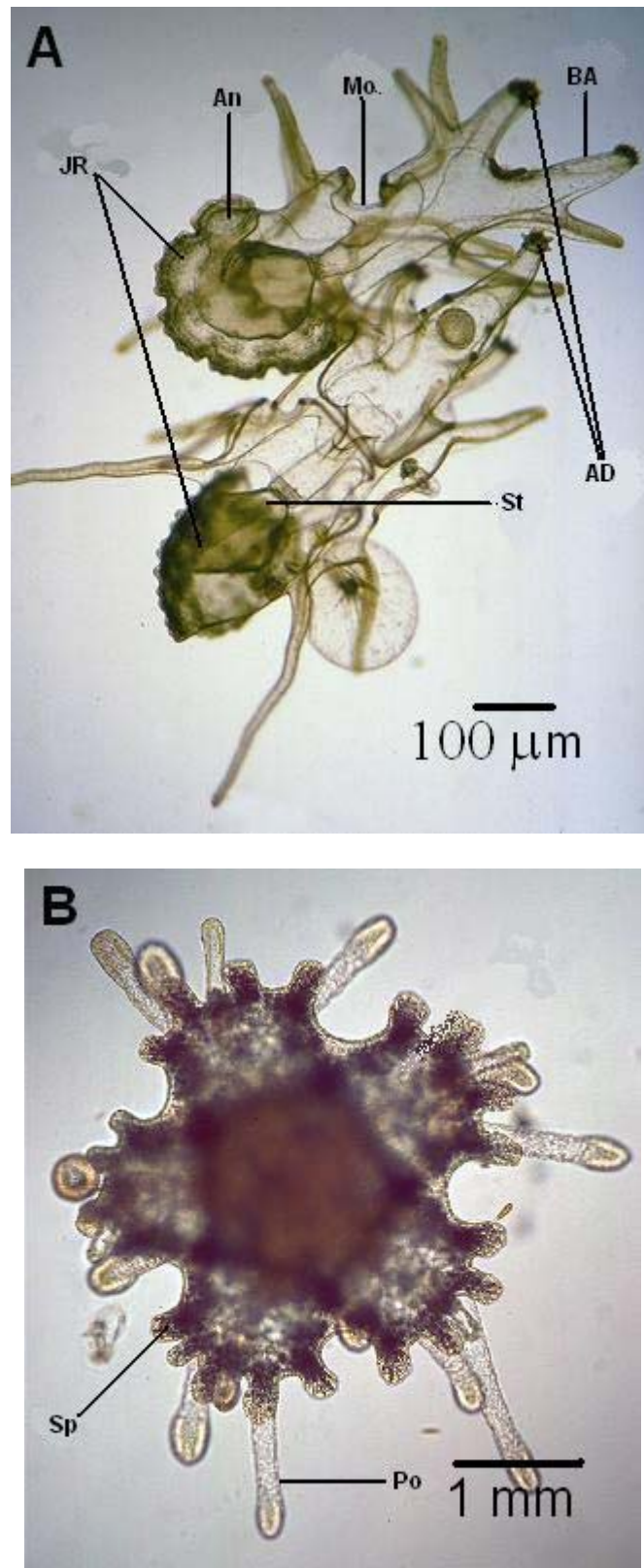
Fig. 2.5. Brachiolaria and Juvenile. **A** Brachiolaria larvae of *Asterias rubens* Linnaeus. **B** Juvenile stage of *A. rubens*. L. Abbreviations: *AD* Adhesive discs; *An* Anus; *BA* Brachiolar arm; *Mo* Mouth; *Po* Podia; *Sp* Spines; *St* Stomach. (Photographs A, B by Jan Parmentier).

independent of habitat or mode of nutrition during development. 2) Developmental patterns can be distinguished by habitat, using the pelagic or benthic feature. 3) The distinction between feeding and non-feeding development provides information about nutrition. All three characters should be used in order to unequivocally describe or classify a developmental pattern.

Eight different developmental patterns can potentially be described using this classification when three characters exist, each with two alternative stages. Of these eight potential patterns only six are known to occur in asteroids:

Indirect development by means of pelagic feeding larvae is common in asteroids and it can involve both bipinnarian and brachiolarian stages (e.g. *Asterias rubens* Linnaeus, Gemmill, 1914) or only bipinnarian stages (e.g. *Astropecten scoparius* , Oguro *et al*., 1976).

Indirect development via pelagic non-feeding larvae can involve a simplified brachiolaria or the barrel-shaped larva.

Indirect development on the benthos by means of a feeding larva possibly occurs in the Antarctic asteroid *Odontaster validus* Fisher, but this is still uncertain (Pearse & Bosch, 1986)

Indirect development via a benthic non-feeding larva is common among brooding asteroid species (e.g. *Ctenodiscus australis* Lütken, Lieberkind, 1926; *Henricia sanguinolenta* (O.F. Müler), Masterman, 1902; *Leptasterias hexactis* Fisher, Chia, 1968).

Pelagic non-feeding direct development has been observed only in *Pteraster tesselatus* (McEdward, 1992). Benthic non-feeding direct development has not been reported in the scientific literature yet, but McEdward & Janies assume it for the

brooding pterasterid, *Pteraster militaris* (O.F. Müler) (Kaufman, 1968; McClary & Mladenov, 1990) based on similarities with *P. tesselatus*.

Strict direct development is extremely rare in asteroids. Direct development involving feeding has not been reported for asteroids. Given the predatory nature of most juvenile and adult asteroids, it is unlikely that transitional developmental stages (mesogens) could acquire particulate food without the use of specialized (i.e. larval) feeding structures. Direct development, with feeding seems to be an unlikely evolutionary option for starfish (McEdward & Janies, 1993).

2.7. Larval cloning in asteroids

Larval cloning is known to occur in all echinoderm classes except crinoids. This supports an earlier conjecture that it might be an ancestral ability of echinoderms (Eaves & Palmer, 2003). The developmental stage of clones at separation ranges from blastulae to fully formed larvae and some clones may not separate until just after the primary larva has begun metamorphosis. This indicates either that larval cloning evolved independently on several occasions or that its mechanisms have diverged extensively from an ancestral mode. Knott *et al*. (2003) were the first to identify field-collected cloning asteroid larvae, and provided evidence that larval cloning is phylogenetically extensive within the Asteroidea. Furthermore cloning occurs regularly and in multiple ways within species that are capable of cloning.

Three distinct modes of larval cloning have been observed in planktotrophic asteroid larvae collected from the field and reared in laboratory including 1) paratomy of the posterolateral arms, 2) autotomization of the preoral lobe, and 3) budding from the larval body and arm tips (Jaeckle, 1994; Knott *et al*., 2003). Clones are able to develop to metamorphosis and may themselves exhibit further larval cloning (Vickery & McClintock, 2000; Kitazawa & Komatsu, 2001). Nevertheless it is not known

whether juveniles derived from larval cloning will develop until they reach sexual maturity, or if larval cloning has only fitness consequences for either the primary or cloned larvae (Knott *et al*., 2003). Balser (2004) stated that cloned larvae also reproduce asexually creating a potential perpetual cycle of cloning, this author suggests that the ecological and evolutionary connotations of this incident is to increase the number of original offspring produced and in consequence to enhance the potential advection of larvae spawned in a single event.

Larval cloning signifies an interesting new element for the life histories of invertebrates. This process presents three potential ecological advantages for the cloning species: 1) an increased fecundity when the conditions are appropriate for optimal growth, 2) an increased probability of settlement after a prolonged larval life, and 3) recycling of otherwise unused or reabsorbed larval tissue (Eaves & Palmer, 2003).

CHAPTER THREE–TEMPERATURE AND PRESSURE TOLERANCES OF EMBRYOS AND LARVAE OF THE ATLANTIC SEASTARS *Asterias rubens* AND *Marthasterias glacialis* (ECHINODERMATA: ASTEROIDEA): POTENTIAL FOR DEEP-SEA INVASION FROM THE NORTH ATLANTIC.

3.1. Introduction

Deep water formation in the world's oceans occurs at three main sites, the Norwegian Sea in the North Atlantic, the northern Mediterranean and the great embayments round Antarctica (Gage & Tyler, 1991; Levin & Gooday, 2003; Tyler, 2003). The deepest water in the world's ocean originates from shallow water around the Antarctic continent where the very dense cold, high saline water (Antarctic Bottom Water)(AABW) is formed by freezing of the surface waters (Mantyla & Reid, 1983; Gage & Tyler, 1991). In the Norwegian Sea, North Atlantic Deep Water (NADW) is formed during winter when high salinity surface water from the North Atlantic cools and sinks to form a deep homogenous water column. This water flows south over the Scotland-Faroes-Iceland-Greenland Ridge sinks and spreads throughout the world's ocean as a deep, cold water mass (Gage & Tyler, 1991). In the Mediterranean, deep water is formed in winter in the Gulf of Lyon by the cooling of surface water by the Mistral (Tyler, 2003). The sill of Gibraltar prevents the incursion of the dense cold water from the North Atlantic resulting in the bottom water temperature in the Mediterranean being ~13°C whilst in most of the world ocean the abyssal temperature is below 4°C. (Sverdrup *et al*., 1942; Tyler, 1995, 2003).

The sites of formation of the deep and bottom waters of the world oceans have been invoked as potential sites of invasion of the deep sea by larvae of shallow water invertebrates. To test this hypothesis, the pressure and temperature tolerances of echinoid embryos from the shallow waters of the N. Atlantic, the Antarctic Peninsula

and the northern Mediterranean have been determined (Young *et al.* 1996a, b, 1997; Tyler & Young, 1998; Tyler *et al.*, 2000). The data from these experiments suggest that the larvae of shallow-water echinoids are sufficiently tolerant of high pressures to allow them to follow an isothermal layer into, at least, bathyal depths and could, subsequently, colonise the deep sea within a single generation (Tyler *et al.*, 2000). Concomitant experiments on bathyal and upper abyssal echinoids suggest that the embryos of bathyal species have the widest tolerance (Tyler & Young, 1998) whilst the embryos of the upper abyssal *Echinus affinis* (Linnaeus) are truly barophilic (Young & Tyler, 1993; Tyler & Young, 1998)

Young *et al.* (1996a) provide data on the pressure/temperature tolerance of developing embryos of the lower bathyal seastar *Plutonaster bifrons* (Wyville Thomson). They found that the highest percentage of normal development occurred at 200atm (≡2000m depth), which is the peak of the species distribution, and virtually no normal development occurred at a pressure corresponding to 3000 m depth. These results indicate that embryonic tolerances could determine the bathymetric limits of distribution for this species.

The seastar species *Asterias rubens* Linnaeus (Fig. 3.1A) is one of the most widespread asteroids of the Northern Hemisphere. It is distributed Cape Cod northwards in the western North Atlantic (Franz et al. 1981; Wares, 2001) and from the Arctic to western France and southern Portugal in the east (Clark & Downey, 1992; Hayward & Ryland, 1995; Wares, 2001) (Fig, 3.1B). This species is found from the intertidal to depths of 900 m (Vevers, 1949; Clark & Downey, 1992), and though normally completely marine, may be found in waters with a salinity close to 10 (Brattstrom, 1941; Segerstrale, 1949; Schlieper, 1957; Nauen, 1978; Sarantchova, 2001). This species is considered to be conspecific with *Asterias vulgaris* (Coe, 1912;

Clark & Downey, 1992), which is widely distributed on the eastern coast of the U.S.A. (Franz *et al.,* 1981).
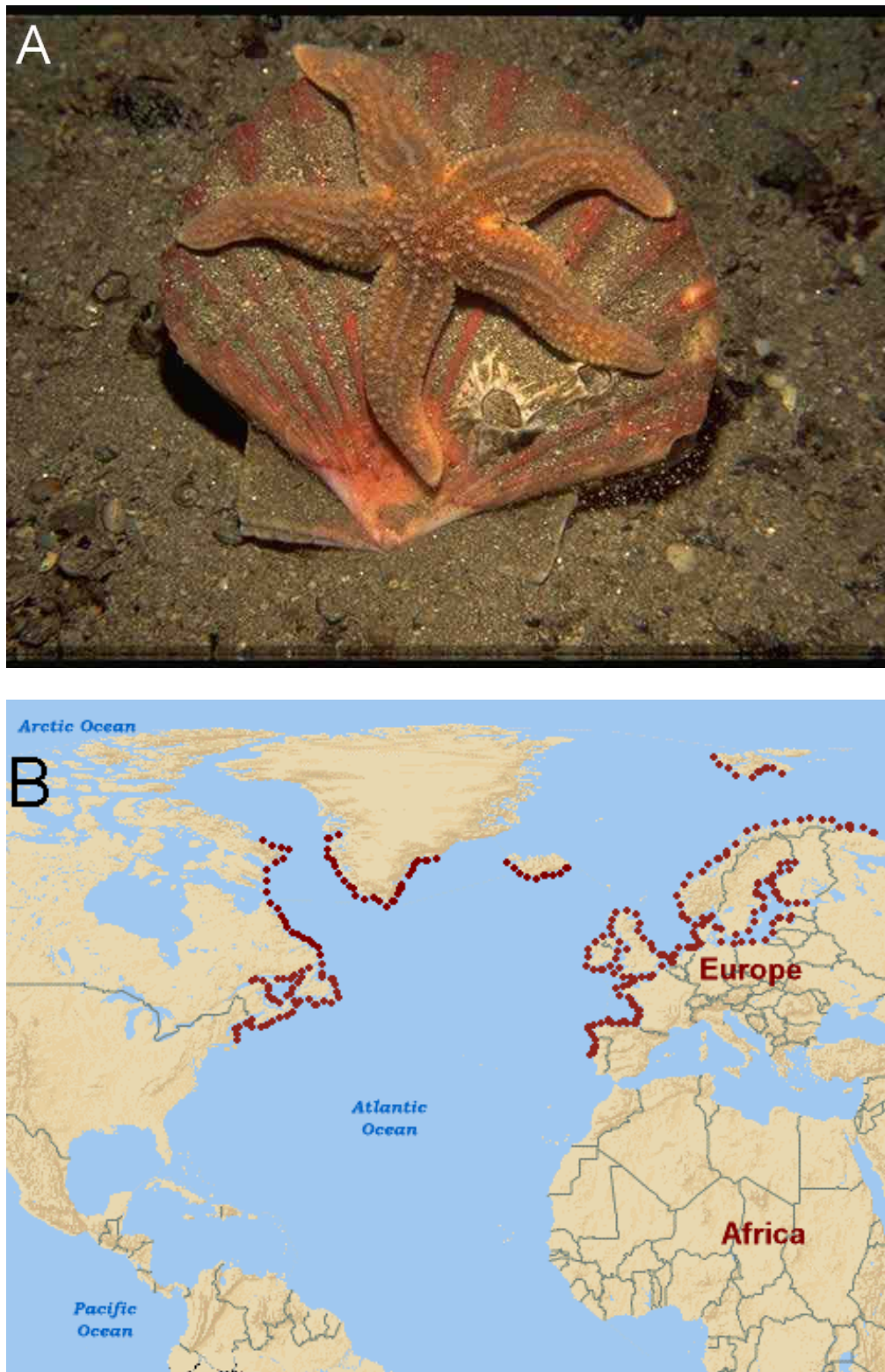
Fig. 3.1. A. *Asterias rubens* Linnaeus on scallop shell. (Image width ca 30 cm. by Sue Scott). **B**. Geographical distribution of *A. rubens*.

Wares (2001) performed phylogenetic and population genetic analyzes from data of populations of *Asterias rubens*, *A. forbesi* (Desor), and *A. amurensis* (Linnaeus) to test the two current hypotheses that explain the speciation of the genus *Asterias* in the Atlantic and he concluded that, although changes in climate and ocean currents (in particular the formation of the Labrador current) were simultaneous with the separation of populations of *Asterias* in the North Atlantic around 3 million years ago, definitive colonization of New England and the Canadian waters by *A. rubens* took place very recently.

A number of aspects of the reproductive biology of *A. rubens* have been studied since Gemmill (1914) accomplished rearing experiments of this species from fertilization to juvenile starfish. Vevers (1949) proposed an index of six stages of maturity in order to explain the reproductive cycle in two populations of *A. rubens* in the English Channel, off Plymouth Sound. Jangoux & Vloeberg (1973) described changes in the size of gonads and pyloric caeca during the annual breeding cycles of organisms taken from the North Sea. Outjeans & Van der Sluis (1979a, b) described the changes in the biochemical composition of the ovaries throughout the annual reproductive cycle, and Walker (1974) described the fine structure of the gonads of *A. vulgaris*. Nichols & Barker (1984) suggested a planktotrophic pelagic life of ~90 days and settlement of larvae on a wide range of substrata. Bipinnaria are present in the plankton mainly between March and April. Late bipinnaria and brachiolaria appear from end of April until early July and late brachiolaria reach a peak in mid-late June, being found occasionally in plankton samples until mid-late July. Diverse authors established that Juvenile *A. rubens* feed carnivorously (Orton & Fraser, 1930; Vevers, 1949; Barnes & Powell, 1951; Hancock, 1955. 1958; Anger *et al.* 1977) but Barker & Nichols (1983) were the first to mention that they do it immediately after the

completion of metamorphosis. Delage (1904) and Barker & Nichols (1983) suggested that larvae of *Asterias rubens* undergo very prolonged development, to allow for wide dispersal under appropriate hydrological conditions.

Marthasterias glacialis (Linnaeus) (Fig. 3.2A) is distributed from Denmark, south to the west coasts of the UK, the North Sea, and as far south on the east side of the Atlantic as the Cape Verde Islands (Penney & Griffiths, 1984). It is also found in the Mediterranean, the Canaries and the Azores (Mortensen, 1927) (Fig. 3.2B). It is not commonly found intertidally but has a depth distribution from just sub-littoral down to a maximum of ~180m (Mortensen, 1927; Madsen, 1950). Although *Marthasterias glacialis* is a common species little is known of its reproduction. Delage (1904) reared the larvae to brachiolaria and described what he called 'parthenogenetic' development. Subsequently, Mortensen (1913) and Gemmill (1916) reared larvae to the brachiolaria stage. Mortensen described that spawning took place in summer and this was refined from June to late September (Boolootian, 1966) and May to June in UK waters (Barker and Nichols, 1983). Barker (1977) determined the length of larval life at ~127 days. Minchin (1987) examined spawning behaviour in relation to sea-water temperature in the southern coast of Ireland.

In the experiments of Barker & Nichols (1983), the larvae of *A. rubens* and *M. glacialis* were in culture for at least 87 days from fertilization to attachment by brachiolaria. However, the larvae took up to 120 days to develop to the advanced brachiolaria stage. Furthermore, larvae that appeared "ready to settle" could be prevented from settlement for an additional two to three weeks without an apparent loss of the ability to attach and complete metamorphosis. Thus both species fall into the category of marine invertebrates with "teleplanic" larvae (Scheltema 1971, 1989) with a long larval life and potentially wide dispersal.

Fig. 3.2. A. *Marthasterias glacialis* (Linnaeus) (Image width ca 45 cm by Bernard Picton). **B.** Geographical distribution of *M. glacialis*.

3.1.1- Chapter objectives

Experiments on the effects of pressure and temperature on larval development in *Asterias rubens* and *Marthasterias glacialis*, were elected to investigate the physiological potential for deep sea invasion by studying the pressure and temperature tolerances of embryos and larvae of two species of North Atlantic asteroids.

The hypothesis being tested is that the early embryos and bipinnaria of *Asterias rubens* and *Marthasterias glacialis* have a similar pressure/temperature tolerance to those of shallow water Atlantic echinoids belonging to the genus *Echinus* (Tyler & Young, 1998) and could use the same pathway for invasion of the deep sea.

3.2- Materials and methods

3.2.1- Field sampling and spawning

The experimental work was carried out in the aquarium of the National Oceanography Centre Southampton (NOCS), during April and May 2002 and 2003. Individuals of *Asterias rubens* were collected from Southampton Water by divers. Individuals *of Marthasterias glacialis* were collected by divers of the Plymouth Diving Centre in Plymouth Sound. All individuals were maintained in running seawater at 15° C until used. To obtain gametes the individuals were placed in separate small plastic aquaria with seawater. In order to induce spawning each animal was injected in the coelomic cavity with 5 ml of a solution of 1×10^{-4} M of 1-methyl adenine (Kanatani, 1969). 15-20 min after the animals were injected, the males started to spawn. The females started to spawn 15-20 min after the males. Eggs were pipetted into a shallow crystallizing dish with seawater and examined under a compound microscope to determine if they had undergone germinal vesicle breakdown and they were ready to be fertilized (Young *et al*. 1996b).

3.2.2- Temperature/pressure effects on fertilized eggs.

1 ml of concentrated sperm was added to a 2 l beaker containing a suspension of eggs in seawater. Successful fertilization was recognized by the appearance of a fertilization membrane as it was monitored using a microscope (Fig 3.3). Excess sperm was removed by allowing the fertilized eggs to settle, decanting off the excess sperm in seawater and replacing with fresh seawater.
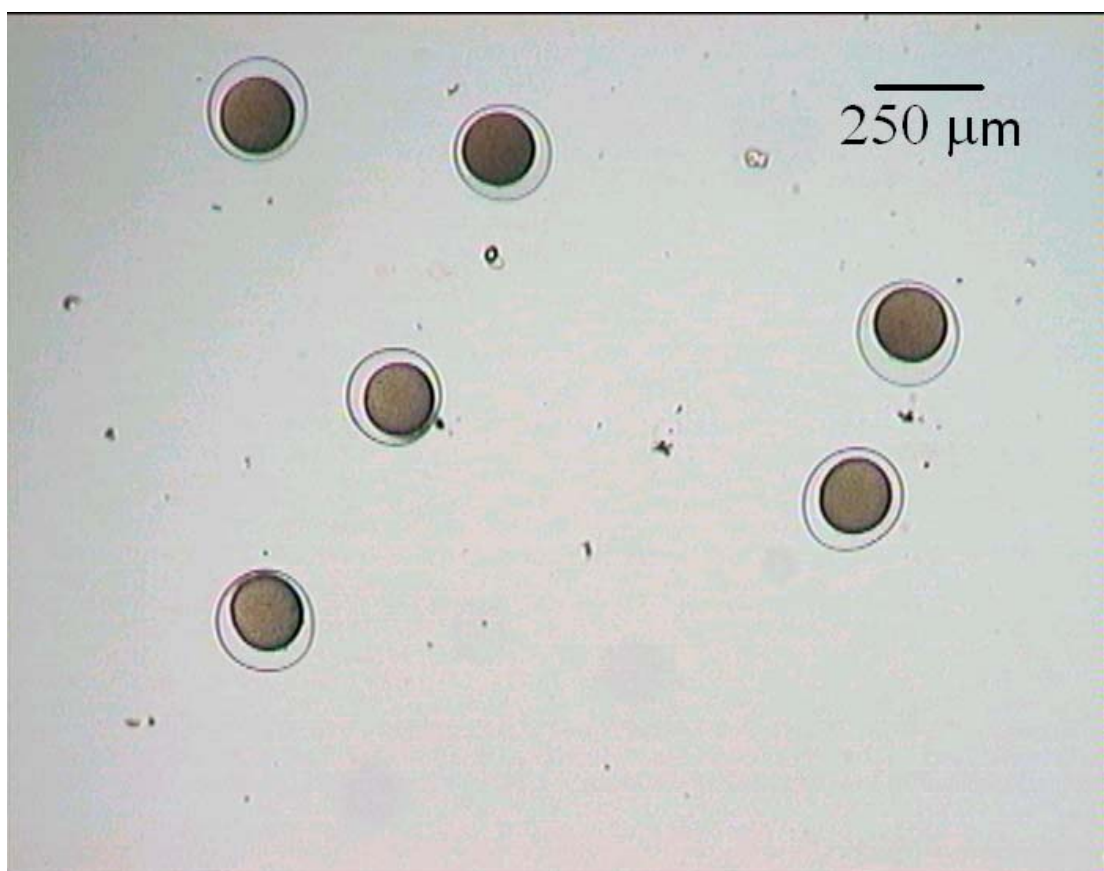
Fig. 3.3. Fertilized eggs of *Marthasterias glacialis* (Linnaeus) as determined by the appearance of the fertilization membrane around the eggs. (Micrograph by Francisco Benitez)

Zygotes in suspension were placed into 8 ml plastic vials that were filled to overflowing with seawater at 15°C and carefully capped to avoid trapping any air.

Three replicate vials were assigned to each pressure/temperature combination in a modified pressure chamber (see Young *et al*., 1996b) (Fig. 3.4 AB).
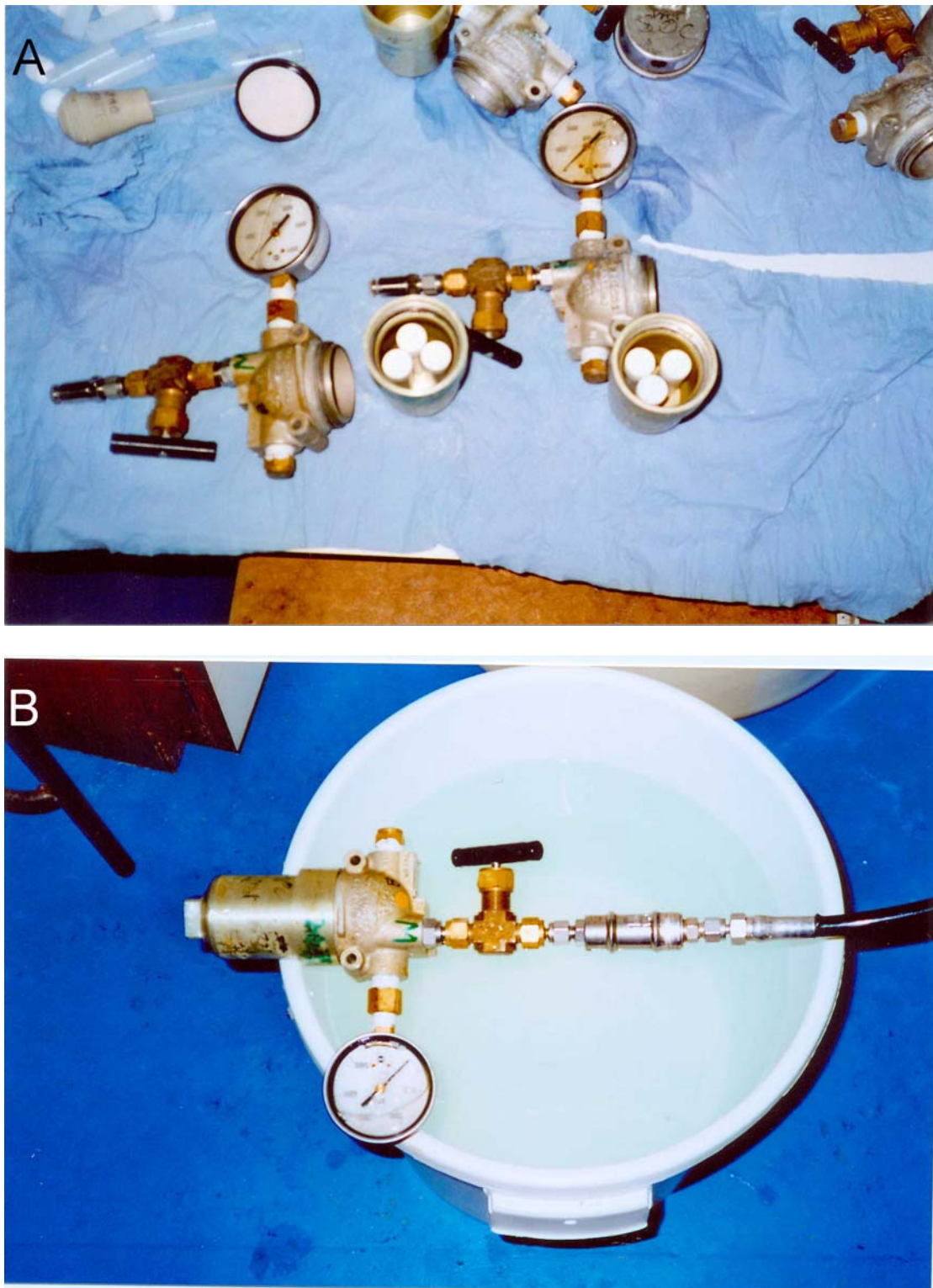
Fig. 3.4. A. Modified pressure chambers containing three replicate 8 ml plastic vials filled with zygotes in suspension in sea water. **B.** Pressure chamber sealed, connected to the pump, and ready to be pressurized (Photograph by Francisco Benitez).

Pressure was applied using an Enerpac hand pump with the pressure vessel under water at 15°C (Fig. 3.5). The cultures were incubated at 5, 10, 15 and 20° C and at 1, 50, 100, 150 and 200 atm. Individual treatments were maintained at temperature either in a constant temperature room or in a temperature-controlled water bath.

Fig. 3.5. Enerpac hydraulic hand pump used to pressurize the modified chambers using fresh water. (Photograph by Francisco Benitez)

Cultures were examined at 6, 12, 24 and 48 h. Pressure vessels were depressurised and the contents of incubations vials were emptied into a counting chamber. Full-sized eggs in each culture were examined under a compound microscope at 10x magnification. The cleavage stage of each normal embryo was noted, and all embryos that had undergone irregular cleavage were counted. Each culture was depressurised, examined and repressurized within 15 mins. At least 50 embryos from each replicate were classified according to embryonic development,

and the data were presented as histograms with mean and standard deviation for each stage of development. Zygotes looking normal with fertilization membranes but no cleavage were classified as uncleaved. Embryos with irregular cleavage or with blebs in the cytoplasm were classified as "abnormal" (Fig. 3.6).

Fig. 3.6. Embryos of Marthasterias glacialis (Linnaeus) that underwent irregular cleavage and two zygotes that remained uncleaved after 48 h of being cultured at 200 atm and 20° C. (Micrograph by Francisco Benitez)

3.2.3- Temperature/pressure effects on larvae.

Embryos were cultured for 20-30 days at 15°C and 1 atm until they became swimming bipinnaria. Three replicate cultures of larvae were subjected to each of 16 pressure/temperature combinations, which included temperatures of 5, 10 15 and 20° C and 1, 50, 100 150 and 200 atm. At least 50 developing larvae from each culture were examined after 24 h and the stage attained noted (Fig. 3.7).
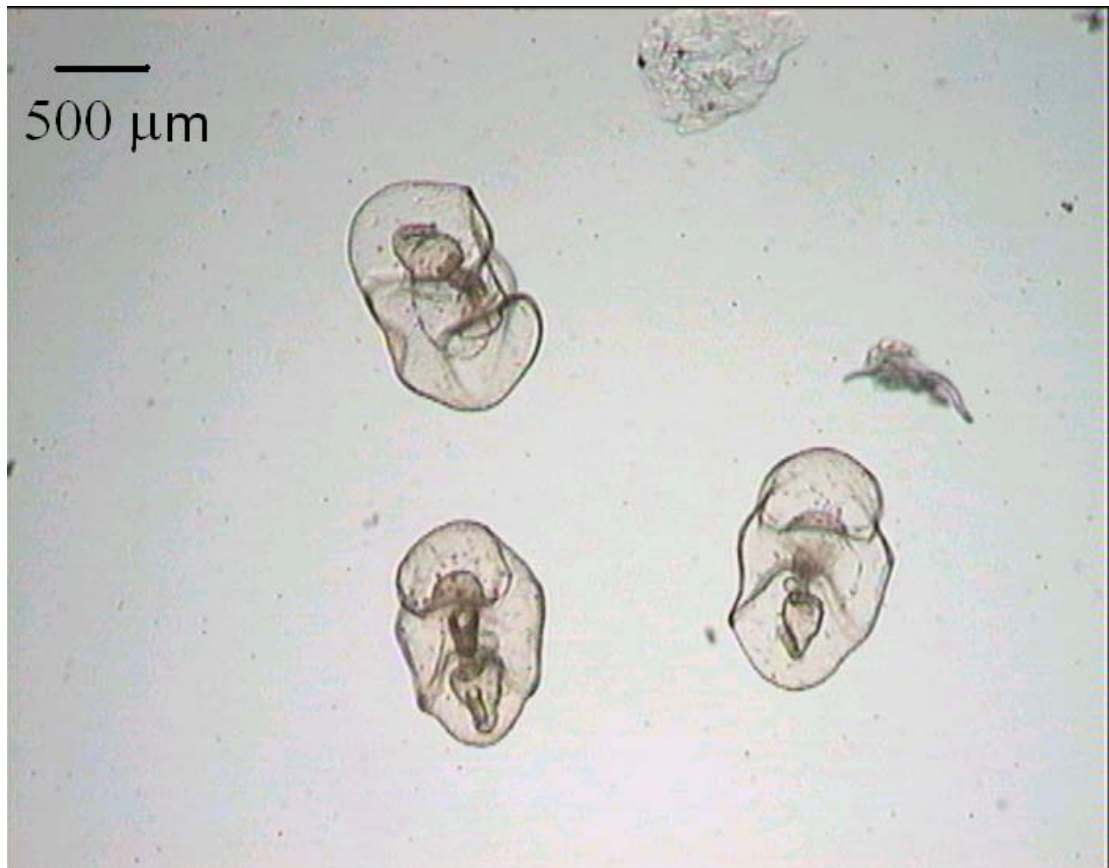
Fig. 3.7. Bippinaria larvae of Marthasterias glacialis observed unaltered after 24 h of being cultured at 200 atm and 10° C. (Micrograph by Francisco Benitez)

3.3- Results

3.3.1- Temperature/pressure effects on fertilized eggs.

Asterias rubens

At 6 h: At 5°C there was almost no evidence of cleavage at any pressure (Fig. 3.8). At 10°C and 1atm, ~40 % of embryos reached the eight- and ~50 % reached the 16-cell stage, at 50 and 100atm around 20% of the embryos were at 4-, 8-, and 16-cell, and ~70 % remained uncleaved, at 150 and 200atm between 80 and 90 % of the zygotes were uncleaved and between 10 and 20 % had an abnormal development. At 15°C and 1 atm around 40 % of the embryos were 16 cell- and ~ 40 % were at 32-cell, less than 20 % of them were abnormal, at 50atm about 50 % of them were 16-cell, almost 20 % were 32-cell and nearly 40 % were abnormal embryos, at 100 and

150atm between 30 and 40 % of the zygotes remained uncleaved and the number of abnormal were approximately 50 %; at 200atm most of the embryos were abnormal. At 20°C at 1 and 50atm between 20 and 30 % of the embryos reached the 16- and 32-cell stages equally and around 60 % were abnormal, from 100 to 200atm around 90 % of them were abnormal (Fig. 3).
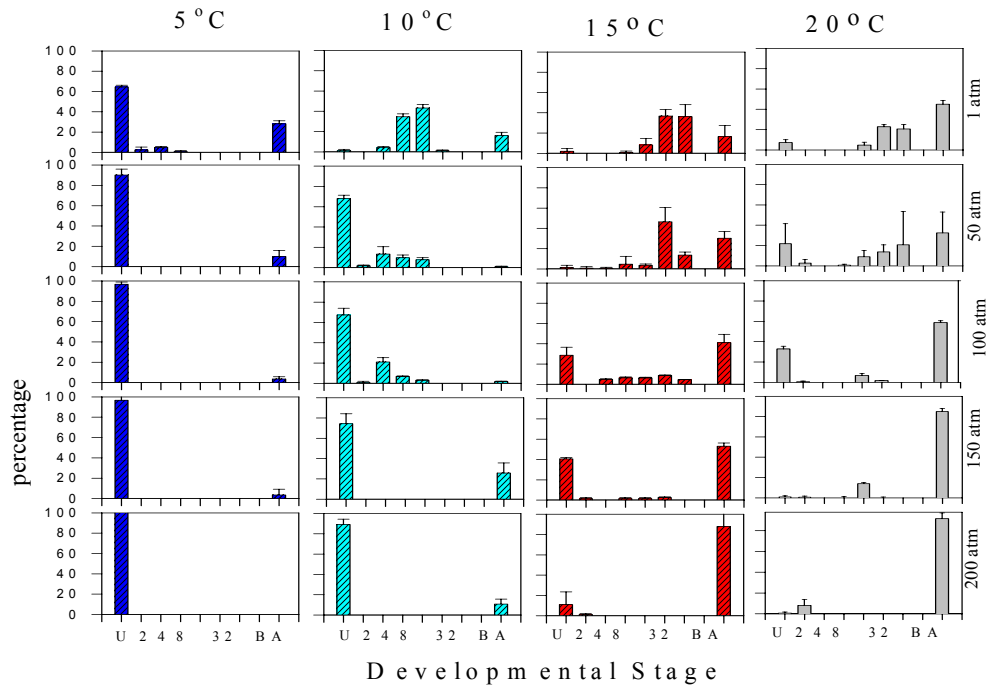
Fig. 3.8. *Asterias rubens* embryos incubated at 5, 10, 15 and 20° C at 1, 50, 100, 150 and 200 atm for 6 h. Histogram represent % mean and SD. Development stages are (U) Uncleaved, 2 to 32 cell, (B) Blastula and (A) Abnormal.

At 12 h: At 5°C and 1atm ~70 % of the embryos reached the 32-cell stage and around 30 % were abnormal, at 50atm the number of abnormal embryos increased to ~40 % and less than 40 % were at the 64-cell stage, around 20 % remained uncleaved, at 100atm almost 50% were uncleaved with the remainder abnormal. At 150 and 200atm around 90 % of the embryos were uncleaved, and ~10 % had abnormal development. At 10°C and at 1 and 50atm nearly 60 % of the embryos reached the 64-cell and between 10 and 20 % reached the 32- cell stage, ~10 % were blastula at 1atm, at 100atm ~40 % of the embryos were 32-cell, around 30 % were 64 cell and ~40 %

were abnormal. At 150 and 200atm all the embryos were abnormal. At 15°C and from 1 to 150atm nearly 50 % of the embryos were blastulae, the number of abnormal embryos was increasing with pressure from ~ 20 % at 1atm to ~50 % at 150 atm, at 200 atm all embryos were abnormal. At 20°C all embryos were abnormal (Fig. 3.9).
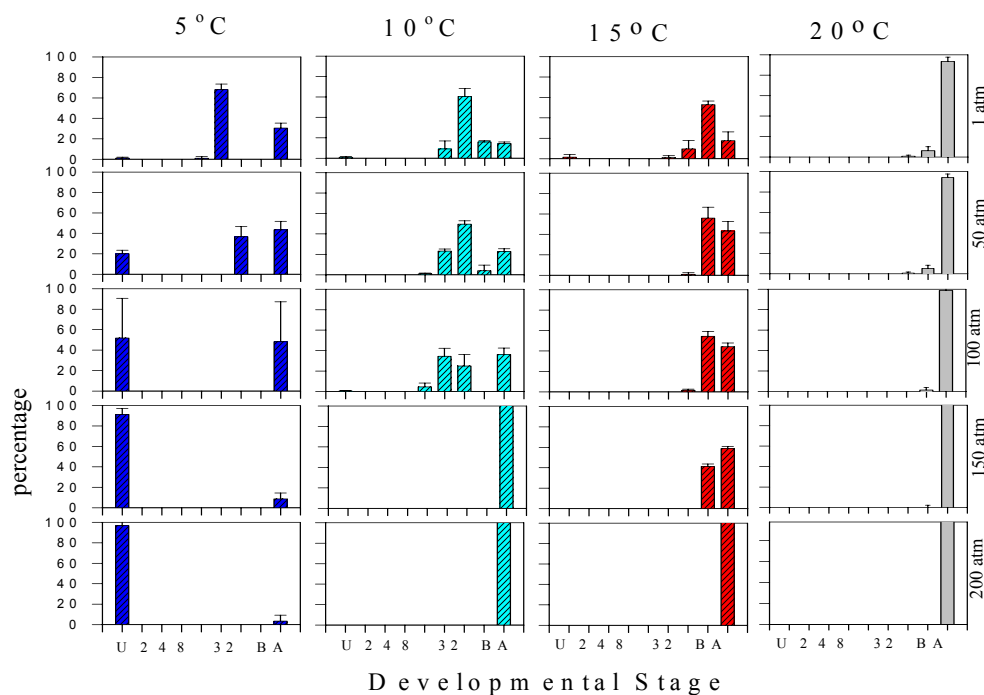
Fig. 3.9. *Asterias rubens* embryos incubated at 5, 10, 15 and 20° C at 1, 50, 100, 150 and 200 atm for 12 h. Histogram represent % mean and SD. Development stages are (U) Uncleaved, 2 to 32 cell, (B) Blastula and (A) Abnormal.

At 24 h: At 5° C and 1atm ~80 % of the embryos were 64-cell and the rest were abnormal, at 50 and 100 atm ~ 80 % were abnormal, the rest remained uncleaved, at 150atm almost 90 % of the zygotes were uncleaved, the rest were abnormal,at 200atm all the embryos were abnormal. At 10°C and 1atm between 60 and 70 % of the embryos were blastulae, ~15 % were 64-cell or abnormal equally, at 50atm ~50 % of the embryos were blastulae, the rest were abnormal, at 100atm ~80 % were abnormal and the rest were blastulae, at 150 and 200atm all the embryos were abnormal. At 15°C from 1 to 100atm the number blastulae was decreasing from ~80

to ~50 %, the rest were abnormal, at 150atm ~60 % were abnormal, the rest were

blastulae, at 200atm all the embryos were abnormal. At 20°C at 1atm >95% of

embryos were abnormal and the rest were blastulae, from 50 to 200atm all the

embryos were abnormal (Fig. 3.10).
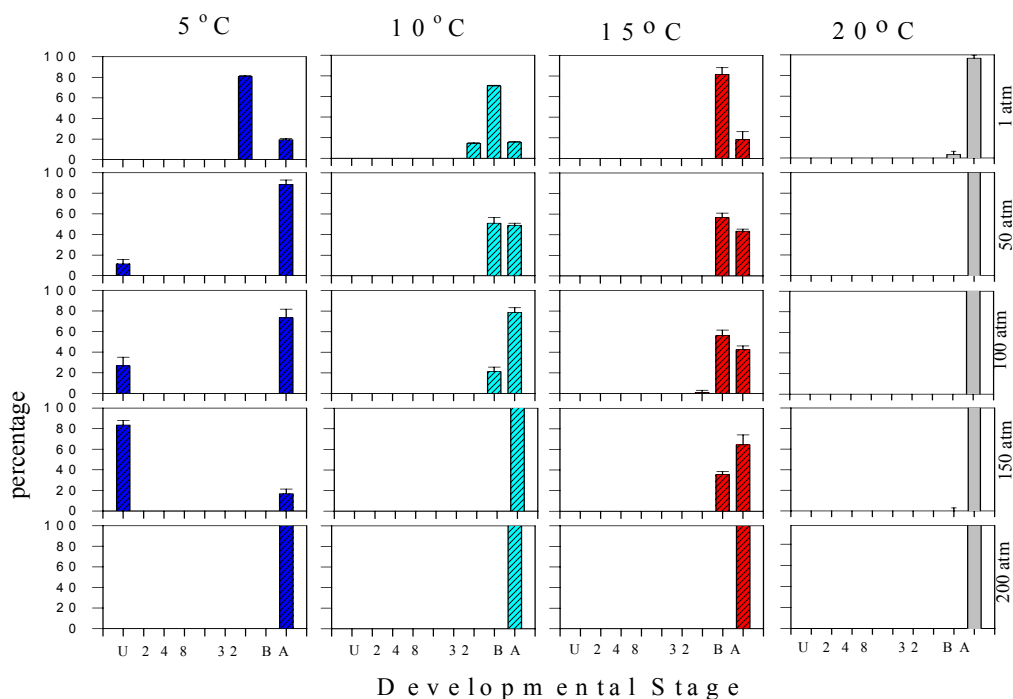
Fig. 3.10. *Asterias rubens* embryos incubated at 5, 10, 15 and 20° C at 1, 50, 100, 150 and 200 atm for 24 h. Histogram represent % mean and SD. Development stages are (U) Uncleaved, 2 to 64 cell, (B) Blastula and (A) Abnormal.

At 48 h: At 5°C and 1atm ~50 % of the embryos were blastulae, the rest were

abnormal, from 50 to 150atm almost 90 % of them were abnormal, the rest remained

uncleaved, at 200atm all embryos were abnormal. At 10°C and 1atm ~60 % of the

embryos reached the late gastrulae stage, the rest were abnormal, at 50 and 100atm

~10 % were gastrulae and the rest abnormal, at 150 and 200atm all embryos were

abnormal. At 15°C and 1atm almost 80 % of the embryos were late gastrula, the

remainder were abnormal, from 50 to 150atm the number of abnormal embryos was

~70 %, the rest were late gastrulae, at 200atm all the zygotes were abnormal. At 20°C

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all the embryos were abnormal and just ~5 % reached the early gastrulae stage at 1atm (Fig. 3.11).
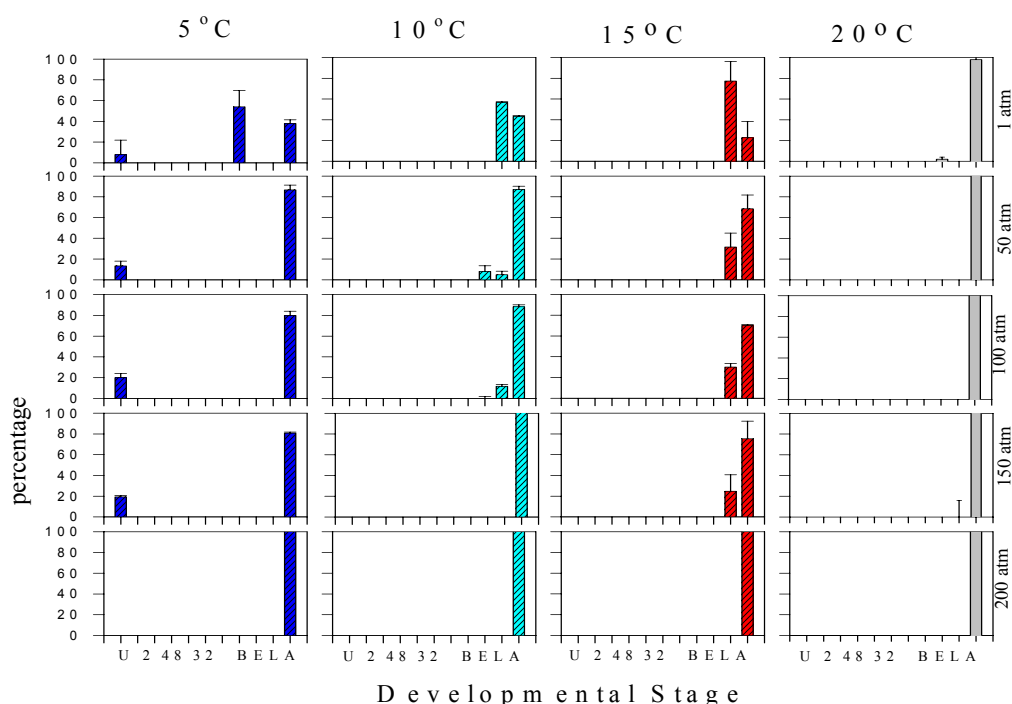
Fig. 3.11. *Asterias rubens* embryos incubated at 5, 10, 15 and 20° C at 1, 50, 100, 150 and 200 atm for 48 h. Histogram represent % mean and SD.   Development stages are (U) Uncleaved, 2 to 32 cell, (B) Blastula, (E) Early gastrula, (L) Late gastrula and (A) Abnormal.

Marthasterias glacialis

At 6 h: At 5°C there was no evidence of cleavage at any pressure. At 10°C and 1atm ~80 % of the embryos were uncleaved, at 50atm ~40 % of embryos were at the 4-cell stage, and the remainder were 2-cell or abnormal. At 100atm ~20 % included 2-, 4-, 8-, and 16-cell, ~50 % were uncleaved and ~30 % abnormal. At 150 and 200atm ~90 % were uncleaved, the rest were abnormal. At 15°C and 1 atm ~80 % of the embryos were at 8-cell, at 50atm ~50 % were 16-cell and ~40 % were abnormal, at 100 and 150atm ~70 % was distributed among 2-, 4-, 8-, and 16-cell stages and ~30 % were abnormal, at 200 atm ~90 % of zygotes were uncleaved and ~10 were abnormal. At 20°C from 1 to 150atm the number of embryos at 32-cell was decreasing from ~70 to ~ 20 %, the number of abnormal embryos increased with the pressure from ~30 % at 1atm to ~80 % at 150 atm, at 200atm ~9 % were abnormal and the rest uncleaved (Fig. 3.12).
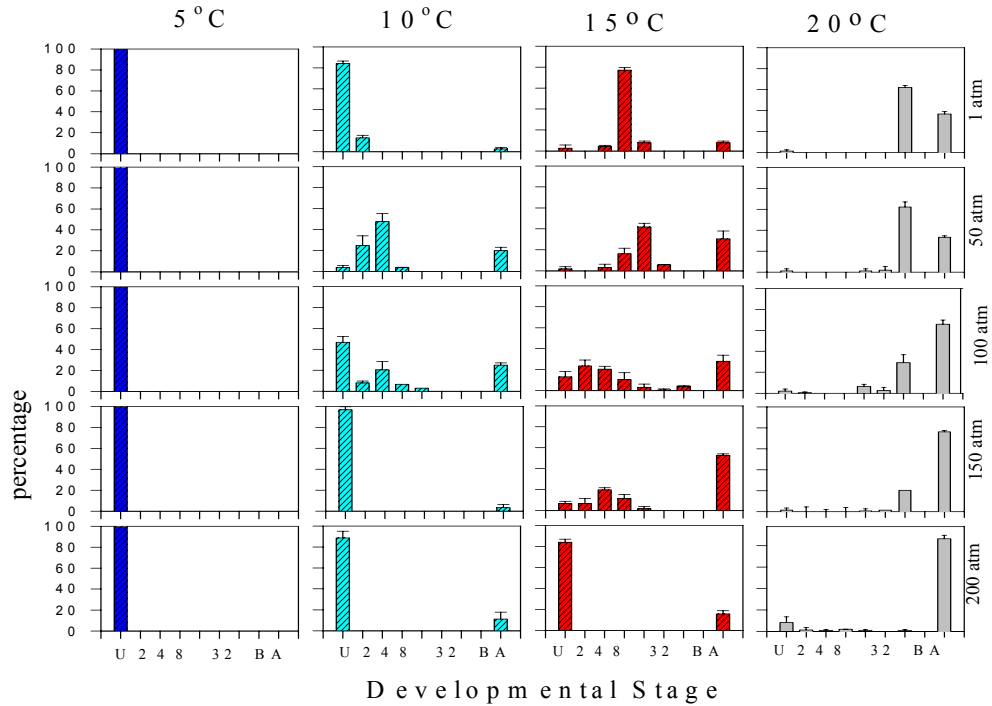
Fig. 3.12. *Marthasterias glacialis* embryos incubated at 5, 10, 15 and 20° C at 1, 50, 100, 150 and 200 atm for 6 h. Histogram represent % mean and SD. Development stages are (U) Uncleaved, 2 to 32 cell, (B) Blastula and (A) Abnormal.

At 12 h: At 5°C almost all zygotes remained uncleaved but showing no irregularities, with just ~5 % abnormal at 1 and 50atm. At 10°C and 1atm ~95 % of embryos remained uncleaved, the rest were abnormal, at 50atm around 40 % embryos were at 2-cell stage, ~50 % remained uncleaved and ~10 % were abnormal, at 100atm ~50 % of the embryos were uncleaved, ~30 % were 2-cell and the number of abnormal increased to ~20 %, at 150 and 200atm all were uncleaved. At 15°C and 1atm ~90 % were 32-cell and ~10 % were abnormal, at 50 atm ~50 % were 32-cell, ~20 % were 16-cell and abnormal equally, ~10 % were at 8-cell stage, at 100 and 150atm ~60 % were abnormal embryos, ~ 20 % were uncleaved and the rest 8- and 16-cell, at 200atm ~90 % of embryos were uncleaved and ~10 % were abnormal. At 20°C from 1 to 100atm between 50 and 60 % reached the blastula stage, ~40 % were abnormal, at 150atm ~70 % of embryos were abnormal, ~30 % were 64-cell and

blastulae, at 200atm 90% of the embryos were abnormal; the rest were uncleaved (Fig. 3.13).
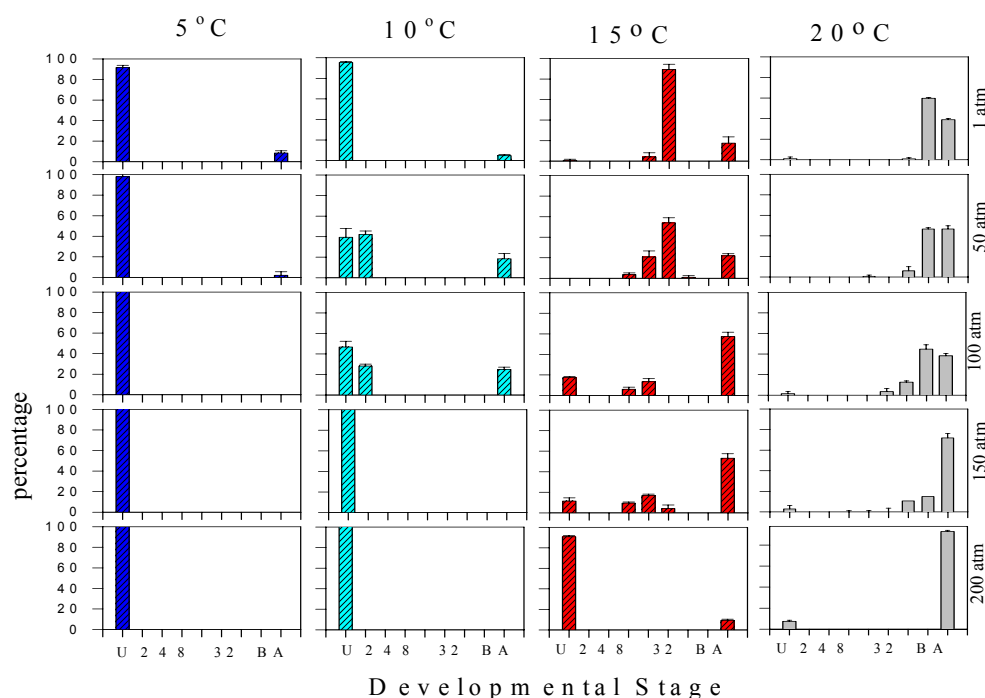
Fig. 3.13. *Marthasterias glacialis* embryos incubated at 5, 10, 15 and 20° C at 1, 50, 100, 150 and 200 atm for 12 h. Histogram represent % mean and SD. Development stages are (U) Uncleaved, 2 to 32 cell, (B) Blastula and (A) Abnormal.

At 24 h: At 5°C and 1atm ~80 % of embryos were abnormal, ~20 % were uncleaved, at 50 and 100atm between 70 and 80 % were uncleaved, between 20 and 30 % were abnormal, at 150atm ~90 % of embryos were abnormal, the rest were uncleaved, at 200atm all the embryos were abnormal. At 10°C and 1atm ~40 % were blastula, ~40 % were 16- and 32-cell, ~20% were abnormal, at 50atm ~80 % were blastulae, ~20 % were abnormal, at 100atm ~40 % were 64-cell, ~10 % were blastulae, ~25% were abnormal and ~25 % were 32- cell, at 150atm ~70% of the embryos were abnormal, around 30 % was a mixture of 8-, 16-, 32- and 64-cell, at 200atm ~70 % were abnormal, the rest were uncleaved. At 15°C and 1 and 50atm ~80 % of embryos were blastulae, the rest were uncleaved; at 100atm ~50 % were

blastulae and ~50 % abnormal, at 150 atm ~60 % were abnormal, ~30 % blastulae and ~10 % uncleaved, at 200atm ~90% were abnormal, the rest were uncleaved. At 20°C at 1atm ~70 % of embryos were early gastrula, the rest were abnormal, at 50atm ~70 % were early gastrula, the rest were abnormal, at 100atm ~90 % were abnormal, the rest were blastulae, at 150 atm ~70 % were abnormal,~ 30 % were early gastrula, at 200atm 70 % were abnormal, 16% were blastula and 14% were uncleaved (Fig. 3.14).
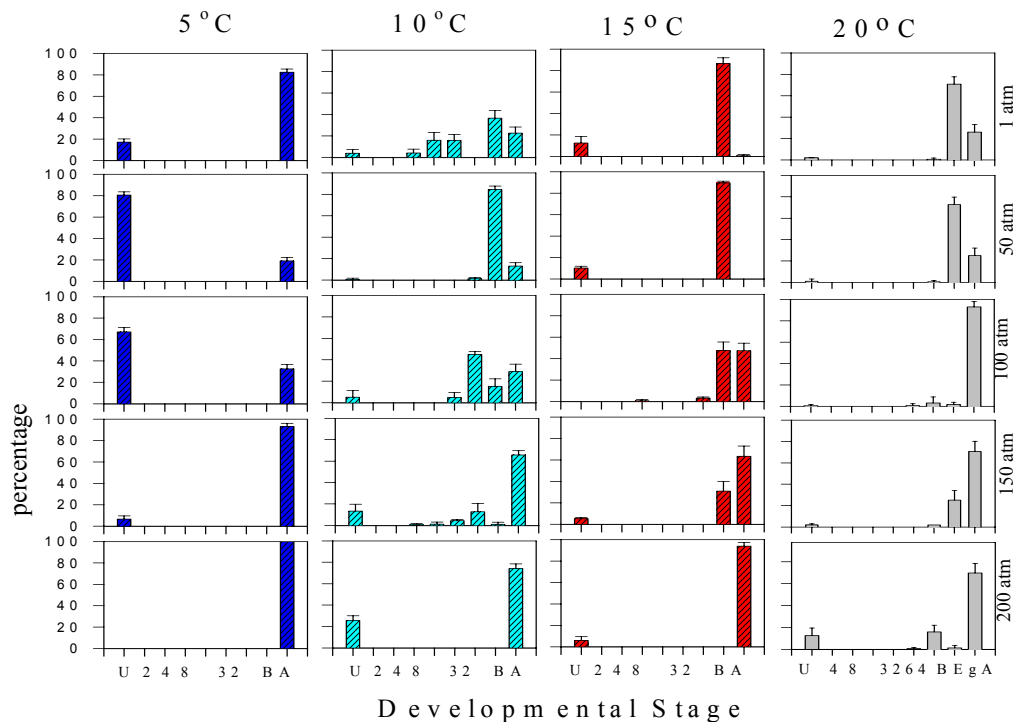
Fig. 3.14. *Marthasterias glacialis* embryos incubated at 5, 10, 15 and 20° C at 1, 50, 100, 150 and 200 atm for 24 h. Development stages are (U) Uncleaved, 2 to 32 cell, (B) Blastula, (Eg) Early gastrula and (A) Abnormal.

At 48 h: At 5°C and 1 and 50atm more than 90 % of embryos were abnormal the rest were uncleaved, at 100 and 150atm ~90 % of embryos remained uncleaved, the rest were abnormal, at 200 atm ~80 % underwent abnormal cleavage and the rest remained uncleaved. At 10°C and 1 and 50 atm ~90 % of embryos were blastula, the rest were abnormal, at 100atm ~60 % of embryos were abnormal, the rest were blastulae and uncleaved, at 150atm all the embryos underwent an abnormal cleavage, at 200atm 60% had abnormal cleavage and 40% were uncleaved. At 15°C and 1 and

50atm more than 90% of embryos were late gastrula, the rest were uncleaved, at 100atm ~60 % of embryos were abnormal and the rest were blastulae, at 150atm ~70 % were abnormal and the rest were gastrula, at 200atm more than 90 % were abnormal, less than 10 % were early gastrula. At 20°C at 1 atm ~90 % were abnormal ~10 % were early gastrula, at 50atm ~60 % were early gastrula, ~40 % were abnormal, at 100atm ~50% were early gastrula and ~ 50% abnormal, at 150atm ~20 % were early gastrula and ~80 % were abnormal, at 200atm all the embryos were abnormal (Fig. 3.15).

A three-way completely crossed ANOVA (main effects: pressure, temperature, species) on percentages of abnormally developing embryos after 40 h revealed not significant interactions among all factors (Table 3.1) indicating that the two species do not respond differently to the various combinations of pressure and temperature.

3.3.2- Temperature/pressure effects on swimming bipinnaria at 24 h.

Asterias rubens

Survivorship of swimming (15 - 20 days) bipinnaria remained high (>70%) after incubation at all the pressure/temperature combinations. The greatest number of surviving swimming bipinnaria was 100% at 10°C/50atm and the lowest was 72% at 15°C/200atm (Fig. 3.16). Survivorship decreased as pressure increased; nevertheless most bipinnariae tolerated a pressure of 200 atm for 24h.

Marthasterias glacialis

Survivorship of swimming bipinnaria (30 days) of *M. glacialis* also remained high (100 %) after incubation at 1atm at all temperatures. At 5°C and 20°C survivorship decrease with pressure increase but remained above 50% and 80%

survival respectively. Survival remained high (more than 90 %) at all pressures at 10 and 15°C (Fig. 3.17).
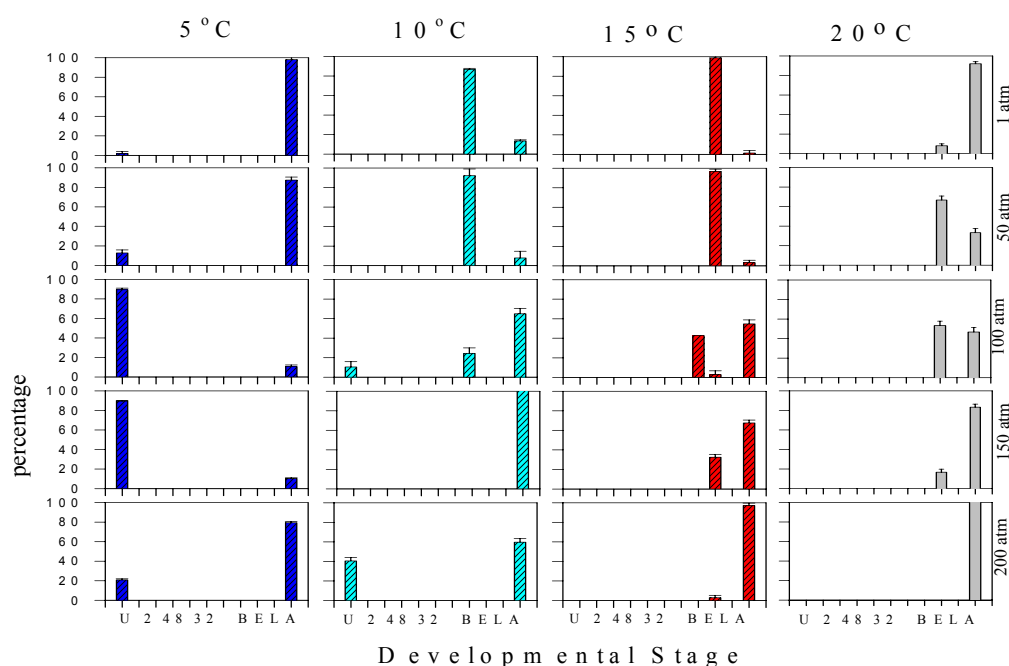
Fig. 3.15. *Marthasterias glacialis* embryos incubated at 5, 10, 15 and 20° C at 1, 50, 100, 150 and 200 atm for 48 h. Histogram represent % mean and SD. Development stages are (U) Uncleaved, 2 to 32 cell, (B) Blastula, (E) Early gastrula, (L) Late gastrula and (A) Abnormal.

3.4- Discussion

Experimental work on the pressure and temperature tolerances of embryos and larvae of shallow water echinoids suggest that they are capable of entering the deep sea along isotherms, but across isobars. In the northern Mediterranean (Young *et al.,* 1997) embryos and larvae of the shallow water echinoids *Paracentrotus lividus* (Lamarck), *Arbacia lixula*, and *Sphaerechinus granularis* tolerated pressures as high as 150 atm at 15° C. Lower temperatures (<11° C) exacerbated the effects of pressure. Living larvae of shallow water Mediterraean echinoids have been collected from depths as great as 400 m (Pedrotti, 1990), indicating that invasion of deeper waters could in fact take place in a single generation.

Those data (Young *et al.,* 1997; Pedrotti, 1990) suggest that such embryos and larvae could colonise the deep-sea under the warm deep-sea conditions that prevailed in the late Mesozoic or early Cenozoic (Menzies *et al*., 1973; Hessler & Wilson, 1983). In the North Atlantic, Tyler & Young (1998) examined the temperature and pressure tolerances of embryos and larvae of the shallow water species *Echinus esculentus* Linnaeus, shallow and bathyal populations of *E. acutus* Linnaeus and lower bathyal populations of *E. affinis* (Linnaeus). Embryos and larvae of both *E. esculentus* and *E. acutus* were unlike the Mediterranean species, developmental arrests and abnormalities did not increase with lower temperatures. Embryos of *E. acutus* var. *norvegicus* from the bathyal zone tolerated a significantly broader range of pressure and temperature than did embryos of *E. acutus* from shallow subtidal habitats and also developed more rapidly at lower temperatures, suggesting that *E. acutus* is a very plastic species and may be currently in the process of invading the deep-sea by slowly adapting to increased pressure.

Source	d.f.	SS	MS	F	*p*
Pressure (P)	4	12.693	2.6675	195.23	< 0.001
Temperature (T)	3	14.239	7.7764	552.67	< 0.001
Species (S)	1	1.526	1.971	110.69	< 0.001
P*T	12	6.453	0.0796	56.43	< 0.001
P*S	4	1.63	0.04351	24.12	< 0.001
T*S	3	1.63	0.7820	47.17	< 0.001
P*T*S	12	0.087	0.1132	7.57	< 0.001
Error	80	8.75	0.0134	-	-

Table 3.1. Three-way analysis of variance for proportion of *Asterias rubens* and *Marthasterias glacialis* that developed abnormally after 48 h of incubation.
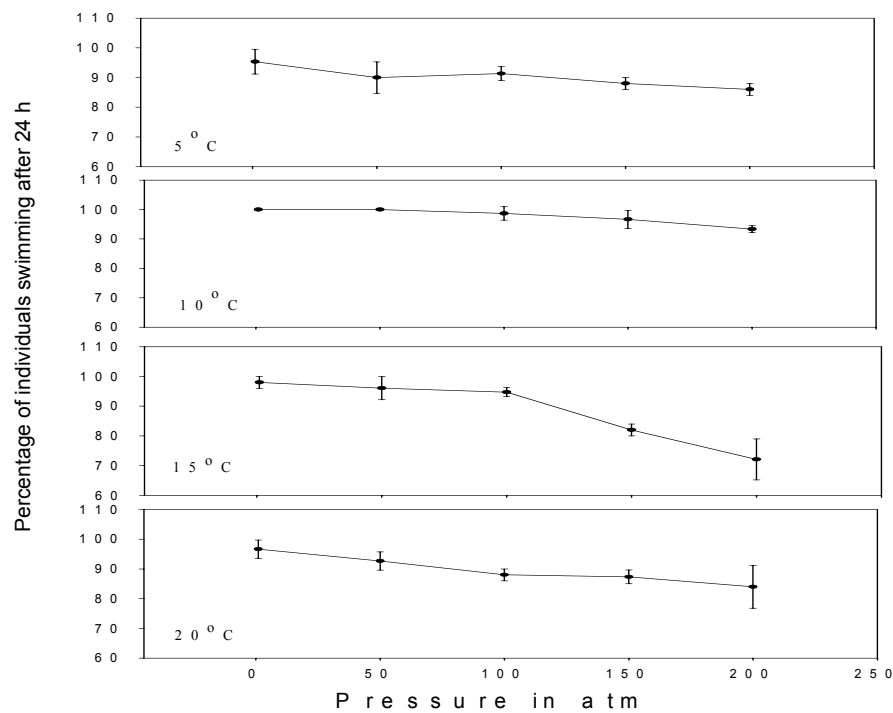
Fig. 3.16. *Asterias rubens* survival of swimming bipinnaria at 5, 10, 15 and 20° C at 1, 50, 100, 150 and 200 atm for 24 h. Data are mean and SD of 3 replicates.

Embryos of the lower bathyal species *E. affinis* were truly barophilic (Young *et al.*, 1993). These data support the hypothesis (Kussakin, 1973; Menzies *et al.*, 1973) that the deep sea could have been invaded during or since the last ice age by larvae capable of tolerating lower temperatures and greater pressures.

In the present study, early embryos of *Asterias rubens* were able to tolerate pressures up to 150 atm at 15° C and 100 atm at 10° C and embryos of *Marthasterias glacialis* were able to tolerate pressures up to 150 atm at 15 and 20° C and up to 100 atm at 10° C. At the lowest temperatures (5° C) there was abnormal embryonic development at 1 and 50 atm, whilst at 100 and 150 atm the development just delayed. Although survivorship of early embryos was variable with temperature and pressure, the survivorship of swimming bipinnaria at all temperature and pressures remained effectively high (> 70 %).
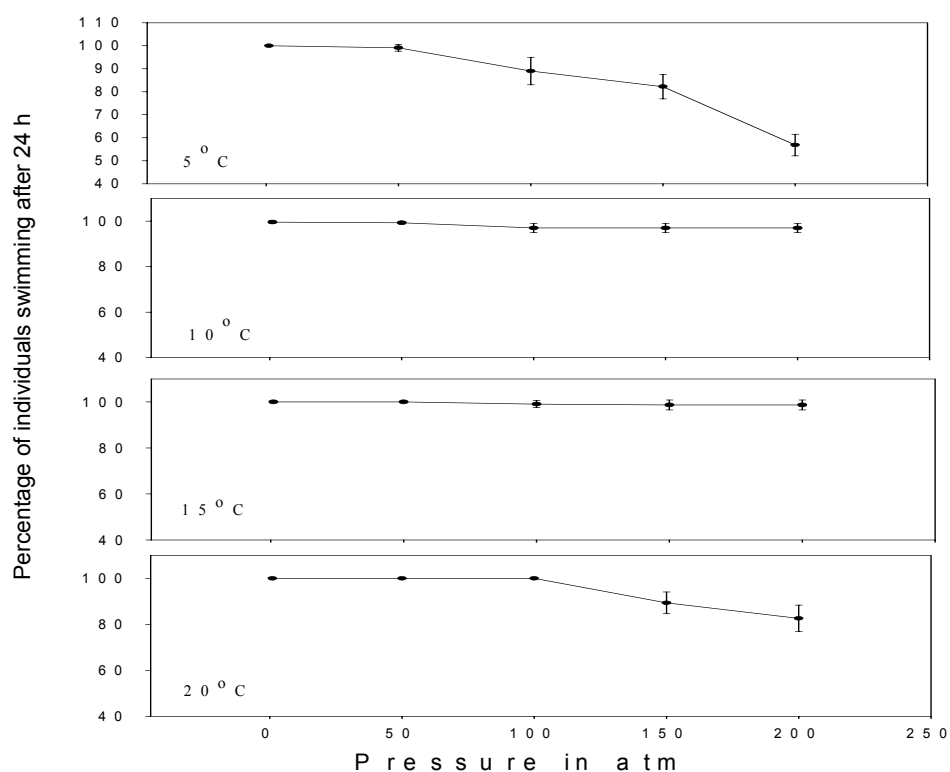
Fig. 3.17. *Marthasterias glacialis survival* of swimming bipinnaria at 5, 10, 15 and 20°C at 1, 50, 100, 150 and 200 atm for 24 h. Data are mean and SD of 3 replicates.

Comparison of *A. rubens* and *M. glacialis* data with those of shallow water and deep-sea Atlantic echinoids suggests that the early embryos of echinoids are more tolerant of pressure/temperature changes but that the later larval stages of asteroids tolerate change more readily than the larval stages of echinoids.

There is increasing evidence for accelerated deep-water formation at the end of glacial periods (Knorr & Lohmann, 2003). During deglaciation, the Atlantic thermohaline circulation becomes more vigorous changing from a weak glacial mode into a strong interglacial mode. There is also recent evidence that there may be a variety of sites of deep-water formation in the N. Atlantic (Pickart *et al.*, 2003). If this more vigorous formation of deep water occurs at this transition period it may be that the warming of surface waters stimulates accelerated reproduction, both through

temperature and primary production and that the resultant larvae using the thermohaline 'conveyer belt, to penetrate the deep sea.

If larvae can tolerate pressures higher than those where adults normally live, then why these species are not found in deeper waters? It is also possible that the pressure/temperature tolerance of different shallow, bathyal and abyssal species may determine their zonation. Howell *et al*. (2002) have shown that the zonation of asteroids varies with depth, with individual species having relatively narrow bands where they are common, but with wide zonation for few individuals.

In a number of species the apparent zonation is wide because juveniles are found outside the adult zone. This event has been observed in the bathyal ophiuroid *Ophiocten gracilis* and the upper abyssal ophiuroid *Ophiura ljungmani* (Gage & Tyler, 1981a, b). Juveniles of both species from the total settlement depth range grow and initiate gametogenesis, but only those individuals settling in the normal adult depth region survive to complete reproduction. These data suggest that in some deep-sea species the post-larvae and juveniles have a wider pressure tolerance than the adults, although survival of juveniles outside the adult zonation is very poor.

Larval settlement (and metamorphosis) is the most critical phase in the life history of any marine benthic species since it involves dramatic changes in morphology, physiology and habitat (Chia, 1989). When settling larvae are incorporated to benthic habitats, they must not only compete with unknown predators and physical factors, but they must also find a suitable settlement site (Stoner, 1994; Bullard *et al*., 2004). Thus the success at settlement achieved by a larva is decisive to the future subsistence of the species at a determined site. The fate of a larva when settling in a new environment is significantly decisive and it can not be completely controlled by the larva itself as reported by Bullard *et al.* (2004). Their results suggest

that settling invertebrate larvae do not avoid settling near established dominant competitors.

It is probable that post-selective forces other than pressure tolerance may exist, such as suitability of habitat or food availability, which eliminates juveniles of *Asterias rubens* and *Marthasterias glacialis* outside the adult range.

The plasticity on the early-life story stages, therefore their ability to tolerate increasing pressure may be cumulative over many generations, until an individual species has successfully adapted to the deep-sea environment. It is possible that this adaptation may have been rapid as a number of deep-sea invertebrate species retain the seasonal growth and reproductive patterns seen in shallow water congeners (Young 2003).

3.5- Recommendations

It is recommended to design a method to measure the pressure experienced inside the plastic vials at the same time as they are located inside the pressurized chambers in order to corroborate that the pressure experienced outside and inside the plastic vials and therefore experienced by the developing embryos is the same.

It is important to perform experiments comparing the effects of pressure on early embryos and larvae using rapid release of pressure and also slow decompression rates, to determine if the depressure/repressure event observed in the present study have a possible effect on the embryos development.

Stumm *et al*. (2001) studied the effect of elevated pressures with rats in laboratory simulating the effects of the high pressure neurological syndrome (HPNS). Their results showed that animals subjected to 61 bars with slow increase in pressure and around two hours of constant high pressure followed by a rapid decompression

suffered depletion in oxygen and died, whereas animals decompressed using slow decompression rates survived. These findings made evident the critical effect of rapid decompression on mammals. Conversely Treude *et al.* (2002) studied the metabolism and decompression tolerance of several species of scavenging lyssianoid amphipods in the deep Arabian Sea. During the experiment the amphipod were decompressed from their ambient at 3950-4420 m depth to atmospheric pressure during recovery. Specimens of the genus *Paralicella* did not survived decompression of more than 300 bar, and because of its limited decompression tolerance this genus might be classified as stenobathic. On the other hand *Abyssorchomene distincta* and *Eurythenes gryllus* had a high tolerance to pressure changes. Both species were recovered without apparent decompression problems and were classified as eurybathic. These results are very important since it is evident that some invertebrate species are able to tolerate decompression without detectable lethal effects; which could be the case of echinoderm embryos and larvae.

CHAPTER FOUR- REPRODUCTIVE FEATURES OF ASTEROIDS IN THE PORCUPINE SEABIGHT AND PORCUPINE ABYSSAL PLAIN, N.E. ATLANTIC AND THEIR RELATION TO THE DEPTH DISTRIBUTION OF THE SPECIES.

4.1- Introduction

A very important objective of ecological research is to explain the evolution of life histories, or more specifically how natural selection modifies reproduction and development in order to generate the patterns that are observed in nature. The relationship between the amount of energy that parents invest per offspring and offspring fitness is one of the fundamental tenets of life-history theory that has been particularly difficult to evaluate empirically. Given that the total resources that the parent allocates to reproduction are limited, there should be an inverse relationship between the investment made in each offspring and the number of offspring that are produced by a parent (McEdward and Morgan, 2001).

Different quantitative models of life-history evolution in marine benthic invertebrates have been produced (e.g., Vance, 1973 a,b; Christiansen and Fenchel, 1979; Pechenik, 1979; Perron and Carrier, 1981; Grant, 1983; Strathmann, 1985; Emlet *et al*., 1987; Havenhand, 1995; Levitan, 1996; McEdward, 1997) in an attempt to describe the effects of natural selection on egg size, presenting some reasonable assumptions about the reproductive and developmental correlates of different parental investment per offspring. The models subsequently predict the direction of evolution of egg size and related life-history traits such as fecundity and larval type under different environmental conditions.

There exists a large amount of published information on the energy content of eggs of different species of marine invertebrates, particularly echinoderms and the

relationship with egg size. Most of the studies have been performed on free-spawning species, which have planktotrophic larval development (Strathmann and Vedder, 1977; Turner and Lawrence, 1979). These data have been complemented with seven species of free-spawning echinoderms with pelagic lecithotrophic larval development by McEdward and Chia (1991).

4.1.1- Fecundity and Egg size

In general terms fecundity refers to the number of offspring produced by a female in her lifetime. Consequently fecundity may be expressed as the number of oocytes, eggs or embryos produced over a certain period (breeding season, year, lifetime) (Extensively reviewed by Ramirez-Llodra, 2002). The analysis of fecundity is very important for studies on reproduction and evolution of life-history because of its relation with energy investment by the parents and other related life-history traits such as egg size. The effect that egg size has on fecundity, fertilization, energy content, parental investment and larval development has been investigated extensively.

The importance of the trade-off between fecundity and egg size in life history is apparent, not only because it represents different ways of partitioning a limited energy resource into offspring production, but also to understand the evolutionary aspects. If fitness is determined as the number of surviving offspring, the fecundity-egg size trade-off is affected by selective pressures through larval mortality, fertilization success, larval development time and survival (Wilbur *et al.*, 1974; Levitan, 1993, 1996; Hadfield and Strathmann, 1996; Podolsky and Strathmann, 1996).

In 1973, Vance proposed a theoretical model to predict optimal egg size with a bimodal distribution corresponding to type of development of the larvae. Considering a continuous reproductive effort, there is an important trade-off between fecundity and energy content per egg; subsequently the model proposed an apparent selection for extreme egg sizes with the production of many small eggs with minimal material or production of very few large yolky eggs. Sewell & Young (1999) performed a re-examination of asteroid and echinoid egg sizes and tested the prediction of bimodality in holothuroids and ophiuroids. Eggs diameters in asteroid species were found to range from 100 to 3500 μm, and the two modes are found in the ranges of 100 to 150 μm and 700 to 1000 μm. The ranges in egg diameter for planktotrophic and lecithotrophic asteroids do not overlap, but egg sizes overlap considerably between lecithotrophic and brooding species (Emlet *et al.*, 1987).

Levitan (1993) proposed a hypothesis to explain the evolution of egg size in marine invertebrates related to the probability of egg fertilization using a model of fertilization kinetics developed by Vogel (1982). The hypothesis proposes that conditions of sperm limitation can select for larger eggs. Consequently, variation in such conditions can contribute to the observed patterns of interspecific variation in egg size, concluding that larger eggs will be fertilized at a greater rate because they provide a larger target for sperm. Podolsky and Strathmann (1996) concluded that the results of Levitan (1993) led to incorrect inferences because he used an interspecific comparison in which other gamete attributes such as egg fertilizability, sperm speed, and sperm half-life co-vary with egg size. As an example they propose that greater zygote production of the echinoid *Strongylocentrotus droebachiensis* relative to its congeneric species results from interspecific differences in egg fertilizability and sperm half-life, not from larger egg size. Podolsky and Strathmann (1996) also

showed that species with internal fertilization and presumably high rates of fertilization display similar patterns of egg variation to those species with freely spawned gametes, thus they suggest that factors other than gamete encounter might be determinant for egg size evolution across different modes of reproduction.

McEdward and Morgan (2001) analyzed the relationship between size of eggs and the energy contained in them using published data for 47 species of echinoderms (Table 4.1). They found that among echinoderms, larger eggs contain more energy, suggesting a general pattern in which energy scales very nearly in direct proportion to the volume of the egg across a significant range of egg sizes, both within and among different modes of development. The only exception is among species with planktotrophic larval development, where there does not appear to be a clear scaling relationship. However, there were wide confidence intervals around the estimated regression parameters in all of the analyses performed by McEdward and Morgan (2001). In addition, in all cases the predictive power of the regression was poor, requiring large differences in egg size in order to produce significantly different predictions of energy content. Therefore they concluded that egg size is of limited value for the quantitative prediction of egg energy content and should be used with caution in life-history studies.

Natural selection is considered to drive the level of egg provisioning towards reproductive strategies with high fitness. If a single maximum is observed in the fitness curve, then selection will be expected to direct towards that best adaptation. However, if the fitness curve possesses another shape, for example curved upward (concave), then an adaptive valley between two optima might exist, and selection will be expected to be disruptive across that region.

Species	Volume	Energy	Dev	Class	Reference
Arbacia Punctulata	0.00022	0.00132	P	E	Strathmann & Vedder, 1977; Turner & Lawrence, 1979; George *et al.*, 1997
Arbacia lixula	0.00024	0.00281	P	E	George *et al.*, 1997;
Strogylocentrotus purpuratus	0.00027	0.00165	P	E	Strathmann & Vedder, 1977
Paracentrotus lividus	0.00041	0.00284	P	E	George *et al.*, 1997
Aspidodiadema jacobyi	0.00049	0.00295	P	E	George *et al.,* 1997
Lytechinus variegatus	0.00061	0.00528	P	E	Turner & Lawrence, 1979
Echinometra lucunter	0.00063	0.00224	P	E	George *et al.*, 1997
Stylocidaris lineata	0.00070	0.00317	P	E	George *et al.*, 1997
Coelopleurus floridanus	0.00080	0.00784	P	E	George *et al.*, 1997
Dendraster excentricus	0.00090	0.00328	P	E	Strathmann & Vedder, 1977
Asterias forbesi	0.00124	0.00796	P	A	Turner & Lawrence, 1979
Archaeopneustes histrix	0.00129	0.00654	P	E	George *et al.*, 1997
Strongylocentrotus franciscanus	0.00144	0.00577	P	E	Strathmann & Vedder, 1977
Pisaster ochraceus	0.00195	0.00783	P	A	Strathmann & Vedder, 1977
Strongylocentrotus droebachiensis	0.00206	0.01218	P	E	Strathmann & Vedder, 1977; Turner & Lawrence, 1979
Strongylocentrotus pallidus	0.00235	0.00904	P	E	Strathmann & Vedder, 1977
Luidia clathrata	0.00245	0.01986	P	A	Turner & Lawrence, 1979
Odontaster validus	0.00257	0.01955	P	A	Shilling & Manahan, 1994
Parastichopus californicus	0.00359	0.00951	P	H	Strathmann & Vedder, 1977
Encope aberrans	0.00359	0.00401	P	E	Herrera *et al.*, 1996
Encope michelini	0.00510	0.04639	P	E	George *et al.*, 1997
Florometra serratissima	0.00742	0.04555	L	C	McEdward *et al.*, 1988;
Clypeaster rosaceus	0.01149	0.02060	P/L	E	Emlet, 1986
Cucumaria miniata	0.06398	0.82539	L	H	McEdward & Chia, 1991
Acodontaster hodgsoni	0.08711	0.97715	L	A	Shilling & Manahan, 1994
Psolus chitinoides	0.09828	1.04957	L	H	McEdward & Chia, 1991
Echinaster sp. *1*	0.19912	2.88073	L	A	Turner & Lawrence, 1979
Echinaster sp. *2*	0.23916	4.69026	L	A	Turner & Lawrence, 1979
Solaster endeca	0.28510	3.55631	L	A	McEdward & Chia, 1991
Echinaster spinulosus	0.31000	3.51600	L	A	George *et al.*, 1997
Solaster dawsoni	0.37250	4.00467	L	A	McEdward & Chia, 1991
Solaster stimpsoni	0.40600	4.52640	L	A	McEdward & Carson, 1987
Mediaster aequalis	0.45990	5.78792	L	A	McEdward & Chia, 1991
Cucumaria curata	0.52360	4.58365	B	H	Turner & Rutherford, 1976
Pteraster tesselatus	0.87000	8.26919	L	A	McEdward & Coulter, 1987; McEdward & Chia, 1991
Peknaster fuscus	0.90478	3.1990	L	A	Shilling & Manahan, 1994
Pteraster militaris	0.90478	10.2000	L	A	McClary & Mladenov, 1990
Henricia leviuscula	1.01000	13.7157	L	A	McEdward & Chia, 1991
Abatus shakeltoni	1.09807	18.1273	B	E	McClintock & Pearse, 1986
Abatus cordatus	1.25983	16.4600	B	E	Lawrence *et al.*, 1984
Anasterias rupicola	1.34636	18.5340	B	A	Lawrence *et al.*, 1984
Anasterias perrieri	2.80616	39.3162	B	A	Lawrence *et al.*, 1984
Abatus nimrodi	4.00310	45.3448	B	E	McClintock & Pearse, 1986
Diplasterias brucei	11.4940	161.297	B	A	McClintock & Pearse, 1986
Notasterias armata	23.2278	143.019	B	A	McClintock & Pearse, 1986

Table 4.1. Egg volume (µl), egg energy content (J egg^{-1}), mode of development (P = planktotrophic; L = lecithotrophic; B = brooded lecithotrophic) and taxonomic class (A = Asteroidea; C = Crinoidea; E = Echinoidea; H = Holothuroidea) for 47 species of echinoderms. (Table taken from McEdward and Morgan, 2001).

Nearly all models of marine invertebrate reproductive patterns are characterized by having concave regions on the fitness curve, with the two major modes of planktonic larval development, planktotrophy and lecithotrophy always lying on opposite sides of the adaptive valley (McEdward and Miner, 2003). From this result, evolution between planktotrophy and lecithotrophy via natural selection would be predicted to be difficult or impossible, nevertheless phylogenetic analyses indicate that evolutionary transitions between these modes have occurred in a number of occasions (Wray, 1995; Rouse, 2000; McEdward and Miner, 2001).

McEdward and Miner (2003) examined the effect of fluctuating food availability on the duration of the planktonic larval period and the number of offspring that survive to metamorphosis in marine invertebrates with planktotrophic development, using fecundity-time models of reproductive strategies. The results showed that when food was abundant, smaller eggs were favoured and fluctuations in planktonic food concentrations affected small-egg strategies more strongly than large-egg strategies, though the variation in fitness was small in relation to fitness differences across egg sizes. Conversely, when food was strongly limited, larger eggs were favoured and fluctuations in planktonic food supply led to variations in fitness that were essentially related to fitness differences inherent in the strategies.

McEdward and Miner (2003) concluded that when the extent of environmentally-caused variations in reproductive success exceeds the differences in fitness among reproductive strategies, the adaptive landscape might be flattened, the intensity of disruptive or directional selection could be reduced and this should facilitate evolutionary transitions between planktotrophy and lecithotrophy or vice versa.

4.1.2- Body Size

The reproductive output (the amount of gametes produced), the reproductive index (the amount of gametes produced per unit body weight), and the reproductive effort (the amount of gametes produced per total amount of production) are all important features of the reproductive cycle of the species. The interspecific variation in body size results in interspecific variation in reproductive output and reproductive index.

Within a species, the relation between size and reproductive output is more direct. A certain minimal size of the body is required before an individual becomes reproductive. This may possibly be the body size that is necessary for the individual to be sufficiently functional in terms of feeding to support gonadal growth. The attainment of adult size occurs rapidly and results from allocating most of the acquired resources to growth rather than to reproduction. The trend seems to be to provide for an ever-increasing reproductive capacity until the adult size is reached.

A major pattern of echinoderms involves their ability to cease growth when extrinsic food limitation exists. For example, *Asterias rubens* can stay as small individuals for months in a "waiting stage" before resuming a growth phase that eventually leads to a size at which reproduction may occur (Nauen, 1978). Some populations of *Echinocardium cordatum* may persist for even years as small individuals without ever becoming reproductive, apparently as a consequence of a low acquisition of nutrients (Buchanan, 1967)

When extrinsic food limitation is less extreme, diverse species of echinoids, asteroids and holothurians do not reach their potential growth rate or maximum size but do reproduce (Lawrence and Lane, 1982). In this situation gonadal and somatic growth seem to have an opposite seasonal relationship. The apparent strategy here is

to redirect nutrients from growth to reproduction, although the size of the reproductive output is not the normal because of the small adult size (Lawrence, 1987).

With few exceptions, the reproductive mechanisms and patterns found in deep-water echinoderms are entirely similar to those found in shallow-water species. Therefore, all known deep-sea species with seasonal reproduction (although these are a few exceptions) produce a large number of small eggs, which are believed to give rise to planktotrophic larvae, and all known species of continuous breeders produce a small number of large eggs, which generate non-planktotrophic larvae (Tyler and Young, 1993). Body size, gonad structure and depth all covary with fecundity in echinoderms (Young, 2003).

The aim of this study is to examine the reproductive biology of the many deep-sea asteroid species found on the continental slope to the west of Europe in order to determine if the reproductive adaptations are a function of depth, distribution or are phylogenetically controlled.

4.2- Material and methods

4.2.1- Collection of data

Data on geographical distribution, maximum adult size, maximum egg size, fecundity and maximum Gonad Index (Table 4.2) were obtained from existing literature for 33 species of asteroids from the Porcupine Abyssal Plain and Porcupine Seabight (North-East Atlantic Ocean) (Fig. 4.1). Depth related distribution of the species was obtained form the work published by Howell et al., (2002). Data on the fecundity, egg size, and G.I. were not available for a number of species from the scientific literature because no studies have been carried out on them. In these cases the reproductive

| Species	Depth of max. abund. (Howell *et al.*) (m)	Depth range (Clark & Downey 1992) (m)	Geographical range (Clark & Downey 1992)	Max adult. Size (mm)	Max. egg size (µm)	Fecundity (Eggs/ind.)	Max. G.I.	References
Stichastrella rosea	150 - 250	4-200	N A	R up to 150	199	1281442	6.1	This study
Astropecten irregularis	350 - 450	10 - 1000	N A	R= 60	110	High	4	Grant & Tyler, 1986
Luidia sarsi	650 - 750	9 - 1300	N A, Med.	R up to 130	64.4	Very high	14.31	This study
Pontaster tenuispinus	650 - 750	around 400 m	NA	R up to 130	800	Low	1.95	Pain *et al.*, 1982
Psilaster andromeda	850 - 950	70 - 1500	NA	R up to 100	950	Low	4.78	Tyler & Pain, 1982
Pseudarchaster parelli	950 - 1050	75 - 2300	NA	R= 48	900	Low	4.8	Tyler & Pain, 1982
Cheiraster sepitus	770 - 1500	485-3700	NA	R up to 65	733	1730	2.11	This study
Brisingella coronata	950 - 1050	100 - 2600	NA, Med.	Diam. 30	1250	60000	2.2	Tyler *et al.*, 1984
Henricia abyssicola	1050 - 1150	1015 - 1380	NA	R up to 48	970	7400	5.58	This study
Poraniomorpha hispida rosea	1250 - 1350	250 - c.1500	NA	R up to 45	546	50986	2.19	This study
Pteraster spp	980 – 4817	1371 – 1774*	NA, SA*	R= 85*	1444*	1446*	2.57*	This study
Zoroaster fulgens	1450 - 1550	220 - 3000	AO	R= 150	950	11000	2.31	Tyler *et al.*, 1984
Plutonaster bifrons	1650 - 1750	630 - 2965	AO, SP	R up to 110	120	1000000	2.27	Tyler & Pain, 1982
Plinthaster dentatus	1650 - 1750	229 - 2910	NA, SA	R= 45	1232	1585	3.15	This study
Persephonaster patagiatus	1650 - 1750	730 - 2000	NA	R up to 105	900	Low	4.19	This study
Solaster sp.	1460 – 1694	0 -599**	NA, SP**	R= 200**	1000**	Low**	3.59**	Gemmill, 1912
Brisinga endecacnemos	1650 - 1750	183 - 2220	NA	Diam 24	1250	60000	5.59	Tyler *et al.*, 1984
Myxaster perrieri	1431 - 2110	around 1300	NEA	R= 75	1507	1984	7.99	This study
Bathybiaster vexillifer	2250 - 2350	1800 - 2000	NA	R up to 140	660	Low	4.78	Tyler *et al.*, 1982
Benthopecten simplex	2250 - 2350	1175 - 3000	NA, SA, SP	R up to 150	950	Low	8.61	Pain *et al.*, 1982
Pectinaster filholi	2250 - 2350	1260 - 4850	NA, SA	R up to 85	850	Low	4.95	Pain *et al.*, 1982
Hymenaster membranaceus	2250 - 2650	128-3239	NEA	R 30 - 45	1100	Low	10.61	Pain *et al.*, 1982
Paragonaster subtilis	2950 - 3050	1845 - 4700	NA, SA	R= 66	900	Low	2.74	Tyler & Pain, 1982
Porcellanaster ceruleus	2950 - 3050	1160 - 6040	Alm. cosmopolitan	R up to 36	600	2800	4.74	Madsen, 1961; This study
Freyella elegans	3850 - 3950	1600 - 4500	NA, SA	Diam. 24	1644	518	4.8	This study
Thoracaster cylindratus	4050 - 4150	2540 - 5990	NA,SA,NP,SP	R up to 103	1091	26881	4.93	This study
Dytaster grandis grandis	4550 - 4650	1000 - 4000	NA	R up to 155	120	1000000	4.25	Tyler *et al.*, 1990
Freyastera benthophila	4550 - 4650	4700 - 5000	NA, SP, IO	Diam. 24	1182	439	3.17	This study
Styracaster chuni	4550 - 4650	2500 - 4800	NA, SA, SP	R up to 64	650	10000	6.4	Ramirez-Llodra *et al.*, 2002
Pythonaster atlantidis	4750 - 4850	3200	NA	R= 165	1561	888	13.78	This study
Styracaster horridus	4750 - 4850	4040 - 5600	NA, SA, SP, IO	R up to 146	650	11143	5.8	Ramirez-Llodra *et al.*, 2002
Styracaster elongatus	4750 - 4850	3310 - 6600	NA, SA, IO	R up to 100	470	16000	6.38	This study
Hyphalaster inermis	4750 - 4850	2280 - 5430	NA, SA, SP, IO	R up to 85	650	9563	4.9	Ramirez-Llodra *et al.*, 2002

Table 4.2. Asteroids used in this study and their reproductive features. Keys: *AO* Atlantic Ocean; *IO* Indian Ocean; *Med* Mediterranean; *NA* North Atlantic; *SA* South Atlantic; *SP* South Pacific. (*specimens collected at 1000 -1500 m identified as *Pteraster personatus*. ** specimens collected at 1400 – 1600 m identified as *S. endeca*)

traits were obtained in the laboratory from fixed specimens archived in the *Discovery Collections* at the National Oceanography Centre, Southampton (NOC). This material was collected at depths from 150 to 4850 m. The samples were obtained over a period of 20 years as part of different programmes. The samples were collected using a semi-balloon otter trawl (OTSB14) and a Granton trawl.

The specimens were first fixed in 10% formalin, and transferred to 70% alcohol for storage.
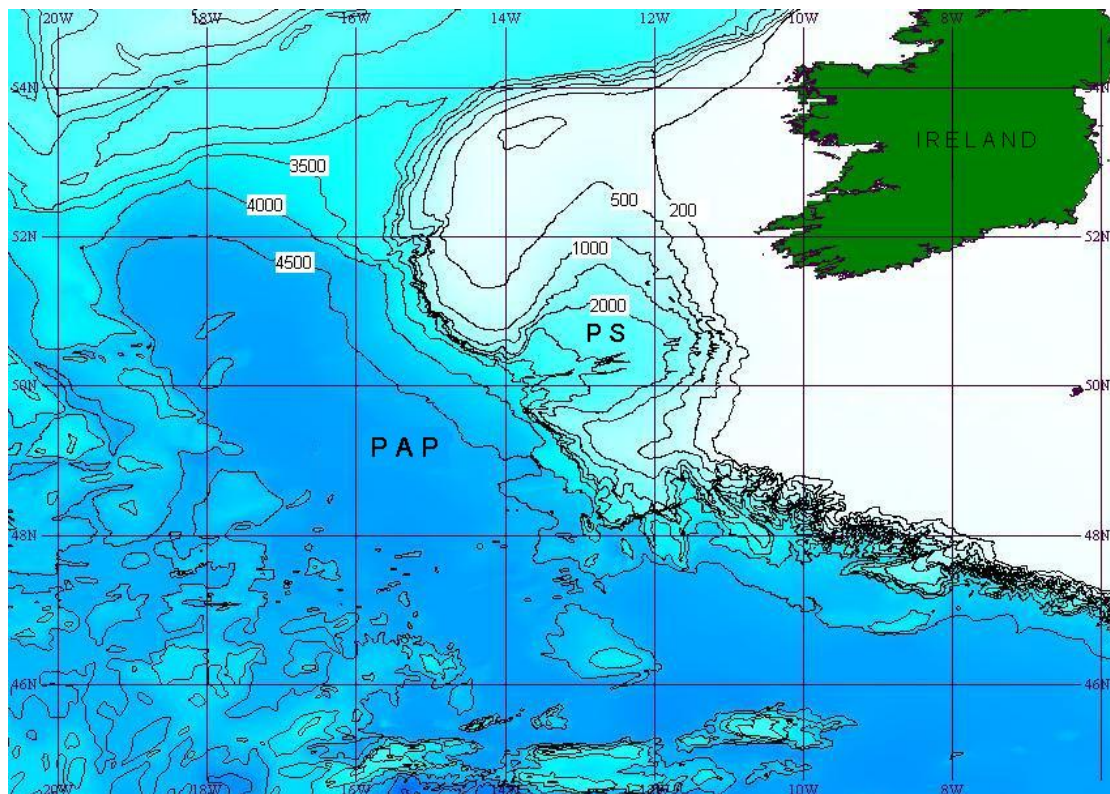
Fig. 4.1. Bathymetric chart of the Porcupine Abyssal Plain (PAP) and Porcupine Seabight (PS), NE Atlantic (Image modified from GEBCO 97).

The gonads of 5-10 specimens per sample were dissected out by opening the oral side of the disc along each radius, previously the whole animals had been damp-dried and weighed. The major radius (R), from the centre of the disc to the tip of the

arm, and the minor radius (r), from the centre of the disc to the centre of the interradial edge, were measured for each specimen to the nearest 0.05 mm.

The gonads of five arms were dissected out from each specimen and damp-dried and weighed separately.

4.2.2- Histology

At first, the ovary volumes were measured to the nearest 0.001 ml by the displacement of fluid method, using a variation of the hydrostatic balance of Mohr-Westphal (Scherle, 1970).

The gonads were dehydrated first, by a whole night submersion in 90 % propan-2-ol and then three, two hours submersions in 100 % propan-2-ol. After that they were cleared by leaving them in Xylene for 24 hours to give the tissues a transparent appearance. Tissue was then embedded by being left for 24 hours in molten histology wax at 70° C. After that the gonads were allocated into moulds and covered with wax in order to create a wax block.Finally the wax blocks were sliced at 7μm sections using a microtome and then stained with haematoxylin and eosin (Figs. 4.2, 4.3).

4. 2.3- Image analysis and estimation of fecundity

At least 100 oocytes sectioned through the nucleus were measured from the histological slides, using an Olympus BH2 compound microscope with camera attachment. Images were captured using Matrox Rainbow Runner and analyzed using SigmaScan Pro version 4 to calculate oocyte diameters ('Feret' diameter, the area if the occyte was a perfect circle, was used). Both stages of oocytes (previtellogenic oocytes and vitellogenic oocytes) were counted for fecundity estimates.

Fecundity is quantified as the number of vitellogenic oocytes per female (actual fecundity) from the mean volume of oocytes and the volume of the ovary in each female. Oocyte volume (OV) was calculated assuming a spherical shape [OV= (4·p·R3)/3] and averaged. The gonadal fluid that solidifies during fixation occupies approximately 15% of the ovary as it was estimated by Ramirez-Llodra *et al.* (2002) for Porcellanasterid asteroids and corroborated in this study with species from the families Asteridae, Astropectinidae, Brisingidae, Echinasteridae, Goniasteridae, and Poranidae. This percentage was subtracted from the total gonad volume.

Fecundity was estimated as follows: V_g = volume of the gonad

V_{pvo} = mean volume of a previtellogenic oocyte

V_{vo} = mean volume of a vitellogenic oocyte

N_{pvo}= previtellogenic oocytes counted in a subsample of 100 oocytes per gonad

N_{vo}= vitellogenic oocytes counted in a subsample of 100 oocytes per gonad

P = ratio between previtellogenic and vitellogenic oocytes): $P = N_{pvo} / N_{vo}$

Assuming that P in a subsample of the gonad is the same as P in the whole ovary, then

$$P = N_{pvo} / N_{vo} = F_{pvo} / F_{vo}; \quad F_{pvo} = P \times F_{vo} \qquad (1)$$

Where F_{pvo} and F_{vo} are the total number of previtellogenic oocytes and vitellogenic oocytes, respectively.

The volume of a gonad is equivalent to the number of oocytes multiplied by their volume:

$$V_g = (V_{vo} \times F_{vo}) + (V_{pvo} \times F_{pvo}) \qquad (2)$$

Replacing F_{pvo} in Eq. 2 with its definition from Eq. 1 we get:

$$V_g = (V_{vo} \times F_{vo}) + (V_{pvo} \times (P \times F_{vo}))$$

$$V_g = F_{vo} \times (V_{vo} + (V_{pvo} \times P))$$

$$F_{vo} = V_g / V_{vo} + (V_{pvo} \times P) \qquad (3)$$

From Eqs.1 and 3 we obtain the estimation for the total number of oocytes in the ovary, or **_potential fecundity_** F:

$$F = F_{pvo} + F_{vo} \qquad (4)$$
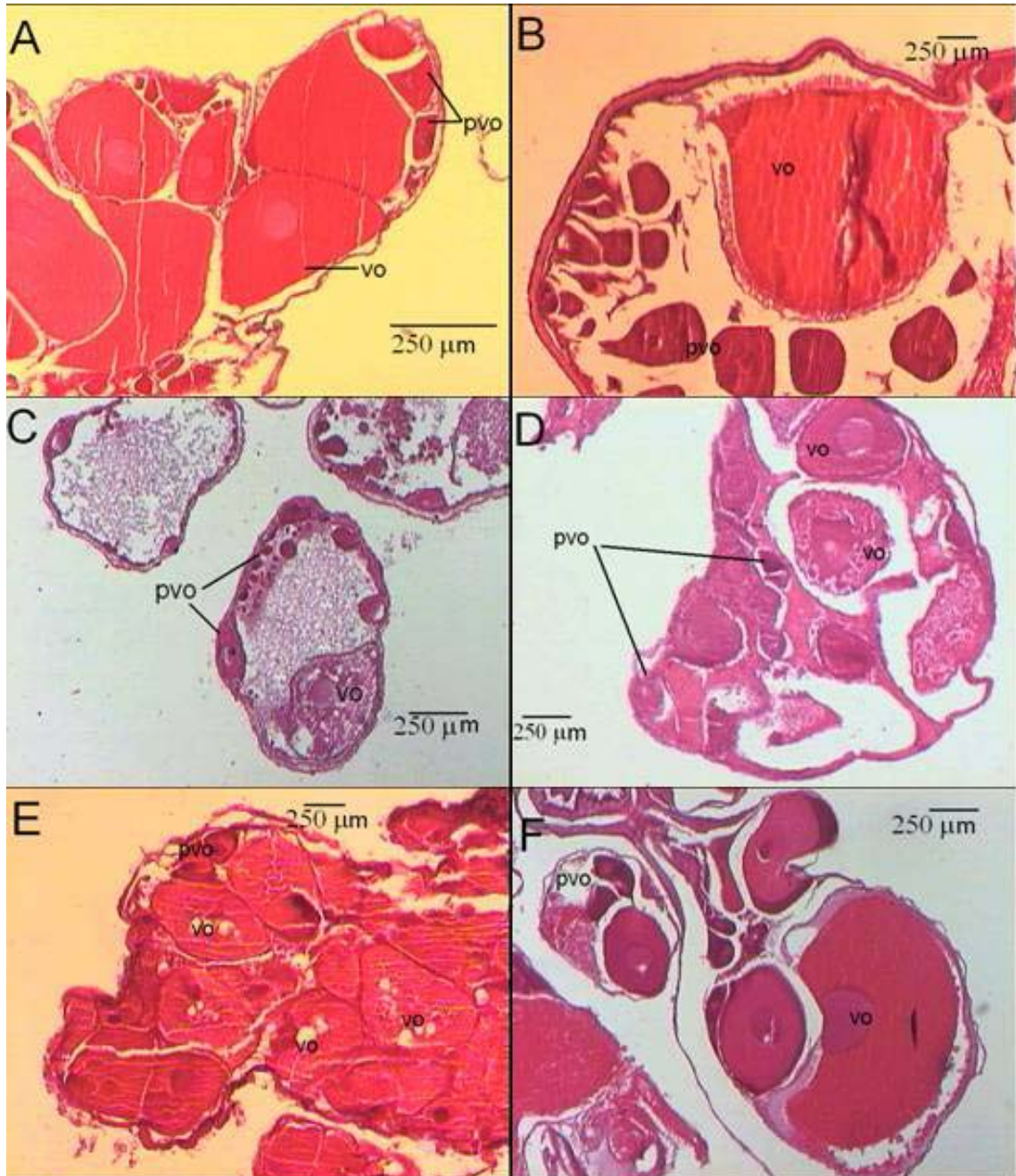
Fig. 4.2. Light histology of gonads of North Atlantic starfish stained with Haematoxylin and Eosin. **A** *Cheiraster sepitus*. **B**. *Freyella elegans*. **C** *Plinthaster dentatus*. **D** *Porcellanaster ceruleus*. **E** *Poraniomorpha hispida rosea*. **F** *Pteraster*

personatus. Abbreviations: pvo, previtellogenic oocyte; vo, vitellogenic oocyte (Micrographs by Francisco Benitez).
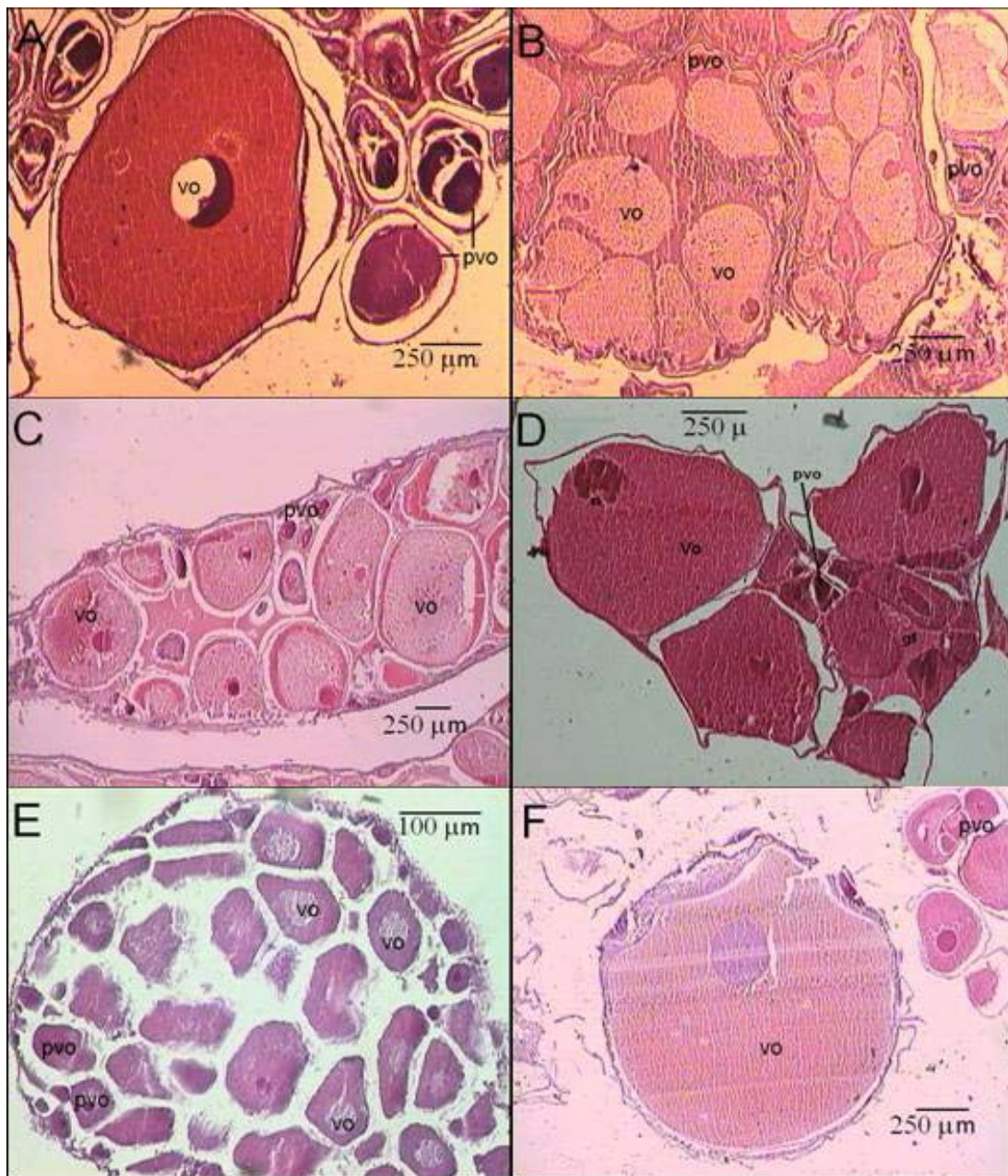
Fig. 4.3. Light histology of gonads of North Atlantic starfish stained with Haematoxylin and Eosin. **A** *Pythonaster atlantidis*. **B**. *Styracaster elongatus*. **C** *Thoracaster cylindratus*. **D** *Henricia abyssicola*. **E** *Luidia sarsi*. **F** *Myxaster perrieri*. Abbreviations: pvo, previtellogenic oocyte; vo, vitellogenic oocyte. (Micrographs by Francisco Benitez).

4.2.4- Statistical analysis

Data on maximum body size, maximum egg size and maximum Gonad Index, were plotted against depth of maximum abundance for all the species, and the resulting graphs were investigated in order to find possible patterns or relations between the reproductive features. The degree to which the variables were related was tested by using the Spearman Rank

Correlation Coefficient (Rs) and the results discussed (Fowler et al., 2000). A non-parametric test was preferred because the data were presented as actual observations, observations converted to ranks and indices; furthermore a parametric test would require the data to be normally distributed and to have homogeneous variances (Fowler et al., 2000).

Although the relatively low number of data would suggest that the data set is not suitable for a multivariate analysis, this analysis was performed with the PRIMER (Plymouth Routines in Multivariate Ecological Research) Version 5 programme (Clarke and Warwick, 2001) to investigate if a pattern could be observed.

Data on adult size, egg size and Gonad Index were analyzed together by the Normalized Euclidean distance measure applied to all the asteroid species. Hierarchical clustering with group-averaged linking and non-metric multi-dimensional scaling using all resulting similarity matrices was performed (Clarke and Warwick, 2001). The groupings identified by both cluster analysis and the MDS plots were investigated using the available data on the species regarding reproductive features determined in this study and depth related distribution in the NE Atlantic established by Howell *et al.* 2002.

4.3- Results

The Spearman's correlation coefficient showed that no correlation exists between depth (over the entire range) and body size (as R) of the species (Rs= 0.056,

df= 31, P>0.05). However, the plot graph indicates that in waters shallower than 1000 m, generally species with large body sizes are found, from 1000 to 3000 m species of all body sizes occur and at depths greater than 3000 m only species with large body size are found (Fig. 4.4).

The correlation between depth and egg size was proved to be not statistically significant (Spearman's Rs= 0.136, *df*= 31, P>0.05) (Fig. 4.5).

The Spearman's correlation coefficient showed that no significant correlation exists between depth and Gonad Index (Rs= 0.297, *df*= 31, P>0.05). Therefore, these reproductive features of the asteroids do not have a direct positive or negative variation in relation to depth (Fig. 4.6).

The results of both Hierarchical cluster analysis and MDS showed species clustering in three groups, according to maximum adult size, maximum egg size and maximum Gonad Index (Figs. 4.7, 4.8).

The first group which was labelled as A contains 10 species which have small adult size. Their major radius (R) ranges from 24 to 85 mm in length, the egg size is large with diameters ranging from 546 to 1444 μm and low Gonad Index, which values range from 2.11 to 5.59.
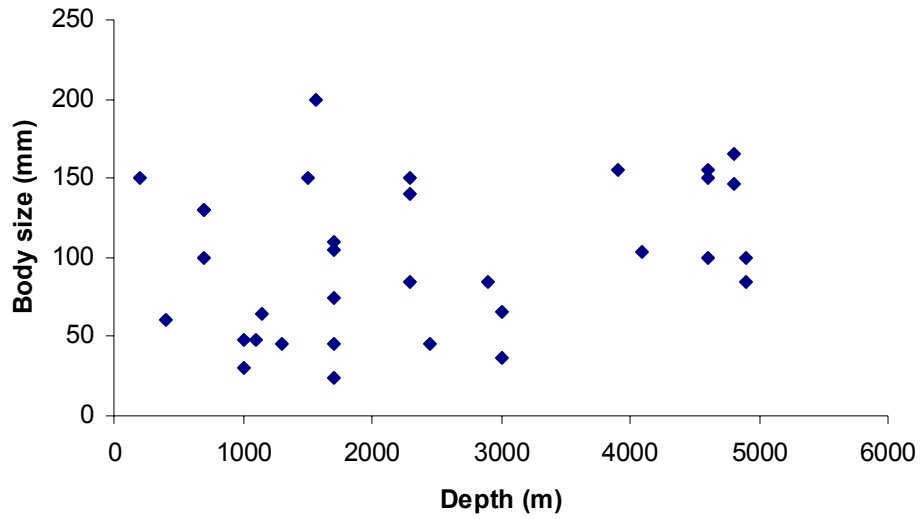
Fig. 4.4. Body size of the asteroid community in PSB and PAP plotted against depth.
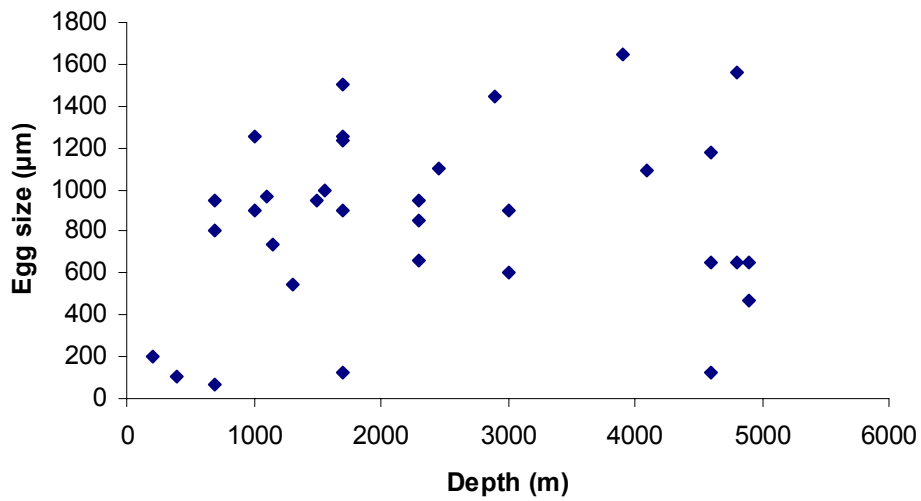
Fig. 4.5. Egg size of the asteroid community in PSB and PAP plotted against depth.
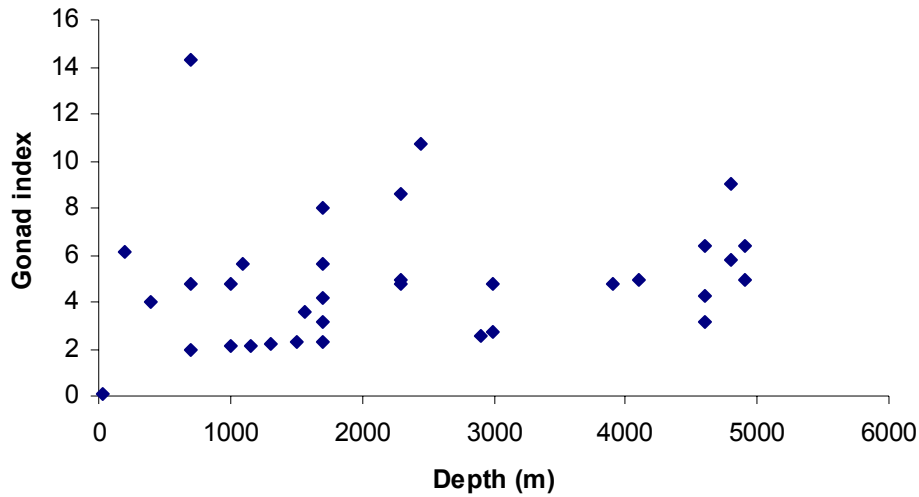
Fig. 4.6. Gonad Index of the asteroid community in PSB and PAP plotted against depth.

In this cluster is found one species from the family Porcellanasteridae (*Porcellanaster ceruleus* Wyville Thomson), one species from the family Benthopectinidae (*Cheiraster sepitus* Verrill) and species from the genus *Pteraster*. All the members of the family Goniasteridae analyzed in this study were clustered in this group, the same result was observed in the families Poranidae, Brisingidae and Echinasteridae.
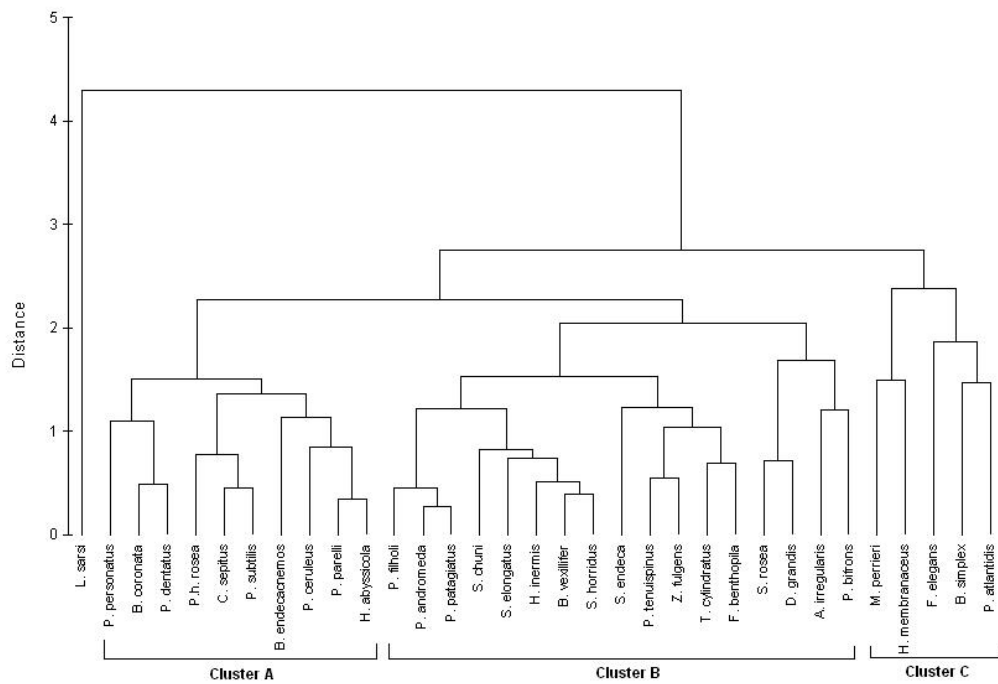
Fig. 4.7. Hierarchical cluster analysis of the data on adult size, egg size and Gonad Index, based on the Normalized Euclidean distance measure.

Group B contains 17 species, which present large adult size (major radius 100-200 mm), large egg size (diameters from 470 to 1182 μm), and low Gonad Index (3.17 to 6.4).

Four species with small egg size were included in this cluster. This occurred because the analysis considered them similar to the other species in having a large adult size and low Gonad Index; however it is evident that two of these species: *Stichastrella rosea* (O.F. Müller) and *Astropecten irregularis* (Pennant) clearly belong to a group of shallow-water species with small egg size, high fecundity and planktotrophic development together with *Luidia sarsi* Düben and Koren, which was separated by the analysis as an outlier because of its very high Gonad Index. These three species are found in the upper slope zone at 150-700 m, as defined by Howell *et al.* (2002).
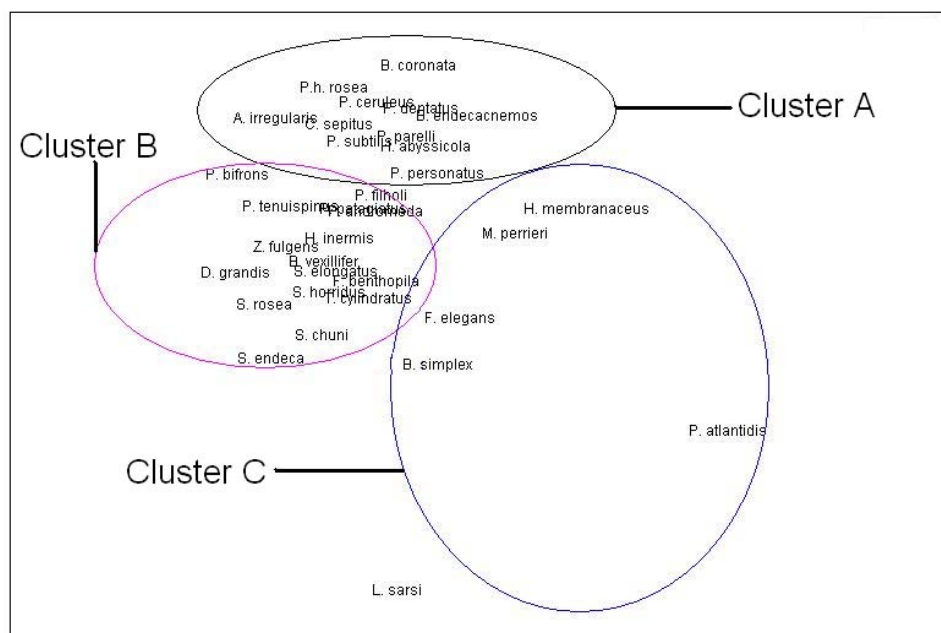
Fig. 4.8. Non-metric multi-dimensional scaling plots for data based on the Bray-Curtis similarity matrix.

Cluster B includes two members of the family Benthopectinidae (*Pontaster tenuispinus* (Düben and Koren) and *Pectinaster filholi* Perrier) and all the species from the families Astropectinidae, Solasteridae, Zoroasteridae, Asteridae and Freyellidae. In general all the members of the family Porcellanasteriade were clustered in this group apart from *Porcellanaster ceruleus* Wyville Thomson, which was considered different from the other species in being, uniquely, a very small-sized species.

Cluster C includes four species which have small adult size with the length of the major radius ranging from 75 to 165 mm, a very large egg size with diameters ranging from 950 to 1644 μm, and High Gonad Index, which values range from 7.9 to 10.6. This cluster includes one species from the family Benthopectinidae (*Benthopecten simplex* (Perrier)), and one species from the family Pterasteridae

(*Hymenaster membranaceus* Thomson). The two species of the family Myxasteridae analyzed in this study were grouped in the cluster B.

After comparing the depth distribution of the species with the results obtained by the cluster analysis, a possible pattern of zonation and reproductive features was found considering the adult size, egg size, fecundity and Gonad Index as follows:

a)   A first zone is found, which coincides with the zonation established by Howell *et al.* (2002). In this first zone situated in the upper slope from 150-700 m depth, large species with shallow-water distribution, small egg size, high fecundity and planktotrophic development are found.

b)   Only two species with large adult size, low G.I., large egg size and low fecundity indicating lecithotrophic development are found from around 800 m depth.

c)   From 700 to 1100 m depth only species having small adult size, large egg size with low fecundity and low G.I. are found, the totality of this species are included in the cluster B recognized by the cluster analysis, this zone coincides with the mid bathyal zone proposed by Howell *et al.* (2002).

d)   A zone of mixed reproductive features on the species was found from ~1100 to ~3000 m. In this zone there are species belonging to the three clusters found in the cluster analysis. Therefore, there are species having large adult size, low G.I., large egg size and low fecundity, there are also species with small adult size, low G.I, large egg size and low fecundity. Finally in this zone are also found species which have large adult size, large egg size, low fecundity, and low Gonad Index. This pattern appears to support the existence of a zone of transition from 1700-3300 m proposed by Howell *et al.* (2002) consisting in part of bathyal species whose ranges extend down into this zone and in part from abyssal species whose ranges extend up into this zone, with few species confined to the transition zone.

e)   In the abyssal zone below 3000 m, species belonging to the cluster B, with large adult size, large egg size, low fecundity, and low Gonad Index are found. *Pythonaster atlantidis* A.H. Clark is the only species included in the group C by the analysis and located in this zone; however this separation seems to be unreliable since this species was considered in group C by the analysis only because of its high G.I., apart from that feature it possesses the characteristic features of the other species in the group B. The only species with small egg size, high fecundity and apparent planktotrophic development grouped in the cluster B is *Dytaster grandis* (Verrill). Coincidentally this species differs from the other as its feeding habits are basically predation and scavenging (Gage *et al.*, 1983 a,b; Tyler *et al.,* 1990).

4.4- Discussion

4.4.1. Body size, fecundity and egg size

One of the major generalizations in population biology is that, within a species, larger body size confers greater reproductive success (Williams, 1975). This generalization is based in part, on evidence indicating that body size and gamete production are directly proportional (Paris and Pitelka, 1962; Rinkevich and Loya, 1979; Suchanek, 1981).

Levitan (1991) discussed that estimating fertilization success, or more generally reproductive success, from gamete production alone can be inappropriate and misleading because while gamete production is correlated positively with body size, individual fertilization success may be a function of population density (Mortensen, 1938; Thorson, 1946; Denny and Shibata, 1989; Strathmann, 1990). Thus, there may be an important trade-off between (a) large size with high individual

gamete production at low population density, and (b) smaller size with lower gamete production at higher population density. The results obtained by Levitan (1991) suggest that influence of population density on fertilization success should be recognized and incorporated into an assessment of the reproductive potential of an organism. These essentially indicate that the influence of body size on fertilization success and reproductive output in shallow-water invertebrates is not important. If a gain in fertilization success balances the cost of reduced gamete production, then small-sized organisms living at high population density may be just as fecund as large organisms living a low population density. However, the converse is true to species living in the deep sea where the low population densities would increase the importance of body size.

The most recent models of life-history theory explain the evolution of patterns of covariation in reproductive traits based on demographic parameters and in relation to variable environmental conditions (Ramirez-Llodra, 2002). The Winemiller-Rose Model is based on a modification and extension of the two-dimensional r-K model (McArthur and Wilson, 1967; Pianka, 1970) by incorporating three major demographic components of fitness (fecundity, survivorship of juveniles and age at maturity). The model implies that trade-offs among life-history traits are based on physiological and ecological constraints that ultimately select for genetic correlations.

In the Winemiller-Rose Model, the r strategy is divided into periodic and opportunistic strategists. The periodic strategists similar to the classical r strategists have high fecundity and low juvenile survivorship. They differ, however, in that they are large, long-lived and have late maturation (Ramirez-Llodra, 2002). The periodic strategy maximizes age-specific fecundity at the expense of turnover time and

juvenile survivorship (small eggs). They inhabit predictable and seasonal environments (Winemiller and Rose, 1992; McCann and Shuter, 1997).

The opportunistic life-history strategy shows most of the classical traits of the *r* strategy, with small body size, early maturation, low juvenile survivorship and short lifespan, although it differs in that fecundity per spawning event is low. The opportunistic strategists maximize population growth through a reduction in the mean time of generation (Ramirez-Llodra, 2002). Early maturation results in a short lifespan and high population yield but diminishes their capability to produce a large quantity of offspring per event and large eggs. However, because of their multiple spawning frequencies, annual fecundity is high and allows for colonization of new habitats. These organisms inhabit highly disturbed and unpredictable environments (Winemiller and Rose, 1992; McCann and Shuter, 1997).

In the Winemiller-Rose model, *K* strategy is redefined as the equilibrium strategy and its boundaries are constrained. The equilibrium strategists have moderate age at maturity, low fecundity and high juvenile survivorship determined by a large egg size. They differ from the classical K strategists in that they have small to medium body sizes (Ramirez-Llodra, 2002). The species that evolve in the equilibrium strategy maximize juvenile survivorship at the expense of fecundity. They inhabit constant environments ((Winemiller and Rose, 1992; McCann and Shuter, 1997).

The production of eggs is a high energy-demanding process. Therefore, the accessibility of food plays an essential role before and during the process of egg production (Eckelbarger, 1986; Chia and Walker, 1991). The process of vitellogenesis consumes a large proportion of the energy intake of an organism, and this requirement

varies depending on the vitellogenic pathway used by the species (Ramirez-Llodra, 2002).

Long-lived species with slow egg production have vitellogenic strategies that are consistent with a continuous or predictable food supply and a relatively stable environment, such as temperate latitude habitats, the Antarctic benthos and abyssal plains (Clarke, 1979; Gage and Tyler, 1991; Eckelbarger, 1994; Eckelbarger and Watling, 1995). In contrast, unstable environments or unpredictable food supply, such as large food falls in the deep sea, boundaries between water masses and sites of vigorous hydrodynamic activity, or ephemeral hydrothermal vents, would select for opportunistic strategies with fast egg production capabilities (Eckelbarger, 1994). Although the process of oogenesis in any species is phylogenetically constrained by ovary morphology, 1) vitellogenic pathways inherent in a species, 2) the digestive structures related to the transfer of nutrients from the somatic organs to the ovaries, 3) fecundity and 4) the quality of offspring, are directly related to the nutritional state of the adult and its resource allocation.

There have been a number of experiments and studies where it has been shown that higher food availability or higher food quality enhances the production of more and/or higher quality eggs. Similarly, a prolonged period of low food availability or quality can reduce fecundity or even stop the production of eggs in species of amphipods (Cruz-Riviera and Hay, 2000), copepods (Razouls et al., 1991; Jónasdóttir, 1994; Williams and Jones, 1999), caridean shrimps (Gorny et al., 1992, Ramirez-Llodra, 2000), polychaetes (Levin and Creed, 1986; Zajaz, 1986; Levin et al., 1987; Grémare et al., 1988; Quian and Chia, 1991, 1992, 1994; Levin and Bridges, 1994; Prevedelli and Vandini, 1998, 1999; Linton and Taghan, 2000; Prevedelli and Simonini, 2000), marine bivalves (Bayne and Worral, 1980; Kautsky, 1982; Bayne et

al., 1983; MacDonald and Thopmson, 1985a, b; Barber et al., 1988; Paulet and Boucher, 1991; Honkoop and van der Meer, 1997), gastropods (Spight and Emlen, 1976; Chester, 1996; Cheung and Lam, 1999;), opisthobranchs (Krug, 1998), echinoids (Vadas, 1977; Meidel and Scheibling, 1998; Beddingfield and McClintok, 1998; Bertram and Strathmann, 1998; Brewin et al., 2000), Antarctic and temperate echinoderms (Shilling and Manahan, 1994), holothurians (Wigham *et al.,* 2003) and asteroids (George et al., 1990; George, 1994; Bosch and Slattery, 1999; Ramirez-Llodra et al., 2002).

4.4.2. Depth allocation of the species and possible causes.

Howell *et al*. (2002) found distinct changes in the vertical distribution of the asteroid fauna in the Porcupine Seabight and Porcupine Abyssal Plain. The present study also found differences in the reproductive traits of the species in correspondence to the zones proposed by Howell *et al*. (2002) and taking into account the effect of the environmental factors in selecting the species with successful life-history strategies, as well as the phylogenetic constrains inherent of the species.

Predator species are more abundant at shallower depths and become scarce with increasing depth (Carey, 1972; Howell *et al*., 2002). Thus the upper slope zone ranging from the shelf break to ~700 m is characterized by large seasonally reproducing predators. They produce small eggs correlated with a large population size, which increases the chances of successful fertilization. It is not necessary to invest energy in producing large eggs because the small eggs produce planktotrophic larvae which feed directly on primary production from surface waters. This upper continental slope zone is characterized by asteroid species which exhibit the life-history periodic pattern established by Winemiller and Rose (1992), of large body

size, high fecundity, small egg size, and semelparity. Nevertheless, other variable demographic features such as lifespan, and age at maturity were difficult to determine because these features have not been documented. The life-history periodic strategy is distinctive of species inhabiting predictable and seasonal environments (Winemiller and Rose, 1992; McCann and Shuter, 1997).

Two large species (*Pontaster tenuispinus* Düben and Koren and *Psilaster andromeda* (Verrill)) with large eggs, low fecundity and low G.I. were found at ~ 650-850 m depth. The reasons why species with these features are found at this specific depth are still unknown and further studies are required in order to understand the factors that determine their distribution. The water mass structure at this depth has been defined by Rice et al. (1991), Van Weering et al. (1998) and New and Smythe-Wright (2001) as the boundary between Eastern North Atlantic Water (ENAW) and Mediterranean Overflow Water (MOW) at ~800 m. It is possible that the reproductive features of these species are influenced in a specific way by the hydrodynamic characteristics of this particular zone, as sharp density differences are known to focus internal wave energy on the continental slope.

From 700 to 1100 m depth only species with small adult size, large egg size with low fecundity and low Gonad Index were found. Howell et al. (2002) defined this zone as the upper bathyal zone, and it is characterized by a rapid succession of species with species displaying narrow total adult bathymetric ranges. The 1100 m boundary of this zone was associated with changes in currents. The main currents around the Porcupine Seabight flow anticlockwise as part of the European Slope Current, which flows northwards (McMahon et al., 1995; New and Smythe-Wright, 2001) (Fig. 6). Above 1000 m the European Slope Current is thought to have seasonal variations in bathymetric range, mean speed, and direction, showing a complete

reversal in flow during spring. Local variability in current speed is also found throughout the depth range of the upper bathyal zone in certain areas of the Porcupine Seabight (Dickson and McCave, 1986; Pingree and LeCann, 1989; Pingree *et al.,* 1999).

Flach et al. (1998) proposed that the variability in the currents certainly have an effect on sediment transport, food supply and larval dispersal and it may affect the zonation of asteroids, particularly suspension-feeding species. The six asteroid species found in this zone and clustered in the same group by the analysis exhibit similar feeding habits, direct or indirectly related to the availability of suspended material. *Brisingella coronata* (G.O. Sars) and *Henricia abyssicola* (Müller and Troschel) are believed to feed on suspended particulated matter (Pawson, 1976; Rowe and Staresinic, 1979; Jangoux, 1982), *Poraniomorpha hispida rosea* Danielssen and Koren basically feed on sessile organisms, such as sponges, hydroids and entoprocts (Clark and Downey, 1994). *Cheiraster sepitus* (Verrill) probably exhibits the characteristic feeding habits of the family Benthopectinidae, which seems to be omnivorous including predation on minute organisms and detritus (Clark and Downey, 1994), and the genus *Pteraster* is likely to feed on sponges as described by Clark and Downey (1994). Many other suspension feeding species have been found to be abundant at around 1100 m (Flach *et al*., 1998), including hexactinellid sponges. Rice *et al*., 1990 proposed that sponge aggregations take place close to regions of the upper continental slope where the bottom topography enhances the tidal current velocities of the near bottom. Thus the asteroid species inhabiting this zone are basically suspension feeders or small predators, which take advantage of the large aggregations of sessile organisms specially sponges. The asteroid species inhabiting this zone exhibit reproductive traits with a high tendency towards the opportunistic

strategy described by Winemiller and Rose (1992) and appear to be related to environmental and population conditions. They possess small body size and low fecundity per spawning event, and aseasonal reproduction with probably multiple spawning frequency, although their large egg size probably follows the general trend in cold waters in order to provide the larvae with energy sufficient for a high survival probability. The opportunistic strategists are distinctive of highly disturbed and unpredictable environments (Winemiller and Rose, 1992; McCann and Shuter, 1997).

The zone between 1100 and 3000 m coincides with the transitional zone proposed by Howell et al., (2002) where boundaries at 2500, 2800 and 3300 m are thought to demonstrate a region of transition between bathyal and abyssal fauna. This region of pronounced faunal change occurs at ~2500 m where bathyal fauna meet, and overlap with, abyssal fauna.

Previous studies have found this overlap in other megafaunal taxa (Vinogradova et al., 1959, Hansen, 1975; Billett, 1991). Vinogradova et al. (1959) claimed this boundary is a well-defined intermediate region at a depth of 2500-3500 m, and they noted the disappearance of large numbers of species at around 3300 m, as well as entire taxonomic groups, to be replaced by a large number of new species, genera and families pertaining only to ocean depths greater than 3000 m.

Within an evolutionary context, bathyal species are likely to have a mixed origin, with some species from the shelf and some originating from abyssal depths (Rogers, 2000). Data for the temperature and pressure effects on the later stages of development of the shallow water asteroids *Asterias rubens* Linnaeus and *Marthasterias glacialis* (Linnaeus) (Chapter 3, section 3.3.2 this thesis) show that all the developmental stages have a potentially wider depth distribution than their respective adults. Therefore, the larvae of shallow water species could survive

transport to deeper waters and some of these species might have been capable of sending colonists to the deep sea.
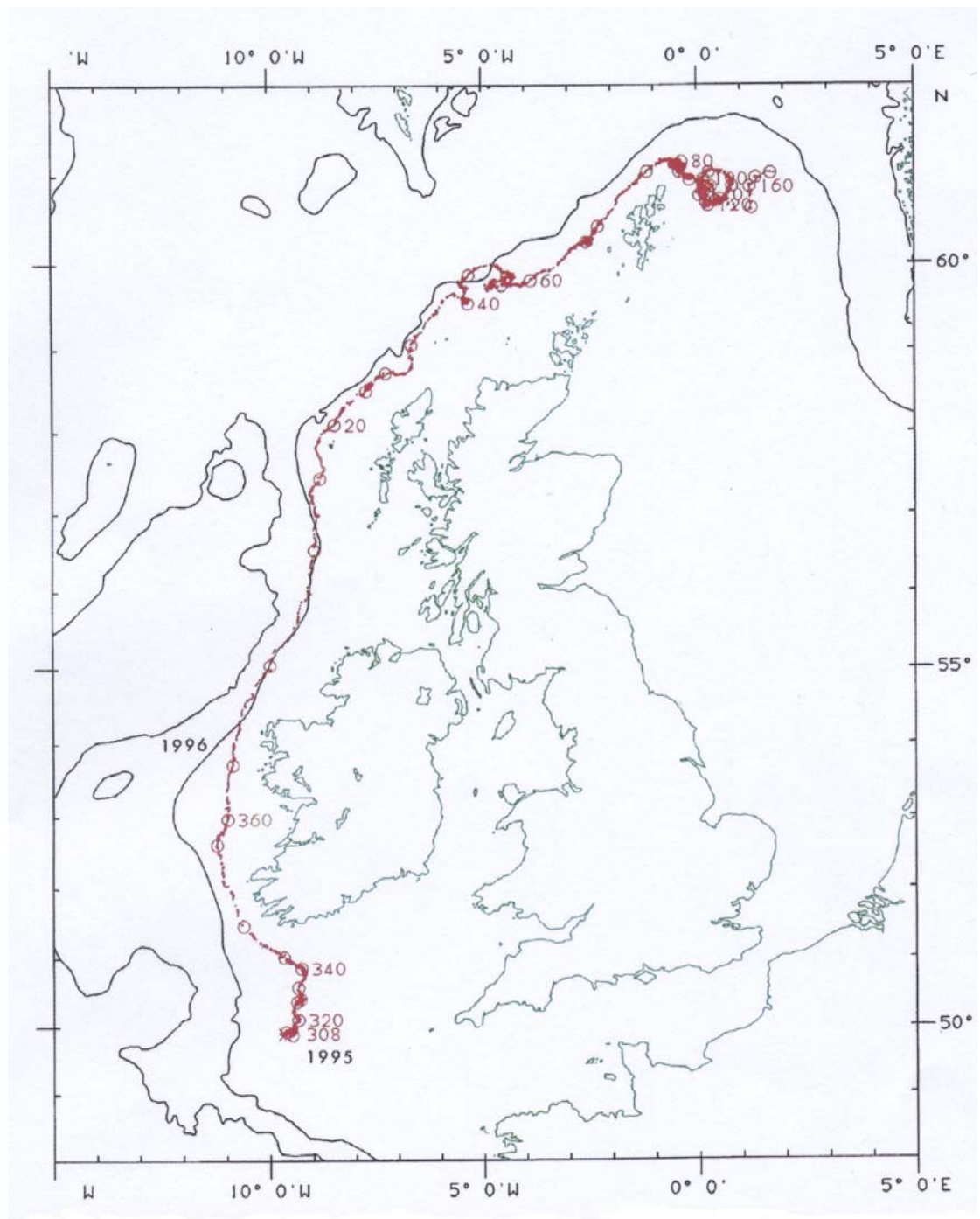
Fig. 6. Lagrangian track (red) from Celtic Sea to west of Norway of a drogued buoy from the programme Argos, covering from 4th November 1995 to 22nd June 1996. (Figure taken from Pingree *et al*., 1999).

It seems that evolutionary features have been more important in defining the asteroid fauna that inhabit this zone. The diverse ecological and environmental features of this zone and the mixed origin of the species allow them to have different reproductive features according to their specific necessities regarding, feeding, population conditions and phylogenetic constraints. Young (2003) considered that as in shallow water, the deep sea contains enough spatial and temporal variability to allow exploitation by species with various strategies of energy allocation.

Below 3000 m a trend is observed that is possibly influenced strongly by environmental factors such as temperature and food quality and quantity, which affect the adults and the larvae. High latitudes and deep waters are characterized by low temperatures. Therefore in these environments there is a tendency to produce larger eggs reducing fecundity in order to provide the offspring with energy sufficient for a longer developmental time (King and Butler, 1985; MacDonald and Thompson, 1985; Lonsdale and Levinton, 1986, 1989; Barber et al., 1988; Mauchline, 1988; Clark and Gore, 1992; Gorny et al., 1992). Conversely, metabolic rates are also reduced and consequently physiological processes demand less utilization of energy. Of the 9 species found below 3000 m in this study, 6 belong to the Porcellanasteridae, a family found entirely in the deep sea. Six of the genera of Porcellanasteridae live in the abyssal depths, and only two genera (*Porcellanaster* and *Eremicaster*) occur at depths less than about 2500 m. One species of *Eremicaster* reaches down into the hadal zone, to a depth of about 7200 m (Madsen, 1961). Therefore it is possible that the reproductive features at this zone are also related to historical factors affecting evolutionary pathways (Vinogradova et al., 1959). This would explain why *Porcellanaster ceruleus* was the only porcellanasterid grouped into another cluster by the analysis as this genus is presumably the youngest abyssal type of porcellanasterid

which has penetrated into the great depths and its features enable this species to inhabit depths above 3000 m where the environmental conditions are different. The base of the continental slope in the NE Atlantic occurs at ~ 3300 m (Rice *et al*., 1991). Howell *et al.* (2002) proposed that this may represent a barrier to abyssal species. They also found a tenuous relation between the depth at which abyssal fauna starts to appear and the depth at which North Atlantic Deep Water (NADW) and Modified Antarctic Bottom Water (MABW) meet between 3000 and 3500 m. Rogers (2000) provided evidence; which suggest that many abyssal groups have originated in the Southern Ocean, where Antarctic Bottom Water originates.

The abyssal zone is inhabited by asteroid species with reproductive features indicative of equilibrium *K* strategists (Winemiller and Rose, 1992), which have low fecundity, large egg size and expected high juvenile survivorship, although their large body size makes them more comparable to the classical *K* strategists (MacArthur and Wilson, 1967; Pianka, 1970), and they are characteristic inhabitants of constant environments (Winemiller and Rose, 1992; McCann and Shuter, 1997).

As Ramirez-Llodra (2002) concluded, the species that have colonized the different deep-sea environments have to be well adapted to their habitat in order to be successful and persistent. In the deep NE Atlantic the habitat has selected for species with specific reproductive traits, which provide them with successful and advantageous life history strategies (Eckelbarger, 1994; Eckelbarger and Watling, 1995) as can be clearly observed in the upper bathyal zone between 700 and 1100 m, where the environmental conditions have selected for small species with low fecundity and large eggs, plus habits related directly or indirectly with suspension feeding. This species exhibit reproductive features associated to the opportunistic

strategy stablished by the Winemiller and Rose model (1992) and the large egg size characteristic of the species inhabiting deep cold waters.

Conversely, phylogenetic and evolutionary factors are also important and seem to be decisive at the deepest waters where basically mainly species belonging to the strict deep-sea family Porcellanasteridae are found. All these species possess a mixture of features proper of classical *K* strategists and the equilibrium strategists proposed by Winemiller and Rose (1992), which enable them to persist in the relatively stable environment with low energy availability that characterizes the great abyssal plains.

CHAPTER FIVE- SYNTHESIS AND CONCLUSIONS

5.1- Factors controlling the bathymetric distribution of species and their effects on early life-history stages of echinoderms

The factors that control the diversity of communities and also the bathymetric distribution of individual species are likely to involve a number of mechanisms that act during the larval stage. These mechanisms may include larval physiological tolerances to temperature and pressure, inherent larval behaviours and the availability of food. Factors that limit depth of occurrence may place limits on the invasion of deeper water by shallow water species, but in most deep-sea animals, such limiting factors for individual species are unknown (Tyler & Young, 1998). In addition, there are only a few physiological experiments on early life-story stages of deep-sea benthic animals. (Dayton *et al*., 1982; Young & Tyler, 1993; Young *et al*., 1996ab, 1997; Tyler & Young, 1998).

Tyler & Young (1998) examined temperature and pressure tolerances of the dispersal stages of congeneric species of echinoids with different bathymetric distributions, and it was inferred that physiological tolerances of the larvae influenced the adult distribution.

Young *et al*. (1996b) experimented with early embryos of 7 littoral species of tropical echinoids from Hawaii and the Bahamas and 3 bathyal species from the Bahamas. In every case, embryos tolerated pressures greater than those of their adult normal distributional ranges, but at temperatures found in shallow water. This suggests that pressure does not set actual depth limits for most species and would not prevent recruitment or invasion of depths as great as 2000 m.

Sewell & Young (1999) concluded that the geographic distribution of the echinoid *Echinometra lucunter* does not appear to be limited by the temperatures at

which normal embryonic/larval development occurs, but by 1) adult temperature tolerances, 2) temperatures needed for growth or spawning, or 3) hydrographic features that limit larval settlement and juvenile survival.

Data from experiments carried out by Tyler *et al*. (2000) showed that the embryonic and larval stages of the Antarctic echinoid *Sterechinus neumayeri* (Müller) are capable of surviving low temperatures in surface waters, but only tolerate higher pressures when water column temperatures are > 0° C. They infer that the larvae of *S. neumayeri* might be capable of penetrating the deep sea through the action of formation of Antarctic Bottom Water in the Weddell Sea, since this pattern of temperature increase is seen during deep water formation.

Only one experiment has been carried out onto pressure tolerances of embryos the bathyal asteroid species *Plutonaster bifrons* (Wyville Thomson) (Young *et al*. 1996a). The greatest percentage of embryos developing normally occurred at pressures equivalent to 2000 m depth, the depth at which the species is most common. No normal development occurred at a pressure corresponding to 3000 m depth. Therefore, embryonic tolerances could determine the bathymetric limits of distribution for this species.

5.2- Effect of hydrodynamic mechanisms on larval dispersal

Developmental mode is not the only factor that might determine dispersal distance, but larval dispersal patterns depend also on hydrodynamic mechanisms and such mechanisms may be different in distinct regions. Therefore, larval dispersal patterns vary between populations according to the relative importance of water advection and diffusion at a local scale. The flow of water in the deep sea is still not fully understood compared to the surface circulation. Therefore, in most cases, it is

not an easy task to predict where larvae released at a particular location will go and how long the journey will take. As an example Campbell & Rowe (1997) described the new species of asteroid *Patiriella paradoxa* Campbell and Rowe, which being a temperate taxon inhabits southern Arabian waters. This fact could only be explained in terms of this species being a relict who evolved to survive continual conditions in this tropical location, supported by the influence of local seasonal upwelling.

Fenaux *et al.* (1994) showed that in the Eastern Alborean Sea, where surface currents form a complex frontal zone, with associated eddies and gyres, the distributions of larvae and postlarvae of echinoderms vary according to hydro dynamic structures. Larvae are numerous in the Atlantic Geostrophic Jet, which passes along the African coast and they are accumulated in an anti-cyclonic gyre to the west of the jet. In the anti-cyclonic eddy of Mediterranean water, north of the frontal zone, the larvae by contrast were scarce.

Wind forcing increases the effect of advection on larval transport and modifies significantly the level of larval retention. Wind-induced currents however, may produce larval transport from one population to another and might be involved in restoration of depleted populations (Ellien *et al.*, 2000).

Marsh *et al*. (2001) demonstrated the prevailing importance of current flow in determining dispersal potential of the tube worm *Riftia pachyptila* at deep-sea hydrothermal vents on the East Pacific Rise and suggested that populations at different vent sites may have different dispersal limits depending on local current conditions. In this region at least, it is apparent that the dispersal distance of *R. pachyptila* was not limited by the physiological performance of the larvae, but by temporal oscillations in the currents and larval loss in their flows.

Larval dispersal from eight populations of the ophiuroid *Ophiothrix fragilis* (Abildgaard) in the English Channel was examined by Lefebvre *et al*. (2003) using an advection/diffusion model. Although larval dispersal and settlement of this species are apparently hydrodynamic constrained in almost all populations larval retention appeared to be sufficient to ensure local recruitment, in spite of short larval life span and/or meteorological conditions.

Nowadays it is recognized that the deep sea is not a single, continuous habitat, but rather a mixture of habitats in which many species have particular specialized requirements depending on where they are situated (Tyler, 1995).

The dynamic, insular and often temporary habitats represented by hydrothermal vents and cold seeps with a rich supply of self-produced (autochthonous) food contrast markedly with, stable, nutrient-poor and extensive abyssal plains, where all energy has its sources from the sea surface. In a similar way, the continental slope and rise include many different habitats, each characterized by specific topographic features, physical characteristics, and food interactions, and each occupied by distinct groups of species adapted to those different conditions. Therefore, reproductive modes and life-history traits of animals show a rich variety of strategies for responding to the diversity of habitats in the deep sea (Young, 2003).

5.3- Larval physiological tolerances of shallow-water asteroids

Eggs of the shallow-water asteroids *Asterias rubens* Linnaeus and *Marthasterias glacialis* (Linnaeus) were fertilized *in vitro* and incubated through the early embryonic cleavages until the larval stage. Early embryos, blastulae, gastrulae, and swimming bipinnaria were subjected to a temperature/pressure regime of 5, 10, 15 and 20°C and 1, 50, 100, 150 and 200 atm.

The results showed that early embryos were able to tolerate pressures up to 150 atm at 15°C and 100 atm at 10°C. Generally, survivorship of *Asterias rubens* swimming bipinnaria remained high (> 70%) after incubation at all the pressure/temperature combinations. The highest number of swimming larvae was 100% at 10°C/50 atm and the lowest was 72% at 15°C/200 atm.

In *Marthasterias glacialis* the highest survival of swimming larvae was 100% at 1 atm/5, 15 and 20°C and 50 atm/15 and 20°C and the lowest was 57 % at 5°C/200 atm. In general, survivorship decreased as the pressure increased; nevertheless the larvae of both species generally tolerated pressures of 200 atm. Furthermore, data for the temperature and pressure effects on the later stages of development suggest that all the larval stages are more temperature/pressure tolerant than the early embryos and survivorship becomes greater with larval age.

All the developmental stages demonstrated to have a potentially wider depth distribution than their respective adults. Comparison of these data with those of shallow water and deep-sea Atlantic echinoids suggests that the early embryos of echinoids are more tolerant of pressure/temperature changes, but that the later larval stages of asteroids tolerate change more readily than the larval stages of echinoids. Therefore, the larvae of the shallow water species *Asterias rubens* and *Marthasterias glacialis* could survive transport to deeper waters and these species may be capable of sending colonists to the deep sea. The plasticity on the early-life history stages, and thus their ability to tolerate increasing pressure, may be cumulative over many generations, until an individual species has successfully adapted to the deep-sea environment. It is possible that this adaptation may have been rapid as a number of deep-sea invertebrate species retain the seasonal growth and reproductive patterns seen in shallow water congeners (Young 2003).

These results are very important since larval settlement (and metamorphosis) is known to be the most critical phase in the life history of any marine benthic species given that it involves essential changes in morphology, physiology and habitat (Chia, 1989).

5.4- Reproductive adaptations of the different deep-sea asteroid species in the North-East Atlantic Ocean.

The present study demonstrates that asteroids can inhabit a greater depth range than most of the population actually exploit. The reasons for the contraction of the depth range of a species are still unidentified. It is probable that post-selective forces may exist, which are responsible for the distribution of the species over such relatively small depth ranges (Howell *et al.,* 2002). Factors specific of a particular zone such as suitability of habitat, food availability, or interactions with other species, which eliminates juveniles are likely to affect the distribution on this local scale rather than potentially lethal factors, such as temperature or pressure.

The present study found differences in the reproductive traits of the species in correspondence to the zones proposed by Howell *et al.* (2002) and taking into account the effect of the environmental factors in selecting the species with successful life-history patterns, as well as the phylogenetic constrains inherent of the species.

The upper slope zone ranging from the shelf break to ~700 m is characterized by large seasonally reproducing predators that exhibit the life-history periodic pattern established by Winemiller and Rose (1992), having large body size, high fecundity, small egg size, single spawning frequency, and possibly low juvenile survivorship. The life-history periodic pattern is distinctive of species inhabiting predictable and seasonal environments (Winemiller and Rose, 1992; McCann and Shuter, 1997).

In general, for all the species other variable demographic features such as lifespan, and age at maturity were difficult to determine because these features have not been documented.

Two species (*Pontaster tenuispinus* Düben and Koren and *Psilaster andromeda* (Verrill)) with large eggs, low fecundity and low G.I. were found at ~ 650-850 m depth. Nevertheless, the reasons why species with these features are found at this specific depth are still unknown and further studies are required in order to understand the factors that determine their distribution.

From 700 to 1100 m depth only species having small adult size, large egg size with low fecundity and low Gonad Index were found. Howell et al. (2002) defined this zone as the upper bathyal zone, and it is characterized by a rapid succession of species with species displaying a narrow total adult bathymetric ranges. The 1100 m boundary of this zone was associated with changes in currents on water masses. Flach et al. (1998) proposed that the variability in the currents certainly have an effect on sediment transport, food supply and larval dispersal and is expected to affect the zonation of asteroids, particularly suspension-feeding species.

The reproductive patterns of the asteroid species inhabiting this zone appear to be related to environmental and population conditions. These species are often basically suspension feeders or small predators. The latter take advantage of the large aggregations of sessile organisms, especially sponges. The asteroids in this zone exhibit reproductive features with trends to the opportunistic strategy, They possess small body size and low fecundity per spawning event, and aseasonal reproduction with probably multiple spawning frequency. However, their large size of possible demersal eggs probably follows the general trend observed in species from cold waters in order to provide the larvae with energy sufficient for a high survival

possibility. The opportunistic species are distinctive of highly disturbed and unpredictable environments (Winemiller and Rose, 1992; McCann and Shuter, 1997).

The zone between 1100 and 3000 m coincides with the transitional zone proposed by Howell *et al*. (2002) where boundaries at 2500, 2800 and 3300 m are thought to demonstrate a region of transition between bathyal and abyssal fauna. Evolutionarily, bathyal species are likely to have a mixed origin, with some species from the shelf and some originating from abyssal depths (Rogers, 2000).

Six of the nine species found below 3000 m in this study belong to the Porcellanasteridae, a family found entirely in the deep sea. It is likely that the reproductive features at this zone are also related to historical factors affecting evolutionary pathways. (Vinogradova et al., 1959). Rice *et al.* (1991) found that the base of the continental slope in the NE Atlantic occurs at ~ 3300 m and Howell *et al.* (2002) proposed that this may represent a barrier to abyssal species.

The abyssal zone is inhabited by asteroids with reproductive features of equilibrium *K* strategists (Winemiller and Rose, 1992), which have low fecundity, large egg size and expected high juvenile survivorship, although their large body size makes them more comparable to the classical *K* strategists (MacArthur and Wilson, 1967; Pianka, 1970). *K* strategists are characteristic inhabitants of constant environments (Winemiller and Rose, 1992; McCann and Shuter, 1997).

5.5- Final remarks

From the earliest days of deep-sea exploration, it was assumed that animals living in the presumably invariable environments of the deep sea should show life-history features and reproductive modes differing from those of their shallow water congeners, but the most recent investigations show that with few exceptions, the

reproductive mechanisms and patterns found in deep-water echinoderms like other taxonomic groups are similar to those found in shallow-water species (Young. 2003). It was also supposed that the rate at which biological processes occur is slower in the deep sea than in shallow waters, but some rates in the deep sea may be similar to or only slightly lower than rates in shallow waters (Gage, 1991; Thistle, 2003).

Recent discoveries and long-term, time-series investigations have made clear that the deep sea, and in particular the deep Atlantic, is a dynamic ocean whose inhabitants undergo environmental variation over an extensive range of spatial and temporal scales. However, the vertical gradients of environmental stability and availability of nutrients that sustained the earliest predictions for the deep sea, still provide a useful framework for considering how natural selection has given form to the life-history attributes of deep-sea animals (Young, 2003).

Today we recognize that the deep ocean is not an isolated system, but interacts with global surface circulation and ocean atmosphere interactions, therefore the high levels of variability exerted by global climate change and disturbances produced by anthropogenic activity on oceanic zones have also an increasing effect on deep waters affecting the biological processes of deep-sea communities.

It is important to remark that in the present study the results showed that temperature is a determinant environmental factor that might allow or prevent the shallow-water species of colonizing the deep sea. The experiments performed with embryos and larvae of *Asterias rubens* and *Marthasterias glacialis* provided evidence that the embryos and larvae have a elevated tolerance to high pressures when the temperature is appropriate. Young *et al*. (1997) also found that larvae of shallow water Mediterranean echinoids tolerate relative high pressures at temperatures that prevail in the modern Mediterranean Sea, those findings give impetus to the

hypothesis that the Mesozoic and early Cenozoic periods were the principal times for the invasion of the deep sea, because during these periods, the water column was warm and isothermal as is the modern Mediterranean (Tyler, 2003). In a global context temperature is an environmental variable which has been experiencing important changes in the last decades and importantly the atmospheric warming also has a similar effect on the ocean, subsequently the processes and interactions observed currently in the oceanic communities could experienced important effects on the patterns currently observed.

A comprehensive knowledge of the ecology of the deep-sea fauna is essential in order to evaluate the level of variability caused in the environment and the possible effects on life-history of the species, more specifically how this disturbance affects the reproductive processes, such as production of gametes and/or larval development, since this are key stages in the life-history of an organism.

Appropriate deep-sea research must be conducted, targeted so as to estimate potential impacts of human activity in order to arrive at the right decisions for an adequate management of resources and conservation of species. In this context studies and experiments on reproduction and larval biology of deep sea organisms are crucial for a better knowledge of the processes driving the communities in the deep ocean.

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