UNIVERSITY OF SOUTHAMPTON

'Reproductive patterns of deep-sea invertebrates related to phylogeny and energy availability'

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ABSTRACT

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REPRODUCTIVE PATTERNS OF DEEP-SEA INVERTEBRATES RELATED TO ENERGY AVAILABILITY

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Reproductive strategies and their interaction with the environment play a major role in the shaping of ecosystems. However, while our understanding of the diversity, biogeography and ecology of deep-sea invertebrates has increased considerably in the last century, little is known about the reproductive biology of most species. This is surprising, because life history strategies integrate information on the genetic, physiological and environmental factors affecting the individuals.

Gametogenesis, and particularly vitellogenic pathways, are genetically determined and constrained by phylogeny. As a result, related species have similar patterns in the early processes of gamete production. In contrast, the reproductive output of an individual is strongly affected by energy availability in the environment. The number of eggs (quantified as fecundity) or their quality (size and contents of the eggs) vary with food quantity and quality and with habitat stability. Fecundity is related directly to other main life history traits, such as age at first maturity, egg size and reproductive effort, and therefore plays a major role in the life history tactics of a species. Fecundity and its relationship with environmental conditions has received special attention in this study.

The hypotheses that 1)- gametogenesis is phylogenetically constrained, and 2)- fecundity and egg size are affected by food availability, were tested for several species of deep-sea invertebrates. Closely related species of caridean shrimps and asteroids from environments with different food availability were studied. Their gonad morphology, gametogenic patterns, fecundity and egg size were analysed.

The reproductive biology of five species of mesopelagic shrimp from the NE Atlantic and three species of hydrothermal vent shrimp from the Mid-Atlantic Ridge was examined and compared. The gonad morphology and gametogenic processes of the eight deep-sea species were similar and characteristic of caridean shrimp. In contrast, the fecundity and egg size varied between species. In the mesopelagic shrimps, fecundity decreased and egg size increased at higher latitudes and deeper depths. These clines of fecundity and egg size are discussed in relation to environmental factors experienced by the adult females and larvae. In the hydrothermal vent shrimps, the three species produced a high number of small eggs. The fecundity of the hydrothermal vent species was significantly higher than that of the small-egged mesopelagic *Acanthephyra* spp.

Three species of porcellanasterid asteroids from three sites (Porcupine Abyssal Plain (PAP), Madeira Abyssal Plain (MAP) and a site off NW Africa (NWA)) with differing food input were analysed. The three species had similar oogenic and spermatogenic patterns, typical of asteroid gametogenesis, at the three sites. They produced a small number of large eggs (~600 μm) in a quasi-continuous rate. The size at maturity and average adult size was significantly smaller in the specimens from the low-food site (MAP). The gonad index of each species was similar at all sites, suggesting that the specimens from MAP invest relatively more energy into reproduction than growth, compared to the conspecific individuals from PAP and NWA. However, the fecundity of MAP specimens was lower than that of PAP and NWA specimens. This lower fecundity might be related to the smaller body size of adult females from MAP. The differences in energy allocation to reproduction are discussed in relation with food availability and quality.

The results from this study confirm that gametogenic processes are predetermined by phylogeny in deep-sea invertebrates. In contrast, fecundity and egg size are free to fluctuate, within species-specific limits, as a response to variability in environmental factors.

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References

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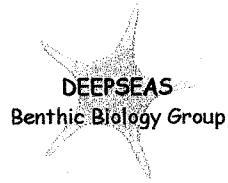
A mis padres, Carmen y Federico,

Y a Hermes

THE DEPTHS OF THE SEA

'For the bed of the deep sea, the 140,000,000 of square miles which we have now added to the legitimate field of Natural History research, is not a barren waste. It is inhabited by a fauna more rich and varied on account of the enormous extent of the area, and with the organisms in many cases apparently even more elaborately and delicately formed, and more exquisitely beautiful in their wonderful phosphorescence (...). And the forms of these hitherto unknown living beings, and their mode of live, and their relations to other organisms whether living or extinct and the phenomena and laws of their geographical distribution, must be worked out.'

Charles Wyville Thomson
1873



CHAPTER ONE- GENERAL INTRODUCTION

1.1- From the 'Azoic zone' to 'hydrothermal oases': a brief history of deep-sea oceanography

With over 50% of our planet below 3000 m of water, the deep sea is the largest ecosystem on earth. The surface waters have played a central role in the development of civilisations, being used for transport of goods and individuals, for fishing and for leisure. We are now entering the depths of the oceans and disturbing the environment both directly (deep-sea fisheries, waste dumping) and indirectly (related to global climate change). But, as a result of technological and economical constraints, very little is known of this vast environment, of its diverse fauna and of its relation with global dynamics, and we are transforming it before we understand it.

The development of deep-sea research as a science is associated with the development of new techniques of navigation, sampling and measuring, and follows the path of great oceanic expeditions. It was during the last two Centuries that these expeditions obtained the first scientific results, which would fascinate and inspire a whole new branch of oceanographers.

The cruise of H.M.S *Beacon* to the Aegean (1841-1842) could be considered one of the first biological deep-sea cruises. Edward Forbes from Edinburgh University joined the ship as a naturalist and made around 100 dredge hauls down to a depth of 230 fathoms (420 m) (Thomson, 1873; Menzies, et al., 1973; Rice, 1986). As the *Beacon* crew dredged deeper fewer species were found, which led Forbes to conclude that no life was present in the oceans below 300 fathoms (~600 m). He named this area the 'Azoic Zone' (Thomson, 1873; Murray and Hjort, 1912; Menzies et al., 1973; Mills, 1983). But already, before Forbes, Sir John Ross, while dredging at 800 fathoms during his exploration for the Northwest Passage in 1818, had collected the ophiuroid *Gorgoncephalus caputmedusae* (as *Astrophyton linckii*) (Menzies, et al., 1973; Tyler, 1980). Later, Michael Sars (1850) published a list with 19 species from waters deeper than 300 fathoms, and his son, George Ossian Sars extended the list to 92 species. Ten years later, the recovery, by Fleming Jenkin, of a submarine cable from 2184 m in the Mediterranean showed the presence of a solitary coral, *Carophyllia borealis*.

With evidence accumulating of an extant and diverse deep-water fauna, Charles Wyville Thomson and W.B. Carpenter encouraged the Royal Society and the

Admiralty to organise a deep-sea expedition. The result was the H.M.S. *Lightning* cruise in 1868 to the NE Atlantic, where, in spite of the bad weather and poor state of repair of the ship, some deep dredges brought a variety of species to the surface. The finding of negative bottom temperatures north of 60°N and of positive temperatures south of 50°N led to the organisation of short cruises on board H.M.S. *Porcupine* to NE Atlantic and to the Mediterranean Sea. During the NE Atlantic cruises, more deep-sea fauna was collected and the unusually low temperatures were confirmed (Thomson, 1873; Murray and Hjort, 1912; Menzies et al., 1973; Mills, 1983; Rice, 1986). Charles Wyville Thomson suggested the existence of a ridge (now called the Wyville Thomson Ridge) separating the North Atlantic water from the subzero Arctic bottom water, and this was later confirmed by Sir John Murray while undertaking a topographic survey of the zone in 1880 on board H.M.S. *Knight Errant*.

With the important discoveries of the *Lightning* and *Porcupine*, W.B. Carpenter's application for a scientific circumnavigation expedition was accepted in April 1872. H.M.S. *Challenger* set sail from Sheerness on December 7th 1872 for her three and a half years cruise of dredging, sounding, measuring and collecting, with C.W. Thomson as chief scientist. The *Challenger* expedition was set up to study the physical, chemical and biological processes in the deep ocean. This circumglobal oceanographic voyage has been considered by many to be the true birth of modern oceanography (Murray and Hjort, 1912; Menzies et al., 1973; Mills, 1983; Rice, 1986).

After the *Challenger* expedition there followed an era of pioneering deep-sea research, involving numerous ships from several countries. The American Alexander Agassiz sampled off the East Coast of North America and in the Caribbean Sea during three cruises aboard the *Blake* (1877-1880) and later off Central America in a cruise aboard the *Albatross* in 1891, focusing on the biological aspects of the deep sea. Alphonse Milne-Edwards and others on board the French ships *Travailleur* (1880-1882) and *Talisman* (1883) worked on deep-sea research in the Mediterranean, around the Azores and in the Sargasso Sea (Menzies et al., 1973; Mills, 1983; Rice, 1986). Between 1885 and 1914, Prince Albert I of Monaco organised, financed and directed several oceanographic cruises in the Mediterranean and NE Atlantic on board the *Hirondelle*, *Prince Alice I*, *Prince Alice II* and *Hirondelle II* (Menzies, 1973; Mills, 1983). In the mid-twentieth century, two circumglobal expeditions were undertaken: the Swedish *Albatross* (1947-1948) and the Danish *Galathea* (1950-1952). The research accomplished during these cruises made important contributions in the

improvement of deep-sea techniques, such as the development by Kullenberg of the now widely used piston corer and single-wire otter trawl, and largely extended the taxonomic knowledge of deep-sea fauna (Menzies, 1973; Mills, 1983). It was during the latter expedition that the crew and scientists of the *Galathea* recovered for the first time animals from the Philippines Trench, at 10190 m depth (Gage and Tyler, 1991).

In the 1960s and 1970s, there was an important change in the approach of deep-sea biological research, switching from descriptive biology to a more ecological, evolutionary and experimental approach. This new line of research was led by American researchers such as Robert R. Hessler, Howard L. Sanders and Frederick Grassle (Hessler and Sanders, 1967; Hessler, 1969; Grassle and Sanders, 1973; Grassle, 1977; Sanders, 1979), and has been increasing since then.

But the conquest of the oceans would not have been complete if man had not developed the ways of entering the deep-sea environment, to observe, explore and experiment *in situ*. Therefore, parallel to the remarkable developments in navigation and oceanographic technologies, there is the history of diving, deeper and longer. It took three hundred years from the diving bells used in the 17th Century for short dives in shallow waters (down to 18 m) to Beebe's Bathysphere in 1930, the first deep water vehicle for observation of the seabed (Beebe, 1939; Busby, 1976; Sweeney, 1970; Welham, 1994; Ballard, 2000).

From there, in little more than 50 years, the advances in deep-sea technology led to research manned submersibles, such as the French *Nautile* and *Cyana*, the American *Alvin* and *Johnson Sea Link I* and *II*, the Russian *Mir I* and *II* and the Japanese *Shinkai 6500*, and Remote Operated Vehicles (ROVs) such as the American *ANGUS* and *Jason*, the French *Victor* and *Epaulard* and the Japanese *Dolphin-3K* and *Kaiko* (Sweeney, 1970; Busby, 1976; Gage and Tyler, 1991; Welham, 1994; Ballard, 2000). Manned submersibles are used for delicate sampling and *in situ* experimentation, allowing for direct observations and for ecological and physiological studies with live specimens (Heirtzler and Grassle, 1976; Geyer, 1977; Gage and Tyler 1991; Young, 1991).

The last two decades have been marked by the discoveries of the hydrothermal vents and cold seeps with their exuberant associated faunas (Corliss and Ballard, 1977; Lonsdale, 1977; Kennicutt et al., 1985; section 2.3 this chapter). After the first descriptions of the hydrothermal habitat and its fauna, sustained by the primary production of chemoautotrophic bacteria, there followed ecological studies aiming to

understand the dynamics, life histories and evolution of these communities. This is a hot topic, made possible by the availability of submersibles and ROVs, and developed in projects such as the European-funded AMORES (Desbruyères et al., 1998), the British BRIDGE (Harrison, 1996), the British-Russian BRAVEX/1994 (Vinogradov and Vereshchaka, 1995) and the American LARVE (C. Young, *pers. com.*).

Also, during the last few decades the non-hydrothermal deep-sea ecosystems have been largely studied under programmes such as the IOS DL benthic biology study of the Porcupine Seabight (Rice et al., 1991), the time-series studies at fixed stations, such as station 'M' in the Rockall Trough (Gage et al., 1980) or station 'M' in the NE Pacific (Baldwin et al., 1998; Drazen et al., 1998), the DEEPSEAS (Rice et al., 1994) and BENGAL (Rice et al., 1998; Billett and Rice, submitted) programmes in the Porcupine Abyssal Plain, and the French EUMELI programme in the NE Atlantic (Sibuet et al., 1993). The main results obtained from these programmes were evidence of seasonal deposition of aggregated phytodetritus to the deep-sea bed and the responses of the benthic fauna to this input of organic matter (section 1.2.2 this chapter).

The exploration of the abyss is one of the great challenges of today, because our knowledge of the ecological, physiological, life history and evolutionary processes driving deep-sea communities is still very poor. With many aspects of deep-sea dynamics being linked to global changes and human activity, public and political interest in deep-sea research is now increasing. The deep ocean is an integral part of the global climatic system, being the biggest buffer for greenhouse gases. It also receives anthropogenic wastes via river discharges, air currents, rain and, lately, also via active dumping of highly toxic or radioactive wastes. Also, exploitation of oil and gases is progressing from outer continental shelves down to the slopes, and with the overexploitation of traditional fisheries, trawlers are fishing deeper down into bathyal depths. However, very little is known on the effects of these disturbances to the benthic communities (Jones, 1992). The deep-sea ecosystem sustains a high biodiversity (Dayton and Hessler, 1972; Grassle and Sanders, 1973; Grassle and Macioleck, 1992; Stuart et al., in press) with many unknown species and habitats. This ecological diversity and richness cannot be protected unless we have a better understanding of the processes driving the communities and of the system as whole.

1.2- The deep-sea environment

1.2.1- Topography and general characteristics

The deep sea is considered to start at the edge of the continental shelf, or below the permanent thermocline, at around 800 to 1300 m, when using hydrographic criteria (Gage and Tyler, 1991). The cold, deep-sea water is formed off Antarctica (Antarctic Bottom Water) and in the Norwegian Sea (North Atlantic Deep Water) by the mixing of cold and saline water masses. The temperature at abyssal depths ranges between −1°C and 4°C, except around hydrothermal vents, the Mediterranean Sea where bottom water is at 13°C, and the Red Sea with bottom water temperature of 21.5°C (Gage and Tyler, 1991; Tyler, 1995). The salinity of deep-water is relatively constant at around 34.8‰ (Menzies, 1965; Gage and Tyler, 1991). The oxygen concentration generally is close to saturation, although it decreases with distance from the origin of deep-water mass formation because of its consumption by metabolic processes (Gage and Tyler, 1991; Tyler, 1995). Light and pressure covary with depth, the former decreasing with increasing depth and disappearing below 1000 m (except bioluminescence), and the latter increasing by 10^5 Pascal (1 atm) for every 10 meters of depth.

With this relative constancy of the physical parameters of deep-water masses, the deep sea was long considered a monotonous cold and dark environment (Thomson, 1873; Menzies, 1965). But the biological, physical and chemical results obtained since the H.M.S. *Lightening* cruise indicated a very different picture. We now have good mapping of the seabed across the oceans, which facilitates our understanding of the dynamics of oceanic and continental crust.

The continental shelf is a shallow area continuous to the continents, at the periphery of the ocean basins. It has a varying width and a relative low inclination, and it ends at the shelf break, at ~200 m depth. At this point, the continental slope deepens into the ocean with a steep inclination, which can be interrupted by submarine canyons and terraces. Deeper, the slope gradient becomes less accentuated on passive margins such as in the Atlantic (Leeder, 1985), and forms the continental rise, which extends from around 2000 to 4000 m depth. The smoother abyssal plains extend from 4000 to 6000 m depth, offering the typical image of wide, flat and featureless extensions, covered with very fine sediment settling from the water column (Gage and Tyler, 1991). Deeper than 6000 m, trenches occur in subduction zones where the oceanic crust is being destroyed, and can attain depths of 11000 m, the deepest points in the oceans.

1.2.2- The abyssal plain environment

The abyssal ecosystem is the largest habitat on earth. It lies beyond the continental slope, between 3000 to 6000 m depth. The abyssal plains of the different oceans are connected globally by the deep-ocean current systems (Menzies, 1965). Most of the ocean floor is covered by deep-water masses originally formed in the Antarctic (Antarctic Bottom Water, AABW) or the Greenland/Norwegian Seas of the Arctic Ocean (North Atlantic Deep Water, NADW). The AABW is formed from the cold surface layers around the Antarctic coast, especially the Weddell Sea. A cold (–0.4°C) and therefore dense water mass sinks to form a circumpolar bottom water with branches that penetrate all the main oceans. In the Atlantic, this bottom water flows north along the west side, but penetrates the east side through major fractures zones along the Mid-Atlantic Ridge.

Because of the relative uniformity in the distribution of temperature, salinity, dissolved oxygen, light and pressure, the abyssal plains have long been considered to be constant and stable environments where the physical and biological processes are unchanged over short and long time-scales. There is evidence now to show that this is not the case for many areas in the deep-sea bed, where physical disturbances occur, causing important biological responses.

In the short time-scale, the flow of cold dense water close to the bottom has semidiurnal and annual tidal variations, and these internal tides are found far out into the ocean (Gould and McKee 1973; Tyler, 1988). The ecological effects of these predictable tidal patterns are not known, but the semi-diurnal variations could be used for orientation by motile organisms and/or feeding purposes, while the annual variations could be used as a cue for synchronised spawning (Tyler and Gage, 1984b, Tyler, 1988).

The abyssal plains are also disturbed by high-energy unpredictable events such as benthic storms, turbidity currents and sediment slides. The benthic storms originate from strong surface currents such as the Gulf Stream, where there is downward transmission of surface kinetic energy from wind stress. These events may last from days to weeks, and cause the suspension, transport and re-deposition of large amounts of sediment (Gardner and Sullivan, 1981; Hollister and McCave, 1984; Hollister et al., 1984; Weatherly and Kelley 1985; Gross et al., 1988). The benthic storms have a daily averaged flow of more than 15 cm s^{-1} (Weatherly and Kelly, 1984) and occur in boundary slope regions and lower continental rises (Hollister and McCave, 1984; Aller,

1989). During a benthic storm, the current changes direction and can double the speed of the internal tides, from 10 cm s^{-1} up to 22 cm s^{-1}. At the same time, the concentration of suspended sediment increases up to 100 times, from less than 500 μg l^{-1} up to 5000 μg l^{-1} in a strong storm (Gross et al., 1988). It has been suggested that the nepheloid layer in the deep-sea might be maintained by the occurrence of a few storms per year (Gross et al., 1988). The unpredictable and very erosive benthic storms, together with the highly energetic turbidity currents (gravity-driven down-slope events such as underwater avalanches) have considerable impact on the deep sea floor. Their disturbance effect is very important in both the redistribution of sediment and the biological responses of the benthic fauna (Hollister and McCave, 1984; Thieste, 1988; Tyler, 1988; Aller, 1989; Gage and Tyler, 1991).

The sediment layer covering the abyssal plains can reach thousands of meters in thickness, giving the popular picture of a flat, monotonous deep-sea bed. These sediments may be clays or biogenic oozes, depending on the productivity of the overlying water masses. Red clays composed by volcanic ash and wind dust are found under the centres of oligotrophic oceanic gyres. The biogenic oozes can be siliceous or calcareous. The siliceous oozes are derived from diatoms in high latitude productive waters or from radiolaria at high productive tropical zones. The calcareous oozes are derived from calcareous foraminifera at productive zones above the calcium carbonate compensation depth (Gage and Tyler, 1991). There are also pteropod oozes rich in aragonite and carbonate, but these are less common because the aragonite is easier to dissolve than the foraminiferan calcite (Gage and Tyler, 1991).

The sedimentary blanket is mainly inorganic, apart from the surface layers that receive the input of organic matter from the water column above. Already in 1880, Moseley suggested that the possible periodic variation of food supply to the deep-sea bed from the surface waters "may give rise to a little annual excitement amongst the inhabitants". But these ideas were not followed, and for almost a century the deep-sea was assumed to be seasonless, receiving a constant and slow input of organic matter in the form of marine snow (Menzel, 1974). It was in the 1980's that the evidence for the seasonal arrival of aggregated phytodetritus on the seabed was collected in the deep NE Atlantic (Billett et al., 1983; Lampitt, 1985; Rice et al. 1986).

The seasonal deposition to the seabed of surface produced organic matter is now considered to be a widespread phenomenon under areas of high surface production (Grassle and Morse-Porteous, 1987; Thiel et al., 1990; Rice et al., 1991, 1994; Santos

et al., 1994; Smith et al., 1997; Baldwin et al., 1998; Beaulieu and Smith, 1998). Because the rapid sinking of phytodetritus prevents its complete utilisation by pelagic grazers, the arrival of this organic matter provides deep-sea communities with a seasonal, high-quality food resource (Gooday and Turley, 1990; Beaulieu and Smith, 1998; Ginger et al., 2000).

Many studies have focused on the responses of the benthic fauna to this input of organic matter (Tyler et al., 1982c; reviewed by Tyler, 1988 and Gooday and Turley, 1990). There is a clear opportunistic response of bacteria, foraminifera and flagellates with a rapid colonisation and utilisation of the phytodetritus (Lochte and Turley, 1988; Turley et al., 1988; Thiel et al., 1990; Gooday, 1993; Pfannkuche, 1993, 1996; Smart and Gooday, 1997; Loubere, 1998). Higher abundance and biomass of macro- and megafauna were found in the Porcupine Abyssal Plain, which receives a strong seasonal phytodetritus signal, than in the more oligotrophic sites of the Madeira Abyssal Plain (Thurston et al., 1994, 1998). The seasonal deposition of organic matter to the seabed can also affect reproduction and there is now evidence for the coupling of seasonal reproduction to phytodetritus falls in several deep-sea species (Tyler, 1988; section 3.2 this chapter).

Another input of organic matter to the seabed of abyssal plains is in the form of large food falls such as macrophytes (*Sargassum, Thalasssia*) or animal carcasses (large fishes, whales), which may provide food, substratum for attachment or shelter for benthic organisms. There are organisms that show an opportunistic response to large food falls, with a rapid colonisation by larvae, early maturity and the production of a high number of eggs, such as the boring bivalves *Xylophaga* and *Xyloredo*, or some capitellid and spionid polychaetes (Wolff, 1979; Turner, 1973; Grassle and Morse-Porteous, 1987).

The abyssal plains are thus far from being constant and featureless environments, and much work needs to be accomplished before we reach a good understanding of the dynamics, interactions and evolution of abyssal ecosystems.

1.2.3- The midwater environment

With the water of the world's oceans representing more than 99% in volume of the biosphere, and the oceans being interconnected by global currents, the pelagic environment is the most extensive on Earth (Madin and Madin, 1995).

The circulation and distribution of water masses in the oceans play an important role in the biogeography and evolution of midwater species. In surface waters of the NE Atlantic, the warm North Atlantic current flows north towards the Norwegian Sea and sinks to form the Subpolar Mode Water (SPMW) in subsurface layers as a consequence of cooling and lateral mixing with polar waters (Van Aken and Becker, 1996). The SPMW forms the bulk of the warm Atlantic water entering the Norwegian Sea and is found above the permanent pycnocline and below the seasonal thermocline. In intermediate layers, Intermediate Water (IW) is found at the oxygen minimum layer in the pycnocline, with temperatures of 6-9°C in the Iceland Basin and Rockall Channel. On the southern slopes of the Porcupine Bank and, to a weaker extent, in the Rockall Channel there is an incursion of Mediterranean Water (MW) with high temperature (8-10°C) and salinity (>35.5) between 800-1200 m (Ellett and Martin, 1973; Lonsdale and Hollister, 1979; Van Aken and Becker, 1996).

The distribution and circulation of the water masses of the deep layers in the NE Atlantic is more complex. In the Norwegian Sea, the cold Norwegian Sea Deep Water (NSDW) is the main source of overflow of dense water from the Norwegian Sea into the Iceland Basin and the Faroe-Bank Channel (Ellett and Roberts, 1973; Van Aken and Becker, 1996). The NSDW interacts with the SPMW during the overflow and forms the Iceland-Scotland Overflow Water (ISOW) (Van Aken and Becker, 1996). As the ISOW leaves the Iceland Basin heading south on the west side of the Rockall-Hatton Plateau, it forms a modified water mass called North East Atlantic Deep Water (NEADW) found near 2600 m depth, with a salinity maximum. In the south of the Iceland Basin, Porcupine Abyssal Plain and Southern Rockall Channel the NEADW is found between the Labrador Sea Water (LSW) and the cold Lower Deep Water (LDW) (Lonsdale and Hollister, 1979; Van Aken and Becker, 1996). The NEADW flows westwards into the NW Atlantic basin, where it joins the Northwest Atlantic Bottom Water to form the North Atlantic Deep Water (NADW). The NADW flows south along the eastern continental slope of North America at 1000 to 5000 m (Gage and Tyler, 1991). It is a relatively warm (9-12°C) but saline (35.3 to 35.5) water mass.

In the Antarctic, the sinking cold surface waters form the Antarctic Bottom Water (AABW) (see chapter one, section 1.2.2). The AABW and NADW cover most of the oceans floor. The NADW is less dense than AABW because of its higher

temperature, and therefore overlies the Antarctic water mass wherever the two meet (Gage and Tyler, 1991).

The pelagic community is self-sustained by autotrophic (phytoplankton) and heterotrophic (bacteria, protozoa and metazoa) organisms. The system is driven by radiant solar energy and nutrient availability in surface waters, and the interactions between organisms determine the energy flow and cycling of organic matter (Smetacek et al., 1987). The intensity of light varies with the seasons and the availability of nutrients is determined by physical (input) and biological (loss) processes.

In temperate and polar waters, there are five phases of production in the upper layers of the oceans (Smetacek et al., 1987). The winter is characterised by a homogenous water column as a result of mixing, with a non-growth phase and low plankton biomass. The onset of the spring bloom is determined by the intensity of vertical mixing during winter (availability of nutrients) and the intensity of solar radiation (radiant energy). There is a diatom bloom with three phases, initiation, exponential growth and sedimentation, terminated by the depletion of nutrients. The spring bloom triggers spawning and growth of copepods, and there is an increase in the herbivorous copepod population and a slow increase in the flagellate population leading to the summer bloom. In summer, the water column is strongly stratified, with a clear thermocline. The summer bloom is characterised by a rapid turnover, with high production, low sedimentation and high remineralisation rates. There is high competition between trophic levels in such a complex system, determining a high diversity and a biological selection for the succession. The summer phase finishes with the breakdown of the thermocline, and is followed by the autumn bloom. This last phase of growth is terminated by a gradually decrease in light availability and an increase in turbulence (Smetacek et al., 1987).

Vertically in the water column, all systems (excepting the chemoautotrophic hydrothermal vents and cold seeps) depend on the photosynthetic biota from the surface layers. This production is transported horizontally by water mass movements and oceanic currents, and vertically by biological process such as migration of organisms, production of faecal pellets, sinking of dead organisms or transport of dissolved organic matter (Smetacek et al., 1987; Van Der Spoel, 1994). Therefore, the pelagic ecotones are horizontally and vertically linked to each other by transport of matter and energy fluxes. In general, upper ecotones are 'producing' while deeper ones

are 'consuming', in the same way as upwelling and divergence systems are 'exporting' while convergence systems are 'importing' (Van Der Spoel, 1994). As a result, the midwater fauna is dependent on surface production and on the transport of organic matter through the food web.

There are two major midwater regions, the mesopelagic zone and the bathypelagic zone. The mesopelagic zone lies between 200 and 1000 m depth, where the sunlight is too weak to allow photosynthesis, but still affects the behaviour of organisms on a diurnal basis. Because of its proximity to the surface layers, this zone sustains a large population of herbivorous organisms and vertical migrators. The bathypelagic zone lies deeper than 1000 m and represents 88% of the entire oceans. There is no sunlight, temperature is low and constant, and food is scarce. This zone supports mainly detritivores, predators, commensals and parasites (Madin and Madin, 1995). The bathypelagic zone is a vast and relatively stable habitat, with few physical barriers and the major oceanic currents exchanging completely the water masses below 1500 m every few hundred years (Madin and Madin, 1995).

This stability of the meso- and bathypelagic zones and the continuity of the ocean basins provide limited scope for isolation and evolution in the mid-water realm (Van Der Spoel, 1994). On a global scale, the distribution of pelagic fauna is determined by the characteristics of the major water masses, with different species adapted to certain combinations of temperature, salinity and dissolved oxygen (Angel and Fasham, 1973, 1974, 1975; Fasham and Foxton, 1979). For example, Crosnier and Forest (1973) noted that the distributions of caridean and penaeid shrimp from the eastern Atlantic Ocean differ from the Mediterranean Sea. These authors proposed that physical (high currents under 100 m at the Gibraltar Strait), chemical (higher temperature and salinity in Mediterranean waters) and geographical (shallow sill depth at the Gibraltar Strait) factors were the cause for the different faunal compositions observed (Crosnier and Forest, 1973). At smaller scales, there are spatial, temporal and physiological aspects (feeding behaviour and reproduction) determining species distributions (Madin and Madin, 1995).

Still, despite the apparent stability and continuity of the oceans, the pelagic habitat supports a relatively high biomass when taking into account the dimensions of the environment. Also, our knowledge of the midwater faunal composition and dynamics is still very poor. The development of new sampling techniques (such as the use of submersibles allowing the collection of delicate gelatinous organisms) and

further research programmes, will increase the number of species identified and enlarge our understanding of the largest environment on Earth.

1.2.4- Hydrothermal vent and cold seep environments

The mid-ocean ridges are very active and dynamic systems found along the centre of the oceans. They are a continuous and branching formation 75000 km long. In the late 1960s, a model of the earth's crustal structure was developed, and the Plate Tectonic theory was established. The dynamic system of lithospheric plates could then be understood, with ocean crust being formed at the mid-ocean ridges and consumed by subduction at the trenches of active margins (Summerhayes and Thorpe, 1996).

Mid-ocean ridges are found at 2.5-4 km depth, occupy about 33% of the ocean floor. They are often segmented by transform faults. In the Pacific, the East Pacific Rise (21°N, 13°N, 9°N, 17°S, 20°N), the Juan de Fuca Ridge (46°N, 130°W) and the Galápagos Rift (0°N, 86°W) are the best known hydrothermal vent fields. In the Atlantic, the seafloor is divided from north to south by the Mid-Atlantic Ridge. Trenches, where ocean crust is being destroyed by subduction, are the deepest parts of the oceans, with depths reaching 11 km at the Mariana Trench. These are part of the active continental margins characteristic of the Pacific, and are associated with seismically active areas (Gage and Tyler, 1991).

Hydrothermal vents were first discovered in 1977 on the Galápagos Rift (Grassle, 1986; German et al., 1995; Van Dover, 2000). The discovery of these new environments was the result of a series of surveys. The diverse studies that led to the discovery of hydrothermal vents include hydrothermal convection by heat-flow mapping (Williams et al., 1974), the description of a chain of mounds by Deep-Tow photography, the detection of clam shells (Lonsdale, 1977), and the detection of ^{3}He anomalies (Weiss et al., 1977; Jenkins et al., 1978; Lupton et al., 1980). Since then, the systematic study of mid-ocean ridges has led to the discovery of new hydrothermal active sites. The number of described, named and sampled hydrothermal vents has increased steadily.

The rate of new crust formation defines different types of spreading centres (Grassle, 1985; Rona et al., 1986; Cann et al., 1994; Patriat et al., 1997):

- *Fast spreading centres* with rates, e.g. 16-18 cm y^{-1} at 20°S on the East Pacific Rise (EPR), and 11-12 cm y^{-1} at 13°N on the EPR.

- *Intermediate spreading centres* (6-7cm y^{-1}) at the Galápagos Rift and 21°N EPR.
- *Slow spreading centres* (less than 2 cm y^{-1}) typical of the Mid-Atlantic Ridge.
- *Ultraslow spreading centres* (~1.5 cm y^{-1}) recently found at the SE Indian Ridge.

Hydrothermal vents on fast spreading centres are confined to a narrow band along the ridge axis in small rift valleys 100-300 m wide and 10-30 m deep. These high temperature vents are located only tens to hundreds of metres apart, forming in some cases chains several kilometres long. Contrasting, slow spreading ridges such as the Mid-Atlantic Ridge have a very different morphology. The rift valley is large, 5-12 km wide, surrounded by steep walls rising up to 1-2 km. Here, the hydrothermal vents are located either on the margin or in the centre of the large median valley, and are spaced hundreds of kilometres apart (Cann et al., 1994; German et al., 1995; Van Dover, 2000).

At spreading centres, the pre-existing crust has a high permeability, caused by cracks and fissures formed during thermal contraction. At these areas, molten rock is injected at 1200°C, which reacts with cold water filtering from the surface above. The reactions that take place produce the high temperature hydrothermal fluids emanating from the vent chimneys made of massive sulphide deposits. These hydrothermal fluids have high concentrations of H_2S, CO_2, Fe, Mn, Cu, Zn, Pb, Ca, but are depleted in Mg and sulphates. The hottest and fastest flow of hydrothermal fluids are found at the 'black smokers', where fluid rises at around 350°C from the hydrothermal chimney at rates of 1-5 m s^{-1}. When mixing with the surrounding cold water, the sulphides precipitate, producing the dark clouds characteristic of fast flow 'black smokers'. On the other hand, if the hydrothermal fluid cools down in the subsurface by mixing with entrained cold sea water, some of the sulphides precipitate before the fluid reaches the surface, giving rise to an intermediate-hot cloudy plume (100-250°C) flowing at tens of centimetres per second, the 'white smokers'. The cloudy aspect of these plumes is caused by white precipitates of barite and silica. The vent fields are also covered by clear metal-free low-temperature diffuse flow (up to 100°C) emanating from cracks and fissures on the sea floor (McDonald et al., 1980; Edmond et al., 1982; Grassle, 1986; Gage and Tyler, 1991; Cann et al., 1994; German et al., 1995). The hydrothermal vent fluid is buoyant, and rises as a plume in the water column while mixing with the surrounding water, until it reaches its neutral buoyant level. In the case of black

smokers, the hydrothermal plume can be found hundreds of metres above the vents (Weiss et al., 1977; Cann et al., 1994; German et al., 1995; Speer and Helfrich, 1995).

The active life of hydrothermal vents is episodic, with life spans of 1-10 years in the Pacific. This was supported by the calculation of heat loss in a balanced heat budget over geological time, by the small volume of sulphide deposits and by the presence of large amounts of dead vesicomyid clam shells (McDonald et al., 1980; Ballard et al., 1982; Turner and Lutz 1984; Desbruyères, 1995). Moreover, the vents present fluctuations in temperature and the emission of hydrothermal fluids (Ballard et al., 1982; Johnson et al., 1988; Tunnicliffe and Juniper, 1990), and this unstability has an important impact in the colonisation and establishment of faunal communities (sections 3.3 and 3.4, this chapter).

The discovery of the hydrothermal vent fauna has been one of the most exciting and unexpected findings in oceanography during the second half of this century. A series of *Alvin* dives during February and March 1977, between the Galápagos islands and mainland Ecuador, showed for the first time the striking hydrothermal vent communities. These ecosystems have often been referred to as deep-sea oases, because of their high biomass and fast growing organisms (Corliss et al., 1979; Turner and Lutz, 1984; Grassle, 1985; Van Dover, 2000). For the first time, samples of large vesicomyid clams, mussels, limpets and tube worms were collected and brought to surface for examination and identification (Corliss et al., 1979). Since then, biological studies at these sites have been systematically undertaken, and both the list of species identified, some new for science, and our knowledge of the vent fauna ecology have increased rapidly.

The hydrothermal vent fauna shows important differences between Atlantic and Pacific vents. The Atlantic vents are dominated by dense aggregations of motile caridean shrimp (*Rimicaris exoculata, Chorocaris chacei* and *Mirocaris fortunata*) and mussel beds (*Bathymodiolus* sp) (Rona et al., 1986; Segonzac et al., 1993; Van Dover, 1995; Van Dover et al., 1988, 1996). In contrast, the Pacific vents have dense communities of large vesicomyid clams (*Calyptogena magnifica*), mytilid mussels (*Bathymodiolus thermophilus*) and vestimentiferan worms (*Riftia pachyptila, Tevnia, Ridgeia, Piscea*) (Lonsdale, 1977; Corliss et al., 1979; Hessler et al., 1985; Desbruyères, 1995). Together with these more conspicuous species, a high number of smaller inhabitants have been identified. These include amongst others, the limpets (*Neomphalus fretterae*) and other gastropods that colonise the tubes or shells of larger

species, the polychaetes (*Paralvinella* sp, *Alvinella pompejana*, *Branchypolynoe seepensis*), the crabs (*Cyanagraea sp.*, *Bythograea* sp., *Segonzacia* sp., *Munidopsis* sp.), the shrimps (*Alvinocaris* sp.), anemones and an ophiuroid (*Ophioctenella acies*) (Grassle, 1985, 1986; Tunnicliffe 1988; Hashimoto et al. 1995; Tyler et al., 1995; Van Dover 1995).

The hydrothermal vent environment is sustained by an autochthonous source of organic matter driven by geochemical energy through chemoautotrophic sulphur-oxidising bacteria. The free-living bacteria grow in direct contact with hydrothermal fluid in a variety of habitats. The bacteria are found below rocky surfaces, on the rocks and walls of chimneys as microbial mats, as episymbionts on external surfaces of vent organisms, or suspended in the hydrothermal plume (Grassle, 1986; Winn et al., 1986; Gage and Tyler, 1991; Van Dover and Fry, 1994). Later studies have shown that not only were free-living bacteria crucial for sustaining such impressive fauna, but also symbiotic bacteria occurred in the tissues of the dominant vent species. Such symbiosis have been described for the vesicomyid *Calyptogena magnifica*, the mytilid *Bathymodiolus thermophilus*, the tube worm *Riftia pachyptila* and the shrimp *Rimicaris exoculata* among others (Cavanaugh et al., 1981; Grassle, 1985; Jannasch and Mottl, 1985; Gage and Tyler, 1991; Tunnicliffe, 1991; Segonzac, et al., 1993).

The chemoautotrophic bacteria are the primary producers in the hydrothermal ecosystem. They use the reduced sulphur-containing inorganic elements from the hydrothermal fluids for chemosynthetic reactions. Sulphur-oxidising bacteria are the most common, as the reactions involving sulphur, sulphide or thiosulphide produce the highest free energy yields. Manganese, methane, nitrites and hydrogen are also oxidised, with oxygen being the electron acceptor (Jannasch and Mottl, 1985; Grassle, 1986; Johnson et al., 1986; Gage and Tyler, 1991). These microorganisms are the primary producers at the base of the hydrothermal vent food chain. They sustain large communities of macro- and megafauna, which show rates of metabolism, growth and reproductive output several orders of magnitude higher than the rates of related species found in the non-hydrothermal deep-sea benthos (Grassle, 1985; Lutz et al., 1994).

The cold seeps are another chemoautotrophic environment located in the Atlantic and Pacific Oceans, as well as in the Mediterranean Sea. They are found from 350 to 6000 m, on both passive (Louisiana slope, Florida Escarpment, continental slope off central California, North Carolina, Gulf of Guinea) and active margins

(compressive margin off Oregon, Nankai Accretionary Prism, Barbados Accretionary Prism, Eastern Mediterranean, Monterey Bay, Japan and Kurile Trench, Aleutian Trench) (Sibuet and Olu, 1998). Cold seeps are characterised by seepage of methane-rich fluid of thermogenic and/or biogenic origin. Production of sulphide by sulphate reduction is also important. There are no significant temperature anomalies (Paull et al., 1984; Sibuet and Olu, 1998). As at hydrothermal vents, dense communities of highly productive invertebrates grow at cold seeps based on chemoautotrophic microorganisms (Paull et al., 1984). The most conspicuous inhabitants of cold seep ecosystems are large vesicomyid clams, mytilid mussels, vestimentiferan tubeworms and cladorhizid and hymedesmiid sponges. This faunal composition is similar to the one found at the Pacific hydrothermal vents, but there are significant differences in species composition, diversity and abundance (Gage and Tyler, 1991; Sibuet and Olu, 1998).

1.3- Reproduction

1.3.1- Early reproductive processes: gametogenesis

Larval development, metamorphosis and settlement are the major processes in reproduction that have important ecological and biogeographical effects. But, leading to the hatching of larvae, there is a whole sequence of reproductive processes. These include meiosis of germ cells, the differentiation, growth and maturation of gametes, spawning events and fertilisation, which might be influenced by both phylogeny and environmental factors.

Because many aspects of life history are constrained by gametogenesis (the production of male and female gametes), this is one of the most important steps in reproduction. The eggs are one of the most valuable single cells in the life history of any species. They are highly specialised, very large cells, which, following activation, initiate the processes of embryogenesis (Eckelbarger, 1983; 1994). It not only gives half of the genetic information and all the mitocondrial DNA to the zygote in sexually reproducing species, but also provides the energetic reserves for embryo development and, in the case of lecithotrophy, sustains the larva until metamorphosis and settlement (Jaeckle, 1995).

With the exception of the Porifera, egg production occurs in the female reproductive organs. These vary in shape and complexity, from loose groups of germ cells found in cnidarians, to the distinct complex organs (ovaries) of the majority of

higher taxa, where different types of cells are organised and have specific roles (Eckelbarger, 1994; Jaeckle, 1995). The production and maturation of oocytes is known as oogenesis. These processes involve the differentiation of germ cells (the oogonia) into previtellogenic oocytes. The previtellogenic oocytes are relatively small with a large nucleus and eccentric nucleolus, and a basophilic cytoplasm that stains purple with the routine staining Haematoxylin and Eosin. The previtellogenic oocytes grow to a species-specific size before undergoing vitellogenesis. The vitellogenesis involves the synthesis and storage of ooplasmic reserves (yolk) in the oocytes and is the longest and most expensive process during oogenesis. The common term of yolk includes a variety of elements, such as lipids, proteins, carbohydrates, pigments, free sugars, free amino acids, nucleotids and nucleic acids (Krol et al., 1992; Eckelbarger, 1994).

There are three major vitellogenetic pathways in invertebrates: autosynthetic, heterosynthetic and mixed. In species with autosynthetic vitellogenesis, there is an uptake of exogenous low molecular weight precursors and the subsequent synthesis of vitellin by the oocyte proteosynthetic organelles. In species with heterosynthetic vitellogenesis, there is a transport of externally synthesised yolk proteins into the oocyte. And finally, the mixed pathway is a combination of the first two (Eckelbarger, 1983, 1994; Jaeckle, 1995).

Because of the time-consuming processes that take place during yolk synthesis in autosynthetic vitellogenesis, these oocytes show a slow growth rate, while oocytes maturing through heterosynthetic production of yolk can show faster growth rates (Eckelbarger, 1986). A continuum is established between species that have heterosynthetic vitellogenesis with fast egg production and autosynthetic vitellogenesis with slow egg production. The consequences of these different patterns in life history strategies are discussed in the following chapter.

The developing oocytes are usually accompanied by accessory cells that play a trophic role during vitellogenesis, and vary in origin, form and function. Somatic follicle cells give mechanical support to oocytes, are involved in synthesis of metabolites and secondary compounds, and might also be involved in the reabsorption of degrading cells. Nurse cells are germ cells in origin, which abort their development and do not complete cytokinesis, maitaining therefore cytoplasmic bridges with the developing oocyte. These connections are used for the transport of metabolites to the oocyte. Finally, nurse eggs are other abortive germ cells, which are then phagocytosed by healthy maturing oocytes as a source of nutrients (Eckelbarger, 1994).

The vitellogenic patterns of an organism are phylogenetically constrained, and are therefore species-specific, having major implications in reproductive traits such as rate of egg production, fecundity and larval development (Eckelbarger, 1983, 1994; Eckelbarger and Watling, 1995; Van Dover and Williams, 1991). At the same time, and because of the high energetic requirement for egg production, the gametogenic processes are affected by external factors, such as food quantity and quality or habitat stability. Low food levels or poor food quality can cause a decrease or even an interruption in yolk synthesis, slowing down the egg production rate and decreasing fecundity (Qian and Chia, 1991; Bridges et al., 1994; Eckelbarger, 1994; Levin et al., 1994, see Chapter 2, this thesis). As a result, even though the vitellogenetic pathways are genetically determined and will not switch from one strategy to another depending on environmental factors, habitat variability can affect the final reproductive output of a species by means of slowing down vitellogenesis or changing the energy allocation to reproduction.

1.3.2- Reproductive patterns of non-hydrothermal vent deep-sea invertebrates

Several decades ago, two major rules concerning reproduction of invertebrates were established and were widely accepted for many years. The first one, known as Orton's rule, suggested that breeding was controlled by sea temperature. Species living in isothermal environments, such as the deep-sea or polar waters, were expected to reproduce continuously (Orton, 1920). The second prediction, Thorson's rule, was that polar and deep-sea species would have direct development because of the high mortality risk associated with a long larval life (Thorson, 1950). Thorson predicted that polar and deep-sea species would have similar early life history strategies with low fecundity and large eggs for direct development.

These ideas were long accepted, but it is now known that the production of large rich eggs in most deep-sea species leads to a lecithotrophic larval development, and that some species produce seasonally a high number of small eggs that develop into planktotrophic larvae (reviewed in Tyler, 1988; Young, 1994). Quasi-continuous gametogenesis with the production of a few large yolk-rich eggs and lecithotrophic development is the most common reproductive pattern found amongst many polar and deep-sea invertebrates (Clarke, 1979; Gage and Tyler, 1991; Young, 1994). In the abyssal plain environment, the low and relative constant energy availability supports a

slow growth rate, continuous production of relatively large eggs and low fecundity. This strategy is common in molluscs, crustaceans and echinoderms.

In 1967, George and Menzies (1967) reported indications of seasonal breeding in isopods from the Scotia Sea. A year later, the same authors gave further evidence for seasonal breeding cycles in deep-sea isopods, suggesting that their cyclic reproductive activity could reflect their shallow water origin (George and Menzies 1967, 1968). However, Rokop (1977) suggested that these data were equivocal, because the samples had been obtained from different depths, locations and years. At the same time, Rokop presented data for evidence of seasonal reproduction in the brachiopod *Frieleia halli* and the scaphopod *Cadulus californicus* from 1350 m depth in the San Diego Trough (Rokop, 1977). Later, the evidence on the seasonal deposition of phytodetritus to the deep-sea bed (Billett et al., 1983; Lampitt, 1985; Rice et al., 1991), and the simultaneous studies on the benthic responses, showed that there were exceptions to the 'rule' in many groups (Tyler et al., 1982c; Tyler, 1988 and Gooday and Turley, 1990).

Seasonal reproduction is commonly associated with an annual reproductive phase synchronised within the population, relatively small eggs and high fecundity. These reproductive patterns are found in the ophiuroids *Ophiura ljungmani* and *Ophiocten gracilis* (Gage and Tyler, 1981a,b; Sumida et al., *in press*), the echinoids *Echinus affinis, E. alexandri, E. acutus, E. elegans* and *Stylocidaris lineata* (Tyler and Gage, 1984b; Gage et al., 1986; Young et al., 1992), the asteroids *Dytaster grandis* and *Plutonaster bifrons* (Tyler and Pain, 1982; Tyler et al., 1990), the bivalves *Ledella pustulosa* and *Yoldiella jeffreysi* (Lightfoot et al., 1979; Tyler et al., 1993), the anemones *Paracalliactis stephensoni, Phelliactis robusta* and *Amphianthus inornata* (Van Praet and Duchateau, 1984; Brondson et al., 1993) and the sponge *Thenea abyssorum* (Witte, 1996).

The fact that several deep-sea invertebrates show seasonal reproduction (or synchronous gametogenesis) has been demonstrated clearly. However, the ways by which these species undergo synchronous reproduction is not so clear, and diverse hypotheses have been proposed. The study of several abyssal echinoderm species from the NE Atlantic show different ways of coupling synchronous reproductive patterns to the seasonal phytodetritus falls. The input of food to the system provides a surplus of energy, which could fuel gametogenesis, providing a seasonal cue to the otherwise relatively constant low-nutrient abyssal plain environment (Tyler, 1988; Gage and Tyler, 1991; Campos-Creassey et al., 1984).

Eckelbarger and Watling (1995) suggested several types of reproductive patterns related to phytodetritus falls that might occur in NE Atlantic invertebrates. In fast egg-producing species, the arrival of a phytodetrital pulse might initiate gametogenesis, with rapid oogenesis followed by a spawning event. In slow egg-producing species, a seasonal organic input can initiate and synchronise vitellogenesis, with a spawning event after a long gametogenic period. It can also synchronise the spawning event when there is a high concentration of food available for the larvae in the water column. In such a case, the reproductive responses and adaptations of each species will reflect their own ancestry, while the phytodetrital pulse only synchronises the pre-established reproductive cycle. Quoting Eckelbarger and Watling (1995): "In those species showing a reproductive response to seasonal organic fluxes, the proximate cause of their seasonal reproductive pattern is the seasonal phytodetrital pulse while the ultimate cause is the vitellogenic mechanism encoded in their phylogenetic history."

1.3.3- Reproductive patterns of hydrothermal vent and cold seep invertebrates

Since the discovery of the hydrothermal vents and their chemosynthetic communities, many studies have focused on taxonomical, physiological and autoecological studies (Tunnicliffe, 1991; Childress and Fisher, 1992; Segonzac et al., 1993; Van Dover, 1995; Gebruk et al., 1997; Tunnicliffe et al., 1998; Van Dover, 2000). However, from the ~500 species described from hydrothermal vents and cold seeps, fewer than ten have been studied for their reproductive biology, and the whole life cycle of a single vent or seep species is not known (Tyler and Young, 1999).

Although the information available is limited, the reproductive patterns of vent and seep species are diverse (reviewed in Tyler and Young, 1999). The oogenic processes are similar to closely related non-vent species and therefore seem to be phylogenetically constrained (Lutz et al., 1984; Van Dover et al., 1985; Bouchet and Warén, 1994; Gustafson and Lutz, 1994; Ramirez Llodra et al., 2000). The vent and seep faunas have physiological adaptations (mainly in nutrition, respiration and thermal adaptations), but a unique hydrothermal life history strategy has not evolved (Van Dover et al., 1985; Tyler and Young, 1999).

Because of the lack of seasonal sampling programmes at vents and seeps, little information is available on the synchrony or seasonality of gametogenesis. However, the oocyte-size frequency distribution of several species gives some information on egg

production patterns. With the continuous energy availability in vents and seeps, rapid growth and continuous reproduction would be possible, and is in fact the case for many species (Lutz et al., 1984; Gustafson and Lutz, 1994; Lutz et al., 1994). However, there is an important diversity of reproductive patterns between species and within congeneric species. For example, in the polychaetes from the genus *Paralvinella, P. pandorae* appears to reproduce semi-continuously, while *P. palmiformis* and *P. grasslei* have discrete breeding periods (McHugh, 1989; Zal et al., 1995). In the giant clams of the genus *Calyptogena, C. kilmeri* seems has seasonal reproduction while the rest of the group (*C. magnifica, C. pacifica, C. soyae, C. lauberi* and *C. phaseoliformis*) all show continuous reproduction (Endow and Ohta, 1980; Berg, 1985; Fiala-Medioni and Le Pennec, 1989; Lisin et al., 1996). Similarly, species of the mytilid bivalves *Bathymodiolus* have different periodicity patterns, with *Bathymodiolus* nov. sp. from EPR and MAR showing a high synchrony, *B. childressi* and *B. puteoserpentis* probably also being synchronous, and *B. thermophilus* and *B. elongatus* having continuous reproduction (Berg, 1985; Hessler et al., 1988; Le Pennec and Beninger, 1997; Comtet and Desbruyères, 1998; M. Baker, pers.com.). In the caridean shrimps *Rimicaris exoculata, Chorocaris chacei* and *Mirocaris fortunata*, the oocyte-size frequency data show the simultaneous development of two cohorts of oocytes, suggesting that there is no seasonality of reproduction (Ramirez Llodra et al., 2000).

Sperm morphology of vent species presents specific physiological modifications that are thought to be adaptations for fertilisation success in a dynamic environment (Eckelbarger, 1994; Tyler and Young, 1999). The release of gametes of vent and seeps organisms seems to ensure fertilisation success in a turbulent environment and protect the developing embryos from the highly toxic hydrothermal fluids. A high number of species have copulation or pseudo-copulation such as alvinellid polychaetes or archeogastropods (Gustafson and Lutz, 1994; Tyler and Young, 1999). Southward and Coates (1989) found sperm masses of the pogonophoran *Ridgeia piscesae* near the gonopores of females, and Cary and co-workers (1989) suggested that the sperm bundles of the vestimentiferan *Riftia pachyptila* would swim from the male to the female tube where the eggs would be fertilised. Also, the dense associations in free spawning species such as the vestimentifera would increase the fertilisation success before the gametes are diluted (Tyler and Young, 1999).

1.3.4- Larval development in the deep sea

An important aspect in ecological studies, which has not always been taken into account, is that most benthic organisms spend a long time of their early life in the water column as planktonic larvae (Giangrande et al., 1994; Ekman, 1996). The study of the larval stage plays a major role in the understanding of life history strategies, biogeography and community dynamics of invertebrates (Eckman, 1996).

In his review of 1950, Thorson classified larval development depending on their nutritional mode and development location. He named planktotrophic larvae as those that feed and develop in the water-column, and lecithotrophic as those that obtain their energy from yolk substances in the egg. The latter were divided into pelagic lecithotrophs, which develop in the plankton, and brooded lecithotrophs, which do not have a planktonic phase.

Nowadays, Mileikovsky's classification (Mileikovsky, 1971, 1974) has been largely adopted. This includes 4 types of development: 1)-pelagic development (in the plankton), 2)-demersal development (near-bottom layer), 3)-direct development (offspring hatch as juveniles) and 4)-viviparity (where the development into juvenile occurs within the parental organism) (Mileikovsky 1971, 1974; Levin and Bridges, 1995). Planktonic propagules can either be planktotrophic or lecithotrophic. Generally, species with a planktotrophic strategy would produce a large number of small eggs (<150-200μm), while lecithotrophs produce a small number of large and rich eggs (>200μm) (Jaekle, 1995; Levin and Bridges, 1995). Planktotrophic larvae feed on bacteria, phytoplankton, zooplankton or detritus, and therefore have well-developed swimming and feeding appendices. Some spend a long time in the water column. On the other hand, lecithotrophic larvae depend on the parental investment in the egg, and obtain their energetic resources from the egg yolk. The first pattern favours wide dispersal, high gene flow and wide geographical range, but mortality is very high. The second pattern implies a high maternal investment in the egg, which enhances the chances of larval survival, but has a lower dispersal potential (but see Gustafson and Lutz, 1994; Shilling and Manahan, 1994) and lower mutation rates (Gage and Tyler, 1991; Levin and Bridges, 1995; Morgan 1995).

A different classification of larval types is based on their dispersal potential and was first proposed by Scheltema (1971). Four categories are found in this classification: 1)- Teleplanic larvae with planktonic periods exceeding two months, 2)-Actaeplanic for larvae that remain in the plankton from one week to less than two months, 3)-Anchiplanic larvae with planktonic phases ranging from a few hours to a few days, and

4)-Aplanic for non-planktonic larvae (Scheltema, 1971, 1989; Levin and Bridges, 1995).

For decades, ideas of deep-sea larval development were seen under Orton's and Thorson's rules (section 1.3.2, this chapter), and there was a common belief that deep-sea and polar species would reproduce continuously and have direct development. There is now evidence of seasonal reproduction in several abyssal species (section 1.3.2, this chapter), and planktotrophic and lecithotrophic larval development are the rule more than the exception in deep-sea invertebrates (Gage et al., 1986; Tyler et al., 1990; Gage and Tyler 1991; Bouchet and Warén, 1994; Pearse, 1994; Sheltema, 1994).

It has long been accepted that planktotrophic larvae have higher dispersal capabilities than lecithotrophic larvae (Levin and Bridges, 1995; Vrijenhoek, 1997). Hydrothermal vent and cold seep organisms would therefore be expected to have planktotrophic development, in order to ensure dispersal and colonisation in discrete, isolated and ephemeral environments (Lutz, 1988; Van Dover et al., 1988; Lutz et al., 1994; Mullineaux et al., 1996). But, with the exception of the mytilid mussel *Bathymodiolus*, most vent and seep species produce relatively large eggs indicative of lecithotrophic development (Lutz, 1988; Tyler and Young, 1999).

There is now evidence that the dispersal potential of larvae is not always directly related to larval type, and to understand the full dispersal capabilities of an organism, physiological and environmental factors need to be taken into account. Shilling and Manahan (1994) showed that several lecithotrophic larvae of Antarctic echinoderms used up to 50% of their energetic reserves in 60 months while this amount of energy was used in only 10 months in planktotrophic larvae. Moreover, the survival time of larvae hatching from eggs of similar size was much larger in polar species than in temperate species (Shilling and Manahan, 1994). The uptake of dissolved organic matter by some polar larvae and the effect of low temperatures of polar and deep-sea waters on reducing metabolic rates, may permit lecithotrophic larvae to have longer planktonic periods under their own nutrient reserve (Lutz et al., 1980; Lutz, 1988; Manahan, 1990; Gage and Tyler, 1991; Gustafson and Lutz, 1994; Shilling and Manahan 1994). There is also evidence of lecithotrophic vent larvae found in the hydrothermal plumes and dispersing in near-bottom flows, suggesting that this larval type could have high dispersal potential through physical transport in water masses (Berg and Van Dover, 1987; Mullineaux et al., 1995, 1996; Kim and Mullineaux, 1998). Moreover, the highly productive hydrothermal habitat is surrounded by vast

volumes of oligotrophic waters, and developing independently of external food availability could have been selected allowing for long dispersal periods in a food-poor pelagic system (Tyler and Young, 1999).

1.4- Aims of the study

The main objective of this study was to analyse the reproductive patterns of a variety of deep-sea invertebrate taxa from environments with different energy availability. Closely related taxa (same species when possible) from different environments were studied, focusing mainly on three reproductive traits: gametogenesis, egg size and fecundity.

The hypotheses to be tested were:

- Gametogenic patterns are genetically determined and therefore phylogenetically constrained.
- Reproductive output, quantified as fecundity and egg size, is not a conservative life history trait and varies with environmental factors. Environments with higher energy availability (higher food quantity or quality) would support a higher reproductive output and *vice versa*.

CHAPTER TWO- FECUNDITY AND RELATED LIFE HISTORY TRAITS IN MARINE INVERTEBRATES: A REVIEW

2.1- Introduction

2.1.1- What is fecundity?

In the last 80 years, the ecological consequences of parental investment per offspring in marine invertebrates has received substantial attention, in order to understand the evolution of reproductive patterns (Orton, 1920; Thorson, 1950; Pianka, 1970; Vance, 1973Stearns, 1992; Levitan, 1993, 1996; Podolsky and Strathmann, 1996). Within the reproductive traits of a species, fecundity is one of the major variables affecting their life history.

In broad terms, fecundity refers to the number of offspring produced by a female. Fecundity may be expressed as the number of oocytes, eggs or embryos produced over a certain period (breeding season, year, lifetime). Most studies refer to fecundity as the number of eggs per female in one breeding season, but life-time fecundity, although difficult to quantify, is of major interest in understanding the life history of a species. Also, in species that have several broods in one breeding season, the term brood size is used to define the number of eggs produced during one breeding event, while fecundity refers to the total number of eggs produced during the whole breeding season (Fonseca-Larios and Briones-Fourzan, 1998).

Anger and Moreira (1998), working with decapods, distinguished three categories of fecundity:

•*Potential fecundity*, defined as the number of oocytes in the ovary, including developing and mature cells.

•*Realised fecundity*, defined as the number of eggs carried in the pleopods.

•*Actual fecundity*, defined as the number of hatched larvae, and therefore related to fertilisation success and embryo mortality.

The terms potential and actual fecundity have also been used in studies of echinoderm reproduction, the former referring to all oocytes in the ovary and the later to oocytes nearing maximum development (Tyler and Gage, 1983; Tyler and Billett, 1987). Here, the definition of actual fecundity varies slightly from the one used for decapods, because the number of fertilised eggs hatching into larvae is very difficult to quantify in free-spawners.

2.1.2- Quantification of fecundity

Amongst invertebrates, two major groups can be identified depending on their spawning patterns. Firstly, the broadcast spawners release their mature gametes into the water column where the eggs are fertilised and the embryos develop into larvae. Secondly, there are species with internal fertilisation where the embryos develop in batches of eggs incubated in brooding structures on the female body.

In brooding species, fecundity can be quantified by counting directly the number of eggs in the brooding chamber. Many decapod crustaceans are good examples, where embryos develop attached to the pleopods of the female, providing very easy access to the total egg mass and therefore allowing for a precise quantification of fecundity. This has made the decapods a very good group for studies on fecundity and related life history parameters (Clarke, 1979, 1987, 1993a,b; Corey and Reid, 1991; Reid and Corey, 1991; Gorny et al., 1992).

Within decapods, two methods have been used to calculate the number of embryos produced by an individual, and this is dependent on the size and number of eggs carried on the pleopods. In species that brood few large eggs, all the eggs can be carefully removed from the abdomen with a spatula and counted directly under a stereomicroscope (Clarke, 1993b). For species that produce a large number of small eggs, the total egg mass can be weighed, and three subsamples of 100 eggs counted and weighed. From the average weight of the three 100 egg aliquots and the weight of the total egg mass, the total number of eggs can be estimated (King and Butler, 1985; Clarke, 1993b). This quantification can be obtained by using wet weight, or by drying the egg mass and the subsamples of eggs for dry weight (Anger and Moreira, 1998; Wehrtmann and Andrade, 1998).

Fecundity estimates in decapod crustaceans are very precise, although some aspects need to be taken into account. Firstly, the number of eggs carried on the pleopods of a decapod is physically limited by the space available on the female's abdomen. Therefore, larger females carry more eggs (Barnes and Barnes, 1968; King and Butler, 1985; Ivanova and Vassilenko, 1987; Mauchline, 1988; Corey and Reid, 1991; Reid and Corey, 1991; Clarke, 1993b). If we were to compare fecundity between species, the number of eggs needs to be expressed in relation to female size, such as number of eggs per 1g of female weight or per 1mm of carapace length (Barnes and Barnes, 1968). Secondly, during embryo development egg size increases and some eggs are extruded from the brood because of space limitation (Gorny et al., 1992;

Ohtomi, 1997; Thessalou-Legaki and Kiortsis, 1997; Wehrtmann and Andrade, 1998). Thirdly, there is often a high variability in the number of eggs carried by large females mainly caused by loss of embryos during sampling. In many species the embryos are not protected but are exposed to the external environment, and in well developed broods embryo loss is common during sampling and sorting (Gorny et al., 1992; Bell and Fish, 1996; Wehrtmann and Andrade, 1998).

In free-spawning species, the eggs are not available for analysis until they are released into the water column. For most species, the timing of the spawning event cannot be predicted and observations in the natural environment is a matter of chance. In the laboratory, there are several techniques that can be used to induce spawning in order to obtain viable gametes for experimentation.

The release of eggs and sperm can be induced by different factors such as changes in the environmental conditions in the laboratory or injection of a spawning-inducing chemical. For example, the abalone *Haliotis tuberculata* or the bivalves *Mytilus edulis*, *Placopecten magellanicus* and *Macoma balthica* can be induced to spawn in captivity by thermal shock (Thompson, 1979; Langton, 1987; Honkoop, 1997; M. Baker, *pers. com.*). The reef-building polychaete *Phragmatopoma lapidosa* is naturally induced to spawn during storms by physical disturbance, and this effect of wave action can be simulated in captivity by extracting the worm from its tube. The naked worms start spawning within a few minutes of having been extracted from their tubes (D. McCarthy, *pers. com.*; E. Ramirez Llodra, *pers. obs.*). Because of the easiness of obtaining viable gametes and experimenting with them, the echinoderms are one of the most utilised groups for reproductive studies on egg and sperm morphology, egg size and number and fertilisation success. In echinoids, an injection of 2 ml of 0.55 M KCl induces the muscles of the gonad wall to contract and ripe males and females will spawn within 5-10 minutes (Young et al., 1997, 1998). In asteroids, the hormone 1-Methyladenine is produced by follicle cells during oogenesis, inducing the resumption of meiosis in primary oocytes and the contraction of gonadal muscles (Kanatani, 1975; Walker, 1980). An injection of 1-Methyladenine in the coelom induces the gametes to finish maturation and ripe specimens spawn between 30 minutes and 18 hours after the injection (McEdward and Colter, 1987). Because of the large number of eggs produced by many broadcast spawners, to quantify fecundity it is necessary to spawn the females in a known volume of water. When all the eggs have been released, a subsample of

known volume (i.e. 1 ml) is taken from a well-mixed egg solution and the eggs are counted under a microscope. The fecundity can be estimated from the number of eggs in the subsample and the total volume of egg solution.

An indirect estimate of fecundity has been used in some reproductive studies of free-spawning invertebrates. This is a non-destructive method consisting in calculating the wet weight of the female before and after spawning. The difference in the pre- and post-spawned weights is used as a relative measure of fecundity to compare gamete production within species in different years, different populations or at different ages (Langton et al., 1987). If the weight of an ovum is a known parameter for the species, fecundity can then be estimated from the weight of gamete produced and the average weight of ova (Barber et al., 1988).

The second group of methods are used for species that cannot be induced to spawn, or when live specimens are not available, e.g. for most deep-sea fauna. In this case, quantifying fecundity is much more difficult, and only a broad estimation can be made. In species that produce a few large eggs or when oocytes are not strongly attached to the gonad, the dissection of the ovary may be sufficient to extract the oocytes. This method has been used in the bivalve *Yoldia notabilis* (Nakaoka, 1994), the corals *Antipathes fiordensis* (Parker et al., 1997), *Goniastrea aspera* (Sakai, 1998), *Acropora intermediata, A. millepora* and *A. hyacinthus* (Smith and Hughes, 1999) and the asteroid *Leptasterias epichlora* (George, 1994a). In the hydrothermal vent polychaete *Paralvinella grasslei*, the oocytes mature free in the coelom and are obtained by extracting the coelomic fluid with a pipette through a small incision in the body wall (Zal et al., 1995). Similarly, the mature eggs of the vestimentiferan *Riftia pachyptila* accumulate at an enlarged extremity of the oviduct and can be extracted with a pipette, allowing to make actual fecundity estimates (C.M. Young, *pers. com.*). In deep-sea holothurians that produce a few large eggs –such as the psychropotid holothurians– or when the gonads are very small –such as in the holothurians *Cherbonniera utriculus, Molpadia blakei* or *Ypsilothuria talismani*–, fecundity has been quantified by clearing the ovary tissues with Histoclear and counting the eggs under a stereomicroscope with transmitted light (Tyler and Gage, 1983; Tyler et al., 1985a; Tyler and Billett, 1987; Tyler et al., 1987).

Finally, the most difficult species to work with in terms of fecundity are those that have oocytes developing in well-organised gonads, where inducing a spawning

event is not possible and where the mature oocytes are not visible through the gonad wall. Some deep-sea asteroids and ophiuroids are good examples. The specimens are generally dead when they reach the surface, and the gametes develop inside large gonads with a thick gonad wall. A histological method has been used to estimate fecundity in such specimens. For example, in the brittle star *Ophiura ljungmani*, Tyler and Gage (1980) estimated fecundity as the average number of oocytes counted from serial sections of a gonad and multiplying this by the number of gonads. In deep-sea asteroids where it was impossible to follow consecutive oocytes through serial sections, a different histological method was used (Ramirez Llodra et al., *in prep.*). This method involves determining the relation between gonad volume and mean oocyte volume. The methodology has been explained in detail in the material and methods section of chapter 5. This indirect quantification of fecundity gives an estimation of the number of eggs that will be spawned in the following reproductive event, but not a precise number of eggs produced. Many physiological processes are involved during gametogenesis that can change the ratio between previtellogenic and vitellogenic oocytes, such as reabsorption or phagocytosis of non-viable eggs. Therefore, the real number of eggs available for fertilisation might be different than the number of mature eggs in the ovaries. Still, in the case of free-spawners where mature eggs cannot be obtained by other laboratory methods, this is a useful estimation of fecundity.

2.1.3- Fecundity and life history theory

Fecundity has long been recognised as one of the main variables in life history strategy. It is a determinant trait, together with growth rate, age and size at maturity, egg size, age- and size-specific reproductive investment, age- and size-specific mortality, larval developmental type and time, and life-span (Wilbur et al., 1974; King and Butler, 1985; Stearns, 1992; Eckelbarger, 1994; Jaeckle, 1995; Hadfield and Strathmann, 1996; McCann and Shuter, 1997). A good knowledge of the life history traits of a species is imperative to understand the effects of natural selection (Stearns, 1992; McCann and Shuter, 1997). Accordingly, many studies have focused on the analysis of life history evolution and its consequences in the adaptation of a species to its habitat.

The life history strategy of a species is composed of a series of reproductive and growth patterns that are the result of selection for an optimal energy allocation between reproduction and somatic growth (Olive, 1985). A study of the evolution of life history

strategies requires the identification of the minimum number of selective pressures that explain the acquisition of the patterns observed, and identifying what causes differences in fitness among life history variants (Wilbur et al., 1974; Stearns, 1992). To understand a pattern of variation in a life history trait and follow its evolution, information is needed on the selective pressures generated by demography (mortality and fecundity), phenotypic and genotypic variation, and the physiological trade-offs found amongst reproductive parameters (Stearns, 1992). However, this amount of knowledge has yet to be obtained for any species, and studies in the evolution of life history vary considerably depending on which parameters are being analysed (Wilbur et al., 1974; Stearns, 1992).

The analysis of fecundity is at the centre of many studies on reproduction and life history evolution because of its relation with energy allocation and with other life history traits such as egg size or brooding frequency. Fecundity is not a highly conservative parameter within species, and varies with nutrition, population density and adult age and size (Eckelbarger, 1986). The data obtained from different taxonomic groups are very diverse, and for decades, biologists have been working on life history models to explain the evolution of reproductive traits such as fecundity, size of eggs or age at maturity. In the next sections, the relationship between fecundity and other life history variables will be discussed, and the different models that have been suggested to explain the evolution of these life history traits will be described. A thorough overview is not possible within the constraints of this thesis, but relevant examples will be presented to illustrate the specific points.

2.2- Relationship between fecundity and other life history variables

2.2.1- Fecundity and egg size

Within egg characteristics, size is probably the parameter that has received the most attention in reproductive studies (McEdward and Carson, 1987). Eggs are amongst the largest cells in an organism and represent the energetic unit invested in the next generation (Eckelbarger, 1986), showing a wide size range within and between species (Herring, 1974b; Clarke, 1979; McEdward and Carson, 1987; Mauchline, 1988; McEdward and Chia, 1991; Clarke, 1993a; Jaeckle, 1995). The effects that egg size has on fecundity, fertilisation, energy content, parental investment and larval development has interested life history biologists and ecologists for decades. The egg size-fecundity trade-off is one of the most important in life history because of its direct relation with

maternal investment in reproduction. Given a constant allocation of energy to reproduction, there is a trade-off between the number of eggs produced and their size. Therefore, species will produce either a small number of large eggs or a high number of small eggs (Thorson, 1950; Menge, 1975; Tyler and Pain, 1982; Olive, 1985; Gorny et al., 1992; Clarke, 1993a; Jaeckle, 1995; Levitan, 1996; Podolsky and Strathmann, 1996; Ohtomi, 1997).

In 1973, Vance proposed a theoretical model to predict optimal egg size, with a bimodal distribution corresponding to larval developmental type. In this model, the energy content of the egg was given a number between zero for eggs that develop into feeding larvae (planktotrophy) and one for eggs that support larval development without feeding (lecithotrophy). If energy invested in reproduction is constant, and we assume a constant mortality and linear relation between egg size and developmental time, Vance proposed a selection for extreme egg sizes. However, intermediate egg sizes are the rule rather than the exception in many planktotrophic species (Levitan, 1996). Sewell and Young (1997) conducted a study to test the hypothesis of a bimodal distribution of egg sizes in echinoderms. Although the species with planktotrophic development form a distinctive group with smaller egg sizes, there is some overlap in egg size between planktotrophic and lecithotrophic/brooding species in the holothurians, ophiuroids, echinoids and asteroids, showing that echinoderm egg sizes need to be seen as a continuum.

In studies following Vance's model, the evolution of egg size in invertebrate species has been related to different pre- and postzygotic factors, where optimal egg size is determined by the relationship between offspring size and offspring fitness. Levitan (1993) suggested that selective pressures could affect egg size evolution at three different phases of a species' life history: 1)- before the embryos or larvae enter the plankton, 2)- during the planktonic phase, and 3)- just after metamorphosis and settlement. Working with three sympatric species of echinoids, selection during the planktonic or the newly metamorphosed phases was rejected, and Levitan proposed a hypothesis to explain the evolution of egg size in marine invertebrates in terms of fertilisation success. Larger eggs would be fertilised at a greater rate, and variation in factors such as adult body size, population density, microhabitat distribution and fertilisation kinetics, which all influence fertilisation success, would allow for different sperm limitation and fertilisation patterns. This, in turn, would account for selection of different egg sizes (Levitan, 1993, 1996).

In answer to Levitan's hypothesis of a prezygotic selection for optimal egg sizes, Podolsky and Strathmann (1996) argued that within a given allocation of energy for reproduction, the increase in fertilisation obtained from larger eggs cannot alone compensate for the decrease in fecundity resulting from the production of larger eggs. These authors proposed that, although larger eggs are fertilised at a greater rate, total zygote production declines because of the loss of fecundity and that therefore, the fecundity-fertilisation trade-off would favour the division of resources into small eggs. They suggest that the advantages of maintaining large eggs are postzygotic, such as larval developmental time and survival. Factors other than gamete encounter, such as egg organic content, sperm life, parental reproductive behaviour or egg chemical attraction for sperm, would then play an important role in fertilisation success and egg size evolution (Podolsky and Strathmann, 1996).

The importance of fecundity and egg size in life history is clear, not only to understand the evolutionary aspects, but also because the biological characteristics relate to the trade-off between number and egg size. Factors such as parental investment (Jaeckle, 1995), food availability and quality (Nichols et al., 1985; Tyler et al., 1993; Campos-Creaesy et al., 1994; Brey et al., 1995; Jaeckle, 1995), fertility (Levitan, 1993, 1996), egg energy content (McEdward and Carson, 1987; McEdward and Chia, 1991; Clarke, 1993a,b; Jaeckle, 1995) or larval developmental time and mortality (McEdward and Carson, 1987; Stearns, 1992; Young and Tyler, 1993; Jaeckle, 1995; Hoegh-Guldberg and Emlet, 1997) are all related in one way or another to egg size and therefore to fecundity. For life history models to have a greater predictive power, it is necessary, although difficult, to incorporate in the model all the above factors, together with environmental heterogeneity and variability (Wilbur et al., 1974; Hadfield and Strathmann, 1996; Podolsky and Strathmann, 1996).

In his model of extreme egg size selection, Vance (1973) suggested a relation between environment and selection for reproductive strategy. When food availability in the planktonic environment is patchy or unpredictable, it would be more efficient to produce a large number of small offspring, while in the case of species that release larvae into a uniform or predictable environment the production of few large eggs would be more efficient. This relation between life history traits and environmental factors has also been expressed in a different way in the concept of *r-K* strategies (MacArthur and Wilson, 1967; Pianka, 1970). In this model, Pianka (1970) suggests a continuum between *r* and *K* selection, and the trade-off between number and size of

eggs plays again a central role. The common traits of a species with *K*-strategy is slow growth, deferred maturity, greater longevity, iteroparity, low fecundity and large yolky eggs. These traits are related to selection for maximum competitive ability in a saturated environment and evolve under density-dependent conditions in stable or predictable environments. Species with *r*-strategy have fast growth, shorter longevity, semelparity, high fecundity and small eggs. These traits correspond to selection for maximum rate of population increase and evolve in density-independent conditions in disturbed environments (Pianka, 1970; Clarke, 1979; Sanders, 1979; McCann and Shuter, 1997). An important prediction of this approach is that species with *K*-strategies have a lower individual annual reproductive effort than organisms exhibiting *r*-strategies (Clarke, 1979). Numerous examples of *K*-strategists with slow growth and production of a small number of large and rich eggs are found in the Antarctic and deep-sea benthos. In these environments, food availability is uniform or with a predictable seasonal pattern and the life history strategies have evolved under a saturated habitat (Clarke, 1979; Pain et al., 1982a,b; Tyler and Gage, 1983; Tyler and Billett, 1987; Tyler et al., 1987; Gage and Tyler, 1991).

The *r-K* theory was very popular during the 70s and 80s, and has been successfully used to explain life history patterns. Still, this model based on differences in mode of population regulation (density-dependent or independent) is too simple to explain the variability found in life histories and many populations do not match the expectancies (Wilbur et al., 1974; Stearns, 1992; Winemiller and Rose, 1992; Giangrande et al., 1994; McCann and Shuter, 1997). In the 90s, many studies of life history evolution have shifted from *r-K* theory to models using demographic parameters as the base of life history evolution (Stearns, 1992; Winemiller and Rose, 1992; Giagrande et al., 1994; McCann and Shuter, 1997). Stearns (1992) discusses these issues and proposes an age-specific (demographic) model for the evolution of life history traits. The demography theory explains the selection of optimal reproductive tactics by the balance between current reproductive output and future reproductive success (~residual reproductive value) (Wilbur et al., 1974; Stearns, 1992; Giangrande et al., 1994; McCann and Shuter, 1997; see below).

2.2.2- Fecundity and demographic parameters

The evolution of life history strategies and their plasticity determine the population dynamics of interacting species. The selection of certain life history traits is

directly related to the demography patterns of a population (Wilbur et al., 1974; Stearns, 1977, 1992; McCann and Shuter, 1997). Demography is the analysis of the effects of age structure on population dynamics and on natural selection, and has been used by Stearns (1992) to explain the evolution of life history strategies.

There are two conditions for natural selection and evolution to occur (Stearns, 1992). First, there must be heritable variability (the genotypic condition). Second, there must be variation in fitness among individuals (the phenotypic condition). Life history evolution under the demography theory assumes that the phenotype consists of demographic traits that are connected and constrained among them by trade-offs. These traits are birth, age and size at maturity, number and size of offspring, growth and reproductive investment, lifespan and death (Stearns, 1992; Winemiller and Rose, 1992). When analysing the evolution of the life history strategy of an organism, we need to know what traits have heritable variability, and how do the variable traits trade-off. Then we can apply the demography theory to understand the selection pressures affecting the phenotype (Stearns, 1992).

There is an age- and size-specific variation in mortality and fecundity connected through demography to variation in fitness. The relationship between fecundity and age depends on the life history strategy of a species. In particular, it is determined by the balance between costs and benefits in the size at first maturity. Early maturing species have a higher probability of reaching maturity simply because of the shorter time needed to reach first reproduction, but have a reduction in future fecundity. In contrast, species with delayed maturity live longer, allowing for 1)- a higher number of reproductive events, and therefore a higher lifetime fecundity, and 2)- a higher quality of offspring and parental care (Stearns, 1980, 1992).

Winemiller (1992) and Winemiller and Rose (1992) proposed a simple model to explain life history evolution. This model is based on an amplification of the *r-K* model, taking into account three important demographic parameters of the life cycle of a species. The Winemiller-Rose model (1992) proposes a continuum in a three dimensional triangle where each vertex reflects a boundary life history strategy. Each one of these three endpoint strategies is defined by three demographic parameters: age at maturity, fecundity and juvenile survivorship. The resulting endpoint strategies are 1)- the periodic strategy, with late maturation, high fecundity and low juvenile survivorship, 2)- the opportunistic strategy, with early maturation, low fecundity but

repeated breeding events and low juvenile survivorship, and 3)- the equilibrium strategy, with moderate maturation age, low fecundity and high juvenile survivorship.

The *r*-strategy is here split into periodic and opportunistic strategists. The periodic strategists share with the classical *r*-strategists the high fecundity and low juvenile survivorship, but differ in that they are large, long-lived and have late maturation. The periodic strategy maximises age-specific fecundity at the expense of turnover time (delayed maturity in order to attain a size sufficient for production of a large clutch) and juvenile survivorship (small eggs). They inhabit predictable and seasonal environments (Winemiller and Rose, 1992; McCann and Shuter, 1997).

The opportunistic strategists have most of the classical *r*-strategists traits, with small body size, early maturation, low juvenile survivorship and short life span, but differ in that fecundity per spawning event is low (although there are multiple spawning events spread over a prolonged annual spawning season). The opportunistic life-history strategy maximises population growth through a reduction in the mean generation time. Early maturation allows for short lifespan and diminishes the capability to produce large clutches and large eggs. Still, because of their multiple spawning events, the annual fecundity is high and allows for recolonisation of new habitats. They inhabit highly disturbed and unpredictable environments (Winemiller and Rose, 1992; McCann and Shuter, 1997).

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

McCann and Shuter (1997) tested the hypothesis that allometric relationships with body size for fecundity and age at maturity in fish differ across the endpoint strategies of the Winemiller-Rose model. They showed that for a given adult weight, the range of ovarian production reflects the range in reproductive strategies available for a population with that average adult weight. These authors proposed the constraints that could be limiting the observed life history strategies. The lower constraints of energy allocation to reproduction were probably determined by demographic traits of population persistence (minimum investment in eggs necessary to exactly replace the

parental pair and minimum investment in egg biomass required to ensure recruitment above a critical value). The upper constraints were determined by energetic and physical factors related to adult survivorship (amount of energy that can be allocated to the ovary, body cavity space for ovarian growth or egg mass attachment) (McCann and Shuter, 1997).

Summarising, the benefits of early maturation are short generation times and higher survival rate to maturity, while the benefits of delayed maturity are a higher lifetime fecundity, higher initial fecundity, and better quality of offspring accounting for a higher larval survival. The selection for early or delayed maturity would depend on an optimal balance between the benefits and costs of the life history traits related to age at first maturity (Olive, 1985; Stearns, 1992; Giangrande et al., 1994; McCann and Shuter, 1997).

2.2.3- Fecundity and female size and age

The relationship between female size and fecundity is a major characteristic of reproduction in crustacean decapods. There is a good positive correlation between the number of eggs carried on the pleopods and the female carapace length, with larger females carrying more eggs (Barnes and Barnes, 1968; King and Butler, 1985; Ivanova and Vassilenko, 1987; Clarke, 1993b; Corey and Reid, 1991; Reid and Corey, 1991). This relationship is a consequence of the physical space limitation between the pleopods for the attachment of the eggs (Barnes and Barnes, 1968; Clarke, 1993b). If the number of eggs produced is quantified as egg mass weight, this variable correlates to carapace volume and female weight (Corey and Reid, 1991a). This linear relationship between number of eggs and female size is important when comparing fecundity 1)- between species of different sizes and 2)- different populations within the same species. Apparent variability in fecundity might merely be a consequence of variability in female size. Therefore, in any group where the number of eggs produced is correlated to female size, fecundity needs to be expressed as number of eggs per unit of female size (i.e. 1g of body weight, or 1 mm of body size). By doing this, the variability caused by differences in adult size at different populations or species is eliminated (Barnes and Barnes, 1968).

A positive correlation between brood size and female size is also found in some opportunistic polychaetes such as *Streblospio benedicti, Ophryotrocha puerilis puerilis* and *Capitella* sp (Levin and Creed, 1986; Qian and Chia, 1991; Bridges et al., 1994;

Bridges, 1996). Opportunistic species have the ability to respond at a population level to environmental disturbance, organic enrichment or unexploited habitats, because they can rapidly converting nutrients into egg production (Eckelbarger, 1986). An increase in food availability can fuel an increase in adult body size, with larger females producing more eggs through a higher number of fertile segments, or with a size-dependent increase in resource acquisition and allocation to reproductive processes (Bridges et al., 1994). *Capitella* sp. and *Streblospio benedictii* reach maximum body size prior to first spawning. The subsequent decrease in body size may account for the decrease in fecundity of *Capitella* sp. (Qian and Chia, 1992), while the constant body size of *Ophryotrocha labronica* throughout its lifespan may account for its constant fecundity (Cassai and Prevedelli, 1999). In opportunistic species, there is a greater egg production at early ages, a decrease in age at first maturity and an increase in adult body size, causing an exponential population growth when resources are being exploited (Bridges et al., 1994). However, Bridges and co-workers (1994) found different reproductive responses to amended sediment in *Streblospio benedictii* and *Capitella* sp. They suggested that even though opportunistic species share similar characteristics in growth and reproductive rates, their life history traits are not identical. Instead, the selection for certain life history patterns may depend on species-specific sensibility to environmental factors such as oxygen level, tolerance to toxic elements, feeding behaviour and digestive physiology (Bridges et al., 1994; Bridges, 1996).

Mean adult body size of a species can also introduce constraints in reproduction, affecting the selection of reproductive traits. Cassai and Prevedelli (1999) working with polychaetes, suggested that the very small body size of *Ophryotrocha labronica* does not allow for semelparity or for the production of a large number of eggs. There is a design constraint in small species, causing selection for the production of a few large eggs in a continuous or semi-continuous rate. In small females, the capacity of egg production is too low and the offspring production in one spawning event would not be enough to maintain the population (Cassai and Prevedelli, 1999).

In molluscs, bivalves have been studied in detail because of the commercial importance of many species. In several scallops, mussels and oysters, while there is an increase in growth rate in the first years and then a decline, the production of gametes increases throughout their lifetime. With increasing age, there is a gradual transition in the allocation of energy from growth to reproduction (Rodhouse, 1978; Griffiths and King, 1979; Thompson, 1979; Kautsky, 1982; MacDonald and Thompson, 1985a,b;

Langton et al., 1987; Honkoop and Van der Meer, 1997; Honkoop et al., 1998). The inner volume of the shell is the upper limit for reproductive production and fecundity is positively correlated to shell size (Rodhouse, 1978; Nakaoka, 1994; Honkoop and Van der Meer, 1997). Because of the significant relationship between body size and fecundity, Honkoop and van der Meer (1997) proposed the use of the easily quantified body mass index to estimate the reproductive output of an individual, as in decapod crustaceans (Corey and Reid, 1991). In the scallop *Yoldia notabilis*, the increase in fecundity is related more strongly to female size than to age. The individuals of *Y. mutabilis* mature one year later in the population from the deeper site (14 m) than shallower population (10 m), but size at first maturity is similar in both populations and coincides with the size at which these bivalves escape predatory pressure by the crab *Paradorippe granulata* (Nakaoka, 1994). The production of eggs is then optimised when adult mortality is reduced, increasing female fitness. The fecundity of the mussel *Mytilus edulis* also depends more on size than on age, growth or production, resulting on maximum gonad production when excess food is available (Kautsky, 1982).

In colonial species, such as corals, analysing reproductive traits can be more arduous because of the modular characteristic of the organisms. First maturity starts when the colony reaches a certain size related to a minimum number of polyps, and fertility is heterogeneous within a colony (Parker et al., 1997; Sakai, 1998; Smith and Hughes, 1999). In the reef-building corals *Monastrea annularis* and *Antipathes fiodensis* there is no homogenous fecundity within a colony, but at the individual level fecundity increases with polyp size (Van Veghel and Kahmann, 1994) and larger colonies produce a higher overall number of eggs (Parker et al., 1997). In the massive coral *Goniastrea aspera*, there is a difference in the fertility of polyps depending on their position within the colony. The marginal polyps are rarely fertile, and function as a buffer against external disturbance, protecting the non-marginal fertile polyps. There is a minimum colony size of 60 polyps before the colony is actively reproducing, and the size of the fertile polyps is larger in reproducing colonies (Sakai, 1998). In corals, an important aspect of their life history is the dispersal and survival of fragments of the colony. Because of the dependence between colony size (or fragment size) and food intake and fecundity, small fragments have a lower probability of survival and/or of reproduction. Smith and Hughes (1999) suggested that the loss of fecundity in small fragments can be a temporal adaptation for survival, with a re-allocation of energy towards growth and repair before the new colony can be reproductively active again.

2.2.4- Fecundity and reproductive effort

Reproductive effort is the total energy used in reproduction, including 1)- the energy allocated to gonad production, 2)- the energy spent in collecting supplementary food during the reproductive period, 3)- territorial and mating behaviour and 4)- parental care (Clarke, 1987; Thessalou-Legaki and Kiortsis, 1997). This is clearly a very difficult variable to quantify with precision and therefore reproductive output is used instead. Reproductive output is quantified as the biomass of reproductive products per unit biomass of the female (Clarke, 1987; Thessalou-Legaki and Kiortsis, 1997; Anger and Moreira, 1998). When the energy available for organisms is limiting, an increase in reproductive effort results in an increase in reproductive output and a reduction in somatic investment. Moreover, because of the trade-off between egg size and number, an increase in reproductive output would be reflected by an increase in fecundity or by an increase in offspring survivorship resulting from a higher investment per individual egg. The reproductive output is therefore defined by two aspects, the quantity of eggs, or fecundity, and the quality of eggs related to offspring survival (Olive, 1985; Bell and Fish, 1996). Species with different larval strategies, that is different egg sizes and number, can have the same overall reproductive investment. The polychaete *Streblospio benedicti* has individuals with lecithotrophic development cohabiting with other individuals of the same species that produce planktotrophic larvae. While the lecithotrophic developing specimens produce eggs six times larger than the planktotrophic ones, the former produce six times fewer eggs. As a result, the reproductive investment is similar in both strategies (Levin and Creed, 1986). Similarly, studies on the fecundity and egg size of *Capitella* sp. showed that individuals from different environments had similar reproductive investment but differed in their fecundity-egg size trade-off (Qian and Chia, 1991).

There is another important trade-off, between reproductive output and residual reproductive value, where residual reproductive value is the lifetime product of survivorship and fecundity. The allocation of resources to growth, survival and reproduction has to be optimised over the lifespan of the organism, and this is affected by the trade-off between current reproduction and the subsequent adult reproductive success (Stearns, 1992; Giangrande et al., 1994). For example, the opportunistic polychaete *Capitella* sp. has a low initial reproductive effort allowing for an enhanced adult survivorship, which in turn results in a longer life-span and a higher number of reproductive events during its life (Qian and Chia, 1992).

The number and quality of eggs produced by an organism during its lifetime vary with adult age. The reproductive effort is maximised at a certain age, depending on the life history strategy of the species. In species with decreasing profit at high levels of reproductive effort, or when there is a higher mortality risk related to investment in reproduction, there would be selection for intermediate levels of reproductive effort. Conversely, when mortality increases at a certain age class, fecundity will be optimised at the previous age range (Stearns, 1992). Also, when an unstable environment increases the adult mortality risk at any time, as in the gravel amphipod *Pectengorammarus planicurus*, point reproductive success will be maximised (Stearns, 1992; Bell and Fish, 1996). Ultimately, it is not the number of surviving offspring in a single brood which is important, but the total number of offspring surviving in the lifetime of the adult, and therefore lifetime fecundity is optimised (Stearns, 1992; Bell and Fish, 1996).

2.3- Variability of fecundity with environmental factors

2.3.1- Fecundity and food availability

The production of eggs is a highly energy-demanding process and food availability before and during gametogenesis is an important factor during the reproductive phase (Eckelbarger, 1986; Chia and Walker, 1991). Vitellogenesis (the synthesis and storage of proteins, lipids and carbohydrates in the growing oocytes) is the longest and most energetically expensive process during oogenesis. There are three major vitellogenetic pathways in invertebrates: autosynthetic, heterosynthetic and mixed (Eckelbarger, 1983, 1994; Jaeckle, 1995; Chapter 1, section 1.3.1 this thesis). These different vitellogenetic mechanisms determine different rates of egg production and the length of the interval between egg-laying events.

A continuum is established between species that have heterosynthetic mechanisms of yolk production, allowing for fast egg production and short periods between breeding events, and species with autosynthetic vitellogenesis causing slow egg production and long periods between breeding events. Long-lived species with slow egg production have vitellogenic strategies that are consistent with a continuous or predictable food supply and a relative stable environment such as temperate latitude habitats, the Antarctic benthos or the 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 or

the ephemeral hydrothermal vents, would select for an opportunistic strategy with fast egg production capabilities (Eckelbarger, 1994). The reproductive capability of a species is phylogenetically constrained by the morphology of the gonad, the vitellogenetic pathways and the digestive structure related to the transfer of nutrients from the somatic organs to the ovaries. Therefore, if there is an increase in food quantity or quality, the gametogenic patterns will be unchanged, but more eggs will be produced, or they will be of a higher quality (Olive, 1985; Eckelbarger, 1986; Jaeckle, 1995; Eckelbarger and Watling, 1995; Bertram and Strathmann, 1998). A low food level may cause a decrease or even an interruption of yolk synthesis and a lowering of fecundity (Barber et al., 1988; Qian and Chia, 1991; Bridges et al., 1994; Eckelbarger, 1994; Levin et al., 1994; Eckelbarger and Watling, 1995).

There is good evidence that food levels and quality are linked with egg production rate, fecundity and egg quality in copepods. Many copepods have opportunistic life history traits, with iteroparity, rapid growth and high fecundity in successive broods. In these species, no food reserves are built up and energy for reproduction is directly derived from food supply (Jönasdóttir, 1994; Williams and Jones, 1999). It has been shown in laboratory and field experiments that food availability plays a major role in the number of eggs produced, the interval between broods and the size of the eggs. An increase in food quantity and/or food quality results in an increase in reproductive output. This is expressed as an increase in fecundity and a reduction in the time between breeding events (Tester and Turner, 1990; Razouls et al., 1991; Jönasdóttir, 1994; Laabir et al., 1995; Bell and Fish, 1996; Pond et al., 1996; Williams and Jones, 1999; Kleppel and Hazzard, 2000). The ability of fast egg production in species with heterosynthetic vitellogenesis allows for a rapid use of energy intake. An increase in food quantity and quality in the environment maintains a high reproductive rate in female copepods by fuelling vitellogenesis and therefore sustains the maturation of successive batches of eggs (Razouls et al., 1991).

This trend of a higher fecundity with smaller eggs at increasing energy availability is found in many crustaceans (copepods, amphipods, caridean shrimp), and reflects the adaptation of the reproductive investment to variable environmental factors. While the overall investment in reproduction is set by the energy available to the female, the investment per embryo is related to the conditions awaiting the pelagic larvae. When food quantity or quality are poor in the environment, the investment per offspring is high allowing for higher survival probability of the larvae. Conversely,

when the energy available in the water column is high, the females invest less per offspring, producing a higher number of lower quality embryos that hatch and develop in a favourable environment. Gorny and co-workers (1992) showed that there is a smaller production of larger eggs in the Antarctic caridean shrimps *Chorismus antarcticus* and *Notocrangon antarcticus* from the Weddell Sea than in the lower latitude populations from South Georgia. Many authors have suggested that there is a need to provide a greater amount of energy for the larvae hatching in the less favourable environments in order to increase the larval survival probabilities (Thorson, 1950; Gorny et al., 1992; Clarke, 1993a).

The polychaetes have often been used for experiments on the effects of environmental factors such as temperature and food in reproductive. Within the family Spionidae several species are poecilogonous, that is multiple developmental types within a species. *Streblospio benedicti* has sympatric specimens producing either lecithotrophic or planktotrophic larvae. Levin and Creed (1986) showed that changes in temperature or food did not cause a switch from one mode of development to the other one, implying that larval type is genetically determined and polymorphic in this species. On the other hand, an increase in food availability and quality caused a higher production of eggs in the specimens with lecithotrophic development. These have a higher amount of heterosynthetic yolk bodies than the specimens with planktotrophic development, allowing for a faster allocation of nutrients to the production of oocytes (Levin and Creed, 1986). Experiments on the short-term reproductive response of *Capitella* sp. fed on different diets also showed that an increase in food ration or quality results in a higher body size, higher fecundity and higher reproductive output (Grémare et al., 1988). Qian and Chia (1991) suggested that opportunistic polychaetes have a high genetic variation in reproductive traits such as fecundity, egg size and adult size, providing flexibility in reproduction and growth in variable environments. These authors presented evidence for the production of a higher number of smaller eggs in a population of *Capitella* sp. feeding on high quality food (squid egg capsules) compared to a population feeding on a lower quality food source (detritus). Also, a recent study shows that salinity and diet affect the survival, fecundity and sex determination of the polychaete *Dinophilus gyrociliatus*. The greater fecundity (higher number of eggs per week and longer reproductive life) was found on the group fed with the higher quality food. Also, individuals kept in low food conditions showed a bias in sex ratio towards

males, probably because of the lower energy requirement for the production of male gametes (Prevedelli and Simonini, 2000).

In marine bivalves, a decrease in nutritive conditions during gametogenesis causes a decrease in the production of eggs, with a lower fecundity or the production of smaller eggs (Bayne et al., 1983; Barber et al., 1988). Barber and co-workers (1988) found that a deep population of the giant scallop *Placopecten magellanicus* in the Gulf of Maine had a reduced production of eggs compared to the shallow-water population. As the egg size was similar in both populations, the result was a reduction in reproductive output of the deep-water scallops without affecting the survival of the larvae. The same results were obtained by MacDonald and Thompson (1985a,b) with different populations of *P. magellanicus* from Newfoundland and New Brunswick. The adult size, somatic growth and reproductive output were lower in the deeper population where temperature, food quantity and quality are lower. These authors proposed that the growth rate and reproductive output are good indicators of suitability of the environment for the individual, because these indices integrate the response of physiological process in an organism. In the intertidal bivalve *Macoma balthica*, a surplus of energy intake allows for higher somatic and reproductive production, and food quantity, quality and temperature, all affect reproductive output (Honkoop and van der Meer, 1997). Also, the mussel *Mytilus edulis* has high variability in fecundity between and within populations in different seasons, caused by geographical or seasonal changes in food availability (Bayne and Worrall, 1980; Kautsky, 1982).

In echinoderms, there is evidence for a higher reproductive output in populations of the echinoids *Arbacia lixula* and *Strongylocentrotus droebachiensis* and the asteroid *Leptasterias epichlora* from rich environments than in populations where food quantity or quality is poor (Bertram and Strathmann, 1998; George, 1994a; George et al., 1990). In deep-sea echinoderms, the most common life history strategy involves the quasi-continuous production of a small number of large eggs with direct or lecithotrophic development (Shick et al., 1981b; Tyler et al., 1982a,b; Gage and Tyler, 1991). Nevertheless, a few species have been found to produce seasonally a high number of small eggs that develop into planktotrophic larvae. It has been suggested that the selection for seasonal reproduction with small eggs, high fecundity and planktotrophic development in the ophiuroid *Ophiura ljungmani*, the asteroids *Plutonaster bifrons* and *Dytaster grandis* and the echinoid *Echinus affinis* is related to the seasonal flux of phytodetritus to the sea bed. This input of organic matter may fuel

gametogenesis and/or be used by the planktotrophic larvae (Tyler and Gage, 1980; Tyler and Pain, 1982; Tyler et al., 1990; Campos-Creaesy et al., 1994).

2.3.2- Fecundity and latitude and depth

The variation of fecundity and egg size with depth and latitude are ultimately related to environmental factors, such as temperature and food quality and quantity that affect the adults and the larvae.

In many crustacean species, deep-water populations are composed of larger individuals resulting in longer reproductive lifespans and the production of larger eggs (Mauchline, 1988). King and Butler (1985) studied five pandalid shrimp from the Pacific with depth ranges between 200 and 800 m and found that specimens from deeper waters produced larger eggs. Nevertheless, the relative brood size and annual reproductive effort were not correlated with depth, indicating that the higher reproductive output of deep-water females is a consequence of their larger size. The production of larger eggs results in the hatching of advanced larvae with a higher survival probability, reducing larval mortality (Herring, 1974b; Omori, 1974; King and Butler, 1985). The larvae of many species that reproduce in deep-waters migrate through the height of the water column to shallow depths. Deep-water larvae are exposed to fluctuations in food, temperature and other environmental factors and to predation during their extended periods in the water column, and larval mortality risk increases with depth (Thorson, 1950; King and Butler, 1985). According to this higher larval mortality risk in deep-water species, it appears to be more efficient to produce a few large eggs with a superior survival rate than a higher number of smaller eggs (King and Butler, 1985). In contrast, Company and Sardà (1997) working on 5 deep-water pandalid shrimp (depth range between 150 and 1100 m) from the Western Mediterranean found no correlation between egg size and depth, but a decreasing brood size with increasing depth. These authors suggest that in the isothermal condition of the Mediterranean below 200 m, the pandalid shrimp do not need to compensate for larval mortality related to temperature gradient with an increase of egg size.

A similar trend to that of deep-water species is found in decapod crustaceans from high latitudes, where females reach a larger size, have delayed maturity and produce larger eggs. The caridean shrimps *Chorismus antarcticus* and *Notocrangon antarcticus* from the Weddell Sea (High Antarctica) mature at a larger size and produce a smaller number of larger eggs than their counterpart populations from South Georgia

(Subantarctic) (Gorny et al., 1992). Also, the snapping shrimp *Betaeus emarginatus* from Chile had a lower reproductive output than other alpheids from lower latitudes (Lardies and Weehrtmann, 1997). These high-latitude species follow a similar pattern to deep-water species, producing a smaller number of larger eggs. The reproductive output decreases with depth and latitude, but the larvae have a better energy provision and therefore survival probability (Clarke, 1979; Gorny et al., 1992; Lardies and Wehrtmann, 1997).

Relations between fecundity and egg size with depth have also been found in bivalves. Reproductive studies on populations of *Placopecten magellanicus* living between 6 and 76 m depth have shown that there is a decrease in fecundity with increasing depth, and this lower production of eggs in deep-water populations has been related to lower temperature and lower food availability (MacDonald and Thompson, 1985b; Barber et al., 1988). The specimens from the deep population (14 m) of the bivalve *Yoldia notabilis* from NE Japan mature one year later than the shallower population (10 m), and age-specific reproductive effort was lower in the former. Nevertheless, size-specific reproductive effort did not show significant differences between populations, suggesting that egg production was related to size and not to age (Nakaoka, 1994). This author proposes that differences in food supply at the two populations affects reproductive effort through differences in growth rate.

2.4- Summary

The term fecundity refers to the number of offspring produced by a female in a certain time unit. This definition varies and is narrowed to adjust to the species studied and needs to be clearly defined in order to obtain the maximum information from the data analysed.

The quantification of fecundity varies depending on the reproductive traits of the species studied. In brooding species, there is easy access to the embryos, allowing for precise quantification of fecundity. In free-spawners, ripe females can be induced to spawn by thermal (or other environmental) shock, or with the injection of spawning-inducing chemicals, and the eggs counted under a stereomicroscope. However, some species cannot be induced to spawn, and in other cases live samples of adults might not be available. In some instances, eggs may be obtained by dissecting ovaries. In species with small gonads or thin gonad walls, the eggs might be visible through the ovary tissue and counted in a microscope with transmitted light. When the oocytes develop in

well-organised ovaries with thick gonad walls, histological methods involving serial sectioning or the relation between gonad volume and oocyte volume can be used for a broad estimation of fecundity.

Fecundity is one of the main variables in life history strategy and is closely related to the other life history traits such as egg size and reproductive effort. One of the main trade-offs in reproductive biology is between number and size of eggs produced. Given a certain allocation of energy to reproduction, there is a negative correlation between fecundity and the quality of the eggs produced. Therefore, species produce either a high number of small eggs or a small number of large eggs. The egg size is related to larval developmental type and time, and in the last three decades several models have been proposed to explain the evolution of egg size and fecundity in invertebrates (Vance, 1973; Winemiller and Rose, 1992; Levitan, 1993; Podolsky and Strathmann, 1996).

Fecundity is also closely dependent on age at first maturity. The demography theory explains the evolution of fecundity traits based on the balance between costs and benefits in the size at first maturity. Early-maturing species have a higher probability of reaching maturity but have a reduction in future fecundity, and species with delayed maturity live longer, allowing for a higher lifetime fecundity.

In many species there is a good correlation between fecundity and female size, with larger females producing more eggs. In some groups such as the decapod crustaceans, the brood size is physically limited by the space between the pleopods where the eggs are carried. In the bivalves, the number of eggs produced is also limited by the inner-shell volume. In polychaetes, larger females have a higher number of fertile segments, allowing for a higher production of eggs.

The reproductive output of a species is defined by two components, the number of eggs (fecundity) and the size of eggs (quality). The allocation of resources to growth and reproduction has to be optimised over the lifespan of the organism, and there is a trade-off between current reproductive output and subsequent adult reproductive success and survival. Ultimately, it is lifetime fecundity that is optimised in the evolution of life history strategies.

Although the gametogenic patterns of a species are phylogenetically constrained, fecundity is not a conservative character and varies with environmental factors such as habitat stability or food quantity and quality. There is evidence in many species that a higher food availability or higher food quality enhances the production of

more and/or better eggs. If the environmental conditions for the larvae are poor or unpredictable, the production of larger eggs with a higher survival probability is selected, while when the conditions are favourable for the larvae, the production of a higher number of smaller eggs is selected.

The effects of environmental factors such as temperature or food availability and quality are reflected in the different fecundity patterns found in different environments. Generally, there is a decrease in fecundity with increasing latitude and increasing depth, often related with less favourable environments with lower energy availability. In some deep-water species, there is a higher reproductive output related to a higher adult body size, and the production of larger eggs allows for a higher survival probability of the larvae in a less favourable environment.

CHAPTER THREE - FECUNDITY AND EGG SIZE OF FIVE MESOPELAGIC CARIDEAN SHRIMP FROM THE NORTH EAST ATLANTIC

3.1- Introduction

3.1.1- The NE Atlantic pelagic environment

The composition and biogeography of mesopelagic faunal assemblages is directly related to the physical characteristics (temperature, salinity, circulation) of the water masses that compose their habitat (Fasham and Foxton, 1979; Sournia, 1994; Legendre et al., 1999).

The distribution of crustacean mesopelagic assemblages in the NE Atlantic is determined by the major water masses, which are characterised by their temperature, salinity, dissolved oxygen and circulation patterns (Angel and Fasham, 1973, 1974, 1975; Fasham & Foxton, 1979). Following a study of the distribution of juvenile and adult decapod crustaceans in the North Atlantic, Fasham and Foxton (1979) proposed seven zones along parallel 20°W, defined by physical factors. In the upper layers, the first horizontal division was made between the water masses over and below the permanent pycnocline, reaching a maximum depth of 200 m at 30°N. In the layer above the pycnocline, a boundary is found at 15°N attributed to the equatorial divergence. Below the pycnocline, the relatively warm water mass is separated horizontally from a deeper cooler layer by the 8°C isotherm and the oxygen minimum layer. Vertically, there is a boundary between 40°N to 53°N caused by the northern frontal system of the subtropical gyre. Below 20°N and between 500 and 1200 m under the oxygen minimum, there is a zone representing the northern limit of the Antarctic Intermediate Water characterised by a salinity minimum. This layer of low salinity overlies the Mediterranean water spreading into the Atlantic and separating the water mass horizontally at about 1000 m. These zones are well correlated with the distribution of decapod assemblages in the NE Atlantic, supporting the physical hypothesis of faunal distribution (Fasham & Foxton, 1979).

3.1.2- Decapod crustacean: taxonomy, distribution and biology

The caridean shrimps are a widely distributed group of decapods, found from shallow to deep water and from tropical to polar seas. Their presence in very different environments, such as hydrothermal vents, cold seeps, deep-sea benthos, mesopelagic

habitat or coastal waters, gives this group a high potential for comparative and ecological studies.

The Infraorder Caridea, Dana 1852, is an extensive and incompletely known group, comprising 15 Superfamilies and 28 families (Chace, 1992; Holthius, 1993). Five deep-water pelagic species found in the NE Atlantic have been considered for this study. They belong to three genera and two families. These species are *Acanthephyra pelagica*, *A. purpurea*, *A. kingsleyi* (F. Oplophoridae), *Systellaspis debilis* (F. Oplophoridae) and *Parapasiphae sulcatifrons* (F. Pasiphaeidae) (Fig. 3.1).

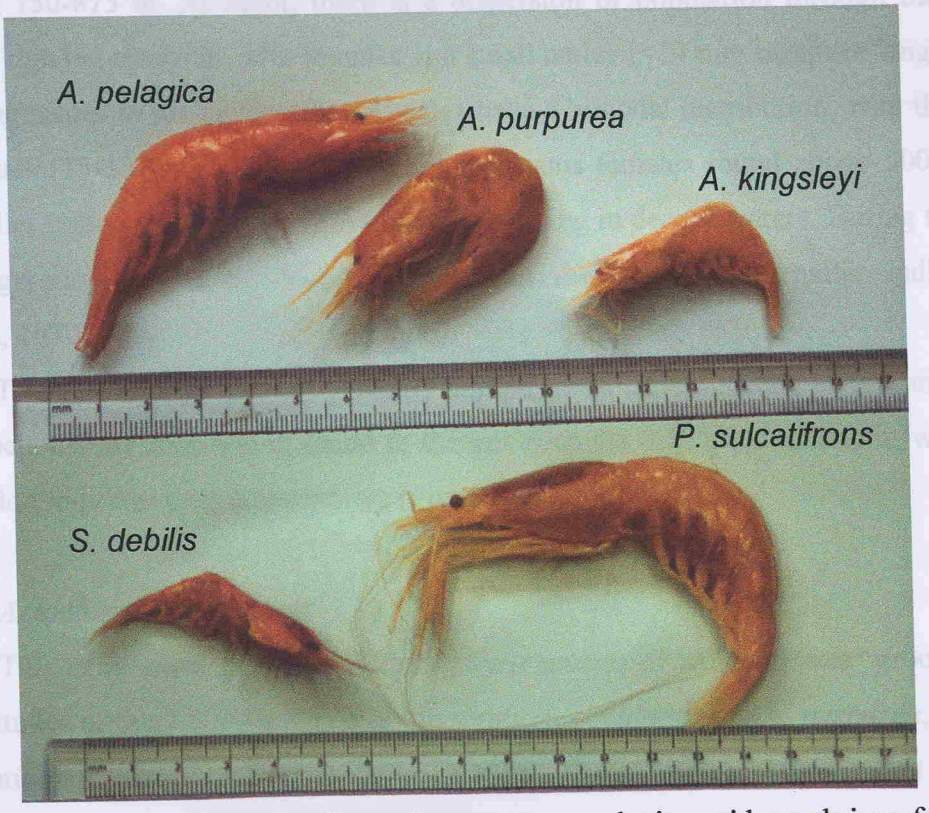


Figure 3.1. Photographs of the five ovigerous mesopelagic caridean shrimp from the NE Atlantic studied in this chapter: *Acanthephyra pelagica*, *Acanthephyra purpurea*, *Acanthephyra kingsleyi*, *Systellaspis debilis* and *Parapasiphae sulcatifrons*.

Acanthephyra pelagica

A. pelagica is found in the Mediterranean Sea, the Atlantic Ocean and the south Indo-Pacific (Foxton, 1972; Crosnier, 1973; Fasham and Foxton, 1979; Christiansen, 1989; Hendrickx, 1989). In the NE Atlantic, *A. pelagica* inhabits latitudes between 40°N and 60°N, and is the deeper living counterpart of *A. purpurea*. *A. pelagica* forms an assemblage of deep mesopelagic shrimps with *Gennadas elegans* and *Parapasiphae sulcatifrons*, with a vertical distribution ranging between 500 and 1500 m, deepening higher latitudes. The upper boundary of the zone inhabited by this assemblage seems to

follow the 8°C isotherm, which coincides with the lower boundary of the area inhabited by *A. purpurea* (Foxton, 1972; Fasham and Foxton, 1979).

Acanthephyra purpurea

This species is one of the most abundant midwater caridean shrimp in the NE Atlantic (Hopkins et al., 1989). *A. purpurea* is mainly distributed between 18°N and 40°N, inhabiting the mesopelagic zone (Fasham and Foxton, 1979). The daytime distribution ranges between 600 and 1000 m, with a broad population maximum between 750-875 m. At night, there is a dispersion of population through the water column, moving upwards, with females and small males (<14 mm carapace length, CL) migrating nearer to the surface. The females have a bimodal distribution, with the large individuals (15-18 mm CL) being mainly ovigerous females found above 500 m and the smaller non-gravid females (8-11 mm CL) staying in deeper waters. During the day, the ovigerous females are found deeper than the non-gravid smaller individuals (Foxton, 1970).

The diet of *A. purpurea* is mainly composed of chaetognaths, euphausiids, fish and copepods, the latter being found in the gut contents only after midnight, when the population migrates to shallow waters (Foxton and Roe, 1974).

Acanthephyra kingsleyi

This is the most abundant *Acanthephyra* species of the 'purpurea' group in the low latitudes of the NE Atlantic. It is the southern counterpart of *A. purpurea*, with its distribution boundary lying in the region of 18°N. During the day, *A. kingsleyi* is found in its deeper range, between 500-700 m, while the whole population migrates to shallower water at night, between 100 to 600 m, with a broad dispersion of individuals (Foxton, 1972; Fasham and Foxton, 1979).

The *Acanthephyra* species reach sexual maturity in the second or third year, and produce broods within the next one or two years (Omori, 1974).

Systellaspis debilis

This is a very abundant species with a wide geographic distribution, found in the Indo-Pacific and Atlantic Oceans, but it is in the latter ocean where most of the catches and studies have occurred (Foxton, 1970; Crosnier and Forest, 1973; Hendrickx

and Estrada-Navarrete, 1989; Hopkins et al., 1989; Allen and Butler, 1994). In the NE Atlantic, *S. debilis* is found in the tropical and subtropical assemblage of upper mesopelagic species, between 10°N and 40°N (Fasham and Foxton, 1979). The depth distribution during the day ranges from 650 to 800 m. The population migrates as a whole to shallower waters at night, with a maximum around 100-200 m. The population is stratified, with the smaller individuals of both sexes in the shallower ranges of the distribution and large females occupying the deeper zones (Foxton, 1970; Hendrickx & Estrada-Navarrete, 1989; Hopkins et al., 1989).

The diet of *S. debilis* is mainly composed of large prey such as chaetognaths, euphausiids and fish (Foxton & Roe, 1974). Similarly to the *Acanthephyra* group, *S. debilis* reaches sexual maturity at two or three years of age, and produce broods within the next one or two years (Omori, 1974).

Parapasiphae sulcatifrons

Parapasiphae sulcatifrons is a cosmopolitan species, occurring in the Atlantic from Greenland to the Gulf of Mexico in the west and to South Africa in the east. In the Pacific, this species inhabits the waters from Japan and Vancouver to South Australia. Although less abundant, it has also been found in the Indian Ocean (Fasham and Foxton 1979; Hendrickx and Estrada-Navarrete 1989; Hopkins et al., 1989; Burukovskii 1993; Allen and Butler 1994).

In the NE Atlantic, *Parapasiphae sulcatifrons* is a deep mesopelagic species, found mostly at high latitudes (40° to 60°N), at depths ranging from 500 to 2000 m. In the NE Atlantic the species is mainly found between 500 to 1500 m (Fasham and Foxton, 1979; Hendrickx and Estrada-Navarrete, 1989; Hopkins et al., 1989; Burukovskii 1993; Allen and Bulter, 1994). Fasham and Foxton (1979), placed *P. sulcatifrons* together with *Acanthephyra pelagica, Gennadas elegans* and *Hymenodora gracilis* in the same assemblage in the NE Atlantic. These species compose an assemblage inhabiting a zone in the water column with its southern boundary around 30°-40°N, coinciding at 1000 m depth with the main outflow of Mediterranean Water. The depth range of the population does not change between night and day, but is deeper at 40°N (700-1500 m) than at 53°N and 60°N (500-1250 m). *Parapasiphae sulcatifrons* is an active predator, feeding on euphausiids, mysids, other shrimps such as *Gennadas, Sergestes* and juveniles of *Acanthephyra,* copepods and chaetognaths.

3.1.3- Reproduction of caridean shrimp

The Order Decapoda is composed by two Suborders: Pleocyemata and Dendrobranchiata. These suborders differ in many respects, including whether their species are brooders (Pleocyemata) or broadcast spawners (Dendrobranchiata) (Clark and Pillai, 1991). The caridean shrimps belong to the Suborder Pleocyemata and therefore brood batches of eggs on the pleopods.

The gonads of caridean shrimp lie under the carapace, overlying the digestive gland. Females have paired ovaries with associated oviducts opening at paired gonopores in the third pereiopods. The ovaries are surrounded by a connective tissue wall, inside which lies the germinal epithelium. The oogonia and follicle cells are derived from this germinal epithelium. Haemal sinuses and blood vessels occur between subunits of oocytes (Krol et al., 1992). Generally, the ovaries of decapod crustaceans lack a muscular layer (Adiyodi and Subramoninan, 1983).

The oogenic processes in decapods comprise a proliferative phase and a differentiative phase (Adiyodi and Subramoninan, 1983). During the proliferative phase, there is a production of clustered oogonia by mitotic divisions in the germinal zone (peripheral or central in the ovary) throughout the life of the female. During the differentiative phase, a sexual response triggers the onset of gametogenesis and the secondary oogonia develop into primary oocytes by meiotic divisions, which move to the growth zone. An unstratified layer of accessory cells surrounds the oocytes. These are the only non-germinative cells within the ovary and are involved in vitellogenetic processes. Primary oocytes stop their first meiotic division in prophase and it is then when growth and vitellogenesis occur. Vitellogenetic processes involve the assembly of organic and inorganic components (water, proteins and lipids) in the oocytes. These provide the nutrients and structural material for tissue formation to the developing embryo. The major protein is vitellin associated with carotenoids, giving the ovaries the characteristic orange-red colour. Vitellogenesis in caridean shrimps occurs in two phases. The first phase involves autosynthesis of lipo- and glycoproteins followed by a resting phase. Secondary vitellogenesis is characterised by autosynthetic and heterosynthetic processes of yolk formation (Nelson, 1991). In mature oocytes, the nuclear membrane disappears, and germinal vesicle breakdown must occur before meiosis continues. Ovulation (escape of mature oocytes from ovarian follicles into the oviducts) occurs when the accessory cells detach from the mature oocytes, and it is then that meiosis continues to the metaphase of primary division (Talbot, 1991; Krol et al.,

1992). At spawning, the cortical rods of the oocytes extrude on contact with water and form a jelly coat around the eggs as they are being transferred to the pleopods (Krol et al., 1992).

Male caridean shrimps have paired testes and associated *vasa deferentia* leading to paired gonopores at the base of the fifth pereiopods. The testes are covered by a thin cortex with an outer epithelial layer and inner connective tissue layer (Krol et al., 1992). During testicular maturation, the germinal layer of spermatogonia develops at the periphery of the seminiferous tubules in the testicular lobes. The germinal spermatogonia multiply by mitotic divisions and differentiate into spermatocytes and spermatids by meiotic divisions during spermatogenesis. Secondary spermatocytes result from the first meiotic divisions and spermatids result from the second meiotic division. The maturing spermatids are sent to the collection tubules and transported to the *vasa deferentia*. (Krol et al., 1992). During spermiogenesis, the spermatids develop into mature spermatozoa, which in decapods are aflagellate, have a decondensed nucleus and a number of spikes. The sperm is packed in spermatophores in the *vasa deferentia*. These are tortuous tubules with a secretary epithelium covered by connective tissue. The secretory epithelium of the *vasa deferentia* aids in gamete transport, maintaining the gametes and secreting the spermatophores (Krol et al., 1992). The spermatophores are stored in the distal zone of the *vasa deferentia* until mating, when the packed sperm is deposited on the female's ventral surface with modified first pleopods (Baeur, 1986).

The spermatophores may be deposited internally in the genital ducts of the female, or externally on the spermatheca. Mating occurs between hard-shelled males and newly moulted females and fertilisation of the eggs occurs at the time of spawning, within 24 hours of copulation (Wickins, 1976; Nelson, 1991). Following fertilisation, the ovigerous females incubate a batch of ovoid eggs on the pleopods, between lateral sclerites that form a brood chamber by enlarging at the prespawning moult. In caridean shrimps, the maturation of the ovary, from oogenesis to ovulation, occurs within a single moult cycle (Adiyodi and Subramonina, 1983). The eggs are attached to the pleopods by sticky egg cases and stalks formed from the oocyte envelope. The embryos grow and develop on the pleopods until they hatch.

Caridean decapods produce a sequence of broods, with the epipelagic and upper mesopelagic species having distinctive reproductive seasons, while the lower meso- and bathypelagic species appear to be reproductively active throughout the year. This

continuous reproduction of deeper-living species could be related to their larvae being independent of phytoplankton production in the euphotic zone (Omori, 1974). However, Company and Sardà (1997) working on five deep-water (150-1100 m depth) pandalid shrimps from the western Mediterranean found that the deepest species had a shorter reproductive period than the shallower species. These authors suggested that the shallower species have access to a year round food source in the neritic zone and this allows an extended breeding season, while the deeper species concentrate the breeding period to coincide with subsurface particulate organic matter maxima (Company and Sardà, 1997).

In crustaceans in general, and in caridean decapods in particular, an important factor related to fecundity is adult body size. Positive correlations are found between carapace length and brood size (Clarke 1979, 1993b; King & Butler, 1985; Bell & Fish, 1996, Stella et al., 1996; Ohtomi, 1997; Thessalou-Legaki & Kiortsis, 1997). This correlation is a result of the physical space limitation between the pleopods for attachment of eggs (King & Bulter, 1985; Corey, 1987; Corey & Reid, 1991; Clarke, 1993b). In consequence, when comparing fecundity between caridean shrimp of different sizes, the number of eggs must be expressed in relation to female weight, in order to avoid the variability related to body size (Barnes and Barnes, 1968; Hines, 1982; Somers, 1991; Clarke, 1993b).

There is also an important trade-off in decapods between egg size and number, which is very clear in caridean shrimp. A species produces either a small number of large rich eggs (i.e. *Systellaspis* spp., *Parapasiphae* spp., *Oplophorus* spp., *Ephyrina* spp.), or a large number of small eggs (i.e. *Acanthephyra* spp., *Meningodora* spp., *Notostomus* spp.) (Herring 1967, 1974a,b; Hopkins et al., 1989; Clarke, 1993a; This thesis chapter two, section 2.2.1). This dichotomy in decapod egg size is constrained by phylogeny, and corresponds to the generic classification of the species (Van Dover and Williams, 1991). Three species belonging to the *Acanthephyra* group that produce a high number of small eggs (*A. pelagica, A. purpurea* and *A. kingsleyi*) and two species that produce a few large eggs (*Systellaspis debilis* and *Parapasiphae sulcatifrons*) have been analysed here.

3.1.4- Chapter objectives

During the last four decades, following a series of RRS *Discovery II* and RRS *Discovery* cruises, a large amount of work has been conducted on pelagic decapods

from the NE Atlantic. The distribution and ecology of pelagic decapods collected during the above cruises were studied (Foxton, 1970a,b, 1972; Fasham & Foxton, 1979). Even so, little is known of the reproduction and life cycle of most deep-water species, mainly because of the difficulty of obtaining deep-living specimens alive.

The reproductive patterns, especially fecundity and egg size, of five caridean shrimp (*Acanthephyra pelagica*, *A. purpurea*, *A. kingsleyi*, *Systellaspis debilis* and *Parapasiphae sulcatifrons*) were analysed. These species live in similar environments in the NE Atlantic, but occupy different depths and latitudes. Even though the gross egg size and number is phylogenetically constrained, there can be variability in fecundity and egg size for a species caused by variations in environmental factors such as food availability, temperature or habitat stability (Bauer, 1992; Chapter two, section 2.3.1 this thesis). The main aim of this study was to quantify the fecundity and egg sizes, and to provide the necessary data for comparisons of the reproductive output within the midwater group and between midwater and hydrothermal vent caridean shrimps (Chapters 4 and 6).

3.2- Material and methods

3.2.1- Study area and samples

The specimens were sorted from the decapod samples of the Discovery Collections located at the Southampton Oceanography Centre. These specimens were collected by the Institute of Oceanographic Sciences Deacon Laboratory between 1970 and 1972, on a transect from 11°N to 60°N along the 20°W meridian (Hargreaves, 1989). Six stations were established, approximately every ten degrees (Table 3.1; Fig. 3.2).

Each station was sampled with a Rectangular Midwater Trawl RMT 1+8 and RMT 25 (Baker et al., 1973, Roe and Shale, 1979) during day and night periods, from the surface to 3000 m. The samples were sorted and fixed on board and stored in preservative fluid (40% Formaldehyde, Propan-1,2-Diol, 1-Phenoxypropan-2-ol and distilled water). In the laboratory, all ovigerous females belonging to the Infraorder Caridea were sorted and identified to species level (Crosnier and Forest 1973; Holthuis 1993).

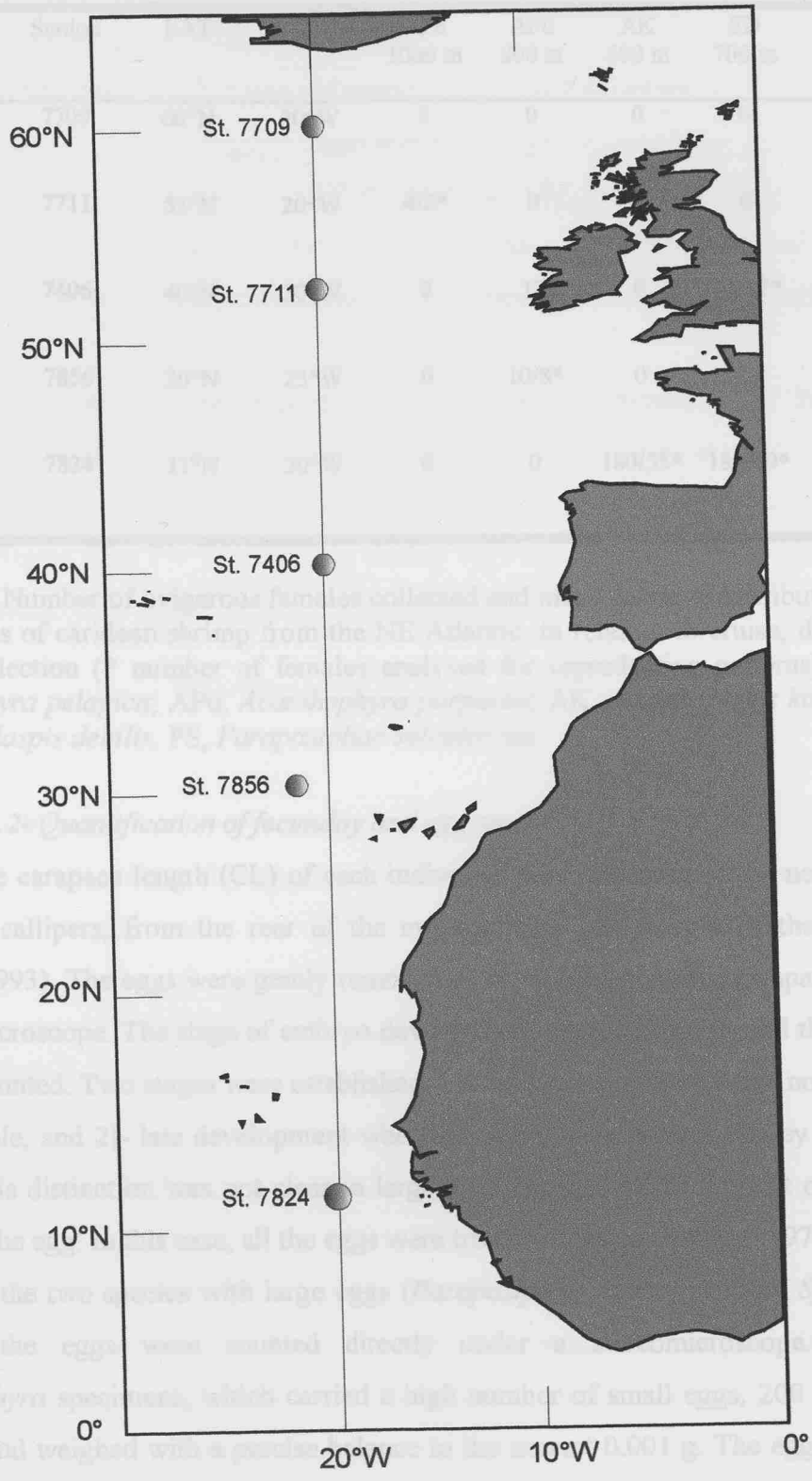


Figure 3.2. Chart of the NE Atlantic showing the six sampling sites along 20°N longitude.

Date	Station	LAT	LONG	APe 1000 m	APu 800 m	AK 600 m	SD 700 m	PS 1000 m
April-May 1971	7709	60°N	20°W	3	0	0	0	22
May-June 1971	7711	53°N	20°W	4/2*	0	0	0	44/38*
Oct.-Nov 1970	7406	40°N	20°W	0	17	0	44/37*	0
March-April 1972	7856	30°N	23°W	0	10/8*	0	19	0
March 1972	7824	11°N	20°W	0	0	180/55*	188/49*	0

Table 3.1. Number of ovigerous females collected and mean depth of distribution for five species of caridean shrimp from the NE Atlantic, in relation to cruise, date and site of collection (* number of females analysed for reproductive patterns). APe, *Acanthephyra pelagica*; APu, *Acanthephyra purpurea*; AK, *Acanthephyra kingsleyi*, SD, *Systellaspis debilis*, PS, *Parapasiphae sulcatifrons*.

3.2.2- Quantification of fecundity and egg size

The carapace length (CL) of each individual was measured to the nearest 0.05 mm with callipers, from the rear of the eye socket to the margin of the carapace (Clarke, 1993). The eggs were gently removed from the pleopods with a spatula under a stereomicroscope. The stage of embryo development was determined and the number of eggs counted. Two stages were established, 1)- early development when no eye spots were visible, and 2)- late development when eye spots were present (Corey and Reid, 1991). This distinction was not clear in large eggs because of the amount of reserves stored in the egg. In this case, all the eggs were treated together (Herring, 1974a).

In the two species with large eggs (*Parapasiphae sulcatifrons* and *Systellaspis debilis*), the eggs were counted directly under a stereomicroscope. For the *Acanthephyra* specimens, which carried a high number of small eggs, 200 eggs were counted and weighed with a precise balance to the nearest 0.001 g. The egg mass was weighed wet (EMWW) after gently removing the excess of preservative fluid with an absorbent tissue. Total egg number was estimated from the weight of 200 eggs and the weight of the total egg mass. The females were weighed wet (FWW) to the nearest 0.001 g. Fecundity was first calculated as *total fecundity*, defined as the number of eggs

carried on the pleopods per female. Secondly, in order to compare the production of eggs between species, fecundity was measured as *size-specific fecundity*, defined as the number of eggs per 1 g of female wet weight.

The reproductive effort was estimated as reproductive output:

$$RO = \frac{Eggs\ Wet\ Weight\ (g)}{Female\ Wet\ Weight\ (g)}$$

Simple regressions of female wet weight, egg mass wet weight and fecundity against carapace length were calculated and the significance of the correlation tested with the Pearson Product Moment.

To calculate the mean egg size, all the eggs were measured for *Systellaspis debilis* and *Parapasiphae sulcatifrons*, and 50 eggs were measured for the *Acanthephyra* species, using an image analysis package (Rainbow Runner/Matrox PC-VCR and Sigma Scan Pro 3). Caridean shrimp eggs are oval, and therefore the length (EL) and width (EW) were measured. The egg volume (EV) was estimated as:

$$EV = EL \times \pi \times \left(\frac{EW}{2}\right)^2$$ (Corey & Reid, 1991).

3.3- Results

The ovaries of the five species were characteristic of caridean shrimps. Paired organs were located under the carapace overlying the digestive gland. In the *Acanthephyra* species, the ovaries were smooth, globular, dark red organs. In *Systellaspis debilis* and *Parapasiphae sulcatifrons* the developing eggs were large and clearly visible in the ovaries.

3.3.1- Acanthephyra spp

3.3.1.1- Adult size

Within the *Acanthephyra* group, a sufficient number of specimens of *A. kingsleyi* and *A. purpurea* were analysed to allow statistical analyses. Only a few specimens of *A. pelagica* were available and the results are given here only as reference, but a larger sample is necessary to conduct statystical analysis (Table 3.1).

The CL of gravid *A. pelagica* ranged from 19.5 mm to 22.1mm (mean = 20.4 ± 0.8 mm) at 60°N, and from 19.0 mm to 21.7 mm (mean = 20.4 ±1.3 mm) at 53°N. There was no statistically significant difference in the mean carapace length of *A.*

pelagica between the two stations (Student's test, t= -0.011, 3df, P>0.05). The data for both locations were combined, giving a mean CL of 20.4 ±1.4 mm.

In the stations sampled at 40°N and 30°N, *A. pelagica* was replaced by *A. purpurea*. The CL of the *A. purpurea* specimens from 40°N ranged from 11.6 mm to 20.1 mm (mean = 16.3 ±0.6 mm), and from 11.6 mm to 16.2 mm (mean = 13.5 ±0.5 mm) at 30°N. In *A. purpurea*, the mean CL of the specimens from 40°N was significantly larger than that of the specimens from 30°N (Student's test, t= 2.825, 23df, P<0.05), and the populations were not combined.

At 11°N, the sample provided a high number of ovigerous *A. kingsleyi*. The CL of ovigerous *A. kingsleyi* ranged from 11.2 mm to 19.3 mm (mean = 13.7 ±0.2 mm).

The mean CL of the two southernmost species, *A. kingsleyi* and *A. purpurea* from 30°N, were significantly smaller than the mean CL of the species in the northern populations, *A. purpurea* from 40°N and *A. pelagica* from 53°N and 60°N (ANOVA, F=17.687, 4df, P<0.0001, Tukey post-hoc test: P<0.001 between *A. kingsleyi* and *A. purpurea* (40°N) and *A. pelagica* (53°N, 60°N), and between *A. purpurea* (30°N) and *A. purpurea* (40°N) and *A. pelagica* (53°N, 60°N); Fig. 3.3).

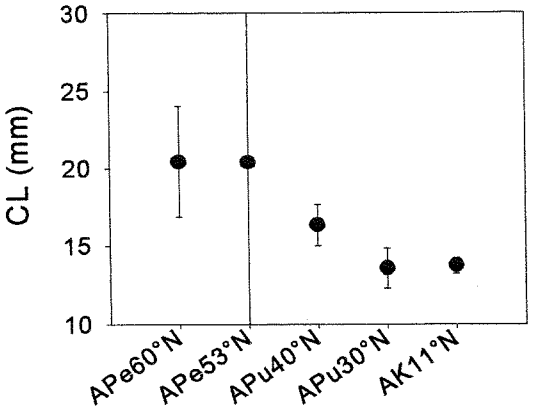


Figure 3.3. Mean carapace length (CL) and 95% confidence limits in five populations of *Acanthephyra*. Ape60°N, *A. pelagica* from 60°N; Ape53°N, *A. pelagica* from 53°N; Apu40°N, *A. purpurea* from 40°N; Apu30°N, *A. purpurea* from 30°N; AK11°N, *A. kingsleyi* from 11°N.

For all three species of *Acanthephyra*, female wet weight was significantly correlated with carapace length (Pearson Product Moment, r=0.99, n=5, P<0.005 for the combined data of *A. pelagica*; r=0.89, n=17, P<0.0001 for *A. purpurea* in 40°N, r=0.94, n=8, P<0.001 for *A. purpurea* in 30°N and r=0.98, n=55, P<0.0001 for *A. kingsleyi*; Fig. 3.4A, B, C & D), indicating that CL is a good measure of body size.

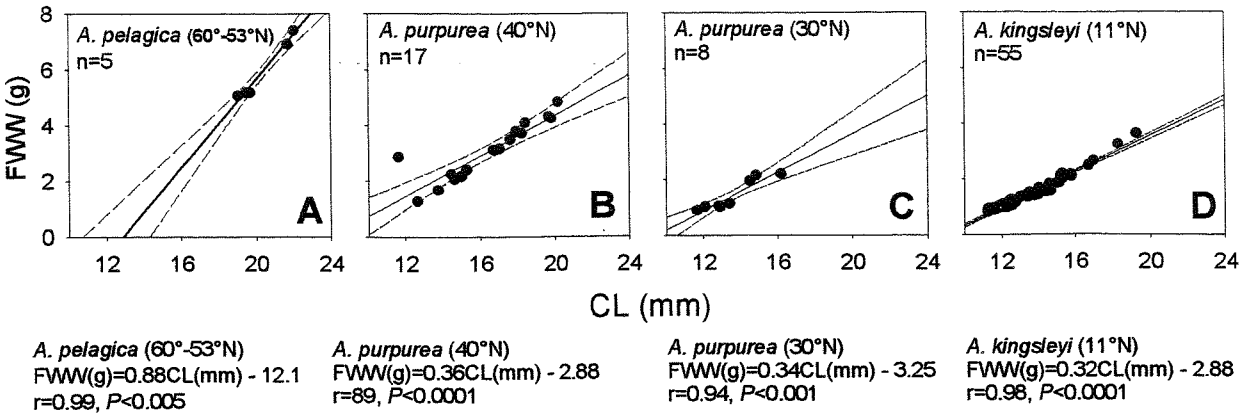


Figure 3.4. Linear regression and 95% confidence limits of female wet weight (FWW) against carapace length (CL) in the *Acanthephyra* group.
A- *A. pelgagica* (combined data of the samples from 60°N and 53°N); B- *A. purpurea* from 40°N; C-*A. purpurea* from 30°N; D- *A. kingsleyi* from 11°N.

3.3.1.2- Fecundity

In caridean shrimps, fecundity varies with female body size. Larger females produce a greater number of eggs. In the five populations of *Acanthephyra* studied, there were significant differences in CL. As a result, fecundity is expressed herein as both, *total fecundity* (number of eggs in pleopods per female) and *size-specific fecundity* (number of eggs per 1 g of female body weight). The latter expression of egg production will be used to compare fecundity within and between species (Table 3.2).

Acanthephyra spp.	Size-specific Fecundity	*Systellaspis debilis*	Size-specific Fecundity	*Parapasiphae sulcatifrons*	Size-specific Fecundity
APe 60°N	239.8 ±72.6	.....	.....	PS 60°N	4.4 ±0.6
APe 53°N	227.0 ±35.4	.....	.....	PS 53°N	4.1 ±0.8
APu 40°N	449.0 ±124.4	SD 40°N	8.0 ±0.4	.....	.....
APu 30°N	446.7 ±221.4	SD 30°N	10.2 ±0.6	.....	.....
AK 11°N	470.6 ±98.6	SD 11°N	10.1 ±0.2	.....	.....

Table 3.2. Mean size-specific fecundity (number of eggs per 1 g of female weight) and standard deviation of five species of mesopelagic caridean shrimp from different latitudes in the NE Atlantic.
APe, *Acanthephyra pelagica*; APu, *A. purpurea*; AK, *A. kingsleyi*; SD, *Systellaspis debilis*; PS, *Parapasiphae sulcatifrons*.

In the few *A. pelagica* available for this study, total fecundity ranged from 834.1 to 2249.5 eggs per female (mean = 1468 ±719.2 eggs per female) at 60°N and from 1275.2 to 1397.9 eggs per female (mean = 1336.5 ±86.8 eggs per female) at 53°N.

The mean size-specific fecundity of *A. pelagica* was 239.8 ±72.6 eggs g^{-1} FWW at 60°N, and 227.0 ±35.4 eggs g^{-1} FWW at 53°N (Table 3.2). There was no significant differences in the mean size-specific fecundity between populations from the two latitudes (Student's test, t= -0.223, 3df, P>0.05). The data for the two populations were combined, giving a mean size-specific fecundity of 234.7 ±54.8 eggs g^{-1} FWW.

In *A. purpurea,* total fecundity ranged from 452.2 to 2878.3 eggs per female (mean = 1366.6 ±609.7 eggs per female) at 40°N, and from 324.0 to 993.3 eggs per female (mean = 701.7 ±236.6 eggs per female) at 30°N. Mean size-specific fecundity was 449.0 ±124.4 eggs g^{-1} FWW at 40°N and 446.7 ±221.4 eggs g^{-1} FWW at 30°N (Table 3.2). There were no significant differences in the mean size-specific fecundity between the populations at the two different latitudes (Student's test, t= -0.033, 23df, P>0.05). The data for the two populations were combined, giving a mean size-specific fecundity of 448.2 ±156.9 eggs g^{-1} FWW.

The total fecundity of the small southern species, *A. kingsleyi* from 11°N, ranged from 330.4 to 1957.4 eggs per female (mean = 761.0 ±342.0 eggs per female). The mean size-specific fecundity was 470.6 ±98.6 eggs g^{-1} FWW (Table 3.2).

The combined data of size-specific fecundity for each species were compared among the three species. There was no significant statistical difference in the mean size-specific fecundity between *A. kingsleyi* and *A. purpurea*, but the mean fecundity of *A. pelagica* was significantly lower (ANOVA, F=9.282, 2df, P<0.0001, Tukey post-hoc test: P<0.0001 between *A. kingsleyi* and *A. pelagica*, and *A. purpurea* and *A. pelagica*; Fig. 3.5). However, the sample size of *A. pelagica* was small and the results should be interpreted cautiously.

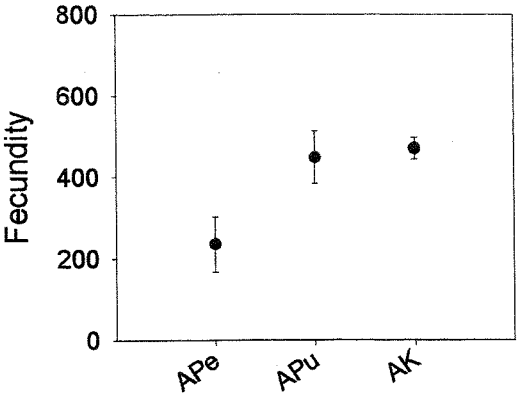


Figure 3.5. Mean size-specific fecundity and 95% confidence limits in three species of *Acanthephyra*. APe, *A. pelagica* from 60°N and 53°N; APu, *A. purpurea* from 40°N and 30°N; AK, *A. kingsleyi* from 11°N.

In the combined populations of *A. pelagica*, there was no significant correlation between total fecundity and carapace length (Pearson Product Moment, r=0.75, n=5, $P>0.05$; Fig. 3.6A). However, the plot of total fecundity against carapace length shows an increase in the number of eggs in larger females. The lack of correlation could be caused by a small sample size with a small range of female sizes not allowing for any possible correlation pattern to show. Total fecundity was significantly correlated with CL in the combined populations of *A. purpurea* from 30°N and 40°N (Pearson Product Moment, r=0.75, n=25, $P<0.0001$; Fig. 3.6B) and in the population of *A. kingsleyi* from 11°N (Pearson Product Moment, r=0.88, n=55, $P<0.0001$; Fig. 3.6C).

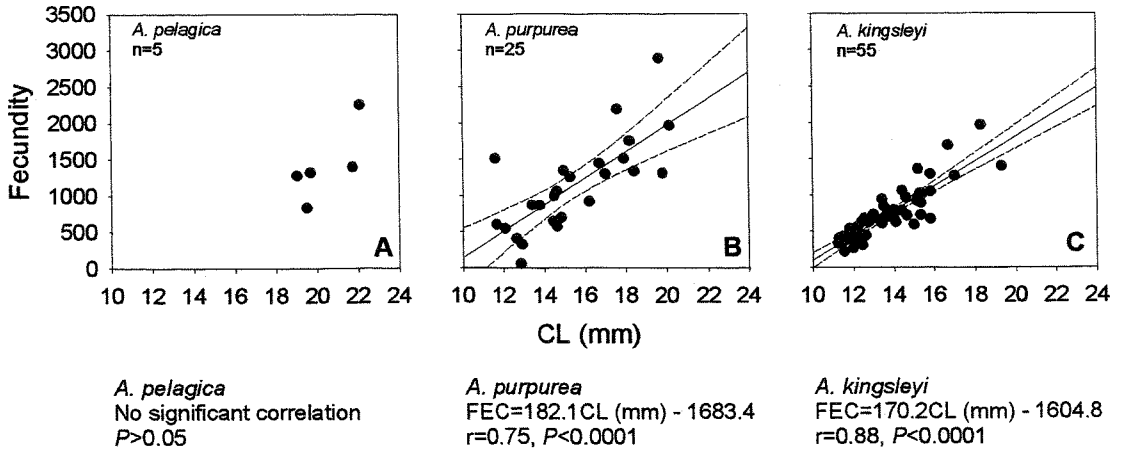


Figure 3.6. Linear regression and 95% confidence limits of fecundity against carapace length (CL) in three species of *Acanthephyra*. FEC, fecundity (number of eggs in pleopods); CL, carapace length (mm).
APe, *A. pelagica* from 60°N and 53°N; APu, *A. purpurea* from 40°N and 30°N; AK, *A. kingsleyi* from 11°N.

3.3.1.3- Egg size

The eggs of the three *Acanthephyra* species studied were small, with lengths ranging between 0.8 and 1.0 mm.

From the 5 specimens of *A. pelagica*, the three females collected at 60°N were carrying early eggs with a mean egg length of 1.08 ±0.01 mm and mean egg volume of 0.51 ±0.01 mm^3, while the two females from 53°N were carrying late eggs, with a mean egg length of 1.27 ±0.03 mm and mean egg volume of 0.78 ±0.05 mm^3 (Table 3.3).

In *A. purpurea* from 40°N, the mean egg length was 0.97 ±0.004 mm for early eggs and 1.0 ±0.003 mm for late eggs, with mean egg volumes of 0.34 ±0.004 mm^3 for early eggs and 0.4 ±0.004 mm^3 for late eggs. The mean egg volume of late eggs was significantly higher than that of early eggs (Student's test, t= 6.802, 298df, P<0.0001; Table 3.3). In *A. purpurea* from 30°N the females were only carrying early eggs, with a mean length of 0.89 ±0.02 mm and mean egg volume of 0.25 ±0.04 mm^3. The mean egg volume of *A. purpurea* from 40°N was significantly higher than that of the females from 30°N (Student's test, t= -3.429, 12df, P<0.05; Table 3.3).

The eggs of *A. kingsleyi* had a mean egg length of 0.9 ±0.04 mm in early eggs and 1.03 ±0.05 mm in late eggs. The mean egg volume of early eggs was 0.25 ±0.005 mm^3 for early eggs and 0.40 ±0.09 mm^3 for late eggs, being significantly higher in late eggs than early eggs (Student's test, t= -9.43, 46df, P<0.0001; Table 3.3).

Acanthephyra spp.	EEV (mm^3)	LEV (mm^3)	*Systellaspis debilis*	EV (mm^3)	*Parapasiphae sulcatifrons*	EV (mm^3)
APe 60°N	0.50 ±0.01	.....	.....	.....	PS 60°N	42.2 ±4.0
APe 53°N	.....	0.78 ±0.05	.....	.....	PS 53°N	38.0 ±6.2
APu 40°N	0.34 ±0.004	0.40 ±0.004	SD 40°N	11.9 ±0.3	.....	.....
APu 30°N	0.25 ±0.04	.....	SD 30°N	11.9±0.3	.....	.....
AK 11°N	0.25 ±0.005	0.40 ±0.09	SD 11°N	14.2 ±0.2	.....	.....

Table 3.3. Mean egg volume and standard deviation of five species of mesopelagic caridean shrimp from different latitudes in the NE Atlantic.
EV, egg volume; EEV, egg volume of early eggs; LEV, egg volume of late eggs; APe, *Acanthephyra pelagica*; APu, *A. purpurea*; AK, *A. kingsleyi*; SD, *Systellaspis debilis*; PS, *Parapasiphae sulcatifrons*.

There were significant differences in the mean egg volumes between species for both stages, early and late eggs (Fig. 3.7). In early eggs, the mean egg volume of *A. pelagica* from 60°N was significantly higher than that of the other three populations (*A. purpurea* from 40°N and from 30°N, and *A. kingsleyi* from 11°N), and the mean egg volume of *A. purpurea* from 40°N was significantly higher than that of *A. purpurea* from 30°N and *A. kingsleyi* from 11°N. There were no significant differences in the mean volume of early eggs between the two southernmost populations (*A. purpurea* from 30°N and *A. kingsleyi* from 11°N) (ANOVA, F=53.769, 3df, P<0.0001; Tukey post-hoc test, P<0.0001 between *A. pelagica* from 60°N and *A. purpurea* from 40°N and 30°N and *A. kingsleyi* from 11°N, and P<0.0001 between *A. purpurea* from 40°N and *A. purpurea* from 30°N and *A. kingsleyi* from 11°N).

In late eggs, the mean egg volume of *A. pelagica* from 53°N was significantly higher than that of *A. purpurea* from 40°N and that of *A. kingsleyi* from 11°N (ANOVA, F=394.597, 2df, P<0.0001; Fig. 3.7).

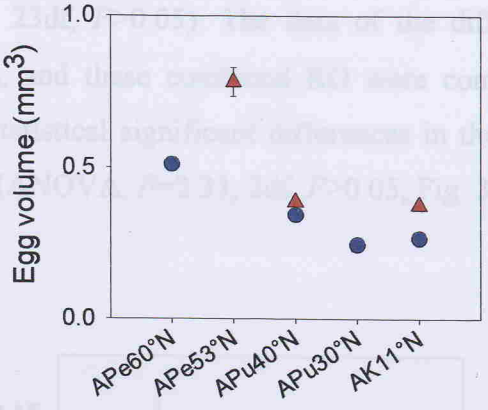


Figure 3.7. Mean egg volume and 95% confidence limits of three species of *Acanthephyra*. Data for early eggs (blue circles) and late eggs (red triangles).
APe, *A. pelagica* from 60°N and 53°N; APu, *A. purpurea* from 40°N and 30°N; AK, *A. kingsleyi* from 11°N.

3.3.1.4- Reproductive output

The reproductive output (RO) ranged from 0.058 to 0.126 (mean = 0.1 ±0.04) in the population of *A. pelagica* from 60°N, and from 0.134 to 0.146 (mean = 0.140 ±0.008) at 53°N. In *A. purpurea*, the RO ranged from 0.062 to 0.139 (mean = 0.1 ±0.02) in the population at 40°N, and from 0.032 to 0.119, (mean = 0.08 ±0.03) at 30°N. In *A. kingsleyi*, the RO ranged from 0.055 to 0.146 (mean = 0.1 ±0.002) (Table 3.4).

Acanthephyra spp.	RO	*Systellaspis debilis*	RO	*Parapasiphae sulcatifrons*	RO
APe 60°N	0.10 ±0.04	.....	.....	PS 60°N	0.05 ±0.03
APe 53°N	0.14 ±0.008	.....	.....	PS 53°N	0.08 ±0.03
APu 40°N	0.10 ±0.02	SD 40°N	0.08 ±0.02	.....	.....
APu 30°N	0.08 ±0.03	SD 30°N	0.07 ±0.02	.....	.....
AK 11°N	0.10 ±0.002	SD 11°N	0.09 ±0.01	.....	.....

Table 3.4. Mean reproductive output (RO) and standard deviation of five species of mesopelagic caridean shrimp from different latitudes in the NE Atlantic.
APe, *Acanthephyra pelagica*; APu, *A. purpurea*; AK, *A. kingsleyi*; SD, *Systellaspis debilis*; PS, *Parapasiphae sulcatifrons*.

There were no statistically significant differences in the mean RO of *A. pelagica* between 53°N and 60°N (t-Student, t= -1.45, 3df, P>0.05). Similarly, there were no statistically significant differences in the mean RO of *A. purpurea* between 30°N and 40°N (t-Student, t=1.84, 23df, P>0.05). The data of the different populations were combined within species, and these combined RO were compared among the three species. There were no statistical significant differences in the mean RO of the three species of *Acanthephyra* (ANOVA, F=2.33, 2df, P>0.05; Fig. 3.8).

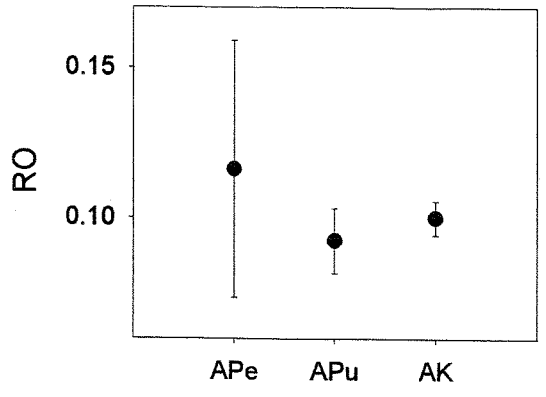


Figure 3.8. Mean reproductive output (RO) and 95% confidence limits of three species of *Acanthephyra*. APe, *A. pelagica* from 60°N and 53°N; APu, *A. purpurea* from 40°N and 30°N; AK, *A. kingsleyi* from 11°N.

3.3.2- Systellaspis debilis

3.3.2.1- Adult size

The carapace length of *Systellaspis debilis* ranged from 11.1 to 14.5 mm (mean = 13.2 ±0.1 mm) in the population from 40°N, from 12.4 to 14.9 mm (mean = 13.2 ±0.1 mm) at 30°N, and from 12.2 to 14.8 mm (mean = 13.5 ±0.6 mm) at 11°N. There were no statistically significant differences in the mean CL between the populations from 11°N, 30°N and 40°N (ANOVA, F=1.710, 2df, P>0.05, Fig. 3.9).

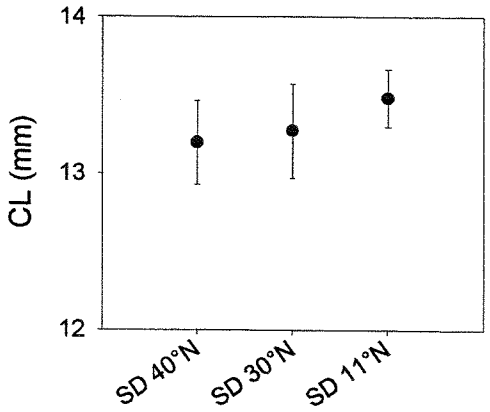


Figure 3.9. Mean carapace length (CL) and 95% confidence limit of three populations of *Systellaspis debilis* (SD) from 40°N, 30°N and 11°N.

The mean female wet weight was 1.3 ±0.2 g at 40°N, 1.1 ±0.2 g at 30°N and 1.3 ±0.2 g at 11°N. There was a positive significant correlation between CL and female weight in the populations at 40°N and 11°N (Pearson Product Moment, r=0.84, n=37, P<0.0001 and r=0.89, n=49, P<0.0001 respectively; Fig. 3.10A & C), but there was no significant correlation in the sample from 30°N (Pearson Product Moment, r=0.41, n=19, P>0.05; Fig. 3.10B). Again, the lack of correlation could have been caused by a small sample size and small range of carapace sizes.

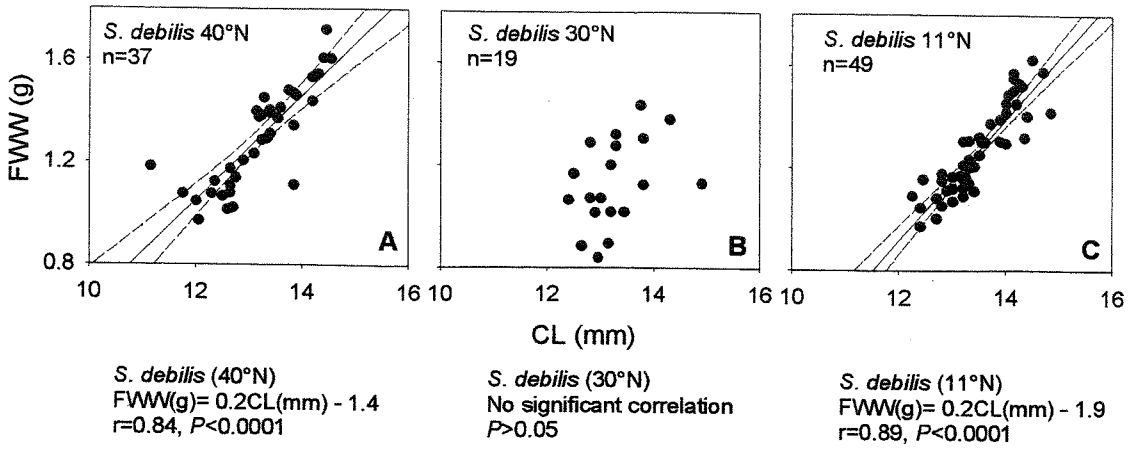


Figure 3.10. Linear regression of female wet weight (FWW) against carapace length (CL) in three populations of *Systellaspis debilis*.
A- *S. debilis* from 40°N; B- *S. debilis* from 30°N; C- *S. debilis* from 11°N.

3.3.2.2- Fecundity

Total fecundity in *S. debilis* ranged from 5 to 18 eggs per female (mean = 10.4 ±3.5 eggs per female) at 40°N, from 6 to 16 eggs per female (mean = 11.3 ±2.7 eggs per female) at 30°N and from 8 to 18 eggs per female (mean = 12.8 ±2.4 eggs per female) at 11°N. The mean size-specific fecundity was 8.0 ±0.4 eggs g^{-1} FWW at 40°N, 10.2 ±0.6 eggs g^{-1} FWW at 30°N and 10.1 ±0.2 eggs g^{-1} FWW at 11°N (Table3.2). There were no statistically significant differences in the mean size-specific fecundity of *S. debilis* between the two southern populations, but fecundity was significantly lower at 40°N (ANOVA, F=11.308, 2df, P<0.0001; Tukey post-hoc test, P<0.005 between *S. debilis* from 11°N and 40°N and between *S. debilis* from 30°N and 40°N; Fig. 3.11).

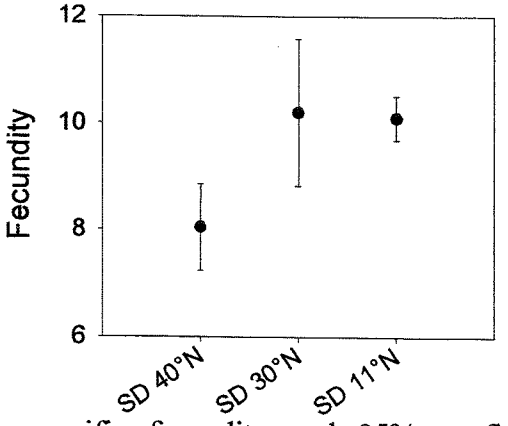


Figure 3.11. Mean size-specific fecundity and 95% confidence limits of three populations of *Systellaspis debilis* from 40°N, 30°N and 11°N.

Total fecundity was positively correlated with CL in the populations at 40°N and 11°N (Pearson Product Moment, r=0.57, n=37, $P<0.005$ at 40°N and r=0.59, n=49, $P<0.0001$ at 11°N; Fig. 3.12A & C). There was no significant correlation between total fecundity and CL in the population from 30°N (Pearson Product Moment, r= 0.25, n=19, $P>0.05$; Fig. 3.12B). This lack of correlation might have been caused by a small sample with a small range of female sizes and by damaged broods. Because the broods are small in *Systellaspis debilis* (between 10 to 12 eggs per brood) the loss of a few eggs represents a high percentage of the total brood, causing high variability in fecundity within a species.

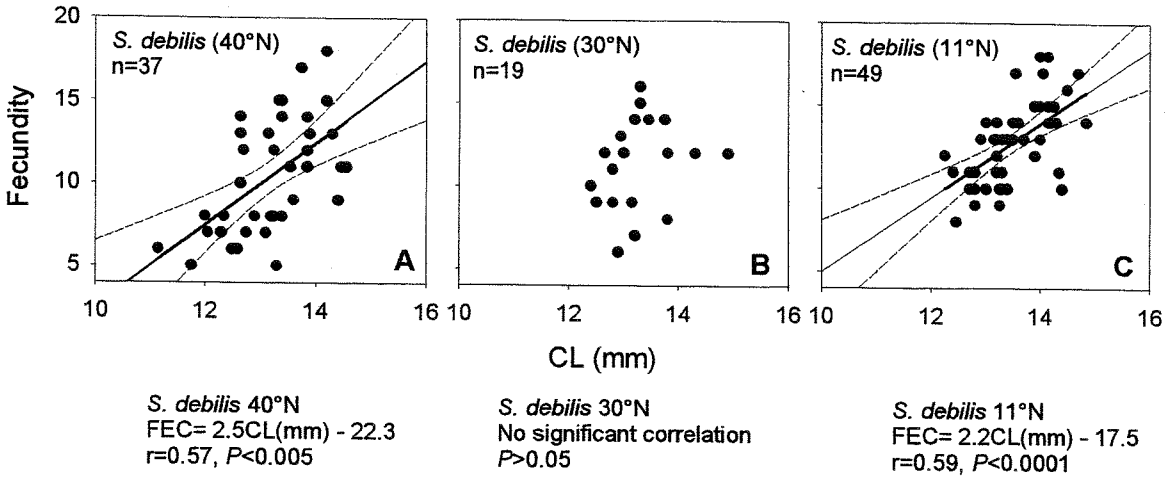


Figure 3.12. Linear regression of total fecundity against carapace length in three populations of *Systellaspis debilis*. FEC, fecundity (number of eggs in pleopods); CL, carapace length (mm).
A- *S. debilis* from 40°N; B- *S. debilis* from 30°N; C- *S. debilis* from 11°N.

3.3.2.3- Egg size

The mean egg length of *S. debilis* was 3.5 ±0.002 mm at 40°N, 3.6 ±0.03 mm at 30°N, and 3.8 ±0.02 mm at 11°N. The mean egg volumes were 11.9 ±0.3 mm^3 at 40°N, 11.9 ±0.3 mm^3 at 30°N, and 14.2 ±0.2 mm^3 at 11°N (Table 3.3). The mean egg volume of the population from 11°N was significantly higher than that of the two northern populations (ANOVA, F= 28.426, 2df, $P<0.0001$; Tukey post-hoc test, $P<0.0001$ between *S. debilis* from 11°N and 30°N and from 11°N and 40°N; Fig. 3.13).

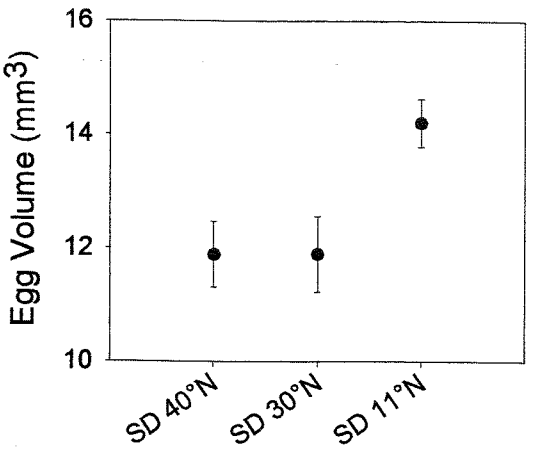


Figure 3.13. Mean egg volume and 95% confidence limits of three populations of *Systellaspis debilis* from 40°N, 30°N and 11°N in the NE Atlantic.

3.3.2.4- Reproductive output

The reproductive output ranged from 0.035 to 0.113 (mean = 0.076 ±0.02) at 40°N, from 0.035 to 0.095 (mean = 0.071 ±0.02) at 30°N, and from 0.064 to 0.118 (mean = 0.093 ±0.01) at 11°N (Table 3.4). The mean RO of the tropical population (11°N) was significantly higher than the mean RO of the two northern populations (ANOVA, F= 17.064, 2df, P<0.0001; Tukey post-hoc test, P<0.0001 between *S. debilis* from 11°N and 30°N and between 11°N and 40°N; Fig. 3.14).

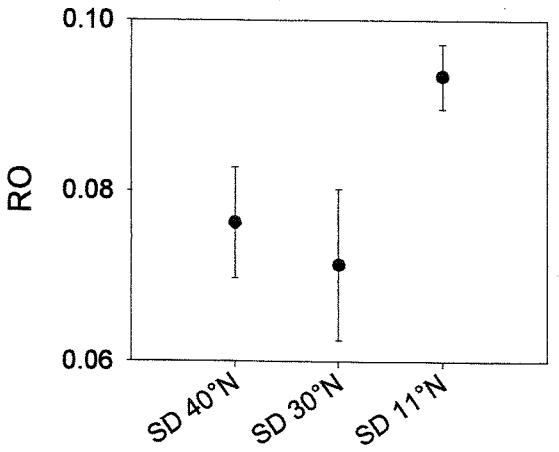


Figure 3.14. Mean reproductive output (RO) and 95% confidence limits of three populations of *Systellaspis debilis* from 40°N, 30°N and 11°N.

3.3.3- Parapasiphae sulcatifrons

3.3.3.1- Adult size

Parapasiphae sulcatifrons was the largest of the species studied and occupies the two most northern stations, 53°N and 60°N.

The carapace length ranged from 22.8 to 26.4 mm (mean = 24.2 ±0.9 mm) in the population from 60°N, and from 21.3 to 26.3 mm (mean = 23.5 ±1.4 mm) at 53°N. The mean CL of the females from 60°N was significantly higher than that of 53°N (Student's test, t= -2.179, 58df, P<0.05; Fig. 3.15).

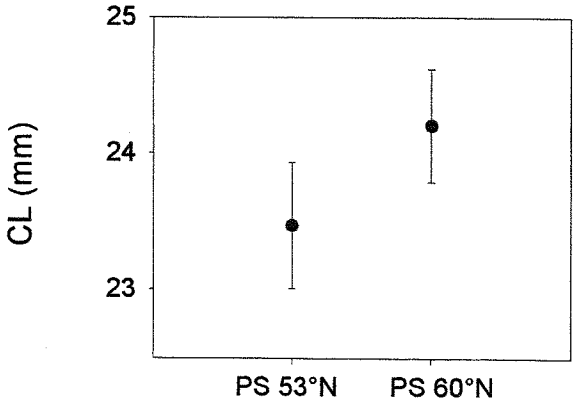


Figure 3.15. Mean carapace length (CL) and 95% confidence limits of two populations of *Parapasiphae sulcatifrons* from 60°N and 53°N in the NE Atlantic.

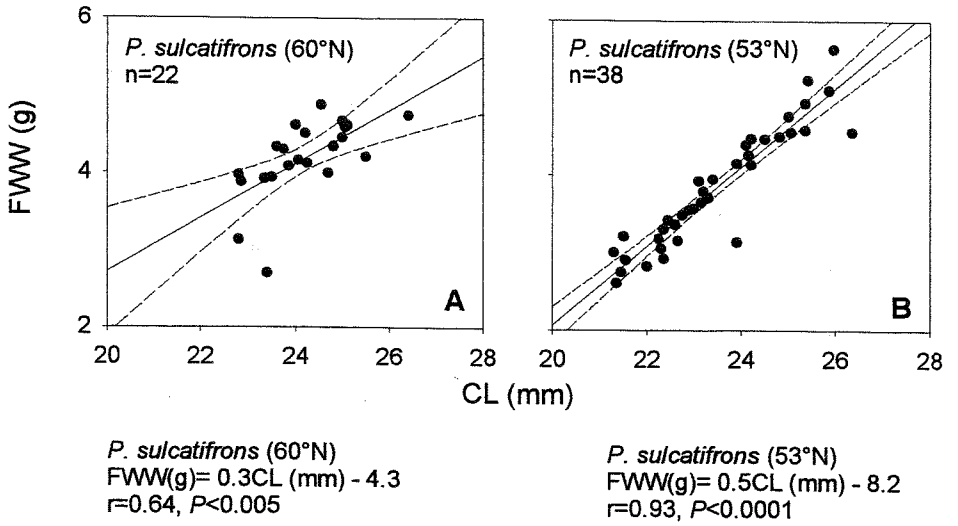


Figure 3.16. Linear regression and 95% confidence limits of female wet weight (FWW) against carapace length (CL) in two populations of *Parapasiphae sulcatifrons* from 60°N and 53°N in the NE Atlantic.

The mean female wet weigh was 4.2 ±0.1 g in the population from 60°N and 3.8 ±0.8 in the population from 53°N. The female wet weight was positively correlated with carapace length at both stations (Pearson Product Moment, r=0.64, n=22, P<0.005 at 60°N and r=0.93, n=38, P<0.0001 at 53°N; Fig. 3.16A & B).

3.3.3.2- Fecundity

Because of the large egg size and lack of brood protection, there was loss of eggs during collection, resulting in many broods containing only a few eggs. In *P. sulcatifrons*, the new developing cohort of eggs is clearly visible through the carapace, and fecundity was estimated by counting the eggs in the ovaries.

In the population from 60°N, the number of eggs in the pleopods ranged from 2 to 17 eggs (mean = 8.5 ±4.6 eggs), while the number of eggs counted in the ovary ranged from 16 to 22 eggs (mean = 18.8 ±1.9 eggs). At 53°N, the females of *P. sulcatifrons* were carrying from 5 to 21 eggs on the pleopods (mean = 12.1 ±4.7 eggs), while there were from 8 to 22 eggs in the ovaries (mean = 15.5 ±3.1 eggs). The number of eggs on the pleopods was statistically lower than the number of eggs in the ovary at both stations (Student t-test, t= -6.7, 25df, P<0.0001 at 60°N; Mann-Whitney, T= 1082.0, 48df, P<0.0005 at 53°N). The mean size-specific fecundity (estimated from ovary counts) was 4.4 ±0.6 eggs g^{-1} FWW at 60°N and 4.1 ±0.8 eggs g^{-1} FWW at 53°N (Table 3.2). There were no statistically significant differences in the mean size-specific fecundity of the two populations at the different latitudes (Student t-test, t= -1.134, 35df, P>0.05; Fig. 3.17).

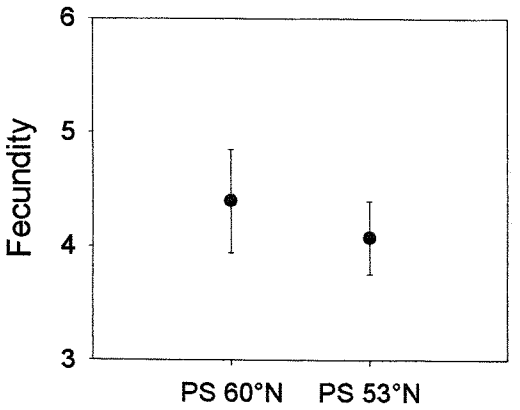


Figure 3.17. Mean size-specific fecundity (number of eggs in the ovary per 1 g of female weight) and 95% confidence limits in two populations of *Parapasiphae sulcatifrons* from 60°N and 53°N in the NE Atlantic.

Total fecundity (as number of eggs in ovary) was correlated with CL in the population from 53°N (Pearson Product Moment, r=0.65, n=25, P<0.005; Fig. 3.18A), but was not correlated in the population from 60°N (Pearson Product Moment, n=17, P>0.05; Fig. 3.18B). The lack of correlation in the latter population could reflect a

small sample size where most of the females had a similar carapace length between 22.8 and 24.8 mm and there was only one larger female with CL = 26.4 mm.

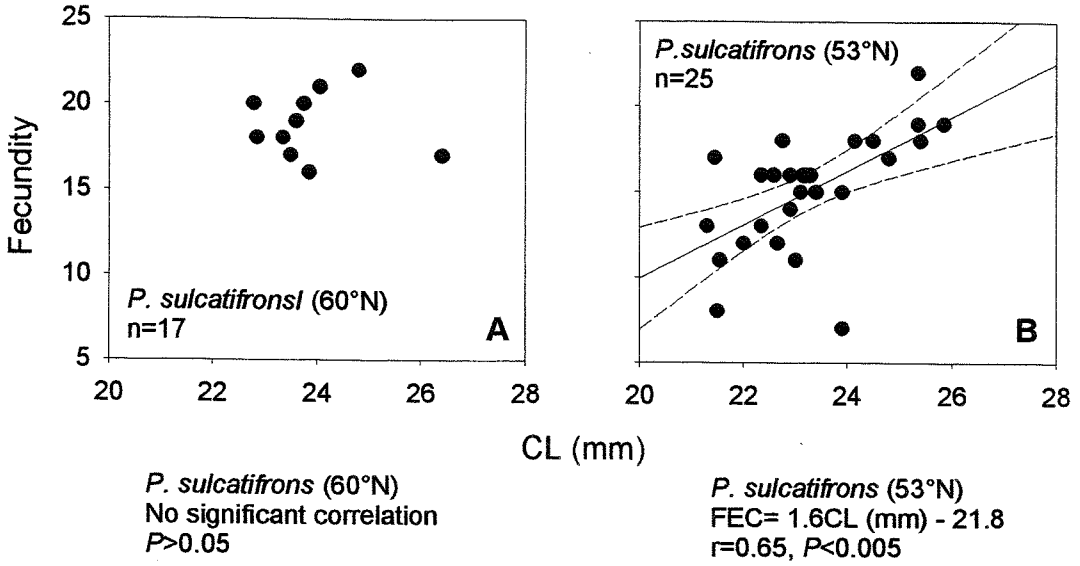


Figure 3.18. Linear regression and 95% confidence limits of total fecundity (number of eggs in ovary) against carapace length (CL) in two populations of *Parapasiphae sulcatifrons* from 60°N and 53°N in the NE Atlantic.

3.3.3.3- Egg size

The mean egg length of *P. sulcatifrons* was 4.7 ±0.2 mm in the population from 60°N, and 4.6 ±0.2 mm in the population from 53°N. The mean egg volumes were 42.2 ±4.0 mm^3 at 60°N, and 38.0 ±6.2 mm^3 at 53°N (Table 3.3). The mean egg volume was significantly higher in the population at 60°N than at 53°N (Student t-test, t=2.335, 40df, P<0.05; Fig. 3.19).

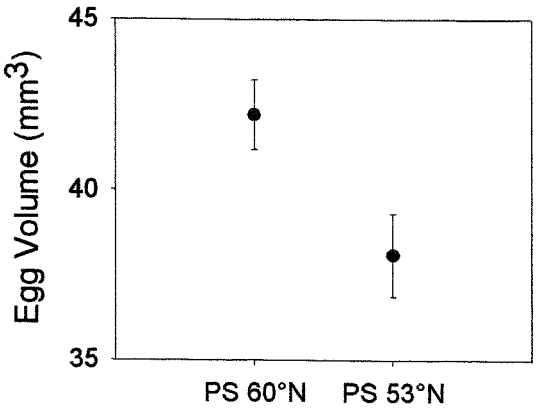


Figure 3.19. Mean egg volume and 95% confidence limits of two populations of *Parapasiphae sulcatifrons* from 60°N and 53°N in the NE Atlantic.

3.3.3.4- Reproductive output

The broods of *P. sulcatifrons* were badly damaged, and the egg mass weight was estimated from the number of eggs in the ovary and the average weight of one egg. The reproductive output was quantified using this estimated egg mass weight and the measured female weight. The mean RO ranged from 0.019 to 0.096 (mean = 0.05 ±0.03) at 60°N, and from 0.01 to 0.128 (mean = 0.07 ±0.03) at 53°N (Table 3.4). The RO of the population from 53°N was significantly higher than that of the population from 60°N (Student's test, t= 2.397, 42df, P<0.05; Fig. 3.20).

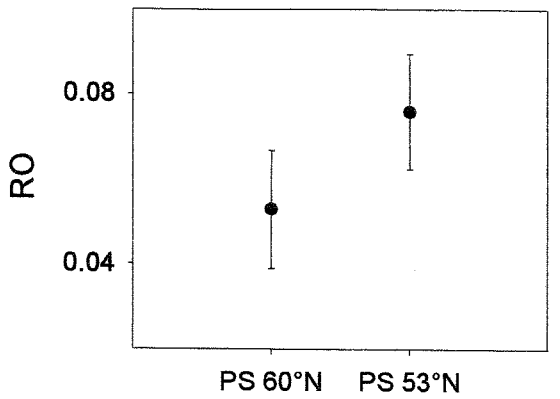


Figure 3.20. Mean reproductive output (RO) and 95% confidence limits of two populations of *Parapasiphae sulcatifrons* from 60°N and 53°N in the NE Atlantic.

3.4- Discussion

3.4.1- Adult size

The five species of deep-water caridean shrimp considered in this study are abundant and widely distributed in the NE Atlantic water column. In these assemblages, there is a decrease in ovigerous female size with decreasing latitude (Fig. 3.21). *Parapasiphae sulcatifrons* and *Acanthephyra pelagica* are the largest, with mean carapace lengths of 23.8 ±0.2mm and 20.4 ±0.9 mm respectively. These species occupy the most northern latitudes and deeper areas sampled, 60°N and 53°N. *A. purpurea* replaces *A. pelagica* between 40°N and 30°N, and is smaller than its northern congener (mean CL= 14.7 ±0.6 mm). *Systellaspis debilis* is found at 40°N, 30°N and 11°N and has similar adult sizes at all latitudes (mean CL= 13.3 ±0.1 mm). Finally, the very abundant *Acanthephyra kingsleyi,* is dominant in the tropical latitudes, at 11°N, and is the smallest of the *Acanthephyra* species, with mean adult size similar to that of *S. debilis* (mean CL= 13.7 ±0.2 mm).

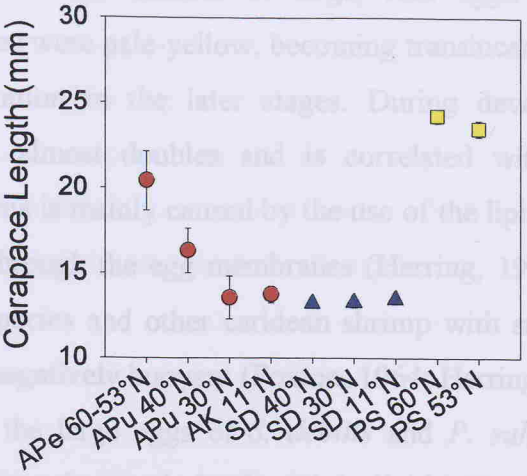


Figure 3.21. Mean carapace length (mm) and 95% confidence limits in five species of mesopelagic caridean shrimp from the NE Atlantic at different latitudes.
APe 60-53°N, combined populations of *Acanthephyra pelagica* from 60° and 53°N; APu 40°N, *A. purpurea* from 40°N; APu 30°N, *A. purpurea* from 30°N; AK 11°N, *A. kingsleyi* from 11°N; SD 40°N, *Systellaspis debilis* from 40°N; SD 30°N, *S. debilis* from 30°N; SD 11°N, *S. debilis* from 11°N; PS 60°N, *Parapasiphaea sulcatifrons* from 60°N; PS 53°N, *P. sulcatifrons* from 53°N.

The increase in body size with increasing latitude and/or increasing depth is a common trend found in caridean shrimps of the families Pandalidae, Hippolytidae, Crangonidae, Nematocarcinidae and Pasiphaeidae (Allen, 1963; Clarke, 1979; King and Butler, 1985; Gorny et al., 1992; Thessalou-Legaki, 1992; Ohtomi, 1997; Company et al., submitted), amphipods (Steele and Steele, 1991), euphausiids and mysids (Mauchline, 1988). The evolution of *K*-strategies (slow growth rate, deferred maturity, low fecundity and production of young of high competitive ability) is a common trait in many deep-water or high latitude species. The low temperatures found at higher latitudes and depths are associated with a lower metabolic rate and females reaching first maturity at larger sizes (Clarke, 1979, 1987; King and Butler, 1985; Gorny et al., 1992; Ohtomi, 1997).

3.4.2- Reproductive patterns

All five species showed typical caridean gonad development. The gonads grow under the carapace over the digestive gland in males and females. With the development of the ovaries, the oocytes are clearly visible to the naked eye, especially in *Parapasiphae sulcatifrons* and *Systellaspis debilis* that produce large, yolky eggs.

Because the amount of energy available for reproduction is limited, there is a trade-off between fecundity and egg size, with species producing either a high number

of small eggs or a small number of large, rich eggs. The small eggs of the *Acanthephyra* species were pale-yellow, becoming translucent and with clear eye spots and limb differentiation in the later stages. During development, the volume of *Acanthephyra* eggs almost doubles and is correlated with a decrease in density (Herring, 1974a). This is mainly caused by the use of the lipidic reserves and an uptake of water and salts through the egg membranes (Herring, 1974a,b). The small eggs of the *Acanthephyra* species and other caridean shrimp with small eggs are denser than large eggs, and are negatively buoyant (Foxton, 1964; Herring, 1967).

Conversely, the large eggs of *S. debilis* and *P. sulcatifrons* were dense and opaque, red-orange in colour and clearly rich in lipids. The large eggs of species such as *Systellaspis deblis, Oplophorus gimaldii* or *Ephyrina hoskynii* are positively buoyant throughout their development because of lipid storage (Herring, 1967, 1974a,b).

The incubation period of the relatively shallow-living *Acanthephyra purpurea* and *A. kingsleyi* is considered to be around three months, while deeper species such as *A. pelagica, A. acutifrons, Notostomus auriculatus* and *Meningodora vesca* have a longer embryo development extending to six months (Herring, 1974a). The duration of incubation also varies with latitude, being longer at higher latitudes and extending up to ten months or more in polar species (Clarke, 1979, 1993a, Clarke et al., 1991). These differences in developmental time are related to the ambient temperature to which the eggs are exposed, with lower temperatures at deeper depths determining a slower development (Herring, 1974a; Thessalou-Legaki, 1992). The incubation time of large eggs is much longer than that of small eggs, being around eight to ten months for the deep-water and large-egged *Systellaspis debilis* and *Ephyrina* (Herring, 1974a). It has been suggested that the slow rate of carbon dioxide and oxygen diffusion in large eggs reduces the metabolic activity of the embryo, decreasing the developmental rate (Lonsdale and Levinton, 1985).

The embryos hatching from large eggs produce larger and more advanced zoea and develop through fewer zoeal stages in the plankton (Herring, 1974a,b; Thessalou-Legaki, 1992, Clarke, 1993a). Because the lipids provide the main energy source for embryonic development in decapods and other crustaceans (Pandian, 1970), species with abbreviated larval development need large energetic reserves for the related extended embryonic development (Herring, 1974b; Thessalou-Legaki and Kiortsis, 1997). In species such as *Acanthephyra* spp, the first zoea have little yolk available but well developed feeding and swimming appendages. In contrast, the first zoea hatching

from large eggs (*Systellaspis debilis, Parapasiphae sulcatifrons, Oplophorus* sp., *Ephyrina* sp. or *Pasiphaea* sp.) have a large amount of yolk reserves and relatively undeveloped mouthparts (Herring, 1974a,b).

Total fecundity was positively correlated with female size (CL) in all species, although in some groups variability was high, caused by small sample sizes and damaged broods. This relationship between fecundity and adult size is common among crustaceans in general and caridean shrimps in particular, and reflects the morphological constraints for ovary growth and egg attachment on the pleopods (Barnes and Barnes 1968; Omori 1974; Clarke 1979, 1993b; Hines, 1982; Somerton and Meyers, 1983; King and Butler 1985; Ohtomi 1997).

Because larger females produce a higher number of eggs, fecundity per gram of female wet weight was used to compare individuals within species and between species of different sizes. In the *Acanthephyra* group, *A. pelagica* is the most northern species and also has the deepest mean depth of distribution (~1000 m). This species had the lowest size-specific fecundity, with 234.7 ±24.5 eggs g^{-1} FWW. The size-specific fecundity of the two other species were not significantly different, with means of 448.2 ±31.4 eggs g^{-1} FWW for *A. purpurea* (mean depth ~800 m) and 470.6 ±23.3 eggs g^{-1} FWW for *A. kingsleyi* (mean depth ~600 m). Conversely, when considering egg volumes, *A. kingsleyi* and *A. purpurea* had smaller eggs (0.28± 0.005 mm^3 and 0.30± 0.015 mm^3 respectively) than their northern and deeper living counterpart *A. pelagica* (0.75± 0.01 mm^3). These data suggest that, within the *Acanthephyra* species studied, fecundity decreases with increasing latitude and increasing depth (Fig. 3.22A) and this decrease in fecundity is associated with an increase in egg size. However, it has been shown that egg size may also increase with increasing female size (Clarke, 1993b), and the increase in egg size with latitude found for the *Acanthephyra* species could in fact be a consequence of the larger body size of ovigerous females at higher latitudes.

The same pattern was found in the two species with large eggs, *Systellaspis debilis* and *Parapasiphae sulcatifrons*, with mean depth distributions at 700 m and 1000 m respectively. Within *Systellaspis debilis*, the two southern populations had a higher size-specific fecundity than the northern one (10.1 ±0.2 eggs g^{-1} FWW in the combined populations from 30°N and 11°N and 8.0 ±0.4 eggs g^{-1} FWW at 40°N) (Fig. 3.22B). Also, the mean size-specific fecundity of *S. debilis* was higher than that of the more northern and deeper-living populations of *P. sulcatifrons* (4.1 ±0.2 eggs g^{-1} FWW

in the combined data from 60°N and 53°N) (Fig. 3.22B). The eggs of *P. sulcatifrons* were significantly larger than the eggs of *S. debilis*, with mean egg volumes of 41.2 ±1.1 mm^3 and 12.6 ±0.2 mm^3 respectively.

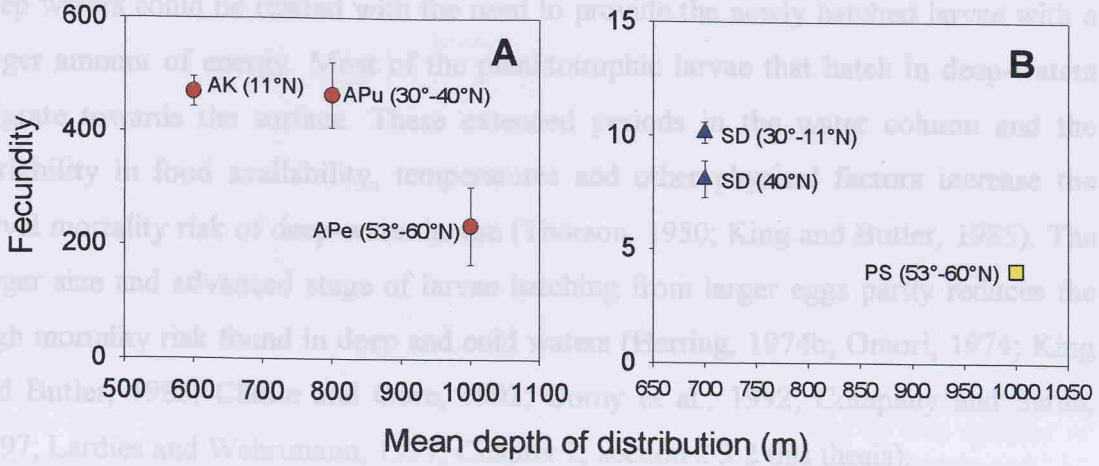


Figure 3.22. Mean size-specific fecundity in five species of NE Atlantic mesopelagic shrimp. A- Small-egged species of the *Acanthephyra* group: AK, *A. kingsleyi*; APu, *A. purpurea*; APe, *A. pelagica*. B- Large-egged species: SD, *Systellaspis debilis*; PS, *Parapasiphae sulcatifrons*.

There seems to be a general cline in fecundity and egg size with latitude, with species living at higher latitudes or deeper water producing a lower number of eggs but with a higher parental investment per embryo. The reasons for these clines are not clear, but it is possible that they are associated with the need to provide a greater amount of energy to the larvae developing at higher latitudes and greater depths (Clarke et al., 1991; Clarke and Gorny, 1992; Gorny et al., 1992). For example, the polar species *Chorismus antarcticus* and *Notocrangon antarcticus* have more conspicuous *K*-strategy characteristics (deferred maturity, low fecundity and large eggs hatching into advanced larvae) than the related temperate species *Pandalus montagui* and *Crangon crangon* (Clarke, 1979). Also, in the polar shrimps *Chorismus antarcticus* and *Notocrangon antarcticus*, the populations from South Georgia (Antarctica) grow to a larger maximum size, attain sexual maturity later and produce fewer and larger eggs than their Subantarctic counterparts (Gorny et al., 1992). Similarly, the deep-water prawn *Pandalus borealis* from high latitudes also reaches larger adult sizes, has late maturity, longer embryo developmental times and larger egg sizes than the populations from lower latitudes (Clarke et al., 1991).

When considering a depth gradient (200 to 800 m), species belonging to the families Pandalidae and Hippolytidae showed longer reproductive lifespans, larger maximum female sizes and larger egg sizes in the deeper populations (King and Butler, 1985; Thessalou-Legaki, 1992). The production of larger eggs at higher latitudes and/or deep waters could be related with the need to provide the newly hatched larvae with a larger amount of energy. Most of the planktotrophic larvae that hatch in deep-waters migrate towards the surface. These extended periods in the water column and the variability in food availability, temperatures and other physical factors increase the larval mortality risk of deep-water larvae (Thorson, 1950; King and Butler, 1985). The larger size and advanced stage of larvae hatching from larger eggs partly reduces the high mortality risk found in deep and cold waters (Herring, 1974b; Omori, 1974; King and Butler, 1985; Clarke and Gore, 1992; Gorny et al., 1992; Company and Sardà, 1997; Lardies and Wehrtmann, 1997; Chapter 2, section 2.3.2 this thesis).

Also, the energy available for the females and for the larvae might select for lower fecundity and larger eggs at higher latitudes and depths. While the overall investment in reproduction is limited by environmental factors experienced by the females before and during ovary maturation, the investment per offspring is related to the conditions found in the plankton by the newly hatched larvae (King and Butler, 1985; Lonsdale and Levinton, 1985; Clarke et al., 1991; Clarke, 1993b). The lower food availability found in deep-waters might constrain the investment in reproduction, and the high larval mortality risk would select for the production of fewer eggs but of a higher quality (larger size) with higher survival probabilities.

Reproductive output (weight-specific gonad production) is an estimation of the reproductive effort of a species. Reproductive effort is an important life history trait representing the fraction of the total energy available to the female that is allocated to reproduction, including collection of extra food, territorial and mating behaviour and caring for the brood and young (Clarke, 1987; Thessalou-Legaki & Kiortis, 1997). In the species of the *Acanthephyra* group, the reproductive output was similar within species at the different latitudes and between species from different latitudes and depths (Fig. 3.23), with a mean RO of 0.09 ±0.002 for the group. In the species with large eggs, the reproductive output was significantly higher in the lower latitude populations. The mean RO was 0.09 ±0.02 for *S. debilis* from 11°N and 0.07 ±0.02 for the combined data of *S. debilis* from 30°N and 40°N. The mean RO of *P. sulcatifrons* was also

significantly higher in the population from the lower latitude, with means of 0.08 ±0.03 at 53°N and 0.05 ±0.03 at 60°N (Fig. 3.23).

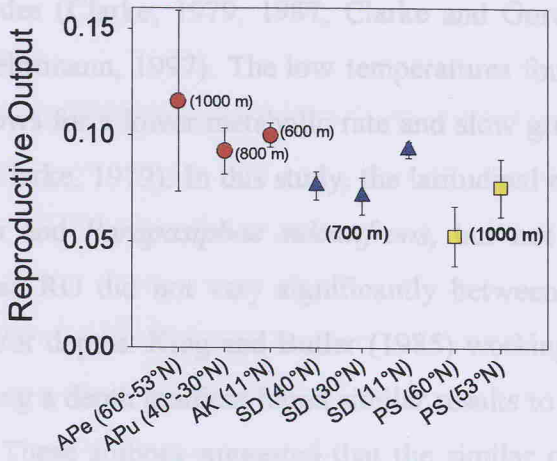


Figure 3.23. Mean reproductive output and 95% confidence limits of five species of mesopelagic caridean shrimp from different latitudes and depths in the NE Atlantic. APe, combined data for the populations of *A. pelagica* from 60°N and 53°N. APu, combined data for the populations of *A. purpurea* from 40°N and 30°N. AK, *A. kingsleyi* from 11°N; SD, *Systellaspis debilis* from 40°N, 30°N and 11°N; PS, *Parapasiphae sulcatifrons* from 60°N and 53°N.

The reproductive output of decapod crustaceans has been considered as one of the most determinant factors in their life history. Data on reproductive output has been used for theoretical considerations of life history strategies (Clarke, 1979, 1987; Hines, 1982; King and Butler, 1985; Ivanova and Vassilenko, 1987; Lardies and Wehrtmann, 1997; Thessalou-Legaki and Kiortsis, 1997), for defining stock units in important commercial species (Morizur et al., 1981), for studies of population variability with habitat variations (Thessalou-Legaki, 1992) and to understand the reproductive biology of species (Somerton and Meyers, 1983).

Ivanova and Vassilenko (1987), described the relationship between fecundity and brood weight with body weight for the Crustacea, and found that the reproductive output had relatively constant values around 0.16 (16% of the body weight is egg weight). Data on RO of caridean shrimps varying from tropical (Anger and Moreira, 1998) to polar species (Clarke, 1979, 1987) and from shallow water (Lardies and Wehrtmann, 1997; Thessalou-Legaki and Kiortsis, 1997) to deep water species (King and Butler, 1985; Clarke, 1987), fit in the range of RO given for the Crustacea group as a whole by Ivanova and Vassilenko (1987). The data for mesopelagic caridean shrimp

from the NE Atlantic are near the range proposed by these authors, although slightly lower.

It has been suggested that there is a latitudinal cline of RO, with higher values of RO at lower latitudes (Clarke, 1979, 1987; Clarke and Gore, 1992; Gorny et al., 1992; Lardies and Wehrtmann, 1997). The low temperatures found at higher latitudes and deeper depths allows for a lower metabolic rate and slow growth with a decreased reproductive output (Clarke, 1979). In this study, the latitudinal cline in RO was found in *Systellaspis debilis* and *Parapasiphae sulcatifrons*, but not in the *Acanthephyra* group, where the mean RO did not vary significantly between the species living at different latitudes and/or depths. King and Butler (1985) working with several species of caridean shrimp along a depth gradient found similar results to the above data for the *Acanthephyra* group. These authors suggested that the similar reproductive output of species from different habitats might reflect a ceiling on parental investment above which adult mortality may be prohibitively high.

CHAPTER FOUR - REPRODUCTIVE BIOLOGY OF THREE CARIDEAN SHRIMP, *RIMICARIS EXOCULATA*, *CHOROCARIS CHACEI* AND *MIROCARIS FORTUNATA*, FROM THE MID-ATLANTIC RIDGE

4.1- Introduction

4.1.1- Mid-Atlantic Ridge vents and faunal composition

Almost ten years were to pass after the discovery of hydrothermal vents along the Galápagos Rift in 1977 before the existence of a hydrothermal vent fauna was demonstrated in the Atlantic Ocean (see chapter 1, section 1.2.4 this thesis). Rona and colleagues (1986) discovered black smokers, massive sulphides and a vent biota in the rift valley of the slow spreading Mid-Atlantic Ridge (MAR) at what is now called the TAG (Trans-Atlantic Geotraverse) vent field. The existence of hydrothermal assemblages was thought to be limited to intermediate-fast spreading ridges, such as the ones in the eastern Pacific Ocean. The hydrothermal vents at TAG were the first of the kind to be reported from a slow-spreading centre (Rona, 1985; Rona et al., 1986; German et al., 1995). Since then, seven hydrothermal vent fields have been sampled for fauna along the MAR south of the Azores triple junction: Menez Gwen (850 m depth), Lucky Strike (1690 m), Rainbow (2250 m), Broken Spur (2900 m), TAG (3650 m), Snake Pit (3480 m) and Logatchev (3000 m) (Fig. 4.1).

While the Pacific vent fauna is mainly composed of the tube worm *Riftia pachyptila,* mussels, clams and other sessile invertebrates, the Atlantic vents are dominated by ubiquitous motile caridean shrimps and bathymodiolid mussels (Rona et al., 1986; Van Dover et al., 1988, 1996; Segonzac et al., 1993; Van Dover, 1995). Together with the dense aggregations of shrimps and mussels, there is an associated fauna found around, or in the proximity of, the vent chimneys. This associated fauna comprises sessile translucent anemones, limpets, the bythograeid crab *Segonzacia mesatlantica* and fishes belonging to the families Moridídae, Chimaeridae and Bythitidae (Gage and Tyler, 1991; Van Dover, 1995; Van Dover et al., 1996).

The systematics of the MAR caridean shrimps has been changing continuously and is still imperfectly understood. The family Bresiliidae originally contained the vent shrimps, until Christoffersen (1986) created the new family Alvinocarididae for the genera *Rimicaris* Williams and Rona 1986 and *Alvinocaris* Williams and Chace 1982.

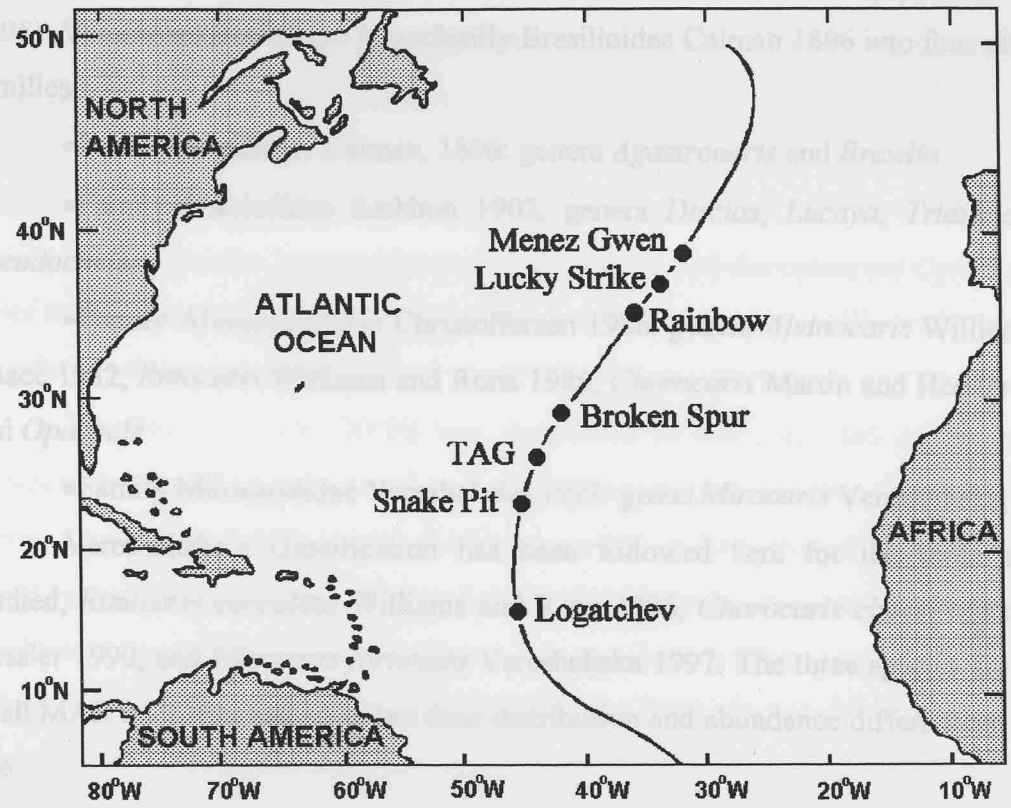


Figure 4.1. Location of hydrothermal vents on the Mid-Atlantic Ridge south of the Azores.

While this new family has been adopted by several authors (Segonzac et al., 1993; Vereshchaka, 1996; Pond et al., 1997b,c; Vereshchaka, 1997; Ramirez Llodra et al., 2000), other authors still use the more conservative approach, keeping the vent shrimps in the family Bresiliidae (Chace, 1992; Gebruk et al., 1993; Holthius, 1993; Murton et al., 1995; Van Dover 1995; Van Dover et al., 1996; Pond et al., 1997a). Recently, Vereshchaka (1997) has created the new family Mirocariidae for the new genus *Mirocaris*, comprising two species -*Mirocaris keldyshi* gen.nov., sp.nov. and *Mirocaris fortunata* renamed from *Chorocaris fortunata* (Martin and Christiansen, 1995). Vereshchaka splits the Superfamily Bresilioidea Calman 1896 into four different families:

•Family Bresiliidae Calman, 1896: genera *Agastrocaris* and *Bresilia*

•Family Disciadidae Rathbun 1902: genera *Discias*, *Lucaya*, *Tridiscias* and *Pseudocheles*

•Family Alvinocarididae Christoffersen 1986: genera *Alvinocaris* Williams and Chace 1982, *Rimicaris* Williams and Rona 1986, *Chorocaris* Martin and Hessler 1990, and *Opaepele*

•Family Mirocarididae Vereshchaka 1997: genus *Mirocaris* Vereshchaka, 1997

Vereshchaka's classification has been followed here for the three species studied, *Rimicaris exoculata* Williams and Rona 1986, *Chorocaris chacei* Martin and Hessler 1990, and *Mirocaris fortunata* Vereshchaka 1997. The three species are found at all MAR hydrothermal sites, but their distribution and abundance differs from site to site.

TAG (26°N) was the first hydrothermal site discovered in the Mid-Atlantic Ridge (Rona, 1985). Its black smokers are located between the floor and the east wall of the rift valley, at around 3650 m depth. The fauna is dominated by the bresiliid shrimp *Rimicaris exoculata*. These shrimps are found covering the chimney walls with densities that can reach up to 2500 individuals m^{-2}. (Van Dover et al., 1988; Gebruk et al., 1993; Segonzac et al., 1993; Van Dover, 1995; Gebruk et al., 1997; Pond et al., 1997b; Gebruk et al., 2000b). The shrimp *Chorocaris chacei* is also found at the chimneys. At the base of the active areas, the bythograeid crab *Segonzacia* sp. and the galatheid crab *Munidopsis* sp. together with the ophiuroid *Ophioctenella acies* are commonly found (Tyler et al., 1995; Gebruk et al., 1997). The gastropod

Phymorhyncus sp. and the anemone *Parasycionis* sp. are abundant at the periphery of the vents (Gebruk et al., 1997).

Broken Spur (29°N) was found in 1993 and the hydrothermal site was first visited by *Alvin* (Murton and Van Dover, 1993). The faunal composition is similar to that of TAG but the biomass is one order of magnitude lower (Gebruk et al., 1997). *Rimicaris exoculata* and *Chorocaris chacei* are found at Broken Spur, but at much lower densities than at the other sites (Van Dover, 1995; Creasey et al., 1996; Copley et al., 1997; Gebruk et al., 1997; Pond et al., 1997a). However, the biomass of fauna other than shrimp is not significantly lower than at other sites. A hypothesis to explain the lack of dense populations of *Rimicaris exoculata* at Broken Spur is that only a small amount of substratum is exposed to the flow of hydrothermal fluids, and this might be a prerequisite for the development of aggregations (Copley et al., 1997). In Broken Spur like in TAG, the crabs *Segonzacia* sp and *Munidopsis* and the ophiuroid *Ophioctenella acies* are abundant at the base of the chimneys, while the gastropod *Phymorhyncus* sp is abundant at the periphery (Gebruk et al., 1997).

The *Snake Pit* site (20°N) was discovered in 1985, at 3480 m, by seafloor photography, and first visited and studied by *Alvin* in 1986 and *Nautile* in 1988. The shrimp fauna at Snake Pit is very similar to the one at TAG (Van Dover, 1995), with dense aggregations of mainly *R. exoculata* inhabiting the warm waters around the black smokers and beehive diffusers. *C. chacei* is found close to *Rimicaris*, but in the cooler waters (Van Dover et al., 1988; Casanova et al., 1993; Segonzac et al., 1993; Van Dover, 1995; Pond et al., 1997 b; Gebruk et al., 2000b). At the base of the chimneys and at the periphery, the fauna is also similar to that of TAG, with abundant *Segonzacia* sp., *Munidopsis* sp., *Ophioctenella acies*, the anemone *Parasycionis* sp. and the gastropod *Phymorhyncus* sp. (Gebruk et al., 1997).

Logatchev (14°N) was discovered using a deep-towed photo-system during the seventh cruise of the RV *Professor Logatchev* in 1993-1994 (Batuyev et al., 1994). The Logatchev area is the southernmost vent site of the MAR, found at 2900-3000 m depth. It has the highest diversity of biotopes and organisms at MAR. There is 1)- an active chimney complex mainly colonised by the mussel *Bathymodiolus* associated with swarms of shrimps (*Rimicaris exoculata, Alvinocaris* and *Mirocaris keldyshi*), 2)- a diffuse flow area in soft sediment mainly colonised by vesicomyid clams, and 3)- a large sulphide mound with smoking craters colonised by a less abundant and less diverse fauna (Gebruk et al., 2000a). The clams found at Logatchev are the first record

of a living population of vesicomyid clams in the MAR (Gebruk et al., 2000a). The crab *Segonzacia* is seen around the mussel beds, while the ophiuroid *Ophioctenella acies* is common on shells and chimney walls and the gastropod *Phymorhyncus* sp. at the periphery of the vents (Gebruk et al., 1997).

The *Lucky Strike* site (37°N) was discovered during the FAZAR expedition, (C. Langmuir, chief scientist, 1992) and first visited by *Alvin* in spring 1993 (Langmuir et al., 1993). The hydrothermal vents at Lucky Strike are located at depths between 1600 and 1700 m. At these sites, the invertebrate fauna is sufficiently different from TAG and Snake Pit to be considered a new biogeographic region (Van Dover et al., 1996). Although most of the taxonomic groups found are the same as at other MAR vent sites, the communities at Lucky Strike differ by the relative abundance and distribution of the species, as well as by the existence of seven new undescribed species (Van Dover et al., 1996). The habitat that was usually occupied by *Rimicaris exoculata* at other vent sites (sulphide surfaces at venting chimneys and cracks in the seafloor where warm water emanates) are here colonised densely by a new species of the mussel *Bathymodiolus*, giving Lucky Strike a very different visual impression from the other MAR sites (Van Dover, 1995; Van Dover et al., 1996; Gebruk et al., 2000b). Most of the mussels from this site contain the commensal polychaete *Branchipolynoe seepensis*, which is an "invisible" but dominant faunal component (Van Dover et al., 1996). As far as the caridean shrimp are concerned, Lucky Strike differs from the other sites by the dominance of the smallest of the three species, *Mirocaris fortunata*. This species was first collected at Lucky Strike, and classified as *Chorocaris fortunata* (Martin and Christiansen, 1995), until Vereshchaka (1997) created the new genus *Mirocaris*. *M. fortunata* is found in patches over the mussel bed and shimmering waters. In some areas, this species is found together with *Chorocaris chacei* (Van Dover et al., 1996; E. Ramirez Llodra, *pers. obs.*), and to a lesser extent with *Rimicaris exoculata* (Desbruyères et al., 1994; E. Ramirez Llodra, *pers. obs.*). Bythograeid crabs and the gastropod *Phymorhyncus* sp. are rare at Lucky Strike. Also, a new species of echinoid, probably *Echinus alexandri* (M. Sibuet, *pers. com.*), has been observed at this site, although it is not common (Van Dover et al., 1996).

Menez Gwen (37°N) was discovered during the DIVA1 cruise and is located on the volcanic segment north of Lucky Strike between 840 and 870 m depth (Desbruyères et al., 1994; Fouquet et al., 1994; Colaço et al., 1998). The faunal composition of Menez Gwen is similar to that of Lucky Strike, dominated by mussel beds of

Bathymodiolus covered by filamentous bacterial mats. There are also small swarms of the vent shrimps *Mirocaris fortunata* and *Chorocaris chacei* and a few bythograeid crabs (Colaço et al., 1998).

Rainbow (36°N) is situated on the western flank of the Rainbow ridge, between 2270 and 2320 m of depth. Contrasting with the other vent fields, Rainbow is situated next to a transform fault, instead of in the middle of a segment. It is one of the most active vent fields in MAR, with ten groups of extremely active black smokers dispersed over the entire vent field (Fouquet et al., 1997). The fauna at Rainbow is in process of being studied, but first observations show an important dominance of the bresiliid shrimp *Rimicaris exoculata* on the chimneys and *Bathymodiolus* sp. at the base (Alyase et al., 1997; Vinogradov, 1999; E. Ramirez Llodra, *pers. obs.*).

4.1.2- Biology of hydrothermal vent shrimp

Rimicaris exoculata (Fig. 4.2A) is found in dense aggregations at all Atlantic vent fields except Menez Gwen. It is particularly abundant at TAG (Gebruk et al., 2000b; P. Tyler, *pers. com.*). This species requires both substratum and a source of hydrogen sulphide to survive (Copley et al., 1997; Gebruk et al., 2000a). *R. exoculata* gains its energy by harvesting ectosymbiotic bacteria that live on modified structures, the scaphagnothites, within the carapace (Van Dover et al., 1988; Gebruk et al., 1993; Segonzac et al., 1993; Rieley et al., 1999). Recent studies have also shown the presence of endosymbiotic gut bacteria that may oxidise polymetal sulphides ingested by the shrimp (Polz et al., 1998). *R. exoculata* has no eyes, but has a specialised dorsal organ that has evolved to be maximally sensitive to low level illumination such as black-body radiation of 350°C vents (Pelli and Chamberlain, 1989; Van Dover et al., 1989; Kuenzler et al., 1997; Gaten et al., 1998a; Jinks et al., 1998). In addition, there are sensillae on the antennae II capable of eliciting a behavioural response at picomolar levels of hydrogen sulphide and to detect sulphide gradients around hydrothermal vents (Renninger et al., 1995; Jinks et al., 1998). It has been suggested that the mesophilic temperatures (>20°C) found close to the vents might be important for the growth of the chemoautotrophic bacteria, and the sensory organs (sensillae and dorsal organ) are believed to be used to maintain position, swimming towards the emanating hydrothermal fluids (Segonzac et al., 1993; Jinks et al., 1998; Gebruk et al., 2000b).

Chorocaris chacei (Fig. 4.2B) is found at all Atlantic vents. This species has eyes and a small dorsal organ (Gaten et al., 1998a; Jinks et al., 1998). They are scavengers and have been reported to be attracted to baited traps. Nevertheless, *Chorocaris chacei* also has episymbiotic microorganisms along its respiratory current pathways (Segonzac et al., 1993; Gebruk et al., 2000b).

Mirocaris fortunata (Fig. 4.2B) is found at all vents from Menez Gwen to Broken Spur. At vents deeper than Broken Spur it is replaced by *Mirocaris keldyshi* Vereschaka 1997. The validity of *M. keldyshi* as a separate species is currently under discussion and, as a result, *M. fortunata* may extend to the deepest vents (Shank et al., 1999). *M. fortunata* is an opportunistic feeder on mussels, shrimp and other invertebrates (Gebruk et al., 2000b). It is also believed to feed on the faecal deposits of *Bathymodiolus* sp. (D. Dixon, *pers. comm.*) and has been observed feeding on mussels damaged by the arm of the submersible during collection (Gebruk et al., 2000b; E. Ramirez Llodra, *pers. obs.*).

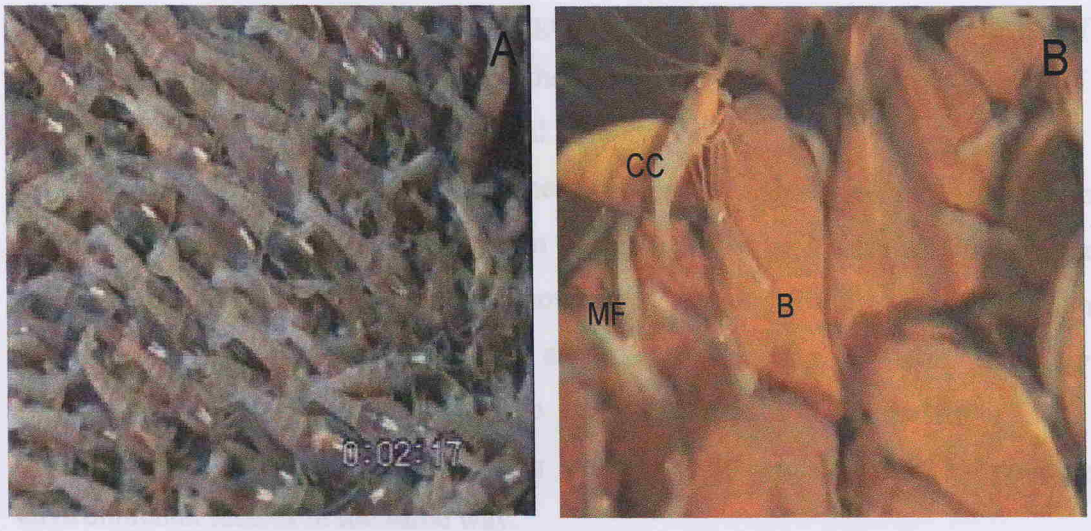


Figure 4.2. *In situ* photographs of hydrothermal vent caridean shrimps.
A- Dense aggregations of *Rimicaris exoculata* at TAG. B- *Chorocaris chacei* (CC) and *Mirocaris fortunata* (MF) over a *Bathymodiolus* (B) mussel bed at Lucky Strike. (Images from Nautile's video, IFREMER, Pico 1998 cruise)

Although there is good information on the taxonomy (Williams and Chace, 1982; Christoffersen, 1986; Williams and Rona, 1986; Martin and Hessler, 1990; Vereshchaka, 1997), trophic ecology (Van Dover et al., 1988; Casanova et al., 1993; Gebruk et al., 1993, 2000b; Segonzac et al., 1993; Pond et al., 1997b,c, 2000a,b; Allen Copley et al., 1998) and sensory studies (Van Dover et al., 1989; Renninger et al., 1995; Charmantier-Daures and Segonzac, 1998; Gaten et al., 1998a,b; Jinks et al., 1998) of the

MAR vent shrimps, little is known about their reproductive biology. Most of the available information on the life history of vent shrimps has been obtained from lipid and stable isotope analyses.

Decapod shrimp require certain essential polyunsaturated fatty acids (PUFA) to be supplied in their diet. These PUFA play a major role in the structure and functioning of cell membranes and need to be provided in substantial amounts during growth and reproduction (Pond et al., 2000a). However, the bacteria on which the vent food web is based do not have the capability of synthesising these components. The main bulk of PUFA in marine systems is produced by phytoplankton and it has been shown that the vent shrimp spend the larval stage in the plankton, during which they feed on photosynthetically derived material (Pond et al., 1997a,b; Dixon et al., 1998). The larval stages of vent shrimp accumulate these essential fatty acids in an important wax ester reserve and mobilise them into phospholipids when needed during growth and maturation (Gebruk et al., 2000b; Pond et al., 2000a). The postlarvae return to the vents to grow and mature, and it has been suggested that high sensitivity to low levels of H_2S and to quanta emitted from the hydrothermal vents allows them to localise the vent fields (Renninger et al., 1995; Gaten et al., 1998b).

Even so, little is known on the early reproductive biology (gametogenesis, fecundity, egg size) of vent shrimps. In order to understand the ecological processes driving a community, a thorough knowledge of the life history of its fauna is imperative. Reproductive patterns such as gamete production, egg size, fecundity and larval development play a major role in the continuity of populations and their adaptation to the environment. These processes do not respond to pressure by environmental factors in the same way.

The vitellogenic pathways, which have major implications in the rate of egg production and larval development mixed (see chapter one, section 1.3.1 this thesis), are phylogenetically constrained, and therefore intrinsic to the species and are not affected by external factors (Eckelbarger, 1983, 1994; Eckelbarger and Watling, 1995). Conversely, the reproductive output (number and size of eggs) is controlled by external factors such as food quantity and quality. Low food levels or poor quality can cause a decrease or even an interruption in yolk synthesis, slowing down the egg production rate and causing a reduction in fecundity (Qian and Chia, 1991; Levin et al., 1994; Eckelbarger and Watling, 1995; Chapter two, section 2.3.1 this thesis).

The characteristics of hydrothermal vents, with localised primary production, high levels of toxic compounds, high temperatures and high temporal and spatial variability (Tunnicliffe, 1991), together with a high energy availability, provide good experimental conditions to test the above postulates.

Most aspects of reproduction have been studied in detail in non-vent caridean shrimp (see Chapter 3 this thesis), and this information provides a base from which to analyse and compare the reproductive patterns of the MAR shrimp.

4.1.3- Chapter objectives

In this chapter, the oogenesis and reproductive output of three species of hydrothermal vent shrimp, *Rimicaris exoculata, Chorocaris chacei* and *Mirocaris fortunata* from the Mid-Atlantic Ridge has been described. The working hypotheses were 1)- that gametogenesis is phylogenetically constrained and therefore will be characteristic of a caridean shrimp, and 2)- that the reproductive output would be enhanced by an environment where there is a continuous and high energy availability. Fecundity and egg size, as well as their implication in dispersal abilities during the larval phase, are also discussed.

4.2- Materials and methods

Three species of caridean shrimp, *Rimicaris exoculata, Chorocaris chacei* and *Mirocaris fortunata* were collected during several cruises along the Mid-Atlantic Ridge hydrothermal vent fields (Fig. 4.1, Table 4.1). Shrimps were sampled by net or by slurp gun attached to the arm of the submersibles *Mir I* and *Nautile.*

R. exoculata was collected from TAG in September 1994 by the submersible *Mir I,* and from Rainbow in June-July 1998 using the submersible *Nautile.* One of the very rare ovigerous *R. exoculata* found collected to date was sampled from Snake Pit during the Microsmoke cruise in November 1995 and was analysed during a visit to Dr. Segonzac in IFREMER, Brest.

C. chacei was collected by *Nautile* from Lucky Strike in September 1997 and June-July 1998. One ovigerous *C. chacei* was collected during the DIVA2 cruise in June 1994 and provided by Dr. Segonzac for this study.

M. fortunata was collected at Lucky Strike in June 1994, September 1997 and June-July 1998, and at Rainbow in June-July 1998.

SPECIES	LOCATION	LATITUDE / DEPTH	CRUISE	DATE	NUMBER OF FEMALES EXAMINED	NUMBER OF OVIGEROUS FEMALES
Rimicaris exoculata	TAG	26°N 3650 m	BRAVEX	Sept. 1994	25	0
	Rainbow	36°N 2250 m	PICO	June-July 1998	25	0
	Snake Pit	23°N 3480 m	Microsmoke	November 1995	1	1
Chorocaris chacei	Lucky Strike	37°N 1690 m	DIVA2	June 1994	1	1
	Lucky Strike	37°N 1690 m	MARVEL	Aug-Sept. 1997	20	0
	Lucky Strike	37°N 1690 m	PICO	June-July 1998	25	0
Mirocaris fortunata	Lucky Strike	37°N 1690 m	DIVA2	June 1994	19	19
	Lucky Strike	37°N 1690 m	MARVEL	Aug-Sept. 1997	25	0
	Lucky Strike	37°N 1690 m	PICO	June-July 1998	37	0

Table 4.1. Location of sampling sites of three species of caridean shrimp along the Mid-Atlantic Ridge, showing cruises, date of collection, number of females examined and number of ovigerous females available.

All material was identified on board and fixed in 5% formaldehyde (BRAVEX material) or 10% formaldehyde (DIVA2, Microsmoke, MARVEL and PICO material) for 5 to 7 days, before being transferred to 70% isopropanol for storage.

In the laboratory, the carapace length (CL) of 20 to 25 females was measured with vernier callipers to the nearest 0.1 mm and the specimens were processed for histology. Whole *M. fortunata* were processed, but only the cephalothorax was prepared for the larger *C. chacei* and *R. exoculata*. The tissues were decalcified in Bouin's solution for 5 days, dehydrated in graded alcohols, cleared in Histoclear and embedded in paraffin wax. Sections were cut at 7 µm and stained with the routine stain Haematoxylin and Eosin.

From all the specimens analysed, only a maximum of nine females per species and sample provided good histological sections and were used for image analysis of the

ovaries. All the oocytes (N = 30 to 160) that had been sectioned through the germinal vesicle were measured (feret diameter) with an image analysis programme (Matrox-Rainbow Runner / Sigma Scan Pro 4). The measurements were grouped into 25 μm classes in order to determine the oocyte size-frequency distribution for each individual. The pooled data for the nine females in each sample were also plotted, except for *Chorocaris chacei* collected during the MARVEL 97 cruise, where only 3 females were available.

The mean size of vitellogenic oocytes was calculated for each individual, and the mean of means calculated for each population. Differences in the mean size of vitellogenic oocytes between the two samples available for each species was tested using the Student's t-test.

The carapace lengths and wet weights of ovigerous females were measured. The eggs carried on the pleopods were gently removed with a spatula and the egg mass weighed wet. The eggs were staged as early or late eggs, depending on the morphological features of the developing embryos. The eggs were measured (egg length (EL) and egg width (EW)) using the Matrox-Rainbow Runner and Sigma Scan Pro 4 software.

The eggs were oval in shape, which is a common feature in caridean shrimp, and therefore egg volume (EV) was estimated as:

$$EV = EL \times \pi \times \left(\frac{EW}{2}\right)^2$$ (Corey and Reid, 1991)

Total fecundity was quantified as number of eggs carried on the pleopods per female. Total fecundity was regressed onto carapace length and the correlation analysed with the Pearson Product Moment Correlation. Size-specific fecundity was also quantified and defined as number of eggs per gram of female weight. This expression of fecundity was used to compare the reproductive output between species and avoid the variation in fecundity caused by differences in female size.

4.3- Results

4.3.1- General patterns of ovary morphology and oogenesis

The ovaries in the three species are similar, situated dorsally under the carapace, overlying the digestive gland. Each ovary consists of several layers of growing oocytes enveloped by a thin gonad wall.

The oogonia proliferate in the germinal epithelium at the periphery of the gonad, developing into previtellogenic oocytes (Fig. 4.3D). The previtellogenic oocytes migrate to the growth zone between large vitellogenic oocytes and undergo vitellogenesis (Fig. 4.3A, C and D). Yolk production is identified by the presence of yolk granules spreading from the periphery of the oocyte towards the germinal vesicle (Fig. 4.3B). The yolk granules occupy most of the ooplasm in mature oocytes. A layer of flattened accessory cells, involved in the vitellogenetic processes, surrounds the vitellogenic oocytes (Fig. 4.3B).

The gametogenic patterns were similar in the three species, although there was some variability in the mean size of the oocyte stages (Table 4.2). However, a processing artefact causing variability between individuals can not be dismissed.

Species	Oogonia	Previtellogenic oocytes	Vitellogenic oocytes
Rimicaris exoculata TAG, September 94	21.9 ±3.4 µm	51.1 ±16.2 µm	166.9 ±48.7 µm
Rimicaris exoculata Rainbow, June 98	25.2 ±3.8 µm	61.2 ±17.1 µm	122.5 ±37.2 µm
Chorocaris chacei Lucky Strike, Sept. 94	26.4 ±5.8 µm	62.5 ±18.9 µm	158.2 ±50.3 µm
Chorocaris chacei Lucky Strike, July 98	31.9 ±5.8 µm	66.8 ±17.6 µm	122.0 ±26.0 µm
Mirocaris fortunata Lucky Strike, Sept. 94	24.1 ±4.3 µm	51.2 ±17.3 µm	149.7 ±56.3 µm
Mirocaris fortunata Lucky Strike, July 98	27.8 ±4.8 µm	58.4 ±17.7 µm	249.9 ±89.5 µm

Table 4.2. Mean size (± standard deviation) of the three stages of oocyte development in *Rimicaris exoculata*, *Chorocaris chacei* and *Mirocaris fortunata* from different samples.

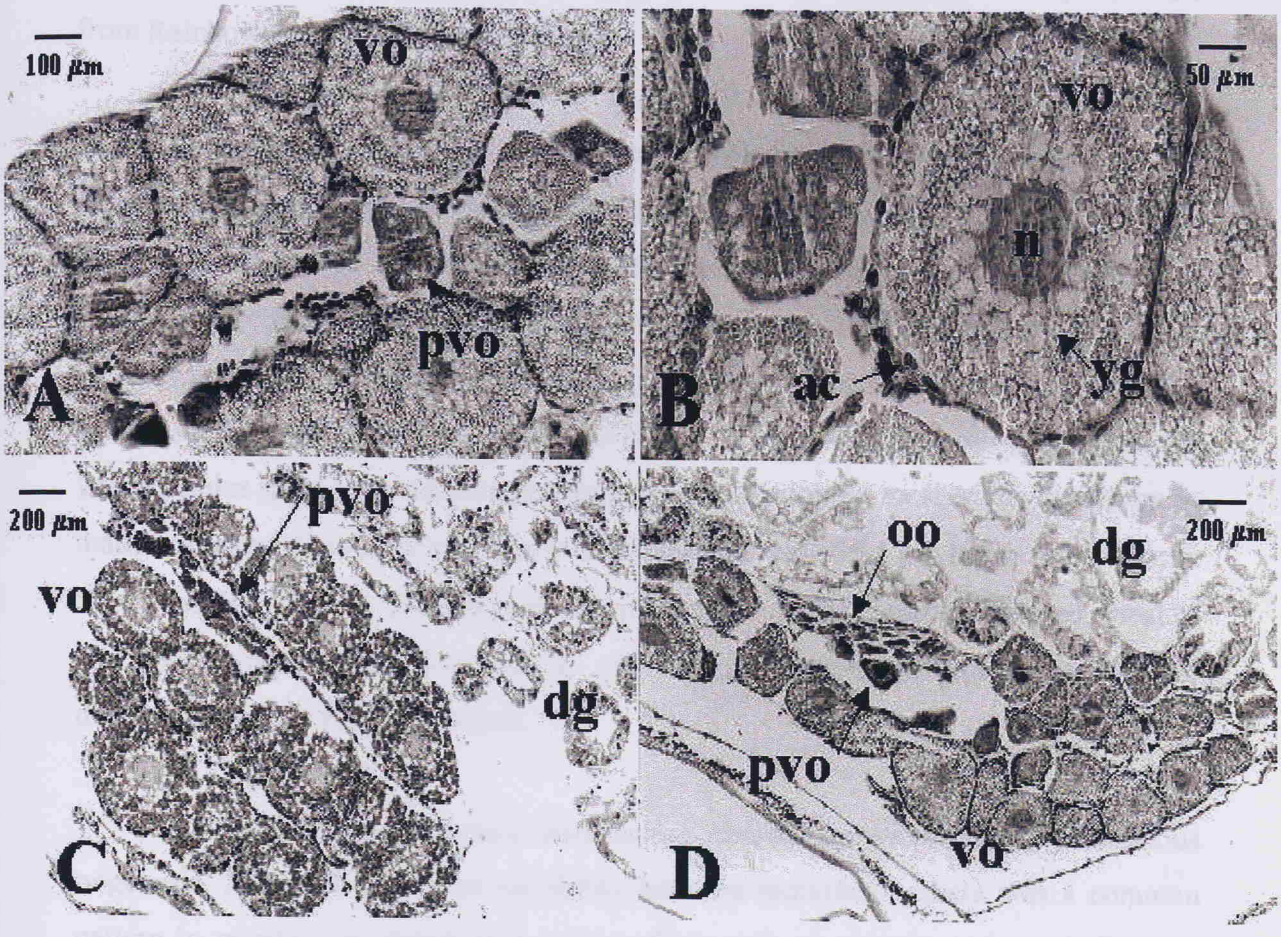


Figure 4.3. Microphotography of ovary sections (Haematoxylin and Eosin).

A- *Chorocaris chacei*: ovary showing vitellogenic oocytes (vo) and previtellogenic oocytes (pvo).

B- Detail of the ovary of *Chorocaris chacei* showing a large vitellogenic oocyte (vo) surrounded by accessory cells (ac) and with yolk granules (yg) spreading in the cytoplasm.

C- *Rimicaris exoculata*: ovary showing previtellogenic (pvo) oocytes developing between vitellogenic oocytes (vo).

D- *Mirocaris fortunata*: section of ovary showing oogonia (oo) and previtellogenic oocytes (pvo) surrounded by large vitellogenic oocytes (vo); dg, digestive gland; n, nucleus.

4.3.2- Oogenesis and oocyte size-frequency distribution of Rimicaris exoculata

Nine females of *Rimicaris exoculata* from TAG (September 1994) and nine from Rainbow (June 1998) were analysed.

The oogonia are small cells of around 20 μm diameter with a dark stained nucleus occupying the entire cell. These cells grow to 25–35 μm diameter and then develop into previtellogenic oocytes. Previtellogenic oocytes are identified by their large nucleus/cytoplasm ratio and basophilic cytoplasm that stains dark purple with Haematoxylin (Fig. 4.3C). Vitellogenesis begins at 85–100 μm, indicated by yolk vesicles appearing at the periphery of the oocytes and a change to acidophilia in the cytoplasm that stains pale pink with Eosin (Fig. 4.3C). As vitellogenesis progresses, the yolk granules spread towards the germinal vesicle, occupying most of the cytoplasm in mature oocytes. The maximum oocyte size observed was 455.1 μm in the females from TAG, and 206.2 μm in the specimens from Rainbow. In the samples analysed, most of the gonad volume was occupied by vitellogenic oocytes surrounding patches of developing previtellogenic oocytes.

The oocyte-size frequency distributions showed no evidence of synchronous oogenesis. Although there was variability between individuals, there was a common pattern in oocyte size distribution, with a first peak of oogonia and previtellogenic oocytes (<100 μm), and most specimens showing a second peak of vitellogenic oocytes (>100 μm) (Fig. 4.4A and C). The pooled data of oocyte-sizes for each sample were used to compare the population oocyte size distribution. The females from TAG in September 1994 (Fig. 4.4B) had gonads with significantly larger vitellogenic oocytes than the females collected at Rainbow in June 1998 (Fig. 4.4D) (Student's test, $t = 2.89$, 13df, $P<0.05$), suggesting that the former were in a more advanced stage of development.

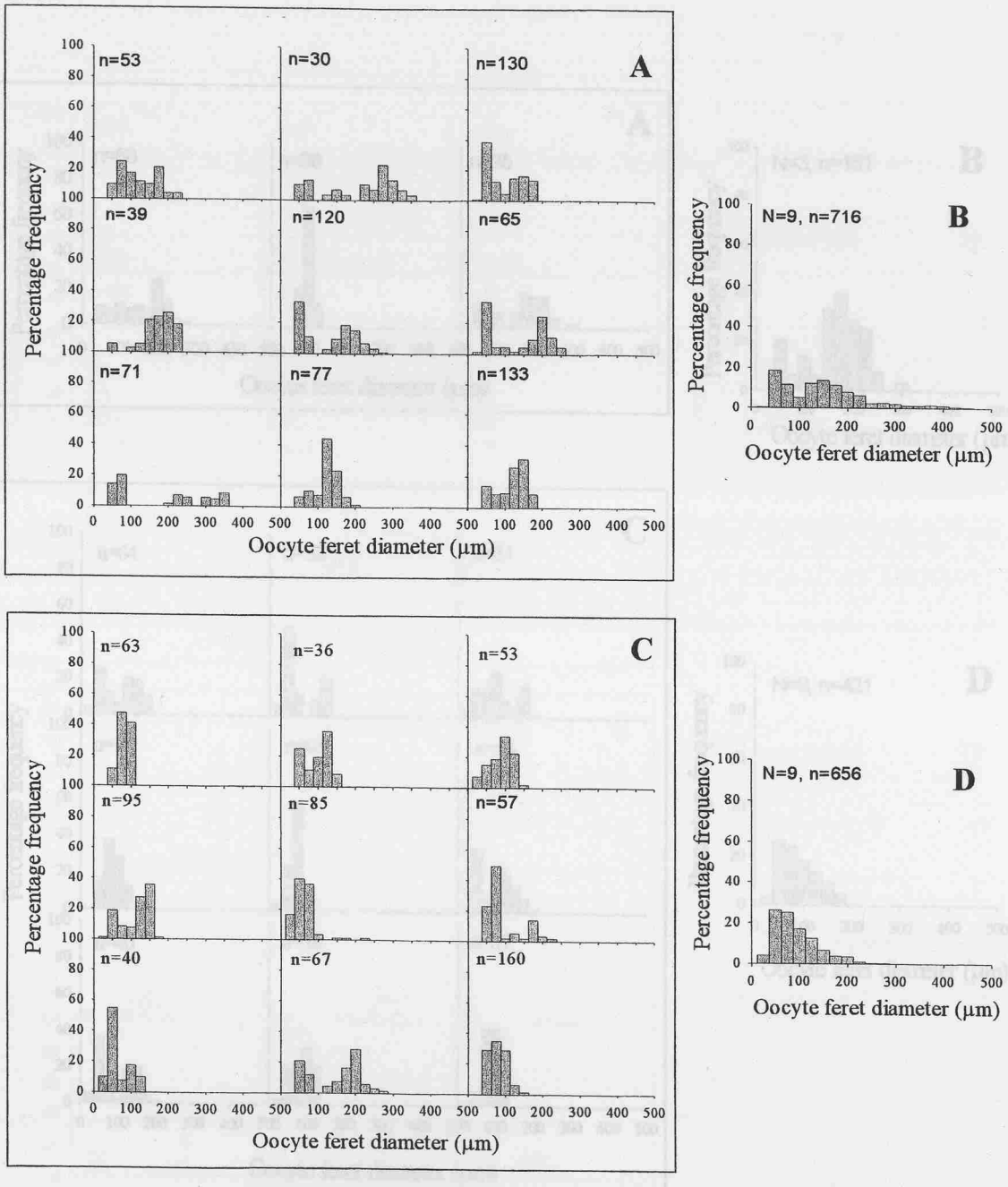


Figure 4.4. Oocyte size-frequency distribution of *Rimicaris exoculata*. A- Oocyte size-frequency of individuals from September 1994. B- Summed oocyte size-frequency distribution for nine individuals of *R. exoculata* from September 1994. C- Oocyte size frequency of individuals from July 1998. D- Summed oocyte size frequency distribution for nine individuals of *R. exoculata* from July 1998. n=number of oocytes measured; N=number of females.

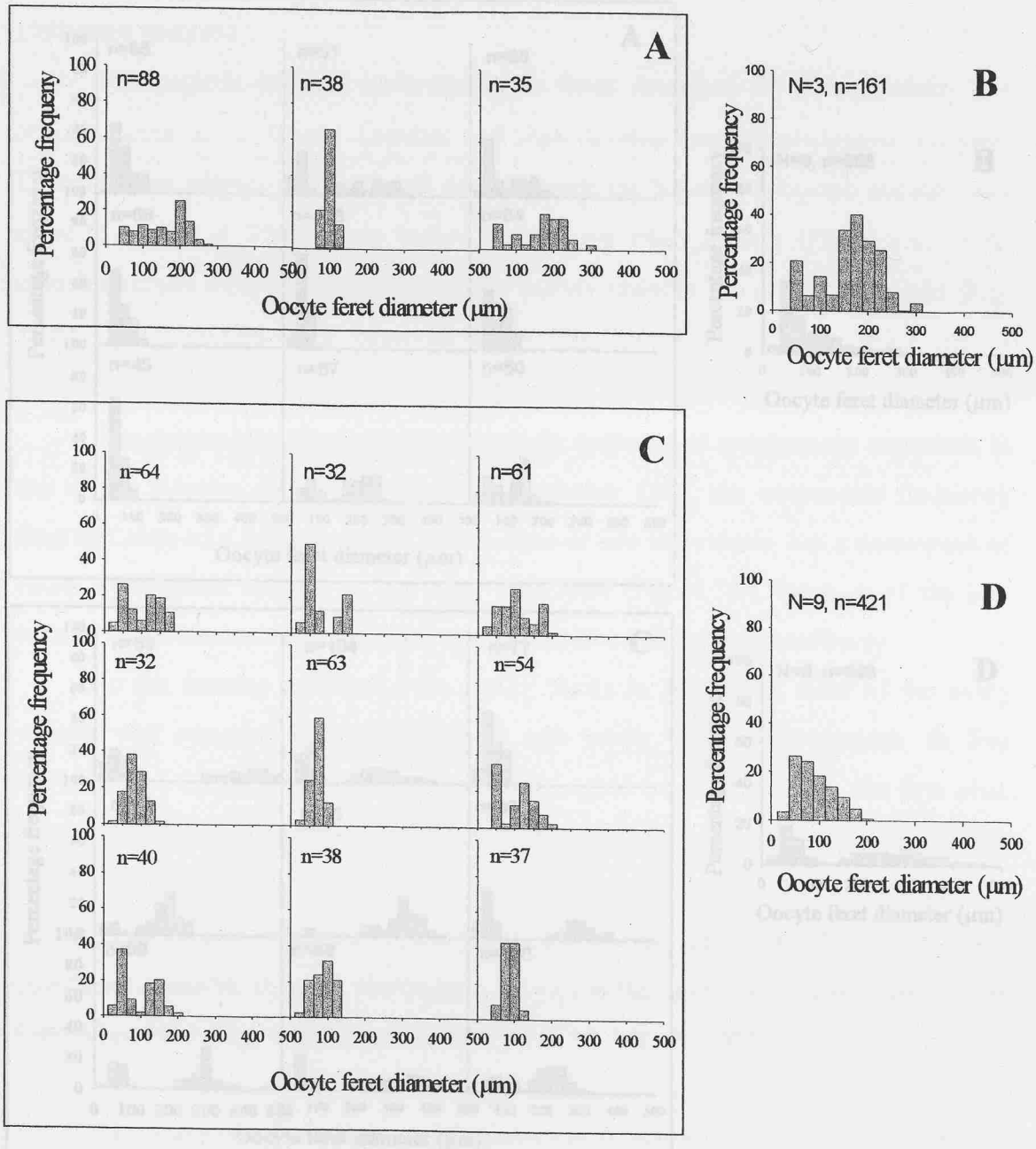


Figure 4.5. Oocyte size-frequency distribution of *Chorocaris chacei*. A- Oocyte size-frequency of individuals from September 1997. B- Summed oocyte size-frequency distribution for nine individuals of *C. chacei* from September 1997. C- Oocyte size frequency of individuals from July 1998. D- Summed oocyte size frequency distribution for nine individuals of *C. chacei* from July 1998. n=number of oocytes measured; N=number of females.

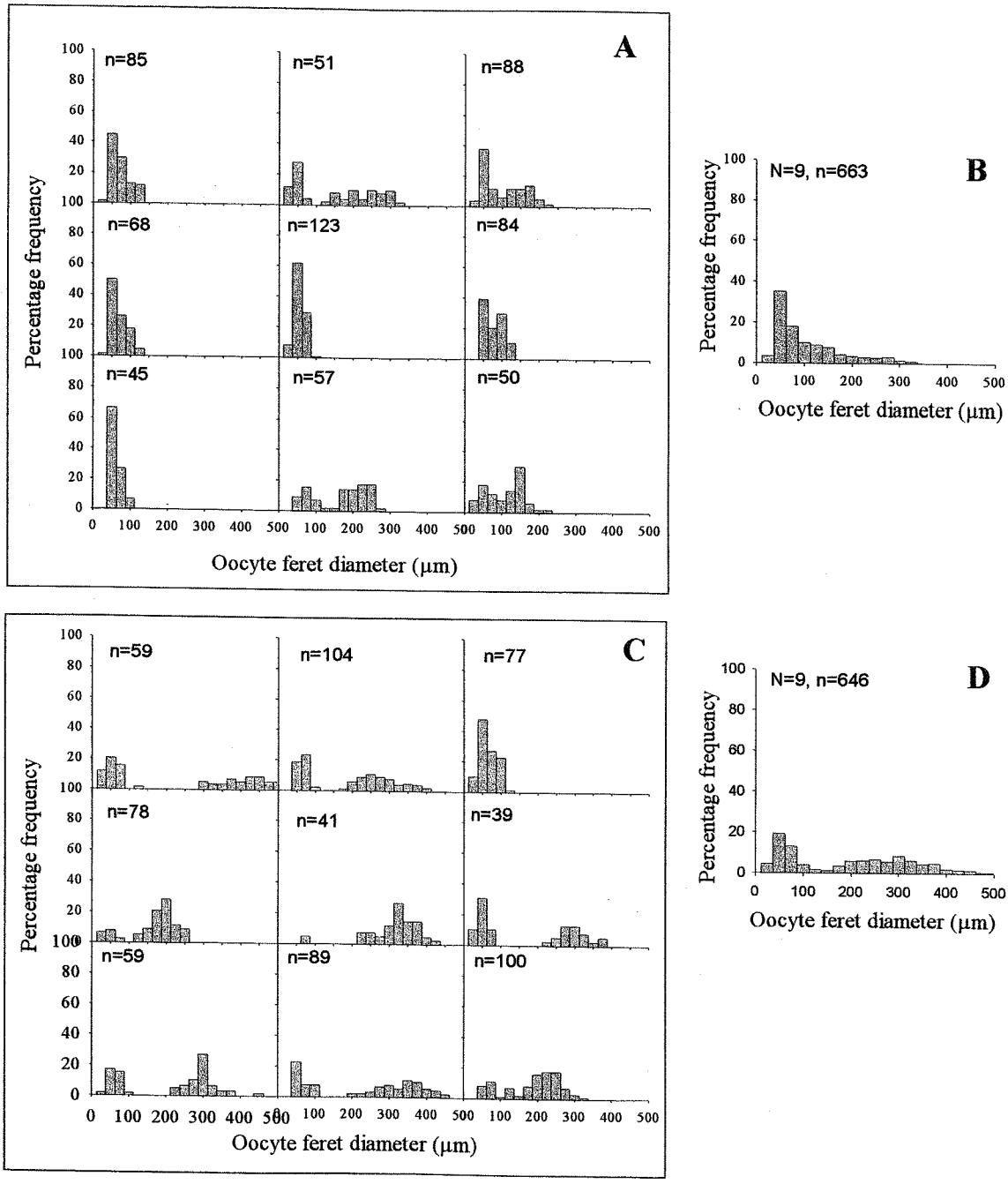


Figure 4.6. Oocyte size-frequency distribution of *Mirocaris fortunata*. A- Oocyte size-frequency of individuals from September 1997. B- Summed oocyte size-frequency distribution for nine individuals of *M. fortunata* from September 1997. C- Oocyte size frequency of individuals from July 1998. D- Summed oocyte size frequency distribution for nine individuals of *M. fortunata* from July 1998. n=number of oocytes measured; N=number of females.

4.3.3- Oogenesis and oocyte size-frequency distribution of Chorocaris chacei

Only three females of *Chorocaris chacei* collected from Lucky Strike in September 1997 provided good histological sections, while nine females from July 1998 were analysed.

The oogenic patterns were similar to those described for *R. exoculata*. The oogonia grow to 30–40 μm diameter and then develop into previtellogenic oocytes. These oocytes migrate to the growth zone between the larger vitellogenic oocytes, and grow to a size of 70–100 μm before undergoing vitellogenesis (Fig. 4.3A). Yolk granules spread from the periphery of the oocyte towards the germinal vesicle (Fig. 4.3B). The maximum size observed for a vitellogenic oocyte was 282.7 μm in September 1997 and 184.3 μm in July 1998.

The oocyte-size distributions showed no evidence of synchronous oogenesis in any of the samples. In the sample from September 1997, the oocyte-size frequency diagrams showed a large spread of oocyte sizes in two individuals, but a dominance of young vitellogenic oocytes in the other individual (Fig. 4.5A). Because of the low number of specimens available, these results should be interpreted cautiously.

In the females collected from Lucky Strike in July 1998, most of the ovary volume was occupied by previtellogenic and young vitellogenic oocytes. In five individuals, a bimodal oocyte-size distribution could be distinguished, the first peak corresponding to previtellogenic oocytes (<100 μm) and the second one to vitellogenic oocytes (>100 μm) (Fig. 4.5C). When comparing the pooled data for each sample, the vitellogenic oocytes from the females collected during July 1998 (Fig. 4.5D) were significantly smaller than the vitellogenic oocytes in the ovaries of females collected in September 1997 (Fig. 4.5B) (Student's test, $t = 6.84$, 9df, $P<0.001$).

4.3.4- Oogenesis and oocyte size-frequency distribution of Mirocaris fortunata

Nine females of *Mirocaris fortunata* collected from Lucky Strike in September 1997 and nine collected from the same location in July 1998 were examined.

The oogenic patterns were similar to those of *R. exoculata* and *C. chacei*. The oogonia develop into previtellogenic oocytes at a size of 25–30 μm diameter. The previtellogenic oocytes grow to 85–95 μm and then undergo vitellogenesis. Yolk granules spread from the periphery of the oocytes towards the germinal vesicle (Fig.

4.3D). The maximum size observed for vitellogenic oocytes was 319.5 μm in September 1997 and 498.9 μm in July 1998.

Here again, the oocyte-size distributions showed no evidence of synchronous oogenesis in any of the samples. The oocyte-size frequency diagrams of individual females from both samples (Fig. 4.6A and C) showed the presence of a pool of previtellogenic and early vitellogenic oocytes in most individuals. Nevertheless, most females had well-developed gonads with a bimodal oocyte size distribution, the first mode corresponding to previtellogenic oocytes (<100 μm) and the second to vitellogenic oocytes (>100 μm). *M. fortunata* was the only species examined that presented significantly larger vitellogenic oocytes in July 1998 (Fig. 4.6D) than in the September 1997 sample (Fig. 4.6B) (Student's test, $t = -4.04$, 14df, $P<0.005$).

4.3.5- Fecundity and egg sizes of M. fortunata, C. chacei and R. exoculata

The mean carapace length of 31 ovigerous *Mirocaris fortunata* analysed was 7.16 ±0.18 mm. The mean female wet weight (not including egg mass) was 0.19 ±0.08 g. There was a significant positive correlation (Pearson Correlation, $r=0.82$, $P<0.001$) between female wet weight and carapace length, indicating that the carapace length was a good measure for body size (Fig. 4.7). From the thirty-one females available, four were damaged and were not included in the following analysis.

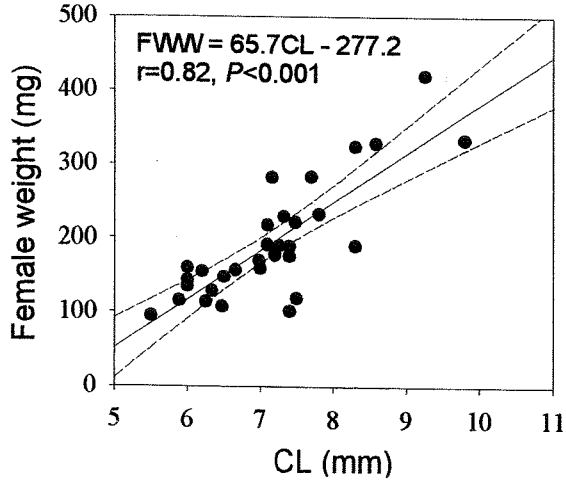


Figure 4.7. Regression of female wet weight (FWW, mg) against carapace length (CL, mm) for *Mirocaris fortunata*, showing the 95% confidence limits, linear regression equation and Pearson's coefficient of correlation.

Three stages of egg development were present in the different broods: early embryos with no eye spots (Fig. 4.8A) and two stages of late development. The first

corresponds to embryos with a clear formation of the abdomen (Fig. 4.8B), and the second to broods with embryos ready to hatch, showing eyespots, clear larval features and the egg membrane breaking down (Fig. 4.8C).

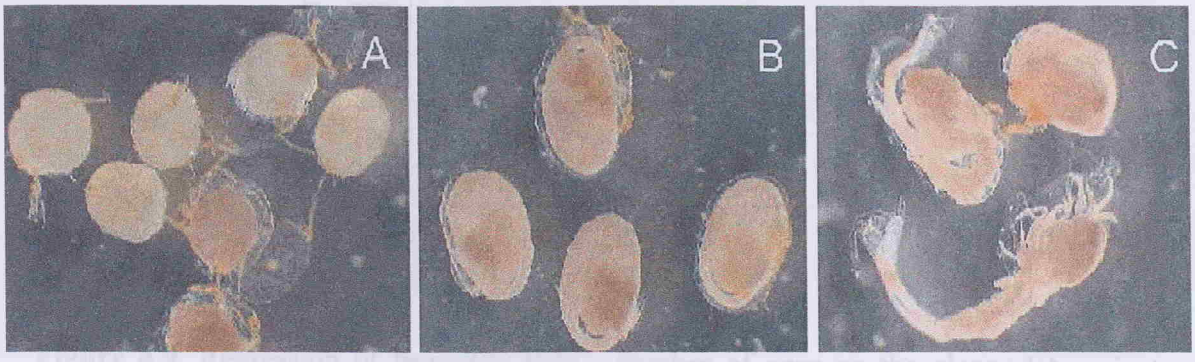


Figure 4.8. Egg stages in the broods of *Mirocaris fortunata*. A- Early eggs; B- Late eggs with late embryos; C- Late eggs with embryos ready to hatch.

Within a brood, the embryos developed synchronously and all the eggs in one batch were at the same stage. In the *M. fortunata* sample, twenty females had broods with early eggs, seven carried late eggs with late embryos and four females were carrying embryos ready to hatch.

The mean egg mass wet weight was 0.023 ±0.02 g. Total fecundity ranged from 25 eggs in females with late broods to 503 in females carrying early embryos. The four females carrying embryos ready to hatch showed evidence of damaged broods and egg loss, and were therefore not included in the analysis. Mean total fecundity (quantified as number of eggs per female in non-damaged broods) was 174.7 ±22.8 eggs per female. There was a significant positive correlation (Pearson Correlation, r=0.79, P<0.001) between total fecundity and carapace length (Fecundity = 77.5 CL (mm) – 335.8) (Fig. 4.9). The eggs of *M. fortunata* were small, with a mean egg volume of 0.21 ±0.08 mm^3 (mean egg length = 0.79 ±0.14 mm and mean egg width = 0.57 ±0.07 mm).

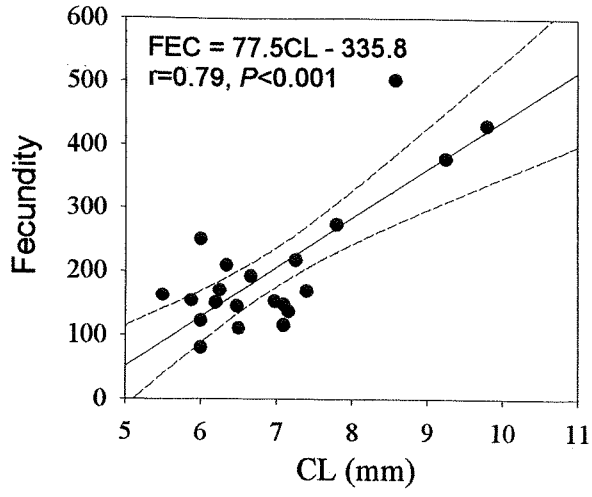


Figure 4.9. Regression of fecundity (FEC, number of eggs on the pleopods) against carapace length (CL, mm), showing 95% confidence limits, linear regression equation and Pearson's coefficient of correlation.

Very few ovigerous *Chorocaris chacei* have been collected to date, and only one specimen collected at Lucky Strike in June 1994 was available for analysis. This female had a carapace length of 16.8 mm, a body weight of 2.26 g and an egg mass wet weight of 0.231 g. The brood consisted of 2510 eggs carried on the pleopods in an advanced stage of development, where the abdomen and appendages of embryos could be distinguished. The mean egg volume was 0.13 ±0.03 mm^3 (mean egg length = 0.70 ±0.1 mm and mean egg width = 0.49 ±0.04 mm).

One of the few ovigerous *Rimicaris exoculata* that have been collected to date was analysed during a visit to IFREMER (Brest, France). This specimen had a carapace length of 16.4 mm, and carried 988 eggs. The female wet weight was 1.64 g and the egg mass wet weight was 0.107 g. The eggs were small (around 0.6 mm in length) and in an early stage of development, with no eye spots or other structural features present. These data are consistent with the observations on two ovigerous *R. exoculata* collected from TAG, one during the NOAA VENTS cruise (August 1985) and one during the BRAVEX cruise (September 1994). The female from TAG had a carapace length of 17.3 mm and the eggs measured 0.62 × 0.72 mm (egg volume = 0.22 mm^3, N=10) (Williams and Rona, 1986). During BRAVEX 94, two ovigerous *R. exoculata* were found in a sample of over 500 specimens, and one was analysed by Copley (1998). This female had a carapace length of 17.7 mm, and was carrying 836 eggs in an early blastula stage of development.

To compare fecundity among the three species, size-specific fecundity was estimated as number of eggs per gram of female wet weight (FWW). The mean size-specific fecundity was 1100.7 ±405.0 eggs g^{-1} FWW for *M. fortunata*, 1112.4 eggs g^{-1} FWW for the only *C. chacei* available and 590.9 ±41.9 eggs g^{-1} FWW for the two *R. exoculata* analysed. The sample sizes were too small to allow for statistical analysis. Nonetheless, the graph of mean size-specific fecundity for the three species suggests that while the reproductive output is similar in *M. fortunata* and *C. chacei*, the size-specific egg production of *R. exoculata* is lower (Fig. 4.10).

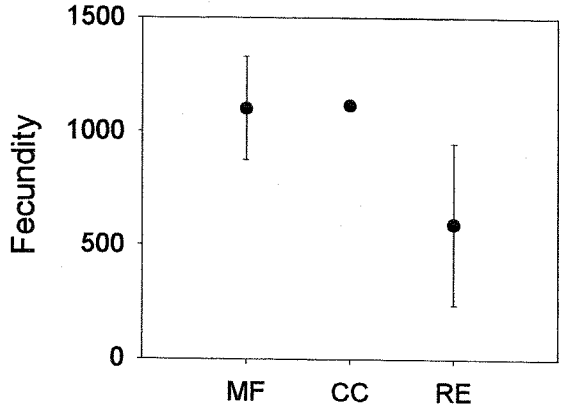


Figure 4.10. Mean size-specific fecundity and 95% confidence limits of three hydrothermal vent shrimp. MF, *Mirocaris fortunata*; CC, *Chorocaris chacei*; RE, *Rimicaris exoculata*.

4.4- Discussion

The ovaries of *Rimicaris exoculata*, *Chorocaris chacei* and *Mirocaris fortunata* are located under the carapace, as in non-vent caridean shrimps, and can be seen through the exoskeleton in fresh specimens. The examination of gonad sections show a similar gametogenic pattern for the three species and characteristic of caridean shrimp. Oogonia (~20–30 μm) proliferate in the germinal epithelium at the periphery of the ovary, developing into previtellogenic oocytes, which then migrate to the growth zone between the large vitellogenic oocytes. These previtellogenic oocytes grow to around 70–100 μm diameter and then undergo vitellogenesis. The yolk granules spread from the periphery of the cell towards the germinal vesicle, occupying most of the ooplasm in mature oocytes. Vitellogenic oocytes are covered by a layer of flattened follicle cells involved in the vitellogenic processes. The maximum size for mature oocytes ranged between 300 and 500 μm.

The oocyte size-frequency diagrams show no evidence of gametogenic synchrony in oogenesis for any of the species studied. All stages of developing oocytes, from oogonia to large vitellogenic oocytes were present in the gonads at a single time. However, there was variability of oocyte-size distribution between individuals. Most females of *Mirocaris fortunata* presented a bimodal distribution corresponding to two different cohorts of developing oocytes, and this, together with the observation that all the embryos within a brood were at the same developmental stage, suggest a periodic production of eggs. Most specimens of *Rimicaris exoculata* and *Chorocaris chacei* showed a more or less accentuated bimodal oocyte-size distribution, but did not have oocytes in the largest classes. Nevertheless, the ovaries contained all stages of oocyte development, with a cohort of young cells (oogonia and previtellogenic oocytes) followed by a cohort of vitellogenic oocytes.

The bimodal distribution of oocyte sizes, as well as the presence of two cohorts of developing oocytes in all specimens, suggest iteroparity and a lack of synchrony in reproduction for the population as a whole. Also, Copley (1998) found that only 8% of 186 females of *R. exoculata* examined had gonads in an advanced stage of development, and 21% of 319 males had vestiges of spermatophores at the gonopores or gonopods. This would suggest a lack of synchrony in gonad development in the population, and the higher proportion of mature males related to females could assure that there are males ready with spermatophores when a female undergoes ecdysis and becomes ready for copulation (Copley, 1998).

An environment, such as hydrothermal vents, where there is a continuous energy supply introduced in the food web by the chemoautotrophic bacteria, can support the asynchronous or quasi-continuous production of eggs. However, Copley (1998) found a polymodal population structure for *Rimicaris exoculata*, indicating a discrete pattern of recruitment, contrasting with the continuous reproductive output that would be expected from a population with an asynchronous reproductive strategy. The ecology of the larval phase may explain this contradiction.

Postlarvae identified as belonging to *R. exoculata, C. chacei* and *Alvinocaris markensis* by ribosomal DNA markers (Dixon and Dixon, 1996; Herring, 1996) have been collected from the water column above Broken Spur. These postlarvae possess a substantial wax-ester reserve, compound eyes and their lipid composition is derived from a diet of photosynthetic material, contrasting with the small or non-existent eyes and bacterial-derived lipids of the adults (Pond et al., 1997a,b,c, 2000a,b; Allen, 1998;

Allen Copley et al., 1998). Also, experiments on the pressure and temperature tolerance of *Mirocaris fortunata* larvae suggest that the upper limit of vertical distribution would be the thermocline while the lower limit would be determined by pressure at ~3000 m, indicating a wide potential for vertical and horizontal distribution of *M. fortunata* larvae (Tyler and Dixon, 2000). These observations suggest a long planktotrophic phase with large migratory abilities towards the surface. The hypothesis of a high dispersal potential during the larval stage is also supported by genetic studies on vent shrimp populations from Broken Spur and TAG, where a high gene flow between the two vent fields with a N_m (number of migrants per generation) of 250 was calculated (Creasey et al., 1996; Shank et al., 1998). Although the production of larvae might be quasi-continuous at population scale, the recruitment could be affected by environmental factors found by the larvae during their extended planktotrophic stage in the water column. Factors such as favourable hydrodynamic conditions or variability in the phytodetritus concentration could be used as settlement cues, causing a discrete recruitment (Copley, 1998).

One of the most intriguing observations on the reproduction of the vent shrimps is the almost complete lack of ovigerous *Chorocaris chacei* and *Rimicaris exoculata* in the samples, while *Mirocaris fortunata* are known to provide gravid females regularly (Van Dover et al., 1996; P. Tyler, *pers. com.*). We believe that *R. exoculata* occupies a habitat peripheral to the vents while brooding, to protect the embryos from hydrothermal fluids and from the risk of mechanical damage caused in the highly active aggregations of shrimps around black smokers (Ramirez Llodra et al., 2000). Gravid females of *Mirocaris fortunata* have been sampled over mussel beds and close to the shimmering waters. They live, however, in less dense populations than *R. exoculata*, with fewer physical interactions between individuals and lower contact with the hot and toxic hydrothermal fluid.

If *R. exoculata* and *C. chacei* move to the periphery of the vents while carrying eggs, samples from these external habitats should confirm their presence. Such a behaviour has been observed in the vent crab *Bythograea thermydron* from 9°N EPR (G. Perovich, *pers. com.*) and in the deep-water crabs of the genus *Geryon*, which move to shallower waters when brooding (Haefner, 1977; Melville-Smith, 1987). An alternative hypothesis would be that the sampling programmes to date have been missing the ovigerous *R. exoculata* because of limitation of submersible diving to the summer and early autumn months. *R. exoculata* could brood their embryos in a different

time of the year than *M. fortunata*, although data on gametogenesis presented here does not support the idea of a seasonal reproduction for any of the species studied.

In crustaceans in general, and in caridean shrimps in particular, an important factor related to the number of eggs is adult body size. A positive correlation is found between carapace length and body size (Clarke 1979, 1993b; King and Bulter 1985; Bell and Fish 1996, Stella et al., 1996; Ohtomi, 1997; Thessalou-Legaki and Kiortsis, 1997; Chapter 3 this thesis). This correlation is a result of the physical space limitation between the pleopods for the attachment of the eggs (King and Butler. 1985; Corey, 1987; Corey and Reid, 1991; Clarke, 1993b). The ovigerous *Mirocaris fortunata* analysed follow this pattern, with a positive correlation between carapace length and fecundity. This correlation was stronger when specimens that had larvae ready to hatch were not included in the data. Broods from these females had very few eggs, ranging from 25 to 60 eggs, while females carrying early broods had a mean fecundity of 193.7 eggs per female. It is possible that egg loss occurs in late stages of development, both naturally and during collection and storage.

Eggs are amongst the largest cells in the organism and show a wide variability between and within species. As explained in chapters 2 and 3, invertebrates produce either a small number of large, rich eggs or a high number of small eggs (Menge, 1975; Clarke, 1993a; Podolsky and Strathmann, 1996). This trade-off between egg size and fecundity is very clear in caridean shrimp (Herring, 1974a,b; Clarke, 1993b; Ohtomi, 1997). The effects and consequences that this range of sizes might have on energy content, parental investment, fertilisation success and larval development is what has been interesting life-history biologists and ecologists for decades.

Vance (1973) proposed a theoretical model predicting an optimal egg size following a bimodal distribution corresponding to larval developmental type (i.e. planktotrophy for small eggs and lecithotrophy for large ones). Later studies suggest that the evolution of egg sizes is related to prezygotic factors, such as higher fertilisation rates in large eggs offering larger targets for sperm (Levitan, 1996), and postzygotic factors, such as larval mortality and developmental time (Podolsky and Strathmann, 1996). In deep-sea species with migrating larvae, the mortality risk while in the water column increases with depth (Thorson, 1950; King and Butler, 1985; see chapter 2, section 2.3.2 this thesis). It has been proposed that these species reduce this risk by producing larger eggs, which will hatch into larger and more advanced larvae with higher survival probabilities during their migratory movements (Clarke, 1979; King and

Butler, 1985; King, 1987; Clarke, 1993a; Chapter 3 this thesis). However, *Mirocaris fortunata* and the few *Chorocaris chacei* and *Rimicaris exoculata* examined have a high number (around 300 for *M. fortunata*, 2500 for *C. chacei* and 1000 for *R. exoculata*) of small eggs (around 0.5 to 0.8 mm in length). These eggs have similar sizes to the eggs brooded by the mesopelagic shrimp *Acanthephyra* spp. (Herring, 1974b; Chapter 3 this thesis). The small size of the eggs, together with the genetic and biochemical data (Creasey et al., 1996; Pond et al., 1997a,b,c; Allen et al., 1996; Allen, 1998; Allen Copley et al., 1998; Shank et al., 1998), suggest a short embryonic development with larvae hatching in an early stage and undergoing a relatively long planktotrophic phase.

Size-specific fecundity is higher in the three vent shrimp than in the small-egged, non-vent species *Acanthephyra* sp. When calculating fecundity as number of eggs per gram of female body weight, *M. fortunata*, *C. chacei* and *R. exoculata* produce 2.5, 2.5 and 1.5 times more eggs respectively than the species of *Acanthephyra* from the NE Atlantic (see chapters 3 and 6 this thesis). The rich hydrothermal vent environment, with chemoautotrophic bacteria providing the trophic web with a high and continuous primary production, can support a high production of eggs in vent shrimp. There is therefore a high number of feeding larvae hatching from these small eggs, which will be able to spend long periods in the water column, incrementing the dispersal and colonisation potential of the species.

Data obtained here suggest that, in the hydrothermal vent caridean shrimps *Mirocaris fortunata*, *Chorocaris chacei* and *Rimicaris exoculata*, the process of oogenesis is conservative and phylogenetically constrained, as it is in many of the vent species studied until now (Van Dover et al., 1985; Tyler and Young, 1999). Conversely, fecundity is enhanced as a consequence of living in a rich environment with a continuous and rich supply of energy. Furthermore, the small eggs developing into planktotrophic larvae capable of long residence times in the water column would allow for the colonisation of new vent fields in an environment characterised by its patchiness and the instability caused by the extinction/reactivation of vent sites (Langumir et al., 1997; Tyler and Young, 1999).

CHAPTER FIVE- REPRODUCTIVE BIOLOGY OF PORCELLANASTERID ASTEROIDS FROM THREE ABYSSAL SITES IN THE NORTHEAST ATLANTIC WITH CONTRASTING FOOD INPUT

5.1- Introduction

5.1.1- NE Atlantic abyssal plains: environment and ecology

The abyssal plains start where the continental rise levels off, at around 4000 m of depth. These are extensive, relatively flat or undulating areas covered with a thick carpet of fine soft sediment, extending from 4 to 6 km of depth (Gage and Tyler, 1991, Chapter 1, section 1.2.2).

The composition and distribution of abyssal fauna is related directly to sediment properties and species interactions. While abundance usually decreases with increasing depth, diversity in the deep sea is high. This high diversity is maintained by small scale heterogeneity created by events such as 1)- phytoplankton deposition, 2)- sinking of wood, seaweed, fish and cetacean carcasses, 3)- disturbances to the sediment by macro- and megafaunal feeding and borrowing (Dayton and Hessler, 1972; Rowe et al., 1982; Gage and Tyler, 1991; Grassle and Maciolek, 1992; Snelgrove and Grassle, 1995).

Meio-, macro- and megafauna are all present at abyssal plains. The meiofauna (size of organisms in the order of microns) is mainly dominated by nematodes and foraminifera, which present a high degree of diversity and most of which have still to be identified (Gage and Tyler, 1991; Lambshead, 1993; Gooday et al., 1996). The polychaetes dominate the macrofauna (size of organisms in the order of millimetres), comprising half to three quarters of the total. Small peracarid crustaceans, molluscs, nemerteans, sipunculans, echiurans and enteropneustes are also abundant (Gage and Tyler, 1991; Grassle and Maciolek, 1992). The megafauna (size of organisms in the order of centimetres) is composed by both errant and sessile fauna. The errant megafauna is dominated by echinoderms, decapod crustaceans and fish, whilst the sessile megafauna comprises mainly crinoids, poriferans and anthozoans and are mostly found on hard substratum (Sibuet, 1979,1984; Lampitt et al., 1986; Gage and Tyler, 1991).

Energy availability and flow play a major role in driving the biological processes of an ecosystem. With the exception of hydrothermal vent communities, which are fuelled by primary production of chemoautotrophic bacteria, all of the deep-

sea fauna is dependent ultimately on primary production in the surface layers of the oceans.

Before the 1980s, the supply of organic matter to the deep-sea bed was thought to be in the form of a continuous and slow sinking detrital material (Menzel, 1974). However, the IOSDL benthic programme in the Porcupine Seabight to the southwest of Ireland gave evidence for the first time on the seasonal arrival of aggregated phytodetritus to the seabed (Billett et al., 1983; Lampitt, 1985; Rice et al., 1991). Nowadays, the deposition to the seabed of surface derived organic matter is known to be a widespread phenomenon in all regions with a highly seasonal surface production (Grassle and Morse-Porteous, 1987; Tyler, 1988; Thiel et al., 1990; Rice et al., 1994; Smith et al., 1997; Baldwin et al., 1998; Beaulieu and Smith, 1998). Because the rapid sinking of phytodetritus prevents its complete utilisation by pelagic grazers, the arrival of this organic matter at the seabed provides deep-sea communities with a high-quality food resource (Gooday and Turley, 1990; Beaulieu and Smith, 1998; Ginger et al., 2000; Witbaard et al., 2000).

During the last decades, the NE Atlantic abyssal plains have been the focus of several multidisciplinary programmes aiming to understand the processes driving deep-sea ecosystems and their relation with the surface layers of the ocean. The European programme DEEPSEAS (Rice et al., 1994) analysed the influence of phytodetritus on the benthic communities of the NE Atlantic by comparing two abyssal sites with contrasting food supply. The northern site was located at the Porcupine Abyssal Plain (PAP). The water column above PAP is subjected to deep mixing down to 500 m during winter. Because new primary production in the euphotic zone depends on the import of exogenous nitrogen from deep-water masses (Williams et al., 1989), the depth of the winter mixing layer plays a major role in surface production and therefore in the input of organic matter to the seabed. This deep-water mixing above the Porcupine Abyssal Plain in winter allows for an enrichment of the surface waters with nutrients. Subsequently, there are significant seasonal changes on surface productivity. The seasonal variations in surface primary production lead to a strong pulse of phytodetritus to the seabed between April and September, following the phytoplankton spring bloom in the surface (Rice et al., 1994). The southern locality of the DEEPSEAS programme was on the Madeira Abyssal Plain (MAP). This location has a winter thermocline at less than 150 m and does not receive a significant phytodetritus signal (Rice et al., 1994; Thurston et al., 1994).

In the 1980s, the Institute of Oceanographic Sciences undertook a series of surveys to study the Great Meteor East site (GME) at 31°17'N, 25°24'W in the Madeira Abyssal Plain (Roe et al., 1987). Further south, the OLIGO station (21°N, 30°W) was established under the French EUMELI programme (Sibuet et al., 1993). Neither of these southern sites is affected by strong seasonal phytodetritus falls (Thurston et al., 1995, 1998; Vanreusel et al., 1995).

The main biological results of the above programmes showed a significantly higher abundance and biomass of megafauna at PAP than at MAP, GME or OLIGO, and the size spectra based on abundance and biomass confirmed the megafauna as a functional group at PAP only (Thurston et al., 1994, 1998). There were also differences in the trophic structure between localities. The detritivores are the main group at all localities, but carnivores are more important at MAP and GME. The deposit-feeders such as holothurians, and macrophagous fish were dominating at PAP, while asteroids and decapod Natantia were more common at the Madeira Abyssal Plain (Khripounoff and Sibuet, 1980; Thurston et al., 1994, 1995, 1998).

The metazoan meiofauna is also affected by organic inputs from the surface, showing a higher abundance, biomass and number of large nematodes at PAP than at the OLIGO station (Vanreusel et al., 1995). The group that shows the most clear and rapid response to input of surface derived organic matter is the opportunistic foraminifera. The abundance and distribution of some foraminifera is closely related to the availability of seasonal phytodetritus falls, with the input of organic matter allowing for a rapid growth and reproduction (Gooday, 1993, 1996; Gooday et al., 1996; Smart and Gooday, 1997). The flagellates and bacteria also respond to surface derived organic matter by using this resource and rapidly increasing in biomass (Turley et al., 1988; Lochte and Turley, 1988; Gooday and Turley, 1990; Pfannkuche, 1993, 1999).

The reproductive processes and early developmental stages of any species are especially sensitive to energy availability in the environment. The energy surplus obtained from a periodic deposition of organic matter can fuel gametogenesis, or can be used as a cue to set-up a biological clock in an otherwise mainly unvarying environment. There is now evidence of seasonal reproduction coupled to seasonal phytodetritus falls in several megafauna species such as the ophiuroids *Ophiura ljungmani* and *Ophiocten gracilis* (Gage and Tyler, 1981a,b; Sumida et al., 2000), the echinoids *Echinus affinis*, *E. alexandri*, *E. acutus*, *E. elegans* and *Stylocidaris lineata*

(Tyler and Gage, 1984b; Gage et al., 1986; Young et al., 1992), the asteroids *Dytaster grandis* and *Plutonaster bifrons* (Tyler and Pain, 1982; Tyler et al., 1990) and the bivalves *Ledella pustulosa* and *Yoldiella jeffreysi* (Lightfoot et al., 1979; Tyler et al., 1993).

5.1.2- Sexual reproduction in asteroids

The asteroids are generally gonochoric, although there are some hermaphrodite species, such as certain species of *Asterina* and *Asterias* (Cognetti and Delavault, 1962; Kanatani and Nagahama, 1983). There are two main types of gonad organisation, single and serial. In single gonads, there are five pairs of proximal tufts of tubules, two at each interradius, with a gonoduct leading to a gonopore. In serial gonads, there are series of tufts of tubules situated along the axis of each ray (Chia and Walker, 1991). In each case, the reproductive system is composed of several units, each unit consisting on a gonad, a gonoduct, a genital branch of the aboral haemal ring and a genital branch of the coelomic (perihaemal) ring (Walker, 1976; Chia and Walker, 1991).

The gonad wall of the asteroids is the most complex of the Echinodermata, consisting on an inner sac and an outer sac separated by genital haemal sinus, or perihaemal sinus (Walker, 1974, 1976; Kanatani and Nagahama, 1983; Chia and Walker, 1991). The inner sac consists in an outer epithelium (non-ciliated cells and myoepithelial cells), an haemal sinus and the inner germinal epithelium. The outer sac is composed by a visceral peritoneum (myoepithelial and neurosecretory cells), an elastic/collagenous connective tissue layer and an inner epithelium (non-ciliated cells and myoepithelial cells). The genital haemal sinus is limited by the inner and outer walls (fibro-granular basal laminae) and filled with ameboid and rounded cells and collagen fibers (Walker, 1974, 1976; Kanatani and Nagahama, 1983; Chia and Walker, 1991). The volume of the fluid in the genital haemal sinus varies with the annual gametogenic cycle, suggesting that it is involved in the transfer of nutrients to germinal cells during vitellogenesis (Walker, 1974, 1976; Kanatani and Nagahama, 1983; Chia and Walker, 1991).

In females, the germinal epithelium consists of germ cells and non-germ cells. Oogenesis starts with the proliferation of oogonia from the germinal epithelium, which differentiate into primary oocytes. These have a large germinal vesicle (germinal vesicle) occupying most of the cell (Schoenmakers et al., 1981; Kanatani and Nagahama, 1983). The primary oocytes (arrested in prophase of first meiotic division)

are arranged in layers lining the ovarian wall and surrounded by follicle cells. The follicle cells are in contact with the developing oocytes through protoplasmic extensions. With the onset of vitellogenesis, the oocytes grow in volume and the cytoplasm becomes acidophilic, staining pale pink with Eosin. At this stage, the follicle cells respond to a gonad stimulating substance produced in the cells of the radial nerves by producing 1–methyladenine (a meiosis-inducing hormone). In primary oocytes, 1-methyladenine induces breakdown of the follicular envelope and the cytoplasmic synthesis of maturation-promoting factor (MPF). MPF causes germinal breakdown and the oocytes finish first meiotic division. These oocytes become free in the ovary and are ready to be spawned (Shoenmaker et al., 1981; Chia and Walker, 1991; Chia et al., 1993). The oocytes are forced out of the ovary by contraction of the muscular layers in the gonad wall. The spawned oocytes continue to undergo meiotic division and discard half of the chromosomes as polar bodies. These mature oocytes are ready to be fertilised. In fully developed oocytes, there is an accumulation of cortical vesicles that migrate from the germinal vesicle towards the oocyte cortex. The cortical vesicles fuse with each other and with the cell membrane and release their contents, forming a mucoplysaccharide layer around the oocyte, the jelly coat (Schoenmaker et al., 1981; Chia and Walker, 1991).

In males, the spermatogenic phase begins with the mitotic divisions of spermatogonia in the germinal epithelium. During the proliferation phase, these spermatogonia form primary spermatocytes that become organised with accessory cells into spermatogenic columns, or colonettes. During their transition through the colonettes, the primary spermatocytes start meiosis until prophase of first meiotic division. The primary spermatocytes are always connected to the potentially nutritive fluid of the genital haemal sinus that passes with somatic accessory cells through the middle of the colonettes (Walker, 1980; Chia and Walker, 1991). Spermiogenesis begins when the follicle cells are stimulated by the gonad stimulating substance. In response, the follicle cells produce 1–methyladenine. This hormone induces the primary spermatocytes to undergo meiosis at the apex of the colonettes and causes breakdown of the adhesive substance binding spermatozoan flagella together. Spermatids arise from the tip of the spermatogenic colonettes and differentiate into spermatozoa that accumulate in the lumen of the testis (Walker, 1980; Chia and

Walker, 1991). The hormone 1–methyladenine also induces the testes wall muscles to contract during the spawning event (Walker, 1980; Chia and Walker, 1991).

Fertilisation in most asteroids is external, although there are some species that brood their young, such as *Leptasterias hexactis*, *L. ochontensis*, *L. groenlandica*, *Pteraster* sp. or *Leptychaster alumus* among others. In free-spawners, the embryos develop by radial cleavage in the water column. There are three larval forms in asteroids, bipinnaria, brachiolaria and barrel-shaped larvae. The bipinnaria larvae are planktotrophic. They have two ciliary bands responsible for locomotion and feeding. The brachiolaria larvae usually derive from bipinnaria larvae by the development of the preoral lobe. This structure is composed of three brachiolarian arms and a central disc used for attachment during metamorphosis. Species that produce large yolky eggs hatch into lecithotrophic brachiolarian larvae. The barrel-shaped larvae have a very simple structure with no obvious larval organs and cilia uniformly distributed throughout the surface (Chia and Walker, 1991; Chia et al., 1993).

During metamorphosis, the bilateral pelagic larva is transformed into a radially symetrical bottom-dwelling juvenile. Metamorphosis occurs when the larva finds good conditions of substratum and micro-environment for settlement (Chia et al., 1993).

5.1.3- Porcellanasterid asteroids: taxonomy and biology

The echinoderms are a conspicuous and abundant group of megafauna in the deep-sea benthos. They are well adapted to the abyssal conditions, with the existence of strictly abyssal genera and families. Because of their abundance, burrowing, feeding and respiration processes, they contribute significantly to the ecology of the deep-sea benthic communities (Sibuet, 1984). Although there is a general decrease in the invertebrate megafauna density with increasing depth in the NE Atlantic, there is also an increase in the proportion of echinoderms related to the total invertebrates megafauna (>80% below 1500 m depth) (Sibuet, 1984; Lampitt et al., 1986; Billett, 1991). There is not a preponderance of any of the four classes (Holothuroidea, Echinoidea, Ophiuroidea and Asteroidea) and their relative abundance varies depending on depth and location.

The two genera of asteroids analysed in this study belong to the order Paxillosida Perrier, 1884 and the family Porcellanasteridae. The porcellanasterids are mainly infaunal asteroids. The Paxillosida have five arms, except for some species of the family Luidiidae, which can have up to eleven arms. The Paxillosida are

characterised by large conspicuous marginal plates forming a well-defined margin along the edge of the disk and arms. The tubefeet are in rows of two, and have narrow to blunt tips lacking a sucking disk. The abactinal plates are paxilliform or reduced to flat naked discs and with widely distributed papular areas. The anus is often lacking and can be replaced by a blind epiproctal cone (Sladen, 1889; Madsen, 1961; Mortensen, 1977; Clark and Downey, 1991). The porcellanasterids form a cosmopolitan deep-sea family unknown from depths shallower than 900 m (Madsen, 1961; Clark and Downey, 1991). They are characterised by the presence of cribriform organs between the marginal plates and the lack of an intestine and anus (Mortensen, 1977; Clark and Downey, 1991).

Little is known on the mode of life of the porcellanasterids, and most of the studies conducted until now relate to taxonomy (Sladen, 1889; Madsen, 1961; Mortensen, 1977; Clark and Downey, 1991) and distribution (Cherbonnier and Sibuet, 1972; Sibuet, 1975, 1976; Sibuet 1979; Gage et al., 1983; Sibuet et al., 1984).

The mud star *Ctenodiscus crispatus* (Paxillosida: Goniopectinidae) is a closely related and well studied infaunal deposit feeder. It has often been used as model for the life habits of deep-sea porcellanasterids (Shick et al., 1981a,b). *Ctenodiscus crispatus* spends the majority of its time borrowed in the upper layers of muddy sediments. The epiproctal cone and the five arms form channels that maintain contact between the animal and the water overlying the sediment surface. A water current is pumped by the cribriform organs to the ambulacral grooves and out through the excurrent channels excavated by the arms. This current has mainly a respiratory function (Shick et al., 1981a). The distended stomach of *C. crispatus* contains mainly remains of phytodetritus and small macrofauna, such as foraminifera and polychaetes, and therefore *C. crispatus* is considered a non-selective deposit feeder (Shick et al., 1981a).

The porcellanasterids follow a similar feeding strategy, ingesting the surface layers of sediment as they burrow and extracting nutrients primarily from sediment detritus, bacteria and meiofauna, of which the foraminifera are the most important. They also may scavenge and predate on larger animals, as well as intake dissolved organic matter through the cribriform organs (Shick et al., 1981a; Clark and Downey, 1991; Billett, 1987). *Ctenodiscus crispatus* and the porcellanasterids live in temporary burrows from which they surface to move away and swallow sediment as they burrow themselves again. There is photographic evidence (Bathysnap) indicating that porcellanasterids may form a new burrow every three days approximately (Billett,

1987). Ingesting surface sediment, they enhance the organic uptake by using the richer surface layers, especially in the low nutrient deep-sea floor (Sokolova, 1958; Shick et al., 1981a).

Even so, little is known about the reproductive biology of the porcellanasterids, apart from the observations of low fecundity, large eggs (~600 μm), and the suggestion of direct development related to the large egg size (Madsen, 1961).

5.1.4- Chapter objectives

In this study, the reproductive patterns of three porcellanasterid asteroids from three different sites in the NE Atlantic have been described. The three species were analysed from the Porcupine Abyssal Plain (PAP), the Madeira Abyssal Plain (MAP) and a site off the NW African coast (NWA) (Fig. 5.2). The PAP and NWA sites receive a strong input of organic matter from the surface, while the MAP is more oligotrophic and there is no evidence of seasonal detrital carpet (see section 5.2.1 for explanation on variability of organic matter input).

The gametogenic patterns, egg size and fecundity will be firstly discussed for the three species and compared with the information available for other deep-sea asteroids. Second, the reproductive output of the three species from the three localities with different food availability were analysed and compared. In invertebrates, the gametogenic pathways are phylogenetically constrained but the reproductive output can be affected by environmental factors such as food quantity and quality (Eckelbarger and Watling, 1995). There were two working hypotheses for this study. First, gametogenesis being phylogenetically constrained, there would be no differences in the oogenic and spermatogenic patterns of the porcellanasterids from the three localities. Second, we would expect to find an enhanced reproductive output in the specimens from PAP and NWA compared to the MAP specimens, related to differences in food availability.

5.2- Material and methods

5.2.1- Species and study area

Three species of asteroids, *Hyphalaster inermis*, *Styracaster chuni* and *S. horridus*, were studied (Fig. 5.1). Both genera belong to the deep-sea family Porcellanasteridae (Paxillosida: Asteroidea) and are common at the NE Atlantic abyssal plains (Sladen, 1889; Madsen, 1961; Clark and Downey, 1992).

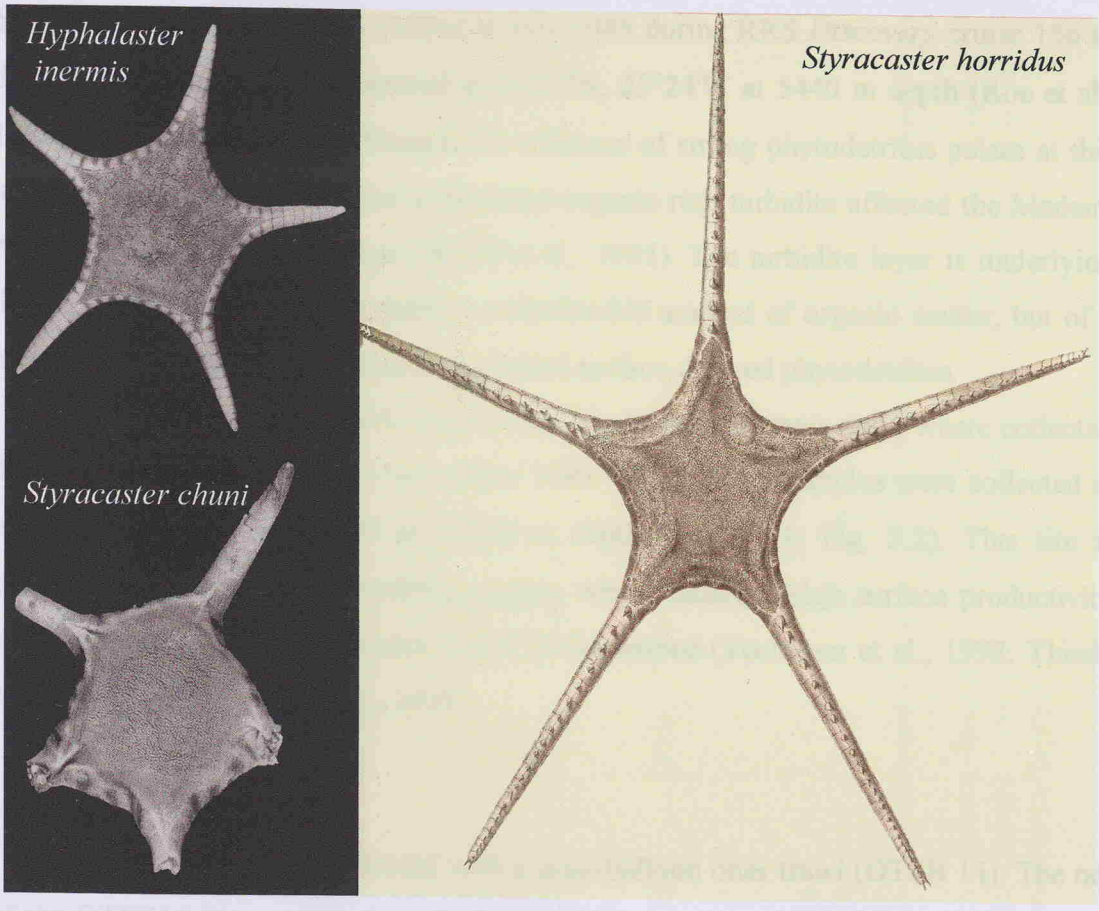


Figure 5.1. Porcellanasterid asteroids from the NE Atlantic. *Hyphalaster inermis* and *Styracaster chuni* (modified from Clark and Downey, 1992), and *Styracaster horridus* (modified from Sladen, 1889).

The Porcupine Abyssal Plain specimens (*Hyphalaster inermis*, *Styracaster chuni* and *S. horridus*) were collected during several RRS *Discovery* and RRS *Challenger* cruises from April 1994 to April 1999. The samples were taken from a single locality centred at 48°50'N, 16°30'W, at 4850 m depth (Table 5.1; Fig. 5.2). The cruises from September 1996 to October 1999 were part of the MAST III-EU funded programme BENGAL (Billett and Rice, submitted; Billett et al., submitted). This northern site receives significant seasonal pulses of phytodetritus between April and September, following the phytoplankton spring bloom in the surface (Rice et al., 1994).

The Madeira Abyssal Plain (MAP) specimens (*Hyphalaster inermis* and *Styracaster horridus*) were collected in July 1985 during RRS *Discovery* cruise 156 to the Great Meteor East site, centred at 31°17'N, 25°24'W at 5440 m depth (Roe et al., 1987) (Table 5.1; Fig. 5.2). There is no evidence of strong phytodetritus pulses at this location (Rice et al., 1994), but a relatively organic rich turbidite affected the Madeira Abyssal Plain ~1000 years ago (Wolff et al., 1995). The turbidite layer is underlying the surface sediments, and contains a considerable amount of organic matter, but of a more refractory nature than freshly deposited surface-derived phytodetritus.

The NW African (NWA) specimens (*Hyphalaster inermis* only) where collected during RRS *Discovery* 243 in November 1999. The asteroid samples were collected at a site located at 15°N 20°W at ~3200 m depth (Table 5.1; Fig. 5.2). This site is characterised by a strong upwelling regime, which causes a high surface productivity and the subsequent organic matter input to the seabed (Tenhaven et al., 1992: Thiede and Jünger, 1992; Lange et al., 1998).

5.2.2- Sampling gear

The samples were collected with a semi-balloon otter trawl (OTSB 14). The net of the OTSB14 has a mesh size of 44 mm in the outer part and 37 mm in the middle and cod end, with a 13 mm inner liner in the cod end (Merret and Marshal, 1981).

5.2.3- Laboratory analyses

For each species at the different sites, the sex ratio, size at maturity, pyloric caecum index, reproductive cycle (gametogenesis and oocyte-size distributions) and reproductive output (gonad index and fecundity) were studied. Also, the population size distribution of *Hyphalaster inermis* from PAP and MAP were analysed.

	Station	Cruise	Date	Number of *Hyphalaster inermis*	Number of *Styracaster chuni*	Number of *Styracaster horridus*
PAP						
48°50'N, 16°30'W 4850 m	53201	C111	April 1994	75	39	4
	12930	D222	Sept. 1996	86	15	4
	13078	C226	April 1997	62	37	3
	13200	D229	July 1997	100	51	8
	54301	C135	Oct. 1997	63	42	0
	13368	D231	Mar. 1998	66	27	2
	13627	D237	Sept. 1998	45	28	0
	54901	C142	April 1999	30	15	0
MAP						
31°17'N, 25°24'W 5440 m	11261	D156	July 1985	246* 90**	119* 50**	0
NWA	13662					
15°N, 10°W 3200 m	13664 13667	D243	Nov. 1999	21	0	0

Table 5.1. Location, cruise and station numbers, dates of collection and number of specimens for three species of porcellanasterid asteroids from the Porcupine Abyssal Plain (PAP), the Madeira Abyssal Plain (MAP) and a site off the NW African slope (NWA). *total number of specimens collected, **number of individuals available for reproductive studies.

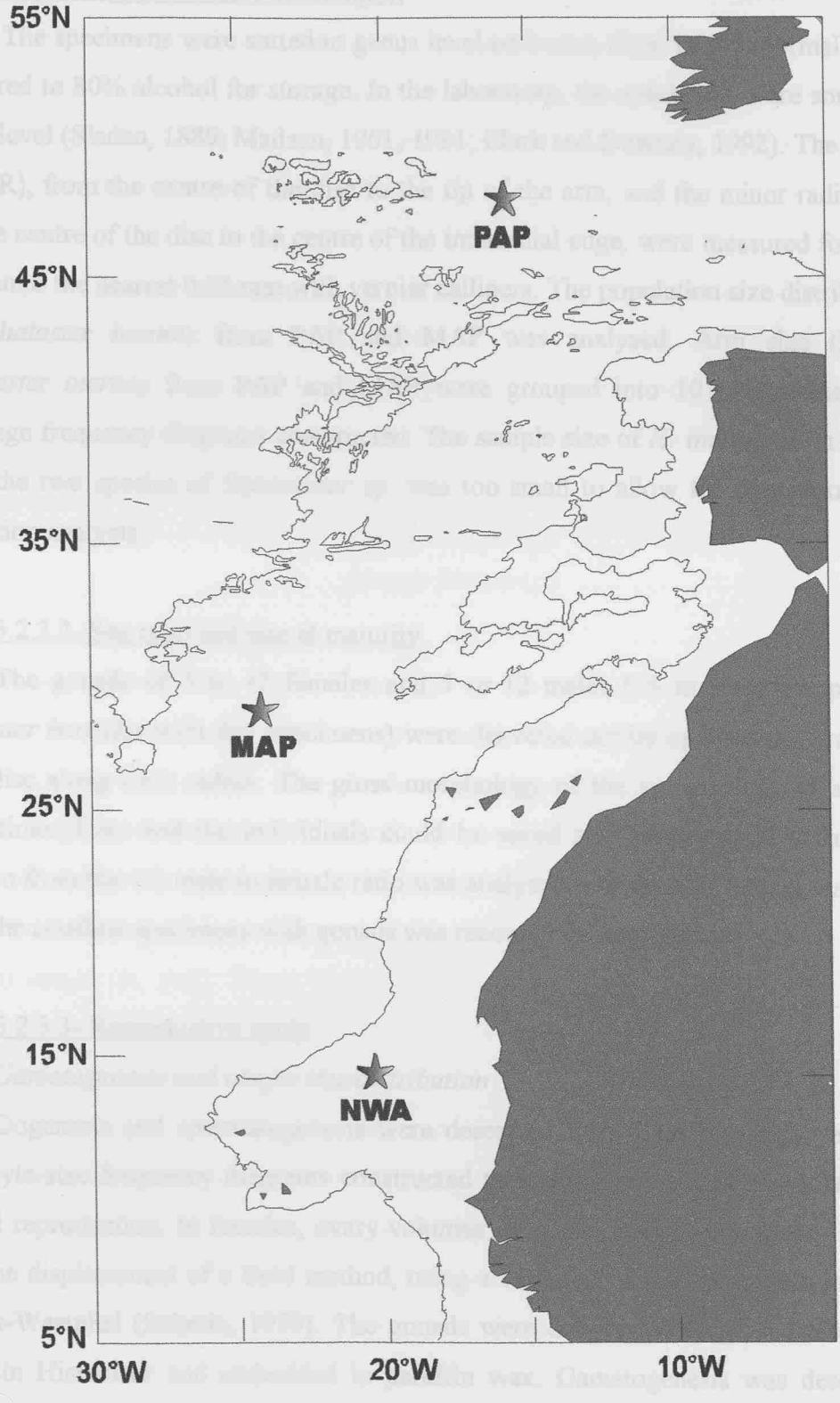


Figure 5.2. Chart of the NE Atlantic showing the three study sites and the 4000 m isobath.

5.2.3.1- Population size distribution

The specimens were sorted to genus level on board, fixed in 10% formalin and transferred to 80% alcohol for storage. In the laboratory, the specimens were sorted to species level (Sladen, 1889; Madsen, 1961, 1981; Clark and Downey, 1992). 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 with vernier callipers. The population size distribution of *Hyphalaster inermis* from PAP and MAP was analysed. Arm size (R) of *Hyphalaster inermis* from PAP and MAP were grouped into 10 mm classes and percentage frequency diagrams constructed. The sample size of *H. inermis* from NWA and of the two species of *Styracaster* sp. was too small to allow for population size distribution analysis.

5.2.3.2- Sex ratio and size at maturity

The gonads of 5 to 12 females and 5 to 12 males (<5 in some samples of *Styracaster horridus* with few specimens) were dissected out by opening the oral side of the disc along each radius. The gross morphology of the gonads showed a clear sexual dimorphism and the individuals could be sexed and the sex ratio calculated. Variation from the 1:1 male to female ratio was analysed with the Chi Square test. The size of the smallest specimens with gonads was recorded for each species.

5.2.3.3- Reproductive cycle

Gametogenesis and oocyte size distribution

Oogenesis and spermatogenesis were described from light histology sections and oocyte-size frequency diagrams constructed to identify any possible evidence of seasonal reproduction. In females, ovary volumes were measured to the nearest 0.001 ml by the displacement of a fluid method, using a variation of the hydrostatic balance of Mohr-Westphal (Scherle, 1970). The gonads were dehydrated in graded alcohols, cleared in Histoclear and embedded in paraffin wax. Gametogenesis was described from 7 μm sections stained with the routine stain Haematoxylin and Eosin. In females, at least 100 oocytes sectioned through the nucleus were measured (Feret diameter) using the image analysis package Matrox Rainbow Runner / SigmaScan Pro 4. The oocyte sizes were grouped into 100 μm classes and oocyte-size frequency diagrams constructed for each individual and for the composite data for each station. The

homogeneity of the oocyte-size distributions of specimens collected during different months at PAP was tested with the G-test of independence (Sokal and Rohlf, 1995). Two slides per male were prepared in order to identify the developmental stage of sperm. Fifty spermatogonia, fifty spermatocytes and fifty spermatozoa were measured and the data averaged for each species.

5.2.3.4- Reproductive output

Gonad index and Pyloric caecum index

Before being processed for histology, the five pairs of gonads from each individual were wet weighed with a precise balance to the nearest 0.001g. The gonad index (GI) of adults was calculated as:

$$GI = \frac{Gonad\,Weight\,(g)}{Female\,Weight\,(g)} \times 100$$

In porcellanasterids, the body weight of an individual varies considerably depending on the amount of sediment content in the stomach and the integrity of the arms, which are long and often broken. In these circumstances, an accurate quantification of body weight was difficult. To avoid this problem, the female weight was estimated from the relationship between body weight and arm length.

The body weight (g) of intact individuals with empty stomach was regressed onto arm length (R, mm). There was a significant positive correlation between arm length and body weight for the three species ($r=0.94$ for *H. inermis*, $r=0.94$ for *S. chuni* and $r=0.98$ for *S. horridus*, $P<0.005$ at PAP, and $r=0.95$ for *H. inermis*, $r=0.90$ for *S. horridus*, $P<0.005$ at MAP; Fig 5.3). The curve was linearised by taking the decimal logarithm of both variables, and the allometric growth curve was defined from these data for each species at PAP and MAP (Table 5.2). The specimens of *H. inermis* from NWA followed the same pattern as the PAP specimens. The weight of every female was estimated from the allometric growth curve and used to calculate the gonad index.

The mean gonad index was compared between males and females for each species using Student's t-test, and between samples within PAP and between samples from the three sites using ANOVA.

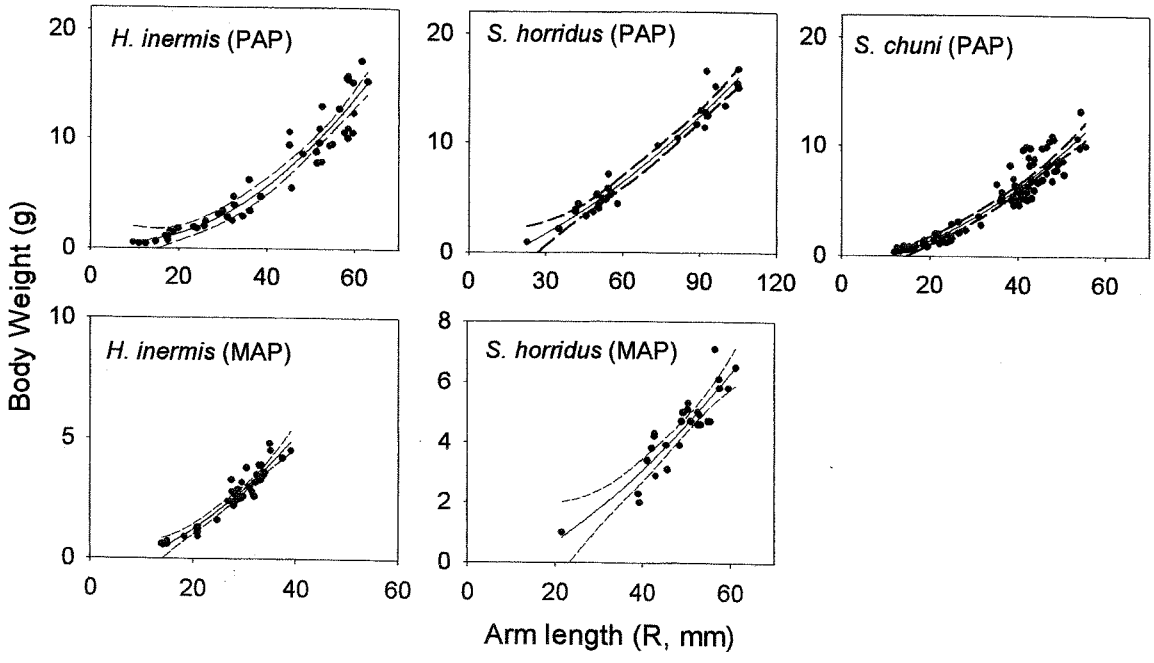


Figure 5.3. Allometric growth curve of *Hyphalaster inermis*, *Styracaster horridus* and *S. chuni* from PAP, and of *H. inermis* and *S. horridus* from MAP.

	Allometric Growth Curve
Hyphalaster inermis (PAP)	$W = 0.00316 \times R^{2.1}$
Styracaster chuni (PAP)	$W = 0.00158 \times R^{2.2}$
Styracaster horridus (PAP)	$W = 0.00501 \times R^{1.8}$
Hyphalaster inermis (MAP)	$W = 0.00158 \times R^{2.2}$
Styracaster horridus (MAP)	$W = 0.00316 \times R^{1.8}$

Table 5.2. Allometric growth curve equations for *Hyphalaster inermis*, *Styracaster* chuni and *S. horridus* from PAP, and for *H. inermis* and *S. horridus* from MAP. W, body wet weight (g); R, arm length (mm)

Because the gonad index and pyloric caecum index (PCI) of asteroids often follow an inverse relationship indicating the transfer of energy from the storage organ to the ovaries and testes, the PCI will be discussed within the reproductive output section, together with gonad index. The pyloric caeca of each individual were dissected out and weighed to the nearest 0.001 g with a precise balance. The PCI was estimated following the same method as for the calculation of gonad index:

$$PCI = \frac{Pyloric\ Caecum\ Weight\ (g)}{Female\ Weight\ (g)} \times 100$$

Fecundity

Fecundity was quantified as the number of vitellogenic oocytes per female (actual fecundity) (Tyler et al., 1984; Tyler and Billett, 1987). Fecundity was estimated from the mean volume of oocytes and the gonad volume for each female. Oocyte volume (OV) was calculated assuming a spherical shape ($OV = \frac{4 \times \pi \times R^3}{3}$) and averaged. The gonadal fluid that solidifies during fixation occupies approximately 15% of the ovary (estimated from ten slides in ten females), and this percentage was extracted from the total gonad volume.

Fecundity was estimated as follows:

V_g = volume of gonad (after subtracting the 15% occupied by the gonadal fluid)

V_{pvo} = mean volume of a previtellogenic oocyte

V_{vo} = mean volume of a vitellogenic oocyte

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

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

P = ratio between previtellogenic and vitellogenic oocytes: $P = \frac{N_{pvo}}{N_{vo}}$

Assuming that P, the ratio between the number of previtellogenic oocytes and vitellogenic oocytes measured in a subsample of the gonad is the same than the ratio between these oocyte stages in the whole gonad, then:

$$P = \frac{N_{pvo}}{N_{vo}} = \frac{F_{pvo}}{F_{vo}}; \quad F_{pvo} = P \times F_{vo} \qquad ①$$

where F_{pvo} and F_{vo} are the total number of previtellogenic oocytes ("fecundity" of previtellogenic oocytes) and total number of vitellogenic oocytes ("fecundity" of 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 ② from ①:

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

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

$$F_{vo} = \frac{V_g}{V_{vo} + (V_{pvo} \times P)} \qquad ③ \textbf{ (Actual fecundity)}$$

From ① and ③ we obtain the estimation for the total number of oocytes in the ovary, or potential fecundity (F):

$$F = F_{pvo} + F_{vo} \qquad ④ \textbf{ (Potential fecundity)}$$

Actual fecundity was calculated for each individual and averaged for each sample. Differences in the mean fecundity between females collected at different months from PAP and between the three locations were tested using ANOVA.

5.3- Results

5.3.1- General gonad morphology

The gonads of *Hyphalaster inermis, Styracaster chuni* and *S. horridus* showed a similar external and internal gross-morphology. There were five pairs of gonads per individual, one pair in each interradius. The gonads were located between the body wall and the stomach, which was often distended with ingested sediment. Each pair of gonads was suspended in the coelom and was attached to the body wall by a short gonoduct opening aborally at the gonopore. The ovaries were bright orange tufts of digitate tubules with large pink oocytes visible in fresh specimens (Fig. 5.4A). The testes were tufts of pale-cream nodular tubules, and in well-developed specimens some tubules extended into the coelom of the arms (Fig. 5.4B).

Microscopically, both ovaries and testes showed the typical gonad wall structure of asteroids (Walker, 1974) with an outer sac and an inner sac separated by the perihaemal sinus (Fig. 5.5A and D).

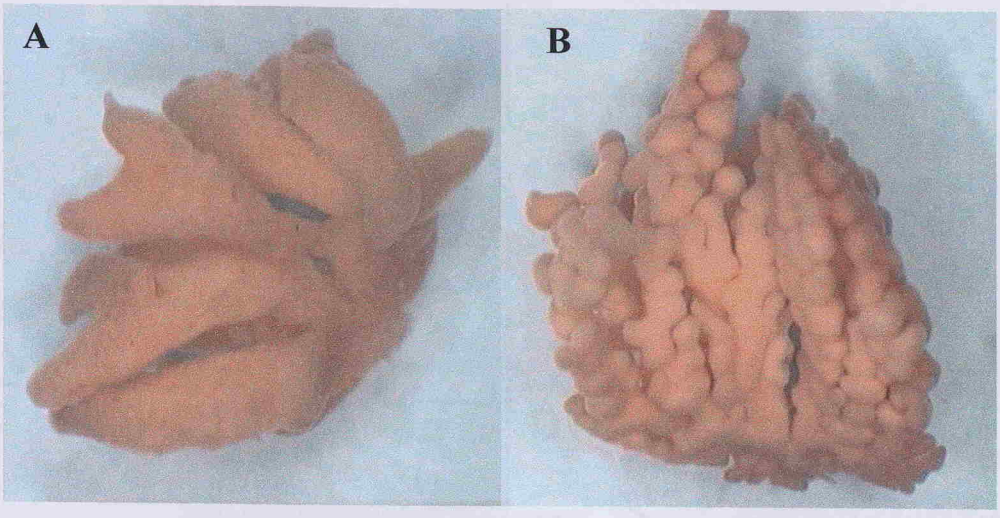


Figure 5.4. External morphology of the gonads of *Hyphalaster inermis*. A- ovary; B- Testis.

5.3.2- Reproductive patterns of Hyphalaster inermis

5.3.2.1- Sex ratio

The sex ratio of *Hyphalaster inermis* from PAP, MAP and NWA did not differ significantly from unity. The total number of sexed specimens from PAP was 302, with 161 males and 141 females (χ^2 = 1.324, 1df, P>0.05). In MAP, the total number of specimens sexed was 44, with 22 males and 22 females (χ^2 = 0.0, 1df, P>0.05). The total number of specimens sexed from NWA was 21 individuals, with 10 females and 11 males (χ^2 = 0.04, 1df, P>0.05).

5.3.2.2- Size at maturity

Juvenile specimens of *Hyphalaster inermis* from PAP grow to a size of around R=31 mm for females and R=35 mm for males before the gonads show any sign of maturing. At that stage, both the testes and ovaries were seen as tiny tufts of white tubules no more than 1 mm long, and showed early stages of gamete development (Fig. 5.5E and F).

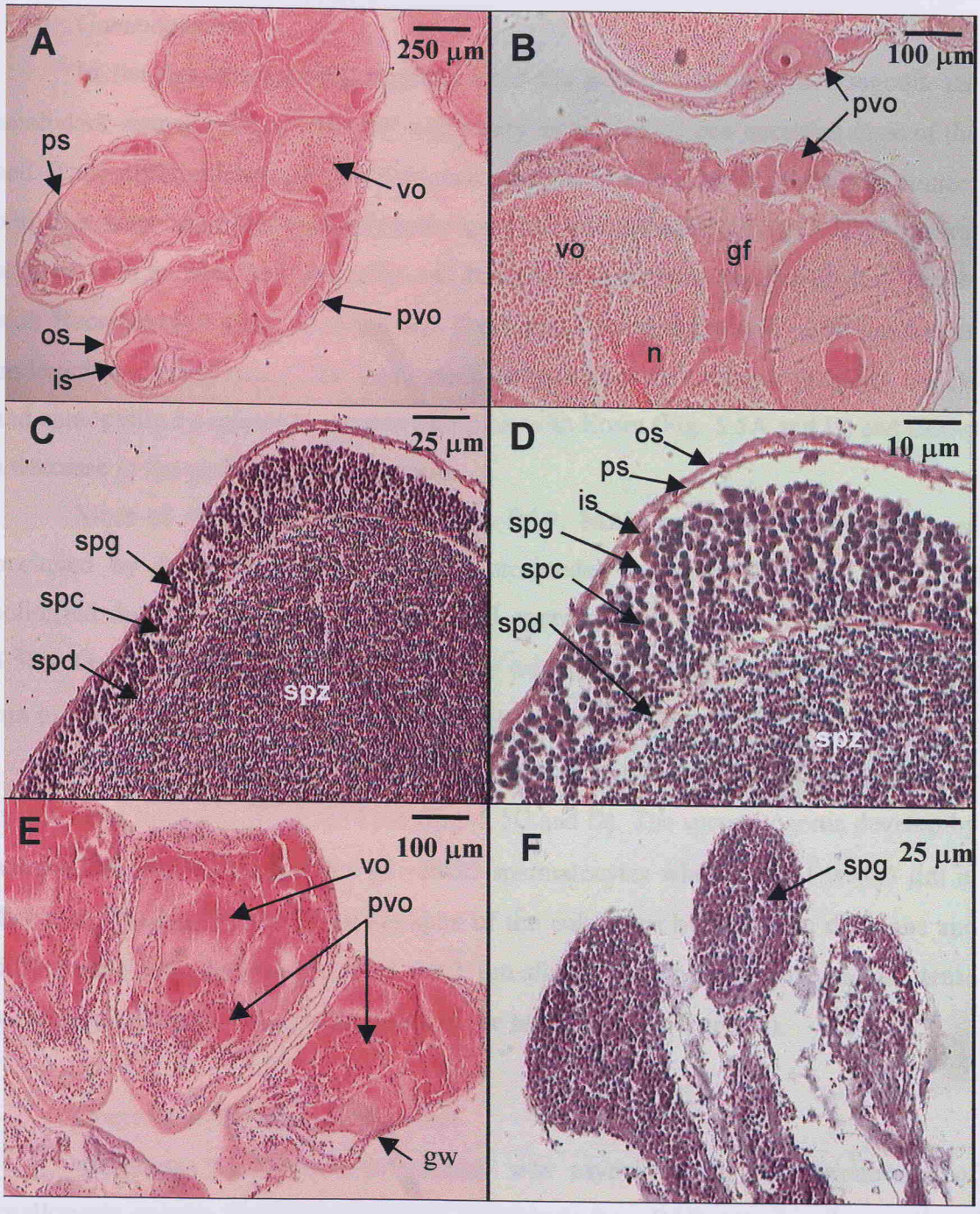


Figure 5.5. Light histology of gonads of *Hyphalaster inermis* stained with Haematoxylin and Eosin. A and B- Ovary of adult female; C and D- Testis of adult male; E- Ovary of young female; F- Testis of young male.
gf, gonad fluid; gw, gonad wall; is, inner sac; os, outer sac; n, nucleus; ps, perihaemal sinus; pvo, previtellogenic oocyte; spc, spermatocytes; spd, spermatids; spg, spermatogonia; spz, spermatozoa; vo, vitellogenic oocyte.

5.3.2.2- Reproductive cycle

Gametogenesis

In females, the growing oogonia lined the germinal epithelium. Oogonia are small dark-stained cells (45-50 μm) with a very large nucleus that occupied most of the cell. They differentiate into previtellogenic oocytes that commonly remain in contact with the inner sac. The previtellogenic oocytes are characterised by a large central nucleus with an eccentric nucleolus and basophilic cytoplasm that stains dark purple with Haematoxylin (Fig. 5.5A and B). These small oocytes grow to ~200 μm before undergoing vitellogenesis. The vitellogenic oocytes have an eccentric germinal vesicle and acidophilic cytoplasm that stains pale pink with Eosin (Fig. 5.5A and B) and shows a decrease in the nucleus/cytoplasm ratio.

Most of the ovary volume, in the PAP, MAP and NWA specimens, was occupied by large vitellogenic oocytes surrounded by gonadal fluid. This fluid solidified during fixation of the tissues and appeared pale pink in the sections (Fig. 5.5B). The maximum size of a mature oocyte was 622 μm in specimens from PAP, 691 μm in specimens from MAP and 510 μm in the specimens from NWA.

In males, the germinal epithelium gives rise to a dense layer of spermatogonia with a mean diameter of 3.4 ±0.4 μm (Fig. 5.5C and D). The spermatogonia develop by mitotic divisions into colonettes of ~20-25 spermatocytes which were 1.9 ±0.3 μm in diameter. Spermatids arise from the apex of the colonettes by reduction divisions and differentiate into spermatozoa (1.3 ±0.2 μm diameter). These accumulate as dense masses of mature gametes in the lumen of the testes (Fig. 5.5C and D).

Oocyte-size frequency distribution

Oogenesis in *Hyphalaster inermis* was asynchronous, with production of vitellogenic oocytes throughout the year. Individuals from PAP, covering the months of March, April, May, July, September, October and November within a 4 year sampling period, as well as individuals from MAP collected in July 1985 and from NWA collected in November 1999 , all showed a broad range of oocyte development at any one time (Fig. 5.6A, B and C).

Although there was variability between individuals within locations, a general pattern in oocyte size distribution was apparent for *Hyphalaster inermis*. All

individuals had a wide range of oocyte sizes covering developmental stages from oogonia to vitellogenic oocytes.

When comparing the composite data of the oocyte size-frequencies for the samples from PAP (Fig. 5.6A), significant differences were apparent between months in the oocyte size distributions (G-test of independence, G=1632.9, 42df, P<0.001). Nevertheless, these diagrams together with the pooled data for the MAP and NWA specimens (Fig. 5.6B and C) showed a similar general pattern.

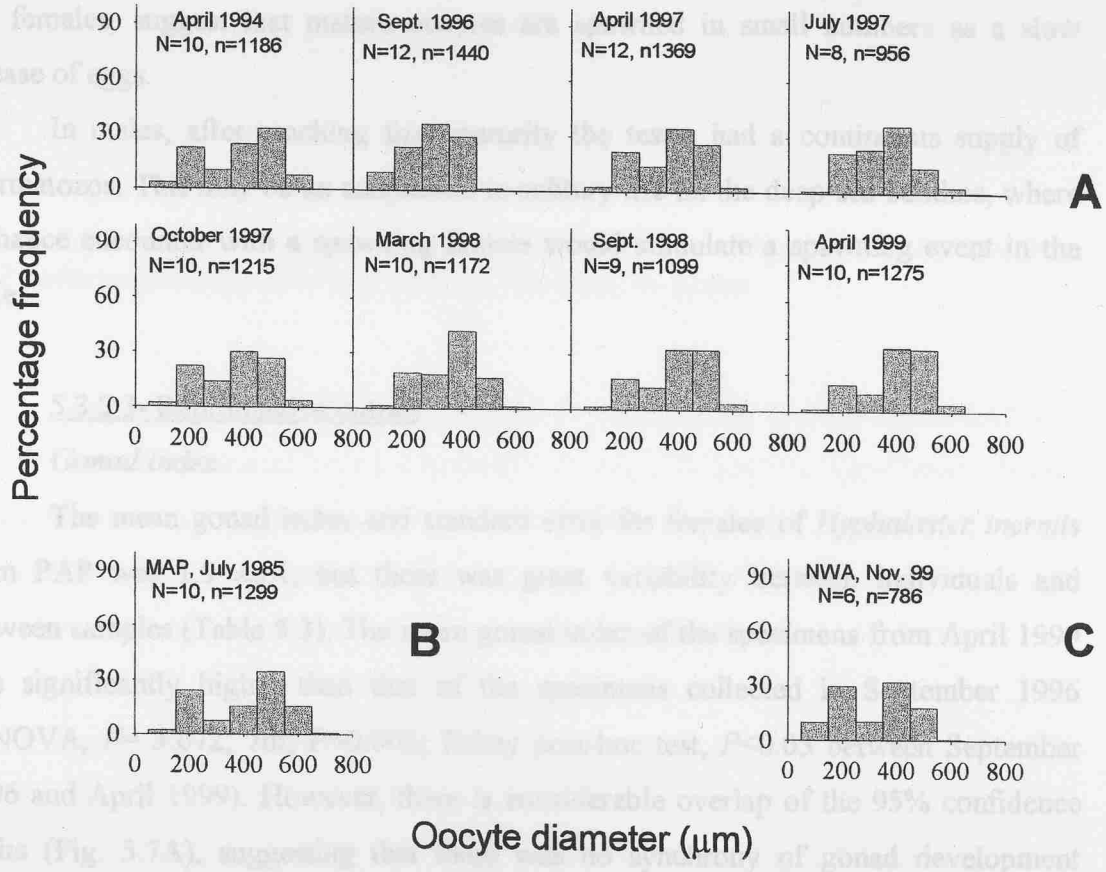


Figure 5.6. Oocyte size-frequency distributions of the composite data of *Hyphalaster inermis* from PAP, MAP and NWA.
A- Oocyte size diagrams for the composite data of each sampling month at PAP; B- Diagrams for the composite data of the MAP specimens; C- Diagram for the composite data of the NWA specimens.
N, number of females analysed; n, number of oocytes measured

There was a wide range of oocyte sizes with a high proportion (~30%) of previtellogenic oocytes (≤ 200 µm) and a peak (~50-75%) in the percentage of large vitellogenic oocytes, mainly in the size classes of 400 and 500 µm. The percentage of oocytes in the largest size classes (≥ 600 µm) was low and accounted only for ~5%.

These data suggest that there is no synchrony in oogenesis within or between samples. In young specimens (R=31 to 40 mm) with ovaries in the first stages of development, the percentage of previtellogenic and early vitellogenic oocytes (≤ 300 μm) was higher than in larger adults. These observations indicated that females reaching first maturity produce a large number of young oocytes that grow and undergo vitellogenesis producing a pool of large vitellogenic oocytes. The presence of a large number of vitellogenic oocytes in the ovaries at any one time, and the small percentage of oocytes in the largest size class, together with the absence of completely spawned out females, suggest that mature oocytes are spawned in small numbers as a slow release of eggs.

In males, after reaching first maturity the testes had a continuous supply of spermatozoa. This may be an adaptation to solitary life on the deep-sea benthos, where a chance encounter with a spawning female would stimulate a spawning event in the male.

5.3.2.3- Reproductive output

Gonad Index

The mean gonad index and standard error for females of *Hyphalaster inermis* from PAP was 1.9 ±0.1, but there was great variability between individuals and between samples (Table 5.3). The mean gonad index of the specimens from April 1999 was significantly higher than that of the specimens collected in September 1996 (ANOVA, F= 3.072, 7df, P=0.005; Tukey post-hoc test, P<0.05 between September 1996 and April 1999). However, there is considerable overlap of the 95% confidence limits (Fig. 5.7A), suggesting that there was no synchrony of gonad development between samples.

The mean gonad index of males was 1.0 ±0.06 (Table 5.3) and was significantly higher than the mean GI of females (Student's t-test, t= -4.984 15df, P<0.0001). The mean gonad index of the specimens collected in April 1999 was significantly higher than that of the specimens from September 1996 and April 1997 (ANOVA, F= 3.232, 7df, P<0.005; Tukey post-hoc test, P<0.05 between April 1999 and September 1996 and April 1997). However, the overlapping 95% confidence limits suggested that there was no synchrony of testis development (Fig. 5.7B).

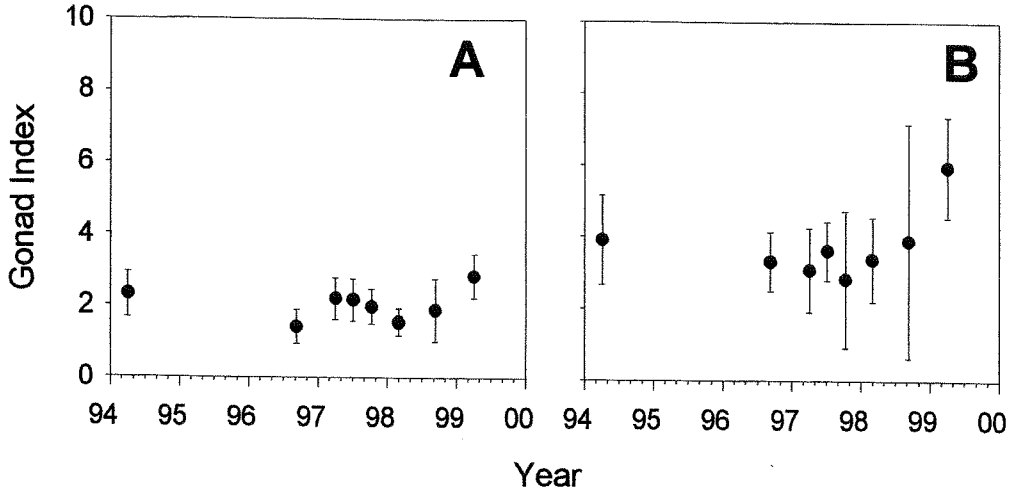


Figure 5.7. Mean gonad index and 95% confidence limits of *Hyphalaster inermis* from PAP collected between 1994 and 1999. A- Females; B- Males

The mean gonad index and standard deviation of *H. inermis* from MAP was 1.93 ±0.4 for females and 3.6 ±1.5 for males (Table 5.3), and was significantly higher in males than females (Student t-test, t= -4.579, 39df, P<0.0001). The mean GI of the specimens from NWA was 2.4 ±1.6 for females and 4.9 ±1.5 for males (Table 5.3), again being significantly higher in males (Student t-test, t= -3.323, 15df, P<0.05).

There were no significant statistical differences in the mean gonad index of *H. inermis* from PAP, MAP and NWA in either females or males (ANOVA, F= 0.959, 2df, P>0.05 for females and F= 5.616, 2df, P>0.05 for males; Fig. 5.8A and B).

	April 1994 PAP	September 1996 PAP	April 1997 PAP	July 1997 PAP	October 1997 PAP	March 1998 PAP	September 1998 PAP	April 1999 PAP	PAP (mean ±SE)	MAP July 1985	NWA Nov. 1999
Gonad Index Females	0.6 ± 0.3	0.4 ± 0.3	0.6 ± 0.3	0.6 ± 0.3	0.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.3	0.7 ± 0.4	0.5 ±0.03	0.3 ± 0.1	0.8 ±0.4
Gonad Index Males	1.0 ± 0.6	0.9 ± 0.5	0.7 ±0.3	0.9 ± 0.4	0.8 ± 0.7	0.8 ± 0.5	1.0 ± 0.9	1.6 ± 0.9	1.0 ±0.06	0.5 ± 0.2	1.3 ±0.4
PCI Females	1.2± 0.4	1.0± 0.3	0.7± 0.3	0.8± 0.2	0.9± 0.4	0.7± 0.2	0.8± 0.3	0.8± 0.2	0.9± 0.03	0.3± 0.1	0.6± 0.3
PCI Males	1.0± 0.3	1.0± 0.3	0.9± 0.3	0.8± 0.2	0.6± 0.3	0.6± 0.2	0.8± 0.3	0.5± 0.2	0.8± 0.03	0.4± 0.3	0.5± 0.3
Fecundity	8202.5 ±2296.5	14288.8 ±8136.5	10470.6 ±4894.9	9750.5 ±7053.3	8095.0 ±3282.4	7157.0 ±3729.7	6772.6 ±2694.5	11774.7 ±4536.0	9732.8 ±601.6	1178.1 ±615.9	14769.1 ±9153.7

Table 5.3. Mean and standard deviation of Gonad Index, Pyloric Caecum Index (PCI) and Fecundity (number of vitellogenic oocytes per female) of *Hyphalaster inermis* from PAP, MAP and NWA.

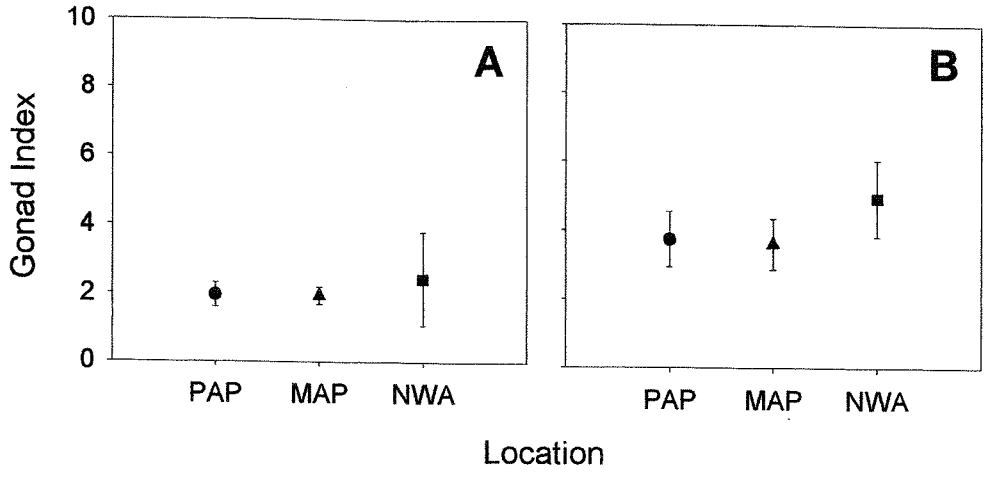


Figure 5.8. Mean gonad index and 95% confidence limits of *Hyphalaster inermis* from PAP (circles), MAP (triangles) and NWA (squares). A- Females; B- Males.

Pyloric Caecum Index

The mean pyloric caecum index (PCI) of female *H. inermis* from PAP was 3.2 ±0.2 (Table 5.3). There were significant differences in the mean PCI of females from PAP between the samples collected in April 1994 and March 1998 (ANOVA, F= 2.645, 7df, P<0.05; Tukey post-hoc test, P<0.05 between April 1994 and March 1998). The high variability within samples (Table 5.3) and the overlapping 95% confidence limits (Fig. 5.9A) suggest that there is no distinct pyloric caecum cycle in females. The same was found in the mean PCI of male *Hyphalaster inermis* from PAP, with a mean of 3.0 ±0.2 and significant differences between months (ANOVA, F= 4.361, 7df, P<0.05; Tukey post-hoc test, P<0.05 between September 1998 and the samples collected in April 1994, September 1996 and April 1997). Again, the overlapping 95% confidence limits and lack of pattern suggest that the PCI of males does not follow a seasonal cycle (Fig. 5.9B).

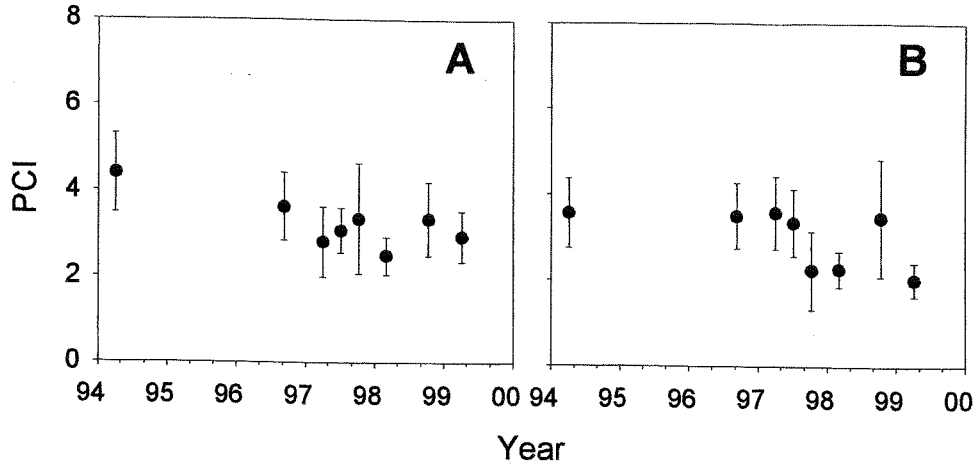


Figure 5.9. Mean pyloric caecum index (PCI) and 95% confidence limits of *Hyphalaster inermis* from PAP collected between 1994 and 1999.
A-Females; B- Males

The mean PCI of *H. inermis* from MAP was 1.8 ±0.8 for females and 2.6 ±1.8 for males (Table 5.3). The mean PCI of the specimens from NWA was 2.2 ±0.9 for females and 2.1 ±1.0 for males (Table 5.3). There were no significant differences in the mean PCI between females and males at any of the three locations ($P>0.05$ for the specimens from PAP, from MAP and from NWA).

There were no significant differences in the mean PCI between specimens at MAP and the specimens from the other two sites, but mean PCI of *H. inermis* from PAP was significantly higher than that of the specimens from NWA (ANOVA, $F=3.972$, 2df, $P<0.05$; Tukey post-hoc test, $P<0.05$ between PAP and NWA; Fig. 5.10).

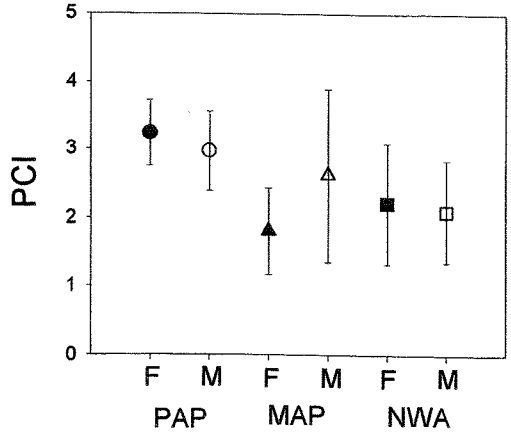


Figure 5.10. Mean pyloric caecum index (PCI) and 95% confidence limits of females (F) and males (M) of *Hyphalaster inermis* collected at PAP, MAP and NWA.

Fecundity

Actual fecundity was estimated as number of vitellogenic oocytes in the ovaries of *Hyphalaster inermis*. In the specimens from PAP, fecundity ranged from 1383 to 25451 oocytes per female, with a mean fecundity of 9563 ±903. There was high intra-sample variability (Table 5.3) and there were significant differences in the mean fecundity between the sample from September 1996 and March and September 1998 (ANOVA, F=2.773, 7df, P<0.05, Tukey post-hoc test, P<0.05 between September 1996 and March and September 1998). However, there was a considerable overlap of the 95% confidence limits (Fig. 5.11A), suggesting that *Hyphalaster inermis* has a similar egg production throughout the year.

The mean fecundity of *H. inermis* from MAP was 1178 ±615 oocytes per female, ranging from 254 to 2033 mature oocytes per female.

The mean fecundity of the specimens from NWA was 14769 ±9153 oocytes per female, ranging between 4722 to 30498 oocytes per female (Table 5.3).

The mean fecundity of *H. inermis* from MAP was significantly lower than that of specimens from PAP and NWA (ANOVA, F=14.474, 2df, P<0.001; Tukey post hoc test, P<0.001 between PAP and MAP, and NWA and MAP; Fig. 5.11B).

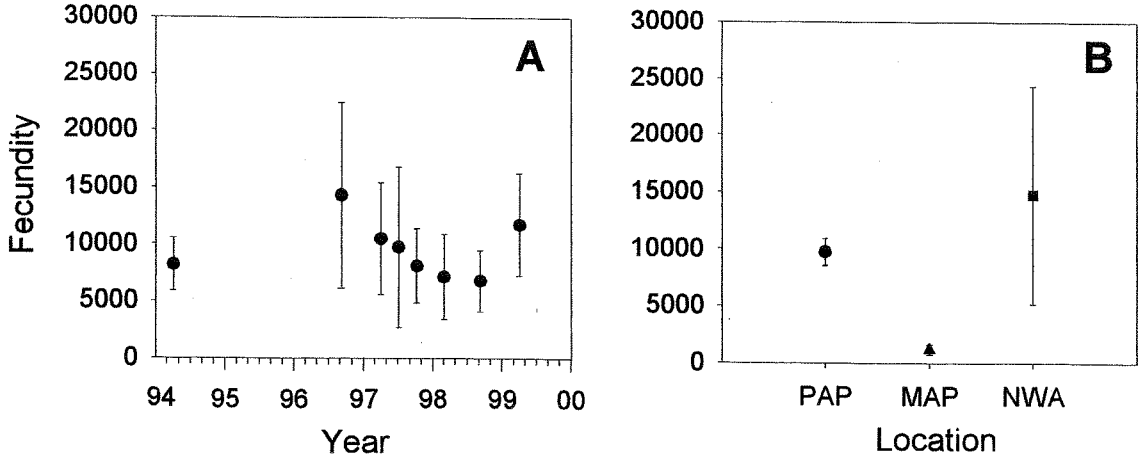


Figure 5.11. Mean fecundity (number of vitellogenic oocytes per female) and 95% confidence limits of *Hyphalaster inermis* from PAP (circles), MAP (triangle) and NWA (square). A- Fecundity of specimens from PAP collected between 1994 and 1999; B- Fecundity of specimens from PAP, MAP and NWA

5.3.2.4- Population size distribution

The population size distribution described below was obtained from small sample sizes (N = 55 to 246). In addition, because of the patchiness of the megafauna on the deep-sea bed, and the low efficiency of the OTSB in sampling infaunal and small specimens (<4 cm diameter), the size distributions presented here represent mainly the distribution of adult *Hyphalaster inermis*. No reliable data were available for recruitment.

The arm radius (R, mm) of the specimens collected in the Porcupine Abyssal Plain ranged from 5.7 to 72.0 mm (mean= 39.2 ±16.2 mm). There is a wide distribution of sizes, and most samples had a weak bimodal distribution (Fig. 5.12A). The first peak of the size distribution corresponds to specimens with R~10-40 mm and the second peak to specimens with R~50-70 mm.

The specimens from MAP showed a unimodal size distribution, with a maximum arm radius of 45.1 mm (mean= 28.6 ±6.1). No specimens had arm radius smaller than 18 mm, and ~95% of the specimens were included in the size classes between 30 and 40 mm (Fig. 5.12B). The mean size of *H. inermis* from MAP was significantly smaller than that from the PAP specimens (Student's test, t= 3.767, 85df, P<0.0001). Specimens with R=30 to 40 mm were young adults with gonads in the first stages of development in PAP, but were fully mature adults in MAP. The sample size of *H. inermis* from NWA was too small to allow for population size distribution analysis and there were no juveniles available, but the data suggested a similar pattern to the PAP population, with a mean size of R = 53.8 ±10.9 mm, ranging from 25.9 to 80.6 mm.

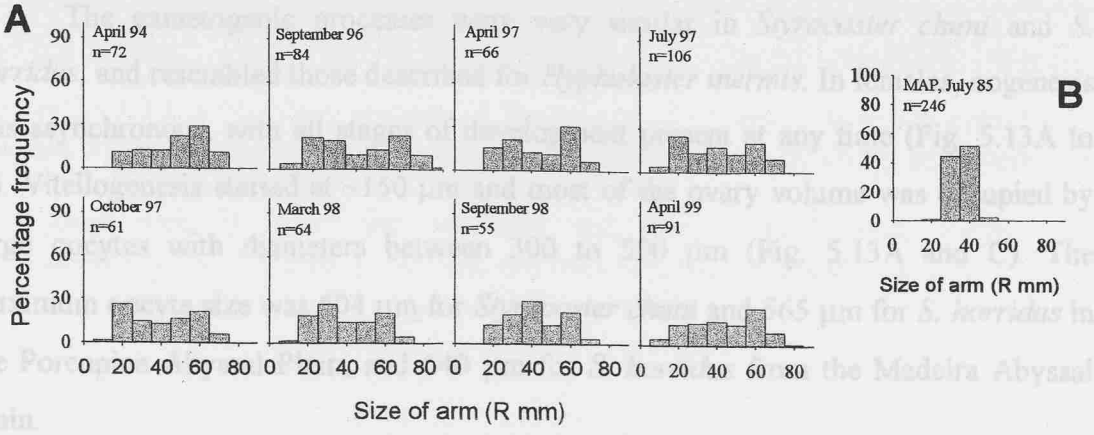


Figure 5.12. Population size distribution of *Hyphalaster inermis* from PAP and MAP. A- Samples collected in PAP between 1994 and 1999; B- Sample from MAP

5.3.3- Reproductive patterns of Styracaster chuni and S. horridus

A sufficient number of *S. chuni* and *S. horridus* were available for reproductive analysis from PAP and MAP, but not from NWA.

5.3.3.1- Sex Ratio

The sex ratio of *Styracaster chuni* and *S. horridus* did not differ significantly from unity in any of the samples from PAP. The total number of *S. chuni* sexed was 178, with 84 males and 91 females ($\chi^2 = 0.32$, 1df, $P>0.05$). Only 20 specimens of *S. horridus* were available from PAP for analysis, of which 9 were male and 11 were female ($\chi^2 = 0.2$, 1df, $P>0.05$).

Similarly, the sex ratio of *S. horridus* from MAP did not differ significantly from the expected 1:1. The total number of individuals sexed was 49, with 22 males and 27 females ($\chi^2 =0.5$, 1df, $P>0.05$).

5.3.3.2- Size at first reproduction

Size at first reproduction of males and females was determined for *S. chuni*, but no young specimens of *S. horridus* were found. Very small gonads, distinguished only as minute tufts of white tubules, were dissected from *S. chuni* specimens as small as R=21 to 23 mm for females, and R=22 to 23 mm for males. When sectioned and analysed histologically, these ovaries and testes showed the presence of developing gametes.

5.3.3.3- Reproductive cycle

Gametogenesis

The gametogenic processes were very similar in *Styracaster chuni* and *S. horridus*, and resembled those described for *Hyphalaster inermis*. In females, oogenesis was asynchronous, with all stages of development present at any time (Fig. 5.13A to D). Vitellogenesis started at ~150 μm and most of the ovary volume was occupied by large oocytes with diameters between 300 to 500 μm (Fig. 5.13A and C). The maximum oocyte size was 604 μm for *Styracaster chuni* and 565 μm for *S. horridus* in the Porcupine Abyssal Plain, and 640 μm for *S. horridus* from the Madeira Abyssal Plain.

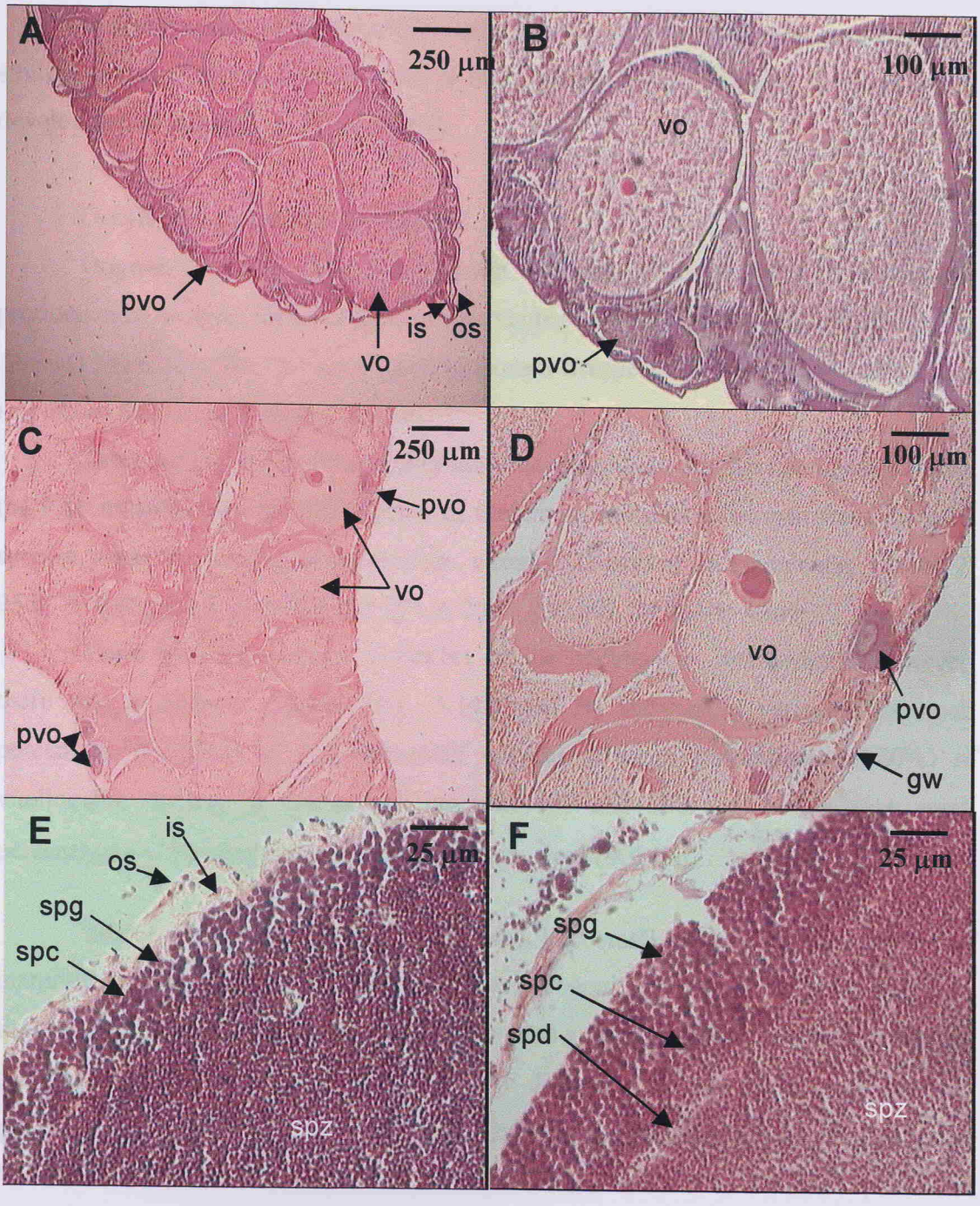


Figure 5.13. Light histology sections of *Styracaster chuni* and *S. horridus* stained with Haematoxylin and Eosin. A and B- Ovary of *S. chuni*; C and D, Ovary of *S. horridus*; E, Testis of *S. chuni*; F- Testis of *S. horridus*.
gw, gonad wall; is, inner sac; os, outer sac; pvo, previtellogenic oocyte; spc, spermatocytes; spg, spermatogonia; spz, spermatozoa; vo, vitellogenic oocyte.

In males, spermatogenesis followed the same pattern in the two species, being similar to that of *Hyphalaster inermis* in both gamete growth and size of the different developmental stages (Fig. 5.13E and F).

Oocyte size-frequency distribution

Oogenesis in the *Styracaster* group was asynchronous, with a continuous production of oocytes. Individuals from the Madeira Abyssal Plain and from Porcupine Abyssal Plain followed the same general pattern irrespective of sampling period, and resembled that of *Hyphalaster inermis*.

There was significant variability between individuals for the two species within the PAP samples, and the distribution of the composite data of oocyte sizes for each species were not homogenous between months (G-test of independence, G=456.2, 35df, P<0.001 for *S. chuni* and G=275.9, 20df, P<0.001 for *S. horridus*).

There was intra-sample variability in the oocyte-size distributions. However, there was a common pattern (Fig. 5.14A and B) showing a variable but small percentage (10-30%) of previtellogenic oocytes, a large percentage (60-80%) of vitellogenic oocytes in the classes between 400 and 500 μm, and a very small percentage (0-5%) of oocytes in the largest size class (600 μm).

Young individuals (R=22 to 40 mm) had small ovaries in the process of maturing, with most of the oocytes at the previtellogenic or early vitellogenic stages. In these specimens, the oocyte size diagrams showed a large number of oocytes in the 100 μm class. The time series samples from PAP did not show any evidence of seasonal gametogenesis in *S. chuni* or *S. horridus*. The oocyte size distribution of *Styracaster horridus* from MAP followed the same asynchronous pattern as the PAP specimens. The oocyte sizes ranged from 50 to 600 μm, and there was a peak in the size classes of 400 and 500 μm representing ~70 to 80% of the oocytes in the ovary (Fig. 5.14C).

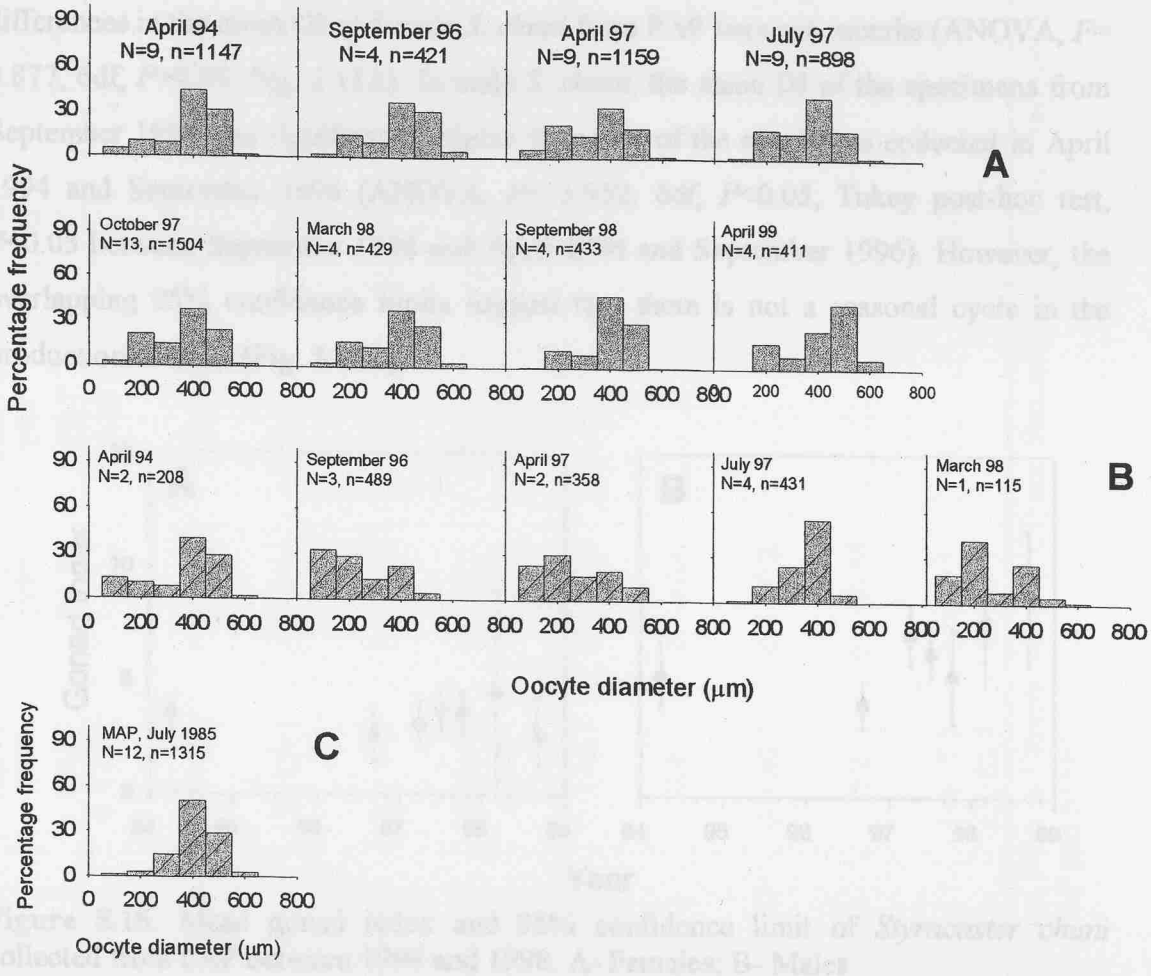


Figure 5.14. Oocyte size-frequency distributions of the composite data of *Styracaster chuni* (plain bars) and *S. horridus* (lined bars) from PAP and MAP.
A- Composite data for the samples of *S. chuni* collected at PAP between 1994 and 1999. B- Composite data for the samples of *S. horridus* collected at PAP between 1994 and 1999. C- Composite data for the sample of *S. horridus* from MAP.

5.3.3.4- Reproductive output

Gonad Index

In the PAP samples, the mean gonad index (GI) of *Styracaster chuni* was 3.3 ±0.2 in females and 6.4 ±0.6 in males, being significantly higher in males than females (Student's test, t= -4.601, 12df, P<0.005; Table 5.4). There were no significant differences in the mean GI of female *S. chuni* from PAP between months (ANOVA, F= 0.877, 6df, P>0.05; Fig. 5.15A). In male *S. chuni*, the mean GI of the specimens from September 1998 was significantly higher than that of the specimens collected in April 1994 and September 1996 (ANOVA, F= 3.952, 6df, P<0.05, Tukey post-hoc test, P<0.05 between September 1998 and April 1994 and September 1996). However, the overlapping 95% confidence limits suggest that there is not a seasonal cycle in the production of testis (Fig. 5.15B).

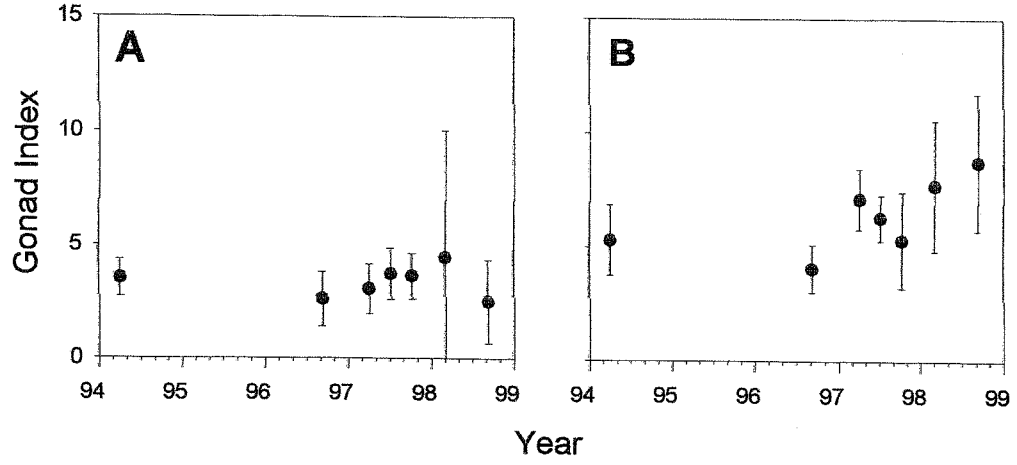


Figure 5.15. Mean gonad index and 95% confidence limit of *Styracaster chuni* collected from PAP between 1994 and 1998. A- Females; B- Males

The mean GI of *S. horridus* from PAP was 5.3 ±0.9 in females and 4.7 ±0.4 in males, with no significant differences between sexes (Student's test, t= 0.605, 13df, P>0.05; Table 5.4). In *S. horridus* from PAP, the sample size from each station was small and did not permit a statistical comparison between months, but the data (Table 5.4) suggest that the gonad index is similar between months, following the same pattern found for *S. chuni* and *Hyphalaster inermis*.

The mean gonad index of *S. horridus* from MAP was 2.9 ±0.3 for females and 5.8 ±0.5 for males (Table 5.4), and was significantly higher in males than females (Student's t-test, t= -5.577, 27df, P<0.0001).

		April 1994 PAP	September 1996 PAP	April 1997 PAP	July 1997 PAP	October 1997 PAP	March 1998 PAP	September 1998 PAP	PAP (mean ±SE)	MAP (July 85)
Styracaster chuni	**Females Gonad Index**	0.5 ±0.2	0.4 ±0.2	0.4 ±0.2	0.6 ±0.2	0.5 ±0.3	0.5 ±0.3	0.4 ±0.3	0.5 ±0.03	.....
	Males Gonad Index	0.7 ±0.3	0.7 ±0.3	1.0 ±0.3	0.9 ±0.2	0.8 ±0.4	1.1 ±0.4	1.3 ±0.6	0.9 ±0.04	.....
	Females PCI	0.7 ±0.3	0.9 ±0.4	0.8 ±0.5	0.8 ±0.4	0.9 ±0.2	0.4 ±0.2	0.7 ±0.4	0.7 ±0.04	.....
	Males PCI	0.5 ±0.2	0.5±0.2	0.6 ±0.3	0.6 ±0.2	0.6 ±0.3	0.5 ±0.1	0.6 ±0.2	0.6 ±0.03	.....
	Fecundity	5469.2 ±2292.6	5510.6 ±2760.5	8349.0 ±750.5	8449.0 ±4350.1	7001.0 ±2618.4	5281.8 ±3179.9	5568.1 ±1868.6	6774.7 ±402.9	.....
Styracaster horridus	**Females Gonad Index**	0.5 ±0.3	0.4 ±0.3	0.6 ±0.4	0.4 ±0.1	.....	0.5	.....	0.5 ±0.06	0.3 ± 0.1
	Males Gonad Index	1.0 ±0.6	0.8 ±0.3	0.9	0.4 ±0.4	.....	.....	.....	0.9 ±0.1	0.7 ±0.2
	Females PCI	0.4 ±0.2	0.8 ±0.07	0.4 ±0.1	0.4 ±0.2	.....	0.2	.....	0.4 ±0.06	0.4 ±0.05
	Males PCI	0.4 ±0.2	0.2 ±0.3	0.2	0.3 ±0.05	.....	.....	.....	0.3 ±0.03	0.3 ±0.07
	Fecundity	7606.0 ±3209.0	10875.1 ±3278.1	10107.0 ±2102.7	15412.1 ±1734.9	.....	8285.0	.....	11143.1 ±1097.3	3613.1 ±1632.2

Table 5.4. Mean and standard deviation of Gonad Index, Pyloric Caecum Index (PCI) and Fecundity (number of vitellogenic oocytes per female) of *Styracaster chuni* from PAP and *S. horridus* from PAP and MAP.

The mean GI of female *S. horridus* from MAP was significantly smaller than the mean GI of female *S. horridus* from PAP (Student's t-test, t= 3.477, 23df, P<0.005; Fig. 5.16). Conversely, there were no significant differences in the mean GI of male *S. horridus* between PAP and MAP (Student's t-test, t= -1.419, 17df, P>0.05; Fig. 5.16).

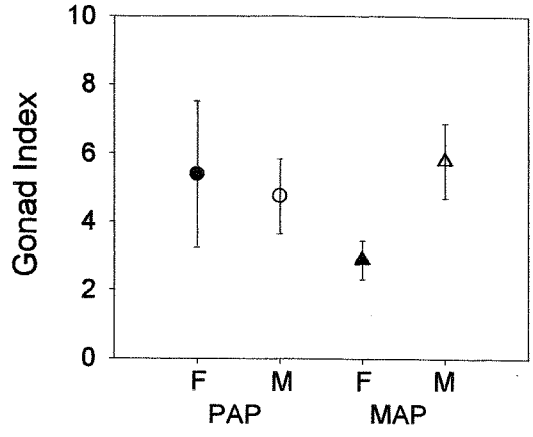


Figure 5.16. Mean gonad index and 95% confidence limits of females (F) and males (M) of *Styracaster horridus* from PAP (circles) and MAP (triangles). Solid symbols: females; open symbols: males

Pyloric caecum index

The mean pyloric caecum index (PCI) of *S. chuni* from PAP was 4.7 ±0.4 for females and 4.6 ±0.3 for males (Table 5.4), and there were no significant differences between sexes (Student's test, t= 2.008, 12df, P>0.05). There were no significant differences in the mean PCI of *S. chuni* from PAP in the different months of collection (ANOVA, F= 1.453, 6df, P>0.05 for females, and F= 0.483, 6df, P>0.05 for males; Fig. 5.17A and B), suggesting that the weight of the storage organ was constant throughout the year.

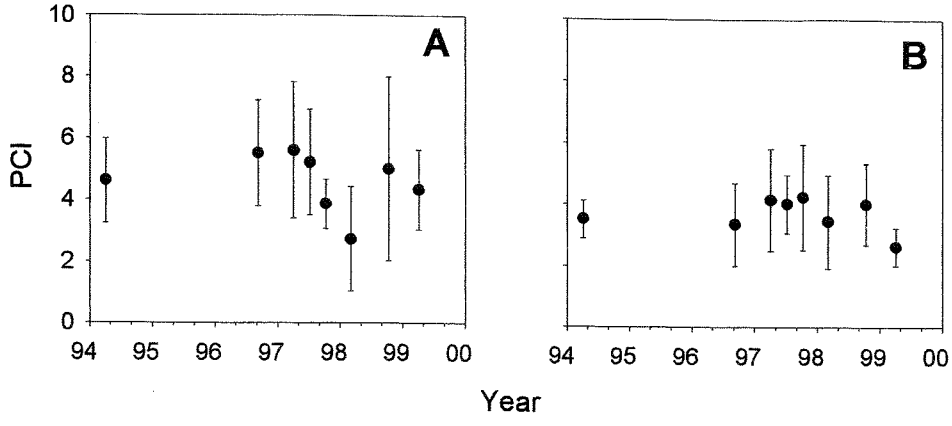


Figure 5.17. Mean pyloric caecum index (PCI) and 95% confidence limits of *Styracaster chuni* collected at PAP between 1994 and 1999. A- Females; B- Males

In *S. horridus*, the sample sizes of the different stations from PAP were too small to permit statistical comparisons, but the few data available (Table 5.4) seemed to follow the same pattern as for *S. chuni* and *H. inermis*. The mean PCI of *S. horridus* from PAP was 2.6 ±0.3 for females and 1.7 ±0.1 for males with no significant differences between sexes (Student' s test, t= 0.674, 18df, P>0.05; Table 5.4).

The mean PCI of *S. horridus* from MAP was 3.0 ±1.3 for females and 2.5 ±1.9 for males, with no significant differences between sexes (t= 0.674, 18df, P>0.05; Table 4). There were no significant differences in the mean PCI of *S. horridus* from PAP and MAP (Student's test, t= -0.778, 19df, P>0.05 for females and t= -1.200, 16df, P>0.05 for males; Fig. 5.18).

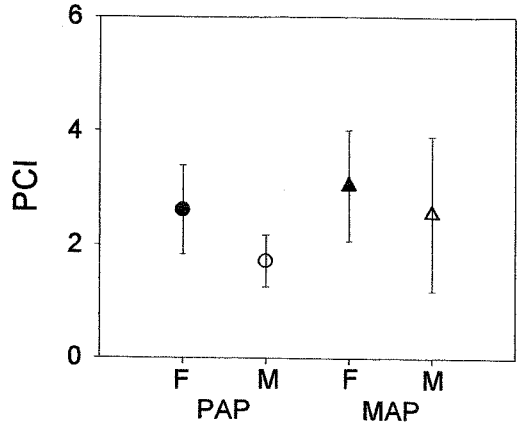


Figure 5.18. Mean pyloric caecum index (PCI) and 95% confidence limits of females (F) and males (M) of *Styracaster horridus* from PAP (circles) and MAP (triangles). Solid symbols, females; Open symbols, males

Fecundity

The two species of *Styracaster* had low actual fecundities of around 5000 to 10000 eggs per female, corresponding with a large egg size.

The fecundity of *S. chuni* from PAP ranged from 1515 to 14367 oocytes per female (mean = 6774 ±402 oocytes per female) (Table 5.4). There were no significant differences in the mean fecundity of *S. chuni* between samples (ANOVA, F=1.864, 6df, P>0.05; Fig. 5.19).

The fecundity of *S. horridus* from PAP ranged from 4403 to 17146 eggs per female, with a mean and standard error for the combined PAP samples of 11143.1 ±1097.3 oocytes per female (Table 5.4). Again, the sample size of *S. horridus* from the different months of collection at PAP was too small for statistical analysis, but the available data seemed to indicate a relatively constant fecundity throughout the year (Table 5.4).

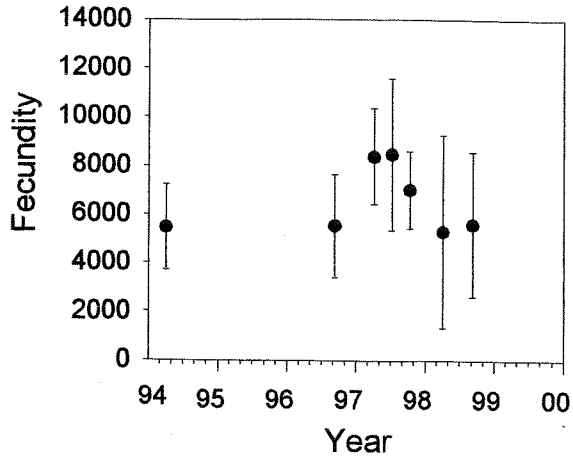


Figure 5.19. Mean fecundity (number of vitellogenic oocytes per female) and 95% confidence limits of *Styracaster chuni* collected at PAP between 1994 and 1999.

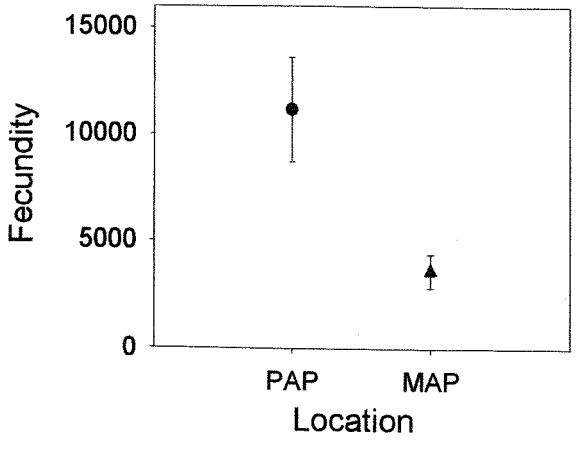


Figure 5.20. Mean fecundity (number of vitellogenic oocytes per female) and 95% confidence limits of *Styracaster horridus* from PAP (circle) and MAP (triangle).

The fecundity of *Styracaster horridus* from the Madeira Abyssal Plain ranged from 254 to 2033 eggs per female, with a mean of 3613 ±1632 eggs per female (Table 5.4). The mean fecundity of *S. horridus* from MAP was significantly lower than the mean fecundity of *S. horridus* from PAP (Student's t test, t=-7.830, 28df, P<0.001; Fig. 5.20).

5.3.3.4- Population size distribution

The sample sizes of *S. chuni* and *S. horridus* were too small for population size distribution analysis, but the mean sizes were 34.5 ±12.8 mm for *S. chuni* from PAP, 66.6 ±24.5 mm for *S. horridus* from PAP and 48.7 ±8.2 mm for *S. horridus* from MAP. The specimens of *S. horridus* from PAP were significantly larger than the specimens from MAP (Student's test, t= 3.717, 85df, P<0.0001).

5.4- Discussion

The reproductive patterns of *Hyphalaster inermis, Styracaster chuni* and *S. horridus* followed a similar trend. There were five pairs of gonads attached to the body wall of the interradius by a short gonoduct. The ovaries were orange tufts of digitate tubules, while the testes were cream-coloured and globular. The sex ratio did not differ from unity in any of the species. Gametogenesis was similar in all three species, starting in individuals of R~30 mm for *Hyphalaster inermis* and R~20 mm for the *Styracaster* species. Oogenesis was asynchronous, with vitellogenesis starting at oocyte size ~150-200 μm. Maximum oocyte size was ~650 μm. The ovary volume was mainly occupied by large vitellogenic oocytes (400-500 μm) in adult specimens at any time and there was a low percentage (~ 5%) of oocytes in the largest size class (600 μm). In all three species, oogonia and previtellogenic oocytes appear to develop continuously into large vitellogenic oocytes and only a number of oocytes seemed to mature and be spawned at any time throughout the year. In adult males, the spermatozoa differentiate from colonettes of spermatocytes and accumulate in the lumen of the testes. Once reaching first maturity, males are always ripe and ready to spawn. This suggests that a chance encounter with a spawning female would stimulate a spawning event in the male, enhancing the fertilisation success in a low density population. This strategy has been indicated for other abyssal asteroids (Pain et al., 1982b; Tyler et al., 1982b, 1984).

Reproduction in deep-sea asteroids shows a wide variety of patterns (Tyler et al., 1982a, 1984). In most deep-sea species, gametogenesis is asynchronous leading to a quasi-continuous production of a relatively small number of large eggs (Table 5.5). These eggs reach sizes between 800 and 1250 μm, indicative of lecithotrophic or direct development. In contrast, a small number of deep-sea species produce a high number of small eggs in a seasonal pattern (Table 5.5). The astropectinids *Dytaster grandis* and *Plutonaster bifrons* have synchronous oogenesis and spawn a large number of small eggs (~120 μm) (Tyler and Pain, 1982; Tyler et al., 1990). The seasonal reproduction of these asteroids is coupled with the seasonal deposition of phytodetritus in the NE Atlantic. The phytodetritus may be utilised by the planktotrophic larvae.

In the three species of porcellanasterid asteroids studied, gametogenesis seemed to be conservative between species and followed the general reproductive characteristics of most deep-sea asteroids, producing continuously a small number of large rich eggs.

The resemblance of the general ecology of the porcellanasterids with the mud-star *Ctenodiscus crispatus* is also found in their reproductive biology. *C. crispatus* has asynchronous gametogenesis, with at least some individuals in the population reproducing at any time during the year. Fecundity is low, and the mature eggs reach sizes up to 400 μm (Shick et al., 1981b; Inger-Britt and Falk-Petersen, 1982). Shick and co-authors (Shick et al., 1981b) also found variations in the reproductive intensity superimposed to the continuous reproduction, with increasing intensity after the spring and autumn phytoplankton blooms. The reproductive patterns of *Hyphalaster inermis*, *Styracaster chuni* and *Styracaster horridus* are similar to that of *C. crispatus*.

The porcellanasterids studied here have asynchronous gametogenesis, produce large eggs and have a low fecundity and a slight variability in the reproductive output between months superimposed on the continuous reproduction. However this variability in the reproductive output of the porcellanasterids could not be clearly related to fluctuations in organic input to the seabed as it was done for *C. crispatus* (Shick et al., 1981b). For asteroids that live burrowed in the sediment, the environment remains quasi-constant throughout the year. The arrival of phytodetritus after the spring bloom provides an extra input of nutrients, which can be used for an increase in the reproductive output.

	Gonad morph.	Egg size (μm)	Fec.	Develop.	Gameto.	Reference
F. Benthopectinidae						
Benthopecten simplex	Single	800-1000	Low	Lecith.	Async.	Pain et al.,
Pontaster tenuispinus	Single	800-1000	Low	Lecith.	Async.	1982a;
Pectinaster filholi	Single	800-1000	Low	Lecith.	Async.	Tyler et al., 1982a
F. Goniasteridae						
Paragonaster subtilis	Single	1000	Low	Lecith.	Async.	Tyler et al.,
Pseudarchaster parelii	Single	1000	Low	Lecith.	Async.	1982a
F. Pterasteridae						
Hymenaster membranaceus	Single	1100	Low	Lecith.	Async.	Pain et al.,
H. gennaeus	Single	1100	Low	Lecith.	Async.	1982b
F. Brisingidae						
Brisinga endecacnemos	Serial	1250	Low	Dir/Lecith	Async.	Tyler et al.,
Brisingella coronata	Single	1250	Low	Dir/Lecith	Async.	1984
Freyella spinosa	Single	1250	Very low	Dir/Lecith	Async.	
F. Zorasteridae						
Zoraster fulgens	Single	950	Low	Lecith.	Seas. (?)	Tyler et al., 1984
F.Astropectinidae						
Bathybiaster vexillifer	Single	900-1000	Low	Lecith.	Async.	Tyler and Pain, 1982;
Psilaster andromeda	Single	900-1000	Low	Lecith.	Async.	Tyler et al.,
Dytaster grandis	Serial	120	High	Plankt.	Seas.	1982b
Plutonaster bifrons	Serial	120	High	Plankt.	Seas.	Tyler and Pain, 1982; Tyler et al., 1990, 1993
F.Porcellanasteridae						
Hyphalaster inermis	Single	650	Low	Lecith.	Async.	Madsen,
Styracaster chuni	Single	650	Low	Lecith.	Async.	1961; This
Styracaster horridus	Single	650	Low	Lecith.	Async.	chapter

Table 5.5. Reproductive patterns of eighteen deep-sea asteroid species belonging to seven families. Gonad morph., gonad morphology; Fec, fecundity; Develop., larval development; Gameto., timing of gametogenesis; Lecith., lecithotrophic; Dir., direct; Plankt., planktotrophic; Asynch., asynchronous; Seas. Seasonal.

The constant, but low, availability of organic matter in the sediment allows for a regular and slow production of eggs in these asteroids, indicative of the most common reproductive pattern found in deep-sea asteroids (Pain et al., 1982a,b; Tyler and Pain, 1982, Tyler et al., 1982a,b; Tyler et al., 1984).

The production of eggs and sperm, larval development, metamorphosis and settlement of juveniles are major reproductive variables that have important ecological and biogeographical consequences. The oogenic pathways in invertebrates are phylogenetically constrained, and therefore are intrinsic to the species and independent of environment variability (Eckelbarger, 1994; Eckelbarger and Watling, 1995). The asteroids analysed in this study are taxonomically closely related, belonging to the family Porcellanasteridae, but have been compared from habitats with a different food supply. The oogenesis and spermatogenesis patterns of the three species were similar. There were no significant differences in gametogenesis when the specimens from PAP and NWA, subjected to high seasonal inputs of phytodetritus, were compared with those from MAP where there is no evidence for a significant input of surface derived organic matter (Rice et al., 1994).

The reproductive output of an individual (gonad production, quantity and quality of eggs), however, is affected by external factors such as food availability and quality or habitat stability (Qian and Chia, 1991; Bridges et al., 1994; Eckelbarger, 1994; Levin et al., 1994; Sheltema, 1994). In this study, two reproductive variables were analysed to test the hypothesis that the reproductive output can be affected by energy availability: gonad production, quantified as gonad index, and fecundity, quantified as the number of oocytes produced per female.

The gonad indices of *Hyphalaster inermis*, *Styracaster chuni* and *S. horridus* from PAP were similar among the different months of collection, suggesting a constant production of gonad throughout the year. In the three species and the three locations (excepting *S. horridus* from MAP), the mean gonad index of males was significantly higher than that of females. This might reflect a higher energy requirement for the production of large rich eggs compared to that of sperm. An other explanation could be related to the need of spawning great quantities of sperm in order to ensure fertilisation success.

The mean gonad index of *H. inermis* did not differ between locations, indicating that this species allocates the same proportion of the total available energy to reproduction, independently of the habitat. However, the specimens from MAP were

significantly smaller than those from PAP and NWA. This suggests that the populations living at the poorer site allocate relatively more energy to reproduction in detriment of somatic growth. In *S. horridus* the gonad index of females from PAP was higher than that from MAP, but there were no differences between locations in males. Again, the specimens from PAP were larger than the specimens from MAP, suggesting that the low food availability is limiting the growth of *S. horridus* at MAP.

Actual fecundity was low, between 5000 and 15000 mature oocytes per female, and did not vary significantly with time within PAP samples for any of the three species. The mean fecundity of PAP and NWA specimens was significantly higher than that of specimens from MAP, which had fecundities between 1000 and 5000 mature oocytes per female.

The large size of mature oocytes (~650 μm) suggests lecithotrophic development of the larvae (Madsen, 1961). During oogenesis, these eggs accumulate an important reserve of nutrients that will sustain the larvae through their non-feeding development in the water-column. Because of the rich nature of the eggs reserve, a high amount of energy needs to be allocated to reproduction in females. Therefore, the energy availability in the environment would be a limiting factor to the production of eggs. The PAP and NWA site have important seasonal inputs of aggregated phytodetritus that fuel an increase in sediment benthic biomass, mainly bacteria and meiofauna (Jangoux, 1982; Gooday, 1988, 1993, 1996; Gooday and Lambshead, 1989; Gooday and Turley, 1990; Lambshead et al., 1995; Drazen et al., 1998). This causes an enrichment of the sediments in which the asteroids are feeding. In contrast, the MAP site does not receive such an input of aggregated organic matter. But the Madeira Abyssal Plain was recently (ca. 1000 years) affected by a relatively organic rich turbidite (Wolff et al., 1995). The turbidite layer underlies the pelagic sediment and its organic matter has a more refractory nature than the aggregated phytodetritus. The small porcellanasterids from MAP might have digestive systems able to extract nutrients from this low-quality food source. The differences in growth and reproductive output among the three abyssal asteroids from PAP, NWA and MAP might be limited more by the quality of food than by the quantity of food available in the sediments.

Similar results of enhanced growth and reproductive output with high food quantity or quality have been found in shallow water asteroids and echinoids. For example, in the asteroid *Leptasterias epichlora*, specimens from an area of high food availability were larger and produced a greater number of larger eggs than in specimens

of the same species from a less favourable area (George, 1994a,b). In two morphs of the asteroid *Echinaster* sp. studied from the Gulf of Mexico, apart from different fecundity, egg size and larval development, there is within-morph variability in reproductive patterns which is attributed to differences in the nutritional stage (Scheibling and Lawrence, 1982). Also, the echinoid *Arbacia lixula* produced larger eggs with a higher protein content at a site of high abundance of high nutritive quality algae than in a site with less food availability (George et al., 1990).

The results obtained here suggest that the arrival of surface derived organic matter to the seabed and the subsequent community responses (meiofauna mainly) can have an important impact in the life history of abyssal asteroids, influencing the energy allocated to growth and reproduction.

CHAPTER SIX: SYNTHESIS, CONCLUSIONS AND FUTURE RESEARCH

6.1- Reproductive biology: constraints, freedom and evolution

Understanding the life history strategies of species within a community is essential to fully determine the structure and functioning of the ecosystem (Stearns, 1992; Eckelbarger, 1994). The process of reproduction ensures the continuity of the species and their evolution. This is the most sensitive stage of a life cycle and plays a major role in the adaptation and establishment of a species in a determined environment.

A life history strategy is composed of several traits (size at birth, age and size at maturity, fecundity, egg size, age- and size-specific reproductive effort and age- and size-specific mortality). These traits are bound together by trade-offs between 1)- current reproduction and survival, 2)- current and future fecundity and 3)- fecundity and egg size. Organisms have evolved different combinations of these traits, resulting in different life history strategies. The evolution of a life history strategy depends on two factors, one genotypic and one phenotypic. First, there has to be genetic variability for the trait in question, determining whether there will be a response to selection. Second, there must be variability in fitness. Fitness is the contribution of a genotype or phenotype to future generations, relative to other organisms in the population. It is a function of the environment in which it is measured (Stearns, 1992). The evolution of strategies can be explained from the combination of traits under the pressure of selective forces, which are mainly generated by the environment. There will be, however, some constraints. These constraints are determined by the available genetic variability and reflect the phylogenetic history of the species (Southwood, 1988; Stearns, 1992). As a result, some reproductive processes are predetermined characteristics of the species (constrained by phylogeny), while others are affected, at ecological times, by environmental factors (adaptation to habitat conditions). The interaction of constraints and adaptation result, at evolutionary times, in the evolution of life history strategies (Southwood, 1988; Stearns, 1992).

6.1.1- Phylogenetic constraints

Many life history traits are determined by ovarian structure and species-specific vitellogenic mechanisms. The interspecific variations in 1)- yolk content of the egg and 2)- the rate at which yolk is synthesised and stored in the egg are limited

by the type of vitellogenic mechanisms of the species. Also, the selective transfer and mobilisation rate of nutrients for growth and reproduction are determined by morphological and physiological constraints of the digestive and nutrient storage system. As a result of the vitellogenic and nutrient mobilisation constraints, the pace of gametogenesis and frequency of spawning, the gross egg size and the related larval developmental type are predetermined characteristics of the species. These traits reflect the phylogenetic history of the species, and restrict the individual responses to environment variability. (Eckelbarger, 1994; Eckelbarger and Watling, 1995; see chapter 1, section 1.3.1 and chapter two, section 2.2.1). The taxonomic level at which oogenesis and spermatogenesis are constrained varies depending on the group (see this chapter, section 6.2, for examples).

6.1.2- Freedom and environmental factors

While the basic gametogenic mechanisms are constrained by ancestry, other reproductive traits are free to vary with environmental factors. The main variable reproductive traits are egg size, egg quality and egg number. Because the production of eggs is a very energy-demanding process, food quality and quantity and habitat stability are the most important factors affecting reproduction (Eckelbarger, 1986; Eckelbarger and Watling, 1995). Many studies in shallow-water species have shown that an increase in food quantity and/or quality affect fecundity and/or egg size within the predetermined limits for these traits in the specific life history pattern (Eckelbarger, 1994; Eckelbarger and Watling, 1995, see Chapter 2, section 2.3.1 for examples). Changes in food level do not appear to affect the timing of reproduction or the duration of oogenesis. If food levels or quality increase, fecundity or the quality of the offspring will also increase, but the overall pace at which the eggs are produced will not change. If nutrient conditions are poor, the number of eggs produced and their quality will decrease, and gametogenesis can cease (Eckelbarger and Watling, 1995).

6.1.3- Evolution and adaptation of deep-sea reproductive patterns

The evolution of different ovarian morphologies and vitellogenic mechanisms, allows for the existence of a diverse range of reproductive patterns among marine invertebrates, both in shallow-water and deep-sea species (Eckelbarger, 1994; Eckelbarger and Watling, 1995). With the basic aspects of ovary morphology, gametogenesis and egg production rate being phylogenetically constrained, it has been

suggested that deep-sea invertebrates have retained ancestral life-history patterns shared with their shallow-water relatives (Eckelbarger and Young, 1992; Bouchet and Warén, 1994; Eckelbarger, 1994; Gustafson and Lutz, 1994; Eckelbarger and Watling, 1995).

The species that have colonised the different deep-sea environments have to be well adapted to their habitat in order to be successful and persistent. However, the specific reproductive traits have not evolved according to environmental factors, but rather the habitat has selected for species with successful and advantageous life history strategies (Eckelbarger, 1994; Eckelbarger and Watling, 1995). For example, the ephemeral and patchy hydrothermal vent habitat appears to have selected for species with the capacity of fast and quasi-continuous egg production sustained by a high availability of nutrients (Gustafson and Lutz, 1994; Eckelbarger, 1994; Eckelbarger and Watling, 1995). Conversely, echinoderms have successfully colonised, amongst other habitats, the low-nutrient abyssal plains. The echinoderms are characteristic slow egg producers, with deposition of nutrient reserves in storage tissues and gradual transfer of nutrients from the somatic storage sites to the gonads during maturation. This reproductive pattern is advantageous in an environment where food availability is low (Eckelbarger and Watling, 1995).

The study carried out during this thesis aimed to obtain a better understanding of the reproductive processes occurring in some deep-sea invertebrates, and on the constraints and environmental factors affecting the evolution of basic reproductive traits such as gametogenesis, fecundity and egg size.

6.2- Constraints on reproductive traits of deep-sea invertebrates

6.2.1- Decapod crustacean

The caridean shrimp are a good taxon for comparative studies of reproductive biology. They are widely distributed and are abundant in a variety of habitats. Moreover, with ovigerous females carrying their embryos on the pleopods, the studies on fecundity, egg size or reproductive output are relatively simple and accurate.

Gonad gross morphology and development were characteristic of the Infraorder Caridea, and were similar in the mesopelagic shrimps and hydrothermal vent shrimps studied during this thesis. The oogonia grow from the germinal epithelium and differentiate into previtellogenic oocytes, which migrate to the growth zone where they

are surrounded by a layer of accessory cells and undergo vitellogenesis. This is the general characteristic oogenic pattern of caridean shrimp (Krol et al., 1992).

In caridean decapods, the early stages of reproduction (gonad morphology and oogenesis) are constrained by phylogeny and do not show special adaptations for 1)- life in deep-water or 2)- the environmental conditions found at hydrothermal vents (high temperature, toxic fluids, high pressure).

In the Crustacea in general, and the caridean shrimp in particular, there is a morphological constraint to fecundity determined by body size. The physical space available for ovary development and for embryo storage on the pleopods imposes a limit on the maximum number of eggs that can be produced and brooded (Corey and Reid, 1991). As a result, there is a positive correlation between fecundity and body size, with larger females producing more eggs than smaller females. This correlation was found in all five mesopelagic and three hydrothermal vent shrimp species.

Additionally, because the production of eggs is a highly energy-consuming process, there is a trade-off between number of eggs and size of eggs. Van Dover et al. (1985) and Van Dover and Williams (1991) found that the egg size was phylogenetically constrained in the galatheid crabs *Munidopsis lentigo* and *M. subsquamosa*, the brachyuran crab *Bythograea thermydron* and the caridean shrimp *Alvinocaris lusca* from hydrothermal vents. Also, different species of the galatheid genus *Munida* produce a large number of small eggs (Wenner, 1982; Hartnoll et al., 1992), while species of the genus *Munidopsis* produce a small number of large eggs (Wenner, 1982; Van Dover et al., 1985). Different species of *Munidopsis* have similar egg sizes in hydrothermal and non-hydrothermal species (Van Dover et al., 1985). Also, different species of the deep-sea crab *Geryon* produce a large number of small eggs (Haefner, 1977; Melville-Smith, 1987; Hines, 1988). In the five mesopelagic shrimp studied here, there was a clear dichotomy in egg sizes, with *Systellaspis debilis* and *Parapasiphae sulcatifrons* producing a few large eggs, while the three species of *Acanthephyra* produced a large number of small eggs. When comparing these results with information on reproductive patterns of congeneric species (Herring, 1967, 1974a, b; Mauchline, 1988), the size of the egg appears to be genetically determined and related at the generic level in species of *Acanthephyra* and *Systellaspis*.

Thus, gametogenesis and gross egg size are determined by phylogeny rather than by the nature of the habitat, and no specific adaptations of these traits are seen for life in the deep sea and hydrothermal vents. The reproductive patterns of deep-sea

species reflect, in most cases, the ancestry of their shallow-water counterparts (Eckelbarger and Watling, 1995), and in the case of decapod crustaceans appear to be related at the generic level.

The three genera of hydrothermal vent shrimp studied here are monospecific, not allowing for intra-generic comparisons. However, *Rimicaris exoculata*, *Chorocaris chacei* and *Mirocaris fortunata*, together with another vent shrimp, *Alvinocaris lusca* (Van Dover et al., 1985) follow a similar strategy by producing a large number of small eggs. The small eggs hatch into planktotrophic larvae with the capabilities of long resident times in the water column. This may allow the larvae to migrate to surface waters where they accumulate essential polyunsaturated fatty acids that are subsequently used for growth and maturation (Gebruk et al., 2000b; Pond et al., 2000a). The planktotrophic larvae also have a high dispersal potential, allowing for the colonisation of new vents in an ephemeral and patchy environment (Creasey et al., 1996; Shank et al., 1998). The production of a high number of small eggs seems to be a highly advantageous strategy for hydrothermal vent shrimp.

Even though *Rimicaris exoculata* lives in very dense aggregations only a few ovigerous females have been collected to date. As a typical caridean shrimp, *R. exoculata* broods its embryos on the pleopods. Ramirez Llodra et al. (2000) suggest that the ovigerous females would move to the periphery of the vents in order to protect their embryos from the very active swarms of shrimp and the potentially highly toxic hydrothermal fluids. A change in the behaviour of ovigerous females is known for other decapods. For example, the hydrothermal crab *Bythograea thermydron* moves to the periphery of the vents when carrying eggs (G. Perovich, *pers. com.*), and the females of the deep-sea brachyuran crabs *Geryon maritae* and *G. quinquedens* move to shallower waters when brooding their embryos (Haefner, 1977; Melville-Smith, 1987).

6.2.2- Echinoderms

Echinoderms, particularly the echinoids, have long been used for reproductive studies, because 1)- the group is widespread, 2)- the individuals are easy to collect and maintain in the laboratory, and 3)- their gametes are easily manipulated.

In echinoderms, the morphology of ovaries is conservative (Walker, 1974, 1976, 1980; Chia and Walker, 1991; Hendler, 1991; Pearse and Cameron, 1991; Smiley et al., 1991) and the vitellogenic mechanisms of oogenesis seem to be similar and related with slow egg production (Eckelbarger and Young, 1992; Eckelbarger, 1994;

Tyler et al., 1994). The most common pattern of reproduction in deep-sea echinoderms is asynchronous gametogenesis with a quasi-continuous production of a few large eggs (Gage and Tyler, 1991). Nevertheless, a few echinoderm species from the NE Atlantic present a seasonal reproductive cycle coupled with seasonal deposition of phytodetritus to the seabed (see chapter 1, section 1.3.1 for examples). The ultimate cause of seasonality in the reproductive cycle of these species is encoded in their phylogenetic history, and the arrival of surface-derived organic matter is the proximal cause determining the individual's internal biological clocks (Eckelbarger and Watling, 1995).

In holothurians, the similarity found in ovary structure and vitellogenic processes between shallow-water and abyssal species suggests that these traits have been highly conserved in evolution (Eckelbarger and Young, 1992). The results of several studies on the reproductive biology of deep-sea holothurians from the NE Atlantic seem to indicate that egg size and fecundity are constrained at family level for many species (Tyler et al., 1985; Tyler and Billett, 1987; Tyler et al., 1987).

Similarly, the basic reproductive traits of echinoids belonging to the family Echinothuridae and to the echiniid genus *Echinus* were similar in both shallow-water and abyssal species (Tyler and Gage, 1984a,b; Gage et al., 1986).

In asteroids, the spermatogenic processes of males are remarkably similar in both shallow and deep-water species. The spermatogonia divide from the germinal epithelium and develop into colonettes of spermatocytes. Spermatids arise from the apex of the colonettes and differentiate into spermatozoa that accumulate in the lumen of the testes (Walker, 1980; Pain et al., 1982a,b; Tyler and Pain, 1982; Tyler et al., 1982b; Tyler et al., 1984; Chia and Walker, 1991; Ramirez Llodra et al., *in prep.*). Once reaching maturity, the testes are always in a ripe stage. It has been proposed that the chance encounter with a spawning female would stimulate a spawning event on the male, enhancing the fertilisation success in the low-density populations found at the abyssal plains. A different strategy used to increase fertilisation success is a pairing behaviour that has been observed in the deep-sea holothurian *Paroriza pallens* and the echinoid *Stylocidaris lineata* (Tyler et al., 1992; Young et al., 1992).

In females, the low food levels of abyssal environments would select for species with nutrient storage organs and slow rates of egg production. This is the case in asteroids, and might explain why this group has been successful in the deep-sea (Eckelbarger and Watling, 1995). The basic oogenic patterns of several abyssal

asteroids from the NE Atlantic show a variety of patterns, but seem to be related at family level for most of the species studied (Pain et al., 1982a; Tyler et al., 1982a; Eckelbarger and Watling, 1995; Ramirez Llodra et al., *in prep.*).

The three asteroids examined here, *Hyphalaster inermis*, *Styracaster chuni* and *S. horridus*, belong to the strictly abyssal family Porcellanasteridae. The oogenic and spermatogenic mechanisms observed correspond to the typical asteroid reproductive pattern. The gonad morphology and gametogenesis were very similar between the two genera and three species at the different sites. These asteroids follow the common reproductive strategy of deep-sea echinoderms, with an asynchronous production of a few large eggs throughout the year. The seasonal arrival of aggregated phytodetritus at PAP did not cause a seasonal variation of gonad production. Moreover, the gross morphology of their gonads, gamete development and size of mature eggs resembled that of the other porcellanasterids *Styracaster elongatus*, *S. armatus* and *Thoracaster cylindratum* (E. Ramirez Llodra, unpublished data). Also, although the porcellanasterid *Porcellanaster ceruleus* has smaller gonads and a simpler oogenesis than the other species (Pain, 1983) the final product is a large egg of ~600 μm (Madsen, 1961). These data suggest that, at least for most of the NE Atlantic porcellanasterids, the reproductive processes are constrained at family level.

6.2.3- Other taxa

Little is known on the gametogenic pathways and egg and sperm characteristics of most deep-sea invertebrates, and even less information is available on comparative studies between close-related species from differing environments (Gage and Tyler, 1991; Young and Eckelbarger, 1994; Tyler and Young, 1999).

Even though polychaetes are the dominant macrofauna in deep-sea sediments and are abundant at hydrothermal vents, their reproductive traits are known only for a few species (Blake, 1993; Tyler and Young, 1999 and references therein for hydrothermal vent species). Shallow-water studies indicate that, contrary to the conservative gametogenesis of decapod crustacean and echinoderms, the polychaetes show a wide variety of reproductive patterns. This would suggest a weak phylogenetic constraint on the evolution of reproductive traits, reflecting the simplicity of their reproductive systems and explaining the diversity of patterns found in both shallow-water and deep-sea species (Eckelbarger, 1983; Wilson, 1991; Giangrande et al., 1994; Copley, 1998; Tyler and Young, 1999).

In molluscs, the reproductive processes and early life history of hydrothermal vent, cold seep and non-hydrothermal species are determined by ancestry and reflect the systematic group to which each species belongs (Gustafson and Lutz, 1994; Bouchet and Warén, 1994; Tyler and Young, 1999).

In a study of the reproductive cycle of three deep-sea demosponges, Witte (1996) found that oogenesis was very similar to that of shallow-water sponges.

6.3- Reproductive variability and energy availability

Although some aspects of the life history of a species are phylogenetically constrained (see above), other traits are variable and reflect environmental forcing. In particular, the number of eggs that can be produced and their quality will depend on the energy (food) available to the female prior to and during oogenesis (Eckelbarger, 1986, 1994). The effects of food quantity and quality on fecundity and egg quality have been analysed experimentally in many shallow-water species, showing that poor food conditions cause a decrease in the number of eggs produced and/or in their quality (see chapter 2, section 2.3.1 for examples).

To test the hypothesis that fecundity in deep-sea species is also affected by energy availability, closely related species were studied and compared from environments with different characteristics in food supply.

6.3.1.- Caridean shrimp

Within the five mesopelagic shrimp from the NE Atlantic, differences in depth and latitude of the population distributions can be translated into differences in food availability.

Surface productivity in the NE Atlantic Ocean differs in polar, temperate and tropical waters. Longhurst and colleagues (1995) divided the oceans into domains and provinces according to data on regional oceanography and subsurface chlorophyll distribution. The five sampling stations analysed for the mesopelagic shrimp fit into four of these biogeochemical provinces: Atlantic Subarctic (station at 60°N), North Atlantic Drift (station at 53°N), East North Atlantic Subtropical Gyre (stations at 40°N and 30°N) and the upwelling region of the Canary Current Coastal (station at 11°N).

The average primary production of these provinces is 302, 240, 122 and 732 g C m^{-2} y^{-1} respectively (Longhurst et al., 1995). This variable primary production causes

differences in secondary production and energy availability for species in higher levels of the food web.

The high fecundity of *Acanthephyra kingsleyi* and *Systellaspis debilis* from 11°N could reflect the good nutrient conditions that are established under upwelling regions, but the overall fecundity data obtained for the five mesopelagic species along the transect in the NE Atlantic do not relate to surface productivity. However, most of the mesopelagic shrimp undergo diurnal migrations and are predators (therefore high in the food web) and the differences in surface productivity and subsequent effects on secondary production could be too small or too attenuated to cause differences in fecundity. Also, other environmental factors such as depth or water temperature might affect the allocation of energy to growth and reproduction, and the selection for certain life history traits (see below).

Organic matter and plankton concentrations decrease with increasing depth in the water column. The low food availability in deep waters could limit the allocation of energy to reproduction decreasing fecundity, while the high larval mortality risk found for larvae that hatch in deep waters and migrate through the water column could select for the production of high quality, large eggs (King and Butler, 1985; Clarke et al., 1991; Clarke, 1993b). In the *Acanthephyra* species studied, fecundity decreased with increasing depth, while egg size increased with increasing depth. Also, in the species that produce large eggs, the size-specific fecundity of *Systellaspis debilis* was lower than that of the deeper-living *Parapasiphae sulcatifrons*. However, a larger sample size, especially for the *Acanthephyra* species, would be essential to obtain robust conclusions about trends in their reproductive biology.

A more significant effect of food availability in energy allocation to reproduction is shown by comparing the fecundity of the mesopelagic *Acanthephyra* species with that of the hydrothermal vent species. The hydrothermal vents have often been considered as deep-sea oases because of the abundance and density of fauna associated with these habitats. The vent fauna is primarily sustained by the production of chemoautotrophic bacteria. These bacteria are either free-living organisms or symbiotic with higher taxa. They provide the food web with a continuous and rich nutrient supply, which accounts for the high biomass found at hydrothermal vents (2 to 8.5 kg wet weight m^{-2} (Gage and Tyler, 1991). Little is known of the chemoautotrophic primary production at the vent ecosystems. Maruyama and colleagues (1998) estimated that the global new production from hydrothermal vent activity corresponds to 1 to

25% of the total imported carbon flow to the deep-sea region. These authors calculated a primary production of 3.3×10^8 kg C y^{-1} for a region in the south East Pacific Rise covering an area of 10286 km^2 (~32.1 g C m^{-2} y^{-1}) (Maruyama et al., 1998). There are no direct data for the primary production at the Mid-Atlantic Ridge region, but the above numbers are given here as an indication of the general high chemoautotrophic productivity of hydrothermal vents.

The three vent shrimp analysed here have a differing trophic ecology, with a decreasing role of symbiotic bacteria from *Rimicaris exoculata* to *Chorocaris chacei* and finally to *Mirocaris fortunata* (Segonzac et al., 1993; Gebruk et al., 2000b). Still, the three species live in a rich environment and have access to abundant food throughout the year. The *Acanthephyra* species and the vent species produce small eggs of a similar size (~0.5mm) and were therefore good comparative subjects for fecundity analysis.

Comparison of the size-specific fecundity of the *Acanthephyra* species with the vent shrimp, showed the fecundity of *M. fortunata*, *C. chacei* and *R. exoculata* was approximately 2.5 (*M. fortunata* and *C. chacei*) and 1.5 (*R. exoculata*) times higher than that of the *Acanthephyra* species (Fig. 6.1).

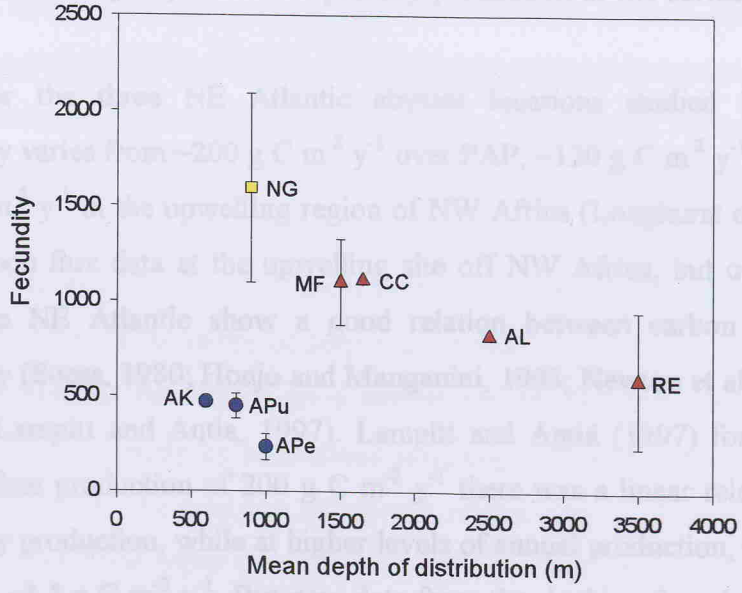


Figure 6.1- Mean size-specific fecundity and 95% confidence limits of eight species of caridean shrimp from different habitats and depths.
Mesopelagic shrimp (blue circles) from the NE Atlantic: AK, *Acanthephyra kingsleyi*; APu, *A. purpurea*; APe, *A. pelagica*. Hydrothermal vent shrimp (red triangles) from the Mid-Atlantic Ridge: MF, *Mirocaris fortunata*; CC, *Chorocaris chacei*; RE, *Rimicaris exoculata*; and from the East Pacific Rise: AL, *Alvinocaris lusca*. Benthic shrimp (yellow square) from an upwelling area in the Indian Ocean: NG, *Nematocarcinus gracilis*.

Figure 6.1 also shows the size-specific fecundity of the Pacific hydrothermal shrimp *Alvinocaris lusca* (Van Dover et al., 1985) and of the benthic shrimp *Nematocarcinus gracilis* from an upwelling area in the Indian Ocean (E. Ramirez Llodra, unpublished data). Both species produce small eggs (~0.5 mm in diameter for *A. lusca* (Van Dover et al., 1985), and ~0.5 and 0.6 mm in diameter for early and late eggs respectively in *N. gracilis* (E. Ramirez Llodra, unpublished data)). The mean size-specific fecundities of *A. lusca* and *N. gracilis* were ~2 and ~4 times higher than that of the *Acanthephyra* species (Fig. 6.1).

The above data would indicate that food availability plays an important role in the allocation of energy to reproduction in deep-sea caridean shrimps. The highly productive environments at the hydrothermal vents, where there is a continuous and important source of energy, supports the high fecundity of vent shrimps. The production of a high number of eggs is important in counteracting the high mortality experienced by the larvae during their long migrations to surface waters.

6.3.2- Asteroids

All benthic abyssal communities (excepting hydrothermal vent and cold seep fauna) are ultimately dependent on primary production at the surface (Thurston et al., 1998).

Over the three NE Atlantic abyssal locations studied here, the surface productivity varies from ~200 g C m^{-2} y^{-1} over PAP, ~120 g C m^{-2} y^{-1} over MAP and to ~730 g C m^{-2} y^{-1} in the upwelling region of NW Africa (Longhurst et al., 1995). There are no carbon flux data at the upwelling site off NW Africa, but other sediment trap data in the NE Atlantic show a good relation between carbon flux and surface productivity (Suess, 1980; Honjo and Manganini, 1993; Newton et al., 1994; Jickells et al., 1996; Lampitt and Antia, 1997). Lampitt and Antia (1997) found that below an annual surface production of 200 g C m^{-2} y^{-1} there was a linear relation between flux and primary production, while at higher levels of annual production, the flux was fairly constant at ~3.5 g C m^{-2} y^{-1}. But new data from the Arabian Sea show fluxes of up to 5.6 g C m^{-2} y^{-1}, which do not support the hypothesis of an upper limit to flux (Honjo et al., 1999). The information on organic carbon fluxes for different sites in the NE Atlantic suggest that the flux at the NW African site will be similar, but probably higher, than the flux at PAP.

Sediment trap data at the PAP site indicate that the annual flux of organic carbon reaching the seabed is 1.15 g C m^{-2} y^{-1} (Lampitt et al., *in press*). The mean flux of organic carbon at the MAP site is 0.41 g C m^{-2} y^{-1} (Lampitt, 1992). However, the differences in carbon flux are not large enough to explain the differences found in the structure and abundance of macro- and megafauna between PAP and MAP (Thurston et al., 1994, 1998). The photographic data indicate that there is a strong seasonal pulse of aggregated phytodetritus arriving to the seabed at PAP, while there is no evidence for such a process happening at MAP (Lampitt, 1985; Rice et al., 1994). Thurston and colleagues (1994) suggested that the nature of organic supply (aggregated, macroscopic and episodic at PAP, and microscopic and quasi-continuous at MAP) plays a major role in the response of the benthic fauna (Thurston et al., 1994; Ginger et al., 2000; Witbaard et al., 2000).

Parallel to the increase in biomass and abundance of macro- and megafauna found at PAP compared to the more oligotrophic MAP (Thurston et al., 1998), there are also differences in some reproductive traits that might be linked to food availability. The seasonal arrival of phytodetritus to the seabed is the proximal cause of seasonal reproductive cycles in some deep-sea invertebrates, but these patterns are ultimately determined by ancestry. In contrast, the size of mature females and the number and quality of eggs are variable reproductive traits related to environmental factors.

Whereas experimental data on variability in fecundity related to food input is abundant and diverse for shallow-water species (see chapter 2, section 2.3.1 for examples), the technological and financial limitations of research in the deep sea have restricted experimental research significantly. As a result, little is known on the effects of food availability in fecundity of deep-sea species.

Variations in food input to a habitat can be induced experimentally and its effects monitored directly by continuous sampling. When this method is not possible, as in the deep sea, the analysis of closely related species from environments with natural different food input can be used instead. The reproductive output of three asteroid species was analysed here, and compared among three abyssal localities with contrasting food availability (see above).

The gonad index (proportion of gonad weight to body weight) was similar among locations for each species, suggesting that there were no differences in reproductive effort. However, the mean body size of the specimens from MAP was significantly smaller and the females reached sexual maturity at a smaller size than the

specimens from PAP and NWA. These data suggest that growth in females from the low-food MAP site is reduced in favour of reproduction, achieving a similar size-specific gonad production than their larger conspecific specimens from PAP and NWA.

The differences in food availability and quality at the different sites also affected the number of eggs produced per female. The mean fecundity of *Hyphalaster inermis* from PAP and NWA was higher than that of the specimens from MAP. Similarly, the mean fecundity of *Styracaster horridus* was higher at PAP than at MAP. These differences in fecundity could be related to the different female sizes among locations. But, because growth is limited by energy availability, the amount and quality of nutrients available in the sediment for these burrowing asteroids would be the ultimate factor affecting their reproductive output (see chapter 5, section 5.4; Ramirez Llodra et al., *in prep.*).

6.4- Final remarks

The study of a community usually follows three stages. First, there is a description of the habitat, flora and fauna. Then follows an analysis of spatial variability in the community and the relations (trophic relations, space competition) among its species. Finally, the dynamics of the community (processes in time and space) are studied. In this last stage, one of the most important aspects is the life histories of the faunal components.

Deep-sea biology and ecology faces strong technological and financial barriers. The progress in our understanding of the deep ecosystems has evolved with the development of new oceanographic techniques and equipment. The main problems confronting deep-sea reproductive biology are 1)- the economical difficulty of repeated seasonal sampling, 2)- the difficulty of obtaining and maintaining live animals, and 3)- the difficulty of *in situ* observation and experimentation (Gage and Tyler, 1991; Tunnicliffe, 1991; Eckelbarger, 1994). As a result, the study of deep-sea biology has been, for almost a century, mainly descriptive, based on the samples that oceanographic ships would bring to surface with trawls and dredges.

The development of deep-water video systems, submersibles and remote operated vehicles, has given the experimental phase of deep-sea research a new perspective. Studies on physiology, trophic relations, species interactions, reproduction and larval migration and settlement have increased drastically (Gage and Tyler, 1991; Tunnicliffe, 1991; Grassle, 1994; Tyler and Young, 1999). However, we still only

understand the life cycle of a very small percentage of the highly diverse abyssal fauna. Some of the basic reproductive patterns (type and timing of gametogenesis, fecundity, size of eggs, fertilisation, larval development and settlement) have been analysed for a few species, but the whole life-cycle is still poorly understood for most of the currently described deep-sea species. This lack of information affects our global understanding of the processes driving and maintaining the deep-sea ecosystems.

The deep ocean is not an isolated system, but interacts with global circulation and climate. Moreover, there is an increasing anthropogenic pressure affecting deep oceanic zones. The effects that global climate change, deep-sea dumping or disposal of radioactive residues, oil exploration and deep-sea fisheries have in deep-sea communities are little understood. Anthropogenic disturbance on deep-sea fauna is increasing at a much faster rate than that of our understanding of the processes driving these communities. It is possible to cause a lethal disturbance to a species by disrupting its reproductive biology by sub-lethal effects. Even when the adult phase survives adverse conditions (biotic and abiotic), if these conditions affect the reproductive processes (gametogenesis and larval development), the long term effect on the species could be lethal an could cause extinction.

A good knowledge of the reproductive biology of deep-sea fauna is essential for an adequate management of resources and conservation of the diverse abyssal ecosystems. For this, the main areas that need addressing in future research programmes include: 1)- size at maturity, 2)- timing of initiation of gametogenesis, 3)- vitellogenic pathways, 4)- fecundity and egg size, 4)- fertilisation kinetics, 5)- larval development, survival and dispersal, 6)- settlement rates and recruitment of postlarvae, 7)- survival of juveniles and 8)- pressure/temperature tolerance of adults and larvae. All these aspects of the life cycle of an individual need to be understood in relation with the phylogenetic history of the species and with the environmental conditions experienced by the population. Comparative studies of closely related species from different environments and of different species from the same habitat would give valuable information on the levels at which phylogeny and the environment affect the reproductive processes. Understanding the sensitivity of the early phases of life cycles is needed to estimate and predict the potential damage caused by anthropogenic disturbance into deep-sea benthic communities.

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