



**Carla Débora
Gonçalves Borges**

**Efeitos da predação pelo homem em características
biológicas das presas**

**Interaction of human foraging behaviour and prey
life-history traits**



**Carla Débora
Gonçalves Borges**

**Efeitos da predação pelo homem em características
biológicas das presas**

**Interaction of human foraging behaviour and prey
life-history traits**

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, Biodiversidade e Gestão de Ecossistemas, realizada sob a orientação científica do Doutor Henrique Queiroga, Professor Auxiliar com Agregação do Departamento de Biologia da Universidade de Aveiro.

Apoio financeiro parcial da Fundação
para a Ciência e a Tecnologia

o júri

presidente

Prof. Dr. Fernando José Mendes Gonçalves

professor auxiliar com agregação do Departamento de Biologia da Universidade de Aveiro

Prof. Dr. João José Roma Passos Pereira de Castro

professor auxiliar do Departamento de Biologia da Faculdade de Ciências da Universidade de Évora

Prof. Dr. Henrique José de Barros Brito Queiroga (Orientador)

professor auxiliar com agregação do Departamento de Biologia da Universidade de Aveiro

Agradecimentos

A presente dissertação é o culminar de um longo percurso nem sempre linear, somente possível graças ao apoio da minha família, amigos, colegas de trabalho e do Prof. Dr. Henrique Queiroga. A todos os meus agradecimentos pela paciência e confiança demonstrada ao longo deste processo.

palavras-chave

Exploração de moluscos, lapas, mudança de sexo, protandria

resumo

O presente trabalho propõe-se divulgar a pesquisa passada e recente no âmbito das consequências da exploração de recursos costeiros pelo homem na biologia das espécies alvo e na comunidade onde estas estão inseridas. A ênfase das pesquisas aqui apresentadas recai principalmente sobre moluscos bentônicos e lapas em particular, dada a ocorrência de protandria (mudança de sexo de macho para fêmea ao longo do ciclo de vida) em algumas das espécies usadas como recurso alimentar. Questões relacionadas modos de reprodução, hermafroditismo e mudança de sexo no mundo animal são igualmente focadas como introdução aos últimos capítulos da dissertação onde se aborda essas questões mais profundamente. No capítulo final são apresentados trabalhos científicos que retratam a importância das áreas marinhas protegidas na conservação de espécies costeiras.

keywords

Mollusc harvesting, limpets, sex change, protandry

abstract

The present work aims to bring to public, past and present research in the costal resources exploitation by Man and its consequences upon target species and the surrounding community. Particular attention is given to benthic molluscs and limpets in particular due to the occurrence of protandry (sex change from male to female during the life cycle) in some of the harvested species. The issues related to reproduction mode, hermaphroditism and sex change in animals are presented first as to introduce later chapters where these subjects are deepened. The final chapter presents relevant research of the use marine protected areas to the conservation of coastal species.

Table of contents

Introduction	1
1. Exploitation and conservation of intertidal and subtidal organisms	2
1.1 Community consequences	3
1.2 The Azorean case	9
1.3 Conservation and management	9
2. Ecology and dynamics of limpet populations	12
2.1 Species distribution along the shore	12
2.2 Population dynamics	12
2.2.1 Population density	13
2.2.1.1 Spawning and recruitment	13
2.2.1.2 Migration and foraging activities	14
2.2.1.3 Mortality	16
2.2.2. Growth	16
3. The evolution of sex	18
3.1 The significance of sexual and asexual reproduction	18
3.1.1 The problem with sex	18
3.1.2 Hypotheses for the advantage of sex: Why reproduce sexually?	19
3.1.2.1 Environmental deterministic hypotheses	19
3.1.2.2 Advantages in heterogeneous habitats	20
3.1.2.3 Hypotheses based on deleterious mutations	21
3.1.3 Evaluating hypotheses for the advantages of sex	21
3.1.4 Evolution of sexes: sperm competition	21
3.2 Sex ratios, sex allocation and sex determination	22
3.2.1 Sex ratios in randomly mating populations	23
3.2.2 The evolution of sex ratios in structured populations	23
3.2.3 Sex allocation, hermaphroditism and gonochorism	23
3.3 The evolution of hermaphroditism among animals	25
3.3.1. The adaptative significance of sequential hermaphroditism in animals	27
3.3.2. Why are hermaphrodites not ubiquitous?	32
4. Sex determination and change in molluscs	34
4.1 The research in <i>Crepidula</i> spp.: factors influencing its sex change	35
4.2 The research in the Patellidae and the Lottidae	49
4.3 Proximal questions on sex change	54
4.3.1 Genetic <i>versus</i> environmental control	54
4.3.2 The physiological pathway to sex change	56
4.4 Evolutionary and adaptative questions about sex change	57
5. Recent insights on the sex change strategies in animals	62
5.1 The mating system and variation in the timing of sex change	62
5.2 Between-population variation in the timing of sex change	62
5.3 No sex change	63
5.4 Early and late sex change	63
5.5 Repetitive sex change	64
6. Proximal cues and variation in the timing of sex change: snapshot of the present and future insights	65
7. Effects of human exploitation upon sequential hermaphroditic prey – the role of marine protected areas	67
References	72

Introduction

Since the beginning of civilization, food resources of coastal areas have been attracting people's attention. With the advance of the industrial revolution at the beginning of last century the pressure on coastal zones has become greater. Since then, besides being prone to increasing exploitation rates, many coastal areas have been destroyed to give space for emerging urbanisations. As a result of human activities both inland and in the coastal zone itself, marine ecosystems and resources are rapidly deteriorating in many parts of the world. The coastal zone also receives pollution, both by direct discharge and via river systems. It is affected by changes in salinity and sedimentation regimes as a result of the damming of rivers and siltation due to deforestation and other land use changes. All this weakens ecosystem resilience and often causes irreparable damages.

All around the world both vertebrates and invertebrates have been gathered for human consumption. Limpets are not exception and many of the stocks are now over-exploited. Since these are keystone grazers that regulate biological interactions in rocky shores through their grazing activity, a drastic reduction of their numbers has wider effects upon the whole community.

This dissertation aims to gather relevant published research on the exploitation of coastal resources and its implications at the community and population level. From an initial general approach to the exploitation of coastal resources the emphasis is then made to limpet exploitation and particularities of their biology having the occurring species in the the United Kingdom as reference. The last chapters will focus on past and current knowledge about sex evolution and change in several groups of organisms having molluscs and limpets in particular as their main focus due to the general occurrence of protandry (individuals been first male and female next) in some exploited populations. The final chapter presents relevant research of the use marine protected areas to the conservation of coastal species.

1. Exploitation and conservation of intertidal and subtidal organisms

From the earliest times, people have inhabited and exploited the shorelines of every continent, as evidenced by shellfish middens and layers at many archaeological sites (McKusic and Warren 1959; Meighan 1959; Waechter 1964; Roubet 1969; Voight 1973; Roche and Texier 1976; Volman 1978; Buchanan et al. 1978, 1984; Schweitzer 1979; Yesner 1984; Buchanan 1988; Cunliffe and Hawkins 1988; Stiner 1994; Yates 1998; Henshilwood 2001; Halketta et al. 2008). Observations have been made on prehistoric middens across the world where the frequent occurrence of a reduction in size of marine molluscs and/or a change in species representation through a shell midden sequence is often taken as an indicator of intensification in exploitation (see Jerardino et al. 1992, Milner et al. 2007, Yesner 1984). Milner et al. (2007) cited examples that include the Danish kitchenmiddens (e.g. Andersen, 1991, 1993; Andersen and Johansen, 1987), the Scottish middens (Mellars, 1978; Russell et al., 1995), the Asturian shell middens (Bailey and Craighead, 2003), the Jomon middens of Japan (Koike, 1986) and the New Zealand middens (Swadling, 1976). Evidence from stratified middens of Quoygreew (Scotland) showed that there was a trend towards the intensification of marine resource from 10th to the 13th centuries due to limpet reduction in size through time and age data that demonstrated a lowering of average age suggesting intensification in gathering rather than environmental influences (Milner et al. 2007).

Today shellfish are heavily exploited in many parts of both the developed and developing world (Raffaelli and Hawkins, 1996) and in the former many shore animals are now gourmet items on the menu. In the third world, subsistence collecting of shellfish has been an important source of protein (see Branch 1975a, Siegfried et al. 1985, Duran and Oliva 1987, Hockey et al. 1988, Lasiak 1992, Kyle et al. 1997) but as populations increase, pressure on resources has also increased and many stocks are now at risk. Moreover, in many countries shellfish collecting has switched from a subsistence activity to a highly profitable commercial operation purveying luxury items (Raffaelli and Hawkins 1996).

Nevertheless it was only during the last decade of the 20th century, that Man come to be considered as a key component in the structure and the dynamics of rocky intertidal and subtidal communities and attempts had been made to quantify effects of human exploitation upon targeted species and upon the shore community (e.g. Moreno et al. 1984, 1986, Siegfried et al. 1985, Hockey and Bosman 1986, Duran et al. 1987). Much of the research was prompted by a pronounced decline in many stocks (see Kay et al. 1982, Bustamante and Castilla 1987). Reasons for these declines centre chiefly around changes, both quantitative and qualitative, in the patterns of exploitation and have come about as a result of, among others factors, the rapid growth of human populations in coastal areas, the replacement of subsistence by commercial exploitation, and technological advances both in methods of collection, processing, storage and transportation

(Eekhout et al. 1992, Santos et al. 1995). Nowadays many species of algae (e.g. *Durviellaea antarctica*, *Iridaea boryana*, *Porphyra columbia* and *Gelidium* spp.) and invertebrates (e.g. the mussel *Perna perna*, the gastropod *Choncholepas concholepas*, and limpets of the genus *Fissurella*, *Patella* and *Lottia*) are taken both for local consumption and export (Paine 1994). In addition to direct collection for food, seaweeds are also harvested to make a variety of products such as agar, carrageenans (see Armisen and Galatas 1987, Stanley 1987) and alginates. The latter end up in a huge range of cosmetics, pharmaceuticals and processed foods such as beer, ice cream, face cream and shampoo (Raffaelli and Hawkins 1996). Shells of many marine invertebrates are collected for ornamental purposes (Wells 1981, Wood and Wells 1988) and many animals are also used as bait in addition to their use as a food resource.

1.1. Community consequences

The effects of humans as top predators on rocky intertidal communities has been the focus of many studies, particularly in South Africa (Branch 1975a, Siegfried et al. 1985, Hockey and Bosman 1986, Hockey and Bosman 1988, Lasiak and Dye 1989, Lasiak 1991, 1992) and Chile (Moreno et al. 1984, 1986; Castilla and Duran 1985; Castilla 1986; Oliva and Castilla 1986; Moreno 1986; Castilla and Bustamante 1989; Bustamante and Castilla 1990), but also in Costa Rica (Ortega 1987) and Australia (Catterall and Poiner 1987). Although some studies have analysed the implications of harvesting in terms of biomass and gonadal outputs (Branch 1975a, Blake 1979, McLachlan and Lombard 1981, Eekhout et al. 1992) most effects of exploitation on populations of intertidal invertebrates were addressed by comparing sites with and without exploitation, before and after human exclusion. One of the most revealing "human exclusion" experiments was initiated in 1982 at Las Cruces (Chile) by J. C. Castilla and his coworkers where access was restricted by a chain link-fence. Duran and Castilla (1989) concluded that their study illustrated a clear case of "cascade effects" (Paine 1980) due to press-perturbation (*sensu* Bender et al. 1984). They observed that exclusion of a top predator (humans) in rocky intertidal resulted in the increase of a keystone predator (*Concholepas concholepas*), and two species of keystone grazers (the limpets *Fissurella crassa* and *F. limbata*). In turn, the increase of *C. concholepas* reduced the cover of the mussel *Perumytilus purpuratus*, a dominant space competitor, favouring settlement of macroalgae in newly available primary space. This state was transient, since macroalgae were subsequently eliminated from the system, most probably due to the grazing action of limpets. After five years, the community at the nonharvested area was dominated by barnacles.

The effect on community structure of excluding critical predators that control the abundance of dominant competitors for primary space (e.g. mussels) has been a recurrent subject in ecological literature (Paine 1966, 1971, 1974). As space is probably the most important resource for intertidal

organisms, especially for sessile species (Connell 1961, Dayton 1971, Paine and Levin 1981, Sousa 1984, Raffaelli and Hawkins 1996), processes involved in the creation of free space are of vital importance to the persistence of many species (Blanchette 1996). Disturbance, the process responsible for the creation of new bare space, provides the space for new colonisers and the hierarchical nature of species interactions dictate the pathway of succession. Disturbance is thus responsible for environmental heterogeneity over time. Predation and grazing are the main agents of biological disturbance. According to classical ecological theory, predation on a dominant competitor for primary space (e.g. limpets or mussels, depending on the community structure) leads to increase species diversity in the community (Paine 1966, 1977; Lubchenco 1978; Branch 1984).

Unlike physical disturbance, predation is considered a selective agent since predators feed preferentially on one or fewer types of prey. Castilla and Duran (1985) realised that on sites where human predators were excluded for two years, the keystone muricid predator *Concholepas concholepas* increased in abundance dramatically, leading to a decrease in the cover of the competitively dominant mussel *Perumytilus purpuratus* and to an increase in species diversity of the primary space occupiers. This result can be explained by the "intermediate disturbance hypothesis" (Caswell 1978, Connell 1978) according to which disturbance through selective predation (which in this case coincided with the maximum predatory impact of *C. concholepas* on *P. purpuratus* having the magnitude of an intermediate level), increases species richness. The intermediate disturbance hypothesis (Caswell 1978, Connell 1978) suggests that at low levels of disturbance, certain competitive species will predominate and hence diversity will be low. At intermediate levels of disturbance, no one species will predominate and diversity will be high. This occurs only as long as disturbance renews resources (e.g. space) at a rate that allows continued recruitment and persistence of species that would otherwise be driven extinct. In this way disturbance must occur with some intermediate frequency and intensity/severity. Under these circumstances, competitive exclusion of species is delayed or never occurs because some areas are routinely set back by "catastrophes" (see Sousa 1979a). As disturbance increases further, only a few highly tolerant or very opportunistic species will occur. As Duran and Castilla (1989) realised, on harvested sites there was no predation of *P. purpuratus* by *C. concholepas* and consequently these competitively dominant mussels retained the primary space and species diversity remained low.

Mobile grazing herbivores are important target species for human exploitation in many systems (Kingsford et al. 1991 and references therein) and limpets have been harvested commercially, (for both local consumption and export) in a number of places, including Hawaii (Kay and Magruder 1977), South Africa (Hockey and Bosman 1986, Hockey et al. 1988, Eekhout et al. 1992, Kyle et al. 1997), the Azores (Martins et al. 1987a,b, Santos et al. 1995) and Madeira (Santos et al. 1989) in Portugal, Chile (Oliva and Castilla 1986, Duran and Castilla 1989), Mexico and California (Pombo

and Escofet 1996). Marine herbivores have often been shown to control the distribution and abundance of their algal prey (Underwood 1979, Branch 1981, Lubchenco and Gaines 1981, Gaines and Lubchenco 1982, Hawkins and Hartnoll 1983, Raffaelli and Hawkins 1996). Herbivores in turn can be regulated by predators such that their potential effects on algae are not always realised (Dayton 1975, Breen and Mann 1976, Simenstad et al. 1978).

Hockey and Bosman (1986) analysed Man's influence on the intertidal community of Transkei where traditionally people have supplemented their diet with marine invertebrates. Comparisons between exploited and protected sites were made in terms of zonation, density, cover (of algae and colonial invertebrates), invertebrate size distributions, species and community structure. They found that the most intensive intertidal exploitation was upon the limpets *Patella* spp. and *Cellana capensis*, which accounted for 79.1 % of all intertidal species in middens. The authors showed that the relative abundance of algae and sessile inedible invertebrates was greater and modal and mean sizes of exploited species were smaller, at exploited sites than at protected sites. Indeed, comparative analysis of size-frequency distributions of limpets (*Crassostrea cucullata*, *Scutellastra granularis* and *Cellana capensis*) from the protected sites revealed a decrease in abundance of bigger sizes due to selective predation.

Hockey and Bosman (1986) concluded that disturbance through selective predation by humans increases species richness, in line with the "intermediate disturbance hypothesis" (Caswell 1978), and also leads to a convergence of community characteristics, whilst unexploited communities have more divergent characteristics. Nevertheless, maintenance of high diversity through biological disturbance in the form of human predation may be undesirable due to the resulting dominance of inedible species and small individuals of targeted species. A management plan was suggested, centred on a rotational "cropping approach" (Hockey and Bosman 1986). Progressive size reduction of depredated species leads to intensified collecting pressure on the remaining individuals, and this, coupled with spatial encroachment of inedible species, inevitably leads to the degradation of the intertidal zone communities as a food resource.

Moreno et al. (1984) demonstrated in a study of the impact of human predation on intertidal invertebrates in southern Chile that reductions in size and density of key grazing gastropods, the limpets *Fissurella* spp., led to an increase of cover of macroalgae mainly the red algae *Iridaea boryana*, which is exported to Japan. This demonstrated how "probably unbeknownst to them, Chilean fisherman are managing the intertidal zone in a way which produces a cash crop they wouldn't otherwise have. Exploitation of algae would be sharply curtailed if Chileans stopped fishing the intertidal" (Moreno et al. 1984, in Paine 1994).

An holistic approach was adopted by Eekhout et al. (1992) to the exploitation of the limpets (*Cymbula gratinata* and *Scutellastra argevillei*) combining measurement of population parameters

in undisturbed and experimentally harvested populations, assessment of the effects of exploitation on the community as a whole and development of quantitative population and economic models, in a way to provide a sound basis for the rational management of living intertidal resources. Empirically derived quotas and minimum size limits for both species were suggested as chief mechanisms for regulating a potentially economically viable and ecologically sustainable fishery. In this study the authors also observed that limpet removal resulted in a decrease in the cover of encrusting coralline algae and an increase in the cover of fleshy macroalgae. Similar results after flood-induced mass mortalities of limpets stocks are documented in Branch et al. (1990). Also an increase in macroalgae cover after natural or experimental removal of grazing herbivores is well documented (Hawkins and Hartnoll 1983 for a review, Raffaelli and Hawkins 1996). In addition several lines of research elsewhere have shown that some encrusting coralline species may depend on the activities of grazers to prevent overgrowth and smothering by other organisms (Steneck 1982, 1991).

Pombo and Escrofet (1996) added other data to the classical way of studying human effects on intertidal community. When studying the effect of exploitation on the limpet *Lottia gigantea* these authors ranked the intertidal gathering using criteria such as gatherers' visiting pattern to the shore; several features of the sites that could explain degrees of exploitation (topography, size of settlements or fishing communities, distance from mainland or major human settlements, habits of consumption of intertidal species by local people) and existence of length protective management programs. Ecological implications of exploitation were explored through the conceptual model proposed by Catterall and Poiner (1987) for assessing the impact of traditional shell gathering on intertidal molluscs. The model uses life history and habitat information to predict the extent to which a given intertidal shellfish population would be either susceptible to depletion or resilient, according to size at maturity, intertidal burying, adjacent subtidal populations, benthic mobility and pelagic larvae. Several studies report a reduction of the size range of populations, decreased abundance and decreased mean size of several limpet species in harvested versus nonharvested sites, or along gradients of human activity (Branch 1975a, Moreno et al. 1984, Oliva and Castilla 1986). Pombo and Escrofet (1996) also verified that mean size of specimens in older middens was significantly larger than in a recent midden, maximum and mean size in artisanal catches of *Lottia gigantea* decreased along the exploitation gradient and the difference between mean size in the catches and in the intertidal population became smaller as exploitation increased. The decrease in mean size as an effective descriptor of the effect of exploitation is reinforced by the decrease in maximum size range of intertidal populations (Branch 1975a), but it may not demonstrate satisfactorily the selection of larger sizes by gatherers (Pombo and Escrofet 1996).

Lasiak (1997) investigated the pattern and intensity of utilization of rocky intertidal reefs along a rural South African coastline. The author based in regular surveys at one particular locality found out that tidal phase had a major influence on activities; during spring low tides approximately 56%

of the visitors were engaged in shellfish-gathering, although factors such as year, season and month of observation had little influence on the densities of the major user-groups, significant differences in the numbers of shellfish-gatherers and walkers were observed during holidays and term-time (Lasiak 1997). Data from additional surveys, conducted at nine study sites, revealed marked regional differences in shoreline utilization; the central region supported the highest numbers of anglers, seaweed-harvesters and shellfish-gatherers. The author concluded that although the intensity of exploitation observed in this region appears to be comparable with that reported previously in popular metropolitan areas it is likely to have a much greater impact on intertidal biota simply because the activities of rural shore-goers are less constrained than those of the urban shore-goers. In previous research in order to assess the putative ecological impact of such unconstrained foraging activities by indigenous coastal inhabitants Lasiak and Field (1995) compared the infratidal macrofaunal assemblages at three exploited sites with those at two adjacent non-exploited sites, based in univariate, graphical and multivariate techniques. The authors stated that two-way nested anovas revealed trends in species richness and diversity not consistent with the presence/absence of exploitation and that the apparent conflict between these results and the expectations based on the intermediate disturbance hypothesis were possibly due to differences in the competitive abilities of "early" and "late" successional species present on the shores. On the other hand, multivariate analysis, in form of dendograms, multidimension scaling (MDS) ordinations and SIMPER routines, derived from Bray-Curtis measures of similarity of abundance and biomass data, provided the most clear-cut discrimination between exploited and non-exploited sites (Lasiak and Field 1995). The authors concluded that the difference in community structure seemed to result from a reduction in the abundance of sessile filter-feeders, increase in abundance of certain seaweed-associated species, and decrease in biomass of exploited species in and associated fauna.

In subsequent work Lasiak (1998) used multivariate analyses, in the form of dendograms, MDS (multidimensional scaling) ordinations, two-way crossed ANOSIMs (analyses of similarities) and SIMPER (similarity percentage) analyses, to examine the influence of human predators on rocky infratidal assemblages. The author compared mean macrofaunal abundance and biomass estimates obtained from samples collected at multiple sites within three 'no-take' marine reserves and at three adjacent exploited localities on the Transkei coast of South Africa. Generally, Lasiak (1998) found that dendrogram and MDS derived from the abundance and biomass estimates revealed two major subdivisions, one representing the sites in the two southern localities, and the other the sites within the central and northern localities of Transkei. Temperate species which were either restricted to or attained higher abundances and biomasses in the southern region appeared to be primarily responsible for this sub-division of sites (Lasiak 1998). The author stated that two-way crossed ANOSIMs indicated that there were significant differences in the assemblages found under the exploited and non-exploited treatments and between the three pairs of locations; while two-way SIMPER analyses suggested that the major differences in the assemblages under the two

treatment regimes were the lower and biomass of sessile filter feeders and microalgal grazers that are dependent on the primary substratum and the greater abundance of phytal-associated species under exploited conditions. These results were consistent with those presented by Lasiak (1999) in her study of the putative impact of the exploitation on rocky infratidal macrofaunal based on multiple-area comparisons.

Being aware of constrains associated with the multivariate analysis previously presented Lasiak (2003) investigated the influence of taxonomic resolution, biological attributes and data transformations on multivariate comparisons of rocky macrofaunal assemblages. Multivariate comparisons of impacted and non-impacted biotic assemblages require decisions to be made about the taxonomic resolution to be used, biological attributes to be measured and whether the contributions of common or combinations of common, intermediate and rare taxa should be emphasised (Lasiak 2003). In order to investigate that Lasiak (2003) compared exploited and non-exploited rocky infratidal macrofaunal assemblages distributed across a biogeographic transition zone on the south-east coast of South Africa, and used two-way crossed ANOSIM tests look for differences in the structure of assemblages among regions of the coast and between exploited and non-exploited localities. Lasiak (2003) found that:

- In tests based on presence/absence, significant differences in assemblages among regions and between treatments were evident only at the species level.
- In tests based on densities and biomasses, regional differences were evident at the species, order, class and phylum level, and were irrespective of whether dominant or combinations of dominant, intermediate and rare taxa were emphasised.
- Significant differences in exploited and non-exploited assemblages were evident in all of the comparisons except those in which numerically dominant higher taxa were emphasised.

Lasiak (2003) concluded that her results do not suggest that the natural environmental gradient influences the fauna more by species replacements than by changes to the proportions of higher taxa, nor do they indicate that natural environmental variations and anthropogenic disturbances modify macrofaunal assemblages at different taxonomic levels. The author emphasized that further studies are needed to establish whether the taxonomic level, at which modification of assemblages occurs, varies with the type of disturbance (e.g. pollution vs exploitation, strong vs weak gradients) or biotic component (e.g. rocky vs soft-bottom assemblages) under consideration (Lasiak 2003).

1.2. The Azorean case

Azoreans have always heavily exploited their littoral and near shore waters, posing considerable problems for both resource management and nature conservation (see Martins et al. 1987a, b; Santos et al. 1995). As in other coastal areas, exploitation pressure increased with the ready availability of cheap snorkelling gear in the 70s. From the two species that occur in the Azores *Patella candei* is mainly exploited on a subsistence or recreational basis, whilst *aspera* has been chiefly exploited by both licensed and unlicensed skin-divers depending on the island concerned (Santos et al. 1995). The former species is found low on the shore and in upper sub-littoral up to 6 meters depth, very exceptionally reaching 15 meters (see Hawkins et al. 1990a,b; Menezes 1991) and *P. candei* inhabits the intertidal, occasionally being found subtidally. Since *P. aspera* was the fifth or sixth most important commercially exploited species in the mid 1980s, especially from S. Miguel Island, most of the conservation measures were addressed to it. In 1989, a ban on all limpets collecting was established for the Central (Faial, Pico and S. Jorge) and Eastern group (S. Miguel, Santa Maria and Formigas). Decline and recovery of the stocks were followed in different islands since the 1980s (Martins et al. 1987, Santos et al. 1989). Santos et al. (1995) reported a commercial fishery crash in 1988 in S. Miguel and very low population levels in the Central group between 1984 and 1987. They concluded that *P. aspera* populations were abundant in the Western group (Corvo and Flores), at the time of the study, where they had never been subjected to heavy exploitation. Recovery in the Central group was observed after various conservation measures but a critical situation was stressed for S. Miguel and Santa Maria, albeit to a slightly lesser extent on the latter. Since then, harvesting has been strictly prohibited in selected areas of the islands, a closed season were established, catches limited to licensed people and minimum sizes for each species were defined. Nonetheless, illegal limpet off-take is frequent and imports from Portugal and Spain constitute an augmented difficulty for distinguishing illegally sold limpets from those claimed to have come from the continent.

1.3. Conservation and management

Managing coastal living resources can be a difficult task since many of the catches are not included in official fisheries statistics. Its management and understanding the population and community consequences of over-exploitation need a broad-based approach (Eekhout et al. 1992). This must encompass studies of the foraging behaviour of people, the density and distribution of collectors, the population biology of target species including life history characteristics, and the role these species play in the community. Such an approach will facilitate understanding the role of people as top predators in many coastal systems and give insights allowing innovative management measures. These could include closed seasons during which all collecting is banned allowing

reproduction to occur combined with rotational or proportional harvesting. Climactic features such as the occurrence of severe winter storms, at least in the case of lower shore species, might considerably curtail any exploitation activities providing a natural closed season that should not be ignored (see Eekhout et al. 1992).

As referred to above, one of the consequences of population over-exploitation is the progressive reduction of depredated species (see Hockey and Bosman 1986, Oliva and Castilla 1986, Pombo and Escofet 1996). This leads to intensified collecting pressure on the remaining individuals and coupled with spatial encroachment of inedible species, inevitably leads to the degradation of the intertidal zone communities as a food resource. Several littoral invertebrates, including limpets, have evolved long-range larval or juvenile dispersal which may be a mechanism for increasing the chances of settlement in favourable environments (Strathmann 1974). Nevertheless, the existence of a pelagic larval stage and the associated capability for dispersal may not be important in the persistence of populations if the refuges for the species are few or far from the sites of intense harvesting. Isolated islands, like The Azores and Hawaii, seem particular prone to recruitment over-fishing because much of the reproductive output is probably dispersed from the islands (Raffaelli and Hawkins 1996). On the other hand, long-distance recruitment, when present, may mask a declining reproductive fitness of a population if relative abundance alone is used as an index of status (Hockey et al. 1986). Whatever the management program adopted it must ensure that maturity can be attained before individuals are noticeable or valuable to harvesters in order not to compromise population output and continuity. This is particularly important for protandrous species, such as limpets, since heavy exploitation of those populations leads to fewer females in the population as larger limpets (predominantly females) are selectively removed. Moreover, under certain situations smaller individuals are probably been removed before they have time to change sex. All this seriously distorts sex ratios and reduces the output of eggs by the population compromising its structure and reproductive rate. In these species (more than in others) catch strategies should include a minimum size in order to guarantee the sustainable use of the resource.

Human activity has probably influenced all environments and species. In this sense no truly pristine situations remain, and all will be increasingly altered by stresses stemming from such direct impacts as exploitation or point-source pollution, or more subtle and less direct effects of global warming: changes in the concentration of atmospheric gasses as the CO₂ that is responsible by the increased acidity of sea water, the elevation of sea levels or the destruction of the ozone layer (adapted from Paine 1994).

To most people, biological diversity is undeniably of value, but it does not always lend itself to the economic valuation upon which policy decisions are usually based. The value of a species can be divided into three main components:

- Direct value of products that are harvested;
- Indirect value where aspects of biodiversity bring economic benefits without need to consume the resource (McNedey 1988);
- Ethical value.

Of these main reasons for conserving biodiversity, the first two, direct and indirect economic value, have a truly objective basis. The third, ethics, on the other hand, is subjective and is faced with the problem that a subjective reason will inevitably carry less weight with those not committed to the conservationist cause. Faced with the impossibility of convincing governments that, according to the ethical view for conservation, every species is of value in its own right, arguments concerning conservation have to be framed in cost-benefit terms in which governments determine their policies (adapted from Begon and Harper 1996).

2. Ecology and dynamics of limpet population

2.1. Species distribution along the shore

In Europe *Patella vulgata* occupies an extensive zone in the littoral region of rocky shores from High Water Neap Tide Level (HWNTL) to Mean Low Water Level (MLWL) (Ballantine 1961, Lewis 1964, Fish and Fish 1989). *Patella depressa* is predominantly present on the middle to lower shore (Fish and Fish 1989). In turn, *Patella ulyssiponensis* occurs on the lower shore and in the sublittoral, also been found in rock pools on the middle and upper shore (e.g. Yonge 1949, Thompson 1979, Fish and Fish 1989). Thompson (1979) discussed how *P. ulyssiponensis* dominates wave beaten shores while *P. vulgata* replaces it under more moderate wave action. *P. ulyssiponensis* is less tolerant of desiccation than *P. vulgata*, and Thompson (1979) defined it as a non-migratory low shore specialist, highly adapted to a specific set of environmental conditions, but lacking the ability to adapt to others. *P. depressa* is not found in places sheltered from the wave action (Evans 1947a,b; Orton and Southward 1961; Fretter and Graham 1976), but is less successful than *P. ulyssiponensis* in extremely exposed places (Fretter and Graham 1976, Thompson 1979). Nevertheless, the degree of overlap between these species varies greatly throughout their geographical range, depending upon the physical and biological conditions of the sites.

2.2. Population dynamics

The population dynamics of limpets, as other benthic molluscs living in a patchy environment, are constrained by the characteristics of their proximal environment. Reviews evaluating variation in population dynamics with habitat variability have suggested the following explanations (e.g. Sutherland 1970, 1972, Lewis and Bowman 1975, Branch 1976, Workman 1983):

- a. Physical factors including wave action or height on the shore, and small scale modifications of topographic features;
- b. Intraspecific competition, and
- c. Intraspecific competition.

These processes influence life history traits such as recruitment, settlement, growth, size, longevity and foraging activity, regulating population performance.

2.2.1. Population density

The density of an adult population is dependent upon the recruitment of juvenile individuals into the population as well as the balance of adult emigration/immigration and mortality of the commonly present ones (Sutherland 1970). Furthermore, species with dispersive (placktonic) larvae, as the patellacean limpets, spatial and temporal variation in the population density are expected to be large (Underwood 1979, Fletcher 1984b). Thus, densities are determined by biological interactions that in turn are dependent upon stochastic mechanisms (e.g. settlement of plancktonic larvae conditioned by hydrodynamism). Next are presented some factors determining that variation on limpet population density.

2.2.1.1. Spawning and recruitment

Most limpet species have a single spawning each year, as typified by *P. vulgata* (Branch 1981). In this species gonadal development begins in July and spawning occurs from October to March (Orton et al. 1956, Thompson 1980, Baxter 1983) being highest in November. The spawning period may vary from year to year and place to place being triggered by rough seas and onshore winds (Fretter and Graham 1976). Indeed Orton et al. (1956) found that the start of maturation occurred earlier in colder localities. *P. vulgata* is sexually dormant (the resting period, where most of the animals cannot be sexed) in spring and early summer (Fretter and Graham 1976), with a maximum of juvenile density being attained between July and December (Lewis and Bowman 1975). *P. depressa* is a summer-breeding species in south-west England (Orton and Southward 1961). The gonads begin to develop in spring (April/May) as temperature rises, and spawning occurs between late July and beginning of September. Orton and Southward (1961) found no resting period in *P. depressa* as it occurs in *P. vulgata*, during which the majority of adult specimens have reduced gonads of indeterminate sex. The same authors indicated temperature raise combined with wave action, as the major cues for gametes discharge on sea-water. Nevertheless, Hatch (1977) and Bowman (1981) stressed the possibility of either a very prolonged spawning period or two discrete releases in one breeding season. *P. ulyssiponensis* has a long breeding period, maturation of gonads usually beginning in May-July and spawning taking place around the beginning of October (Orton 1946, Dodd 1956, Orton et al. 1956, Southward and Dood 1956, Thompson 1979) with a resting phase in January (Fretter and Graham 1976). Orton et al. (1956) and Orton and Southward (1961) suggested that although the development of the gonad in *P. vulgata* and *P. depressa* respectively is well related with temperature, the act of spawning is triggered by violent onshore storms. Thompson (1979) also found *P. ulyssiponensis* spawning during the autumn gales. Hence, it seems likely that spawning cannot take place until a population is sufficiently mature, but after that stage is reached, the first strong gale will trigger spawning (Thompson 1979).

Spat have been found to prefer pools or damp situations (Orton 1929, Jones 1948, Lewis and Bowman 1975). So, damp crevices and small pits are important to settling spat and both the availability of such micro-habitats and the observer awareness of it determine the recruitment recorded (Lewis and Bowman 1975). Branch (1975a) observed greater numbers of recruits of a number of *Patella* species at lower levels on the shore and factors as increased relative abundance of wet patches (Orton 1929) or increased time for settlement (Sutherland 1970) were proposed as enhancing low-shore recruitment. Recruitment also occurs at mid/high-shore levels and factors such as the influence of other species become more relevant. For example, Lewis and Bowman (1975) found that mussels, elongate barnacles and a light cover of algae modulate settlement. While mussels promote settlement mostly in high- and mid-shore levels, their beneficial influence decrease with the reduced space available as their cover increases. Mussels provide shelter and retain humidity better than barnacles, but in the absence of mussels, barnacle cover has been found to be preferential to bare rock (Lewis and Bowman 1975). Limited amounts of *Fucus* spp. reduce desiccation in mid- and high-shore levels and promote settlement. Baxter (1983) pointed out that *P. vulgata* spat prefer a cool, damp environment such as that provided by fucoid cover. However, a heavy perennial cover may have a screening and dislodgement effects. Lewis and Bowman (1975) recorded a general increase in juvenile numbers after removal and exclusion of all existing adults in a specific area.

2.2.1.2. Migration and foraging activities

Immigration and emigration of limpets within a study area depend greatly in their foraging activity. The latter is strongly regulated by the physical patterns of the substratum (e.g. presence of crevices or large cracks that prevent dispersion), competition for food (Branch 1975a) and risk of desiccation or exposure to wave action and hence dislodgement (Branch 1975b, Underwood 1979, Lui 1994).

Vertical migration upshore is widely seen in juvenile *Patella vulgata* from low-shore recruitment areas, and usually takes the individuals away from density related problems such as food competition. Horizontal movements are less common as their main purpose would be to search for better feeding areas. Furthermore, if individuals were to migrate horizontally it would result in competition with different populations of limpets, which could be disadvantageous. However, such movements can probably be induced in experimental removal areas, although horizontal migration is more likely to be undertaken by non-homing limpets and gastropods (Underwood 1979).

Many authors agree that each foraging excursion can be defined as having three distinct phases (Hartnoll and Wright 1977, Little et al. 1988, Evans and Williams 1991): an outward travelling phase; a prolonged foraging and grazing phase, and a terminal phase of returning to home scar. Immigration or emigration might occur if favourable sites are encountered during these excursions.

However, Chelazzi et al. (1994) mentioned that limpets in fact graze constantly during their foraging excursions. Hill and Hawkins (1991) and Della Santina et al. (1995) reported longer foraging excursions in April than November and high-shore limpets moving further away from the home scar in spring than in autumn. Della Santina et al. (1995) suggested that these seasonal differences might have to do with variation in food availability.

Through limpet grazing, large areas of rocky shore are maintained in a primary stage of succession (Evans and Williams 1991). Hence, shore dynamics is also influenced by the grazing intensity and distribution over time and space. The removal of such important grazers as limpets allows algae proliferation. Indeed, dramatic changes in algal communities were recorded following exclusion of grazers (e.g. Paine and Vadas 1969, Lubchenco 1978, Hawkins 1979) although the long term effects may be reduced algal diversity (Raffaelli 1979). On the other hand removing limpets may also temporarily enhance the recruitment of barnacles on newly cleared surfaces (Sousa 1979b).

Larger *P. vulgata* individuals have been found performing longer excursions for longer periods of time than smaller ones (Little et al. 1988, Della Santina et al. 1995) while low-shore limpets have been seen to move further than those from the high-shore (Della Santina et al. 1995). Longer foraging excursions on the lower shore could be a result of higher intraspecific competition occurring at that level (Branch 1981) and also a consequence of having a higher metabolic rate (Davies 1967).

Homing to a fixed scar is well known in limpets (including the genus *Patella*) and is often considered a way to reduce desiccation (Branch 1981). However, the fact that many low-shore and even subtidal species present well-developed scars and homing behaviour, suggests that desiccation cannot be the only factor promoting that behaviour. In general, small individuals do not home, territorial limpets home rigidly and homing is more precise in high-shores species/individuals (Branch 1981). The mechanism of scar formation seems to be a conjunction of a chemical and physical action. Indeed, chemical solvents have been encountered in the pedal mucus glands and mantle edge of limpets. So, suggestions have been made that initially rocks are dissolved and then scraped away by the radula (see Branch 1981). The mechanism of homing to the scar is highly controversial. Although many limpets follow their outward trails home to the scar, homing limpets removed from their scars and replaced nearby on the rock still succeed in finding it (Davis 1885, 1895, Cook et al. 1969). This demonstrates that the simple trail-following is not the only explanation of the homing process. External clues such as recognition of local rock topography and inertial navigation were suggested as also allowing the limpets to recognise the direction of the scar (e.g. Bohn 1859, Galbraith 1965).

2.2.1.3. Mortality

Mortality is commonly defined (in field studies) as including documented deaths (e.g. death from predation, age or disease) and questionable deaths where a limpet is missing but the reason is unknown. Organisms known to prey on limpets include starfish, gulls (*Larus* spp.), oystercatchers (*Haemantopus* spp.) and crabs. Additional causes of death include summer heat, intra- and interspecific competition for food (Fletcher 1984b, Lui 1994), and mechanical destruction by movements of boulders, stones and pebbles during severe storms (Lewis and Bowman 1975). Newly settled individuals, spat and early juveniles are the most susceptible to mortality. They are easily washed-out from settlement refugia (Bowman 1985) and particularly sensitive to environmental stress, canopy shading and sweeping effects (Connell 1961, Brawley and Johnson 1991, Minchigton and Scheiling 1991). Death by desiccation has been proposed as a consequence of their attempts to move into adult habitats (Thompson 1980).

2.2.2. Growth

Availability of algae affects the growth rate of limpets, and in temperate areas algal cover is often more abundant in the winter and lower in summer (Sutherland 1970, Workman 1983, Hill and Hawkins 1991). Thus, faster growth rates would be expected in winter and lower ones in summer. As this is not the case, other factors rather than merely food availability seem to regulate limpet growth. Whilst the availability of food may be suggested as a limiting factor affecting growth, other factors resulting in intraspecific variations in size have been proposed. These include tidal height (Vermeij 1972, Branch 1981); intraspecific competition (Sutherland 1970, Underwood 1979, Creese 1980); interspecific competition (Lewis and Bowman 1975) and wave exposure (Thompson 1979, 1980).

When relating growth rate to gonad weight increases, slow growth rate has been correlated with periods of increases of gonadal weight (Creese 1980, Fletcher 1984a). In several studies growth rate of immature *P. vulgata* were constant from March to November whilst the growth rate of larger (mature) animals was greater during late spring/early summer. The growth rate decreased progressively from June to November where the minimum was attained in gonadal development (Baxter 1982, Hawkins and Hartnoll 1982).

The influence of biological habitat on growth is such that changes in surrounding species will result in changes over time in the growth and size characteristics of limpet populations from the same physical point on the shore (Lewis and Bowman 1975, Thompson 1980, Hartnoll and Hawkins 1985). Comparing habitats, the growth rate and maximum size of *P. vulgata* were found to be greater on bare rock and least among closely packed barnacles. Lewis and Bowman (1975) suggested differences in moving and foraging within these habitats as a possible explanation.

However, the configuration of the barnacle populations in terms of obstructions to limpet grazing has been proposed as more important than its absolute density (Thompson 1980). On the other hand growth in *Fucus* dominated areas was found to be higher than on adjacent bare rock (Ballantine 1961, Choquet 1968, Lewis and Bowman 1975, Hawkins and Hartnoll 1982), probably due to food availability. As in other animals, as age increases the growth rate of a limpet decreases, i.e. smaller individuals increase relatively faster than larger ones (see Thompson 1979, Baxter 1983, Lui 1994). On the other hand fast growth is often correlated with reduced longevity. Branch (1981) suggested that the act of growth might in itself lead to a shortened life expectancy. Rapid growth and early sexual maturity may exhaust reserves so that any subsequent shortage of food cannot be tolerated. Rapid body growth may also reduce the amount of energy devoted to defensive structures such as the shell and hence increase the chances of death (Branch 1981).

The potential size range at any time of year can be considerable for *P. vulgata*. For example in April fast-growing, early settling individuals (September/October) that emmerged in late autumn can have grown up to 6-7mm while later settlers may be just emerging at 2-3mm. However, in the British Isles juveniles rarely exceed 12-15mm shell length by the end of their first year (Bowman 1981).

The growth rates in *P. ulyssiponensis* have been found to be slower than *P. vulgata* (Thompson 1979, Bowman 1981) and less variable (Thompson 1979). Fischer-Piette (1941) reported annual increments in shell length of 2.0-4.5mm at 20-30mm shell length, and 2.0-3.5mm at 20-30mm. Thompson (1979) reported a mean annual increment declining from 7mm in the youngest individuals to about 5mm in six-year-old limpets (Thompson 1979). Thompson (1979) suggested that the growth of *P. ulyssiponensis* is relatively insensitive to environmental variation. *P. ulyssiponensis* may have little ability to take advantage of particularly favourable conditions and would be outgrown by the more flexible *P. vulgata* but under less favourable conditions (e.g. under violent wave action) it may be better in maintaining a steady growth rate (Thompson 1979).

The data existing to *P. depressa* indicate an even slower growth rate than the two species previously mentioned. Bowman (1981) noted that it is likely that 5mm individuals may be nearly three years old and 11mm limpets could belong to at least three different age groups due to the considerable overlap of age groups. Growth rates of intertidal gastropods have been found to increase when densities are experimentally reduced (Frank 1965, Sutherland 1970, Haven 1973, Underwood 1976). Lewis and Bowman (1975) when investigating that correlation in *P. vulgata* suggested that the grazing area available to each animal is more important than the effective density. Other factors being equal, increase in tidal height usually results in a decrease in growth (Ballantine 1961, Sutherland 1970). Lewis and Bowman (1975) noted that low-shore populations of *P. vulgata* have the highest growth rates, mid-shore intermediate growth rates and high-shore the lowest ones.

3. The evolution of sex

Reproduction is said to be the purpose of an individual's life in transferring its genes to the following generations, consequently providing the continuity of the species. Despite the essential similarity of reproductive processes in animals and plants, the conditions of sexuality and the organisation of the life cycle are extremely varied. Species can differ from each other in a great manner of what may be their "life-cycle traits", often collectively and somewhat anthropomorphically described as their reproductive strategy. These differences greatly affect patterns of genetic variation, and therefore the responses of populations to natural selection and other factors of evolution. Mutation and recombination have been cited for more than a century as indispensable for the survival of species.

3.1 The significance of sexual and asexual reproduction

Sexual reproduction is widespread but many organisms can also reproduce asexually, that is, produce offspring without recombination of genetic material. Offspring produced in this way will have a genetic constitution which is virtually identical to that of the parental organism. In other words, some species can include both sexual and asexual genotypes. Sex (singamy), often but not always, involves outcrossing (recombination), whereby the genomes of two individuals are partly or wholly combined.

Asexual reproduction can take place either by:

- Vegetative propagation (parthenogenesis) in which an offspring arises from a group of cells, as in plants that spread by stolons, or by subdivision of an existing body into two or more multicellular parts (e.g. budding and fission in many invertebrates); or
- Apomixis in which offspring arise from unfertilized egg. Meiosis is suppressed, and the offspring are genetically identical to their mother except for new mutations that may have arisen in the cell lineage from which the new individual arose.

Species in which individuals are either female or male are termed dioecious (Plants) or gonochoristic (Animals). An individual that can produce both kinds of gametes is named cosexual or hermaphroditic.

3.1.1. The problem with sex

A traditional explanation of the existence of recombination and sex is that it increases the rate of adaptive evolution of a species, either in a constant or a changing environment. As proposed by

August Wiesman in 1889 sex is adaptative because it provides the variability required for evolution. Nonetheless, recombination destroys adaptative combinations of genes. The major cost associated with sexual reproduction is that it reduces the efficiency with which genes are transmitted between generations, since only half of the genome of the offspring of sexually reproducing individuals is contributed by each parent. This is the cost of meiosis. Therefore, the rate of increase of an asexual genotype is approximately twice as great as that of a sexual genotype. In the long-term, the evolution of asexuality might doom a population to extinction, but in the short term, generation by generation asexuality has a two fold advantage over sexuality, as cost of recombination or of meiosis (see Smith 1978, Bell 1982, Bell and Koufonpanou 1986, Stearns 1987, Charlesworth 1989 for details). Other costs are associated with the need to allocate resources to sexual display and courtship, and with the higher rates of mortality often incurred whilst finding a mate.

3.1.2. Hypotheses for the advantage of sex: Why reproduce sexually?

Many of the earlier theories suggested that sexual reproduction had a long-term advantage to the species or taxa, but conceded a short-term advantage relative to individuals with the capacity for asexual reproduction solely. These are group selection theories and since then other theories have been developed in which the selective advantages of sexual reproduction are perceived as a characteristic of the individual, not of the group or taxa (e.g. Bell 1982). In order to account for the persistence of sex, rather than apomixis, its advantages must be great enough to offset its two fold cost. Although many evolutionary biologists (e.g. Smith 1978, Stearns 1987, Michod and Levin 1988) would grant that the origin of recombination may have been due to its role in DNA repair, they also believe that the evolution of meiosis, distinct mating types or sexes and the maintenance of sex in most species are attributed to other causes involving variation and selection. Kondrashov (1993) distinguished hypotheses that propose some immediate benefit to the individual organism's progeny from hypotheses that invoke variation and selection. The leading hypothesis for an immediate benefit of recombination is that molecular recombination facilitates repair of damaged DNA (Bernstein and Bernstein 1991). The presumed advantage of recombination is that it reduces the frequency of nonoptimal alle combinations and can potentially increase the frequency of superior combinations. Some of the proposed hypotheses for the advantage of sex are now summarily presented.

3.1.2.1. Environmental deterministic hypotheses

In environmental deterministic hypotheses, selection acts not on new mutations but on existing genetic variation, by reorganizing it into new combinations. This is particularly true when reorganization of a polygenetic character is favoured by stabilizing selection, providing an increase in individual fitness. This can provide a long-term advantage to sex (a higher rate of adaptation of

the population), but a short-term advantage as well, because sexual parents are likely to leave more surviving offspring than asexual parents.

3.1.2.2. Advantages in heterogeneous habitats

Spatial, rather than temporal variation in the environment can also provide a strong advantage to sex (Ghiselin 1974, Williams 1975, Bell 1982). Heterogeneity of resources, might favour recombination in two related ways:

- If offspring disperse at random into small patches of habitat where competition occurs, and only one or a few of the individuals best adapted to conditions in a patch survive (Williams 1975). A sexual female has many different offspring genotypes, but an asexual female has numerous copies of the same genotype. Thus, there is a greater probability that the sexual female will have more surviving offspring.
- Within an apparently homogeneous habitat patch, genotypes can differ in their use of resources. If siblings compete, then a patch of habitat can sustain more progeny from a sexual family than from an asexual one, because asexual siblings compete more intensely (Bell 1982, Price and Waser 1982).

The selective advantage of sexual organisms arises from the ability of their diverse offspring to compete in the structurally complex world of saturated environment where there is intense competition. All sexually produced offspring are slightly different and the uniform offspring of an asexually reproducing animal will not be able to supplant them from all the different habitats that they can occupy. Bell (1982) developed a theory which he named "the theory of the tangled bank" where he suggests that sexual reproduction would predominate in stable complex environments. Asexual reproduction would be expected to occur where species are maintained below the carrying capacity of the environment and opportunities for ecological opportunism exist, i.e., disturbed and simple environments. This would occur because the overall fitness of the diverse offspring of sexually reproducing animals in a complex world is greater than that of the uniform offspring of asexually reproducing animals. Asexual reproduction is more efficient in the colonization of new environments where fast reproduction more than genetic variability is needed. Indeed, when environments are saturated, the competition for resources increases. Selection is intensified since genetic variability due to recombination and sexual reproduction provides the diversity upon which natural selection can act.

3.1.2.3. Hypotheses based on deleterious mutations

Several hypotheses postulate that the advantage of sex lies in ridding populations of deleterious mutations which arise at a high rate. Muller (1964) proposed a hypothesis that has been named "Muller's ratchet" where he stressed the role of recombination in a sexual population on the reconstitution of the least mutation-laden classes of genotypes by generating progeny with new combinations of favourable alleles. In an asexual population the zero- and one-mutation classes cannot be regenerated due to the reproductive constraints. Although Muller's ratchet may describe a long-term advantage for recombination, it has a potentially strong effect only in rather small populations, and is not clear that it can provide a short-term advantage.

Another hypothesis advocated by Kondrashov (1993) can apply to large populations since it does not require genetic drift. It assumes that deleterious mutations interact, so that each "genetic death" rids the population of more than one deleterious mutation, and fewer genetic deaths are required to improve the population's mean fitness than if the mutations were purged individually. With a sufficiently high mutation rate per genome, this process can provide a strong short-term advantage for a gene that promotes sex and recombination (Feldman et al. 1980). Other theories suggest that sexual reproduction is maintained as a mechanism which prevents potential cellular parasites from successfully invade all the offspring of an organism, as it would occur if organisms only reproduced asexually. Hurst (1990) has suggested that a strong division into two gametic sizes reduces the possibility of transferring cellular parasites during fertilization.

3.1.3. Evaluating hypotheses for the advantages of sex

Such a difficult conundrum turns out to have many proposed solutions. Some evidences are consistent with several hypotheses, but do not prove any of one of them as the major explanation of sex. Some evidence for the advantage of sex in heterogeneous environments includes the geographic distribution of related asexual and sexual taxa (Levin 1975, Glesener and Tilman 1978, Bell 1982, Bierzychudek 1987). Bierzychudek (1987) described experiments that provided evidence for the lower competition between genetically diverse siblings, by using different combinations of resources, than apomictically produced siblings. Evidence for a high deleterious mutation rate and on synergistic effects of such mutations was confirmed by Kondrashov (1993) which gives strength to the advantage of sex in purging populations of deleterious mutations.

3.1.4. Evolution of sexes: sperm competition

Since the late 70's and early 80's it has become clear that sperm competition is a powerful selective force (Parker 1970, 1984, Smith 1984), shaping life-history characteristics such as body size, morphology, physiology, behaviour and even the evolution of the two sexes. Phylogenetic

evidence indicates that anisogamous organisms (with gametes of different sizes) have evolved from isogamous (with gametes of equal sizes) ancestors. It is also true that in most organisms there exist two forms: males, which produce tiny gametes (microgametes); and females, which produce much larger gametes (macrogametes). Explaining the origin of distinct sexes requires an explanation for the evolution of large versus small gametes. There have been various suggestions.

The earliest was that the rate of fusion in an external medium is maximized when the fusing gametes are very different in size (Kalmus 1932, Scudo 1967). Models by Charlesworth (1978), Hoekstra (1987) and others (e.g. Parker et al. 1972) show that anisogamy would evolve if one genotype is favoured because the large size of its gametes enhances the survival of the offspring, and another because it can make many gametes. Parker et al. (1972) developed a theory that deals specifically with the role of gamete competition in the evolution of males and females. It showed by computer simulation that in some hypothetical externally fertilizing ancestor, coexistence of males and females would be an evolutionary stable strategy (ESS; Smith 1982) when there is a high advantage in provisioning the zygote, and that producing microgametes is the ESS when the advantage of provisioning is weaker. In a population in which all individuals produce gametes of equal size (isogamy) two gamete sizes (anisogamy) are favoured if the relationship between zygote fitness and zygote size is accelerating. Large gametes result in zygotes that survive well, but can be produced only in small number because they are costly. On the other hand small gametes contribute little to zygote survival but obtain vastly more fusions. Starting from an anisogamous population, disruptive selection quickly produces two sexes (males and females) because small-gamete producers and large-gamete producers are simultaneously favoured; producers of intermediate sized gametes quickly become extinct.

Additionally to sperm competition, sperm choice also has its influence in species variability and consequentially in natural selection pathway. Females in many species can choose what sperm to accept by selecting the mates that most attract them. Genotypes associated with brighter colours, larger body sizes or agonistic behaviour are often preferred.

3.2. Sex ratios, sex allocation and sex determination

Among organisms with distinct female and male sexual functions (defined by gametes of different sizes), some are dioecous/gonochorists (have separate sexes), and some are hermaphroditic or cosexuals, with either simultaneous or sequential female and male functions. The theory of sex allocation has been developed to explain this variation (Charnov 1982, Bull and Charnov 1988, Frank 1990). Some of the major points of this theory will be presented next.

3.2.1 Sex ratios in randomly mating populations

Defining sex ratio, as the proportion of males in a population, and individual sex ratio as the sex ratio in the progeny of an individual it is important to distinguish the latest concept from the sex ratio in a population, the population sex ratio. In many species it has a value of 0.5 (1:1).

Fisher (1930) was who first provided the explanation to understanding why a genotype with a sex ratio of 0.5 (1:1) should have an advantage over any other. He realised that because every individual has both a mother and a father, females and males must contribute equally, on average, to the ancestry of subsequent generations, and must therefore have the same average fitness. In a large, randomly mating population, the fitness of a genotype that produces a given individual sex ratio depends on the population sex ratio which in turn depends on the frequencies of the various sex ratio genotypes. That is, the evolution of the sex ratio is governed by frequency dependent selection, which will favour genotypes whose progeny are biased toward that sex that is the minority in the population as a whole. The Fisher's theory holds that the fittest genotype allocates equal resources to daughters and sons, and so a genotype that produces a sex ratio of 0.5 represents an ESS.

3.2.2 The evolution of sex ratios in structured populations

Species which mating occurs mainly within small groups descended from one or a few founders often have sex ratios different from 0.5, the ratio predicted by Fisher's theory. Usually they have a preponderance of females. Hamilton (1967) explained such "sex ratios" by what he termed "local mate competition": Whereas in a large population a female's sons compete for mates with many other females' sons, they compete only with one another in a local group founded by their mother. Nevertheless they compete largely with one other if the group was founded by just few females. Hamilton reasoned that the founding females' gene would be propagated most efficiently by producing mostly daughters with only enough sons to inseminate them. So, groups founded by female-biased genotypes contribute more individuals and genes to the population as a whole than groups founded by unbiased genotypes. The difference among groups in production of females increases the frequency, in the population as a whole, of female-biasing alleles (for evidence supporting this theory see Werren 1980, Herre 1985, 1987).

3.2.3 Sex allocation, hermaphroditism and gonochorism

Closely related to the evolution of sex ratio is the topic of sex allocation. Why in many taxa are all resources allocated to one or the other sex, in other words, why do some taxa have separate sexes? What evolutionary factors affect how hermaphroditic species allocate resources to female

and male function? These include the division of resources into eggs and sperm by simultaneous hermaphrodites and the timing of sex change in sequential hermaphrodites.

Some animals and plants switch sex function once during their lifetimes (sequential hermaphrodites), from male to female (Protandry) or vice-versa (Protogyny). Whether it is advantageous to begin as one sex or the other depends on how reproductive success as a male or as a female changes as a function of an individual's age or size. If the gain in reproductive success via female function increases more steeply with these characters than the gain via male function, protandry is likely to be advantageous; if the reverse occurs then protogyny is expected. Sequential hermaphrodites include unrelated animals such fishes, polychaetes and molluscs. For example, the blue-headed wrasse *Thalassoma bifasciatum* is a protogynous hermaphrodite; fish start reproductive life as females when small and only change to being male when large (Warner et al. 1975). Nonetheless in the anemonefish *Amphiprion akallopisos* there is a sex change in the opposite direction; when small individuals are male and then change to female when large. Like in the wrasses, sex change is socially controlled. If the female is removed, the male is then joined by a smaller individual, so he changes sex and lays the eggs while the newcomer functions as a male (Fricke and Fricke 1977). Considerable effort was devoted to the description and the understanding of sex change phenomenon since the beginning of last century (e.g. Orton 1909, Coe 1935, 1938a, 1953, Bacci 1947, Chipperfield 1951, Ghiselin 1969, fishelson 1970, Harrington 1971, Policansky 1982) but until recent findings presented ahead many queries were still unclarified.

The theory of whether it is advantageous to be hermaphroditic or gonochoristic depends in part on whether the species is a strict out-breeder or is capable of self-fertilization (selfing). In an outbreeding species, allocation of an individual's resources to one sex function (e.g. egg production) decreases allocation to the other sex function, i.e. there is a trade off. So, fitness accrued through either function increases as allocation to that function increases, but it may not increase linearly (Fig. 1).

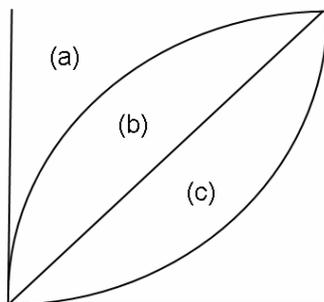


Fig.1- The theory of sex allocation: Reproductive success of an individual as function of the fraction of resources allocated to one sexual function (e.g., female) rather than the other. Increasing allocation to that sexual function may yield decelerating (a), linear (b) or accelerating (c) gains in reproductive success. (After Thomson and Brunet 1990).

If greater allocation to either sexual function yields diminishing returns, in terms of fitness, the relationship between fitness accrued through male function and that accrued through female function is convex (Fig.1-a): greater total fitness can be obtained by playing both sex roles than by

playing exclusively one. Thus selection favours hermaphroditism. In hermaphrodites, the optimum allocation to female versus male function is that combination that maximizes the number of offspring produced via female and via male refunction respectively. If conversely, reproductive success via either male or female functions increases more than linearly with increasing allocation to that function, the relationship between fitness obtained through male and female sex function is concave (Fig.1-c) and hermaphrodites have lower fitness than either "pure sex". Thus gonochorism should evolve.

One factor that may affect the shape of the trade-off in reproductive success an individual achieves through either sexual system (hermaphroditism or gonochorism) is the cost of developing the structures required for the two sex functions. If male and female functions require different structures, a pure male or female pays the cost of developing only one set of structures, whereas a hermaphrodite pays both costs and thus possibly has lower fitness.

3.3 The evolution of hermaphroditism among animals

The necessity to reproduce at all costs should favour the development of selfing wherever the environment is such that the transfer of gametes between individuals is hindered. It appears that selfing has originated convergently in adaptation to special conditions, for it is often facultative. Even though selfing is quite detrimental on the whole, it is more advantageous to self-fertilize than not to reproduce at all (Darwin, 1876). Hermaphroditism might predispose an organism to selfing but this does not tell us why the sexes became united in the first place. Ghiselin (1969) proposed three models to explain the origin of different types of hermaphroditism in animals:

The low density model invokes the advantage of being able to mate with any other member of the species where the probability of encountering a suitable mate is low (such as low mobility caused by sessility, or low population density). It predicts the frequent occurrence of hermaphroditism and other sexual arrangements (as self-fertilization and close association between members of the two sexes) among sessile organisms, deep-sea animals and parasites. Besides those, other adaptations could evolve in response to the same selective influence like vegetative propagation (Parthenogenesis) and apomixis (Ghiselin 1969). This model does not always suffice to account for many instances of hermaphroditism and is not workable for sequential hermaphroditism, such as protandry or protogyny.

The size advantage model is the only one that applies to animals exhibiting sequential hermaphroditism in populations not subjected to chronic or periodic low densities. It explains sequential hermaphroditism as occurring "where an individual reproduces most efficiently as a member of one sex when small or young, but as a member of other sex when it gets older or larger (Ghiselin 1969). Indeed, when the reproductive functions of one sex are better discharged by a

small animal, or those of the other sex by a larger one, if as it grows it assumes the sex advantageous to its current size than its reproductive potential increases. Eggs are more expensive to produce than sperm and the larger the female, the greater the number of eggs that could be produced. In benthic marine invertebrates with a free-swimming larval stage, an individual that happened to reach, without much delay, a suitable spot for post-larval development, could grow rapidly and be ready to function as a female, with greater reproductive potential than later, small arrivals. An individual delayed in finding a suitable locality could still reproduce when small, but could do so better if male than female (Ghiselin 1969). Ghiselin (1969) pointed out that this model predicts protandry where young stages must hunt for a suitable environment and protogyny when there is a sexual selection for largest males or when males care for the young.

The gene dispersal model is based upon the idea that individuals may be adapted to the genetical environment (the population genetic structure), as affected by the prevailing conditions of gene flow. In the inbreeding version it is hypothesized that where motility is restricted, sibling crosses become more frequent. Protandry and protogyny would help to prevent inbreeding by self-fertilization, although a similar phenomenon as merogony (in which an individual has offspring all of the same sex) would have the same effect. The sampling-error version is based upon the fact that genetical environment is also influenced by genetic drift and analogous phenomena, as numerical excess of one sex, in small populations (Wright 1938). Hermaphroditism is conceived as an effective way of minimize those deleterious consequences. Both versions predict modifications in the sexual system, wherever a change in ecology reduces gene flow between the component populations of species. As both inbreeding and sampling error have as consequence the reduction on genetic variability, hermaphroditism would compensate for both of these effects of limited gene dispersal (Ghiselin 1969).

As basis to his comparative study, when testing the proposed models, he used examples available on the literature. The possible pathways for hermaphroditism were prompted based in the advantages that would accrue to the individual rather than to the population. He reviewed conditions in which there had been a shift in habitat or way of life (like elimination of larval stage in some gastropods) possibly correlated with the evolution of the hermaphroditic stage. A version of Bock's principle of multiple pathways of adaptation (Bock 1959) was providentially invoked, as it states that when a group of organisms adapts to a particular selective influence, it may be done by adopting divergent mechanisms which deal with the same functional problem. Thus, for example, where the low density model should be expected to apply, as in parasites and sessile organisms, simultaneous hermaphroditism and phenomena of the dwarf-male type should be seen, although other factors (e.g. inbreeding and merogony) might interfere to prevent the evolution of either or both.

With his systematic review Ghiselin (1969) found evidence to his proposed models in several taxa. For example, he related that: The sexual biology and way of life of the Ctenophora were strictly consistent with the low density model since all are hermaphrodites either simultaneous or protandrous and able to store sperm (which has much the same functional effect). As they are pelagic or benthic with little motility as adults, but invariably have some motile period in their life cycle, Ghiselin (1969) found no appliance of the gene dispersal model. According to him the low density model can also be applied to the Trematoda, which are simultaneous hermaphrodites, except for two families in which males and females live in close association. Protandry observed in Polychaeta was explained by the gene dispersal model, while in the Gasteropoda where it is quite frequent among various unrelated groups of intertidal limpets (Coe 1938a, 1953, Bacci 1955, 1965 Hoagland 1975, Wright 1989, Wright and Lindberg 1982, Collin 1995, Warner at al. 1996, Hobday and Riser 1998) it was suggested to occur in line with the size advantage model.

3.3.1. The adaptative significance of sequential hermaphroditism in animals

Before Ghiselin's (1969) size-advantage model was proposed, virtually all explanations of the role of sequential hermaphroditism in the life of a species suggested benefits that might accrue to the population rather than to the individual. For example, Rosen (quoted in Atz 1964) postulated that the advantage in protogyny might lie in concentrating females in early ages, and that this would increase the total zygote production of the population. Nikolski (1963) and Noe (1969) also attempted to explain hermaphroditism in fishes as due to the correct positioning of males and females in age classes so that the reproductive output of the population is maximized. In addition, Noe (1969) proposed that sequential hermaphroditism is used as a population control mechanism, with the mean transformation age shifting earlier or later to provide fewer or more females, respectively, in times of overly high or low population density. Nevertheless, as suggested by Warner (1975) the high overall zygote production which may occur under conditions of hermaphroditism should be viewed as an advantage rather than a positive adaptation. So, explaining the evolution of hermaphroditism must be done on the basis of the advantage it confers to the individual. The adaptative value of traits should not be viewed as being "good for the population", but "for good of the individual", or more precisely "for the good of the gene" (Krebs and Davies, 1993).

With the advent of Ghiselin's size advantage model as explaining sequential hermaphroditism, different authors attempted to explain its evolution by other mechanisms rather than the referred one. Warner (1975) investigated how spawning behaviour, age structure and the schedule of female fecundity interact to produce selection for sequential hermaphroditism. Although the examples taken from the literature used to model the theoretically optimum schedules for sex change mentioned shrimps and teleost fishes, he prompted that the results should also apply to other animal groups. In order to do this he proposed that selection pressure for sequential

hermaphroditism could exist if spending part of the mature life span as a male and part as a female yields a higher life time expected reproductive potential than that for nonhermaphrodites. His results suggested that protandry may be selected for in populations when individuals mate randomly, as long as female fecundity increases with age for at least the first few years of the mature life span. The pressure would be stronger as faster as fecundity increases with age, being in concordance with Ghiselin's size advantage model. Selection for protogyny was suggested when inexperience, male dominance, mate selection or territoriality lead to a differential in male expected fecundities in succeeding ages, or when female fecundity decreases with age. Mate selection was pointed out as the strongest pressure for protogyny under conditions of low mortality and constant or decreasing age-specific female fecundities. In most of the studied cases were found biased sex ratios. Such deviations from the expected 1:1 ratio (Fisher 1930, Leigh 1970) are due to the fact that selection in sequentially hermaphroditic populations does not equalize parental expenditure on the offspring of the two sexes, nor does it ensure equal expected lifetime reproductive potentials for males and females (Warner 1975). The population sex ratio is the result of selection favouring individuals that change sex within an optimum age range which as Warner (1975) referred maximises their own expected lifetime reproductive potentials. On his report he also stressed the idea suggested by Wenner (1972) that rather than overall sex ratios, the important parameter in sequentially hermaphroditic populations is the size- or age- specific sex ratio pattern. Nonetheless, Warner (1975) also mentions that in populations where there is some mating success of young males, male fecundity at early ages may still be high enough to minimize selection pressure for changing sex (for some examples see Gadzil 1972, Warner et al. 1975, Warner and Hoffman 1980). Indeed, Warner et al. (1975) found cases in fish where there is male dimorphism. Some members of a population are primary males with one type of mating system, while others are protogynous males, with different behaviour. As animals react more to size than to age of their conspecifics in mating interactions, Warner (1975) suggested growth rate of the individual as an important parameter to consider in sequential hermaphroditism. This idea was developed by Iwasa (1991) in one of his proposed alternative models for sex change evolution. Using dynamic programming, he showed that the ESS under an enhanced mortality or reduced growth rate of reproductively active individuals may include an extended nonreproductive period intervening between male and female activities, in addition to the sexual difference in the way fertility increases with size or age. This led him to refute the model of sexual strategy suggested by Charnov (1986) in which sex change is considered not to be evolutionarily stable, under the argument that Charnov's results were due to a restriction of a strategy set he had assumed on his calculation. Charnov (1986) had concluded that when reproductively active individuals suffer a higher mortality than nonreproductive ones, separate sexes can evolve despite a strong sexual difference in the size dependence of fertility. According to Iwasa (1991) evidence for sex changers who have an extended nonreproductive period may be implicit as the "early sex changer" reported for several coral fishes (see Moyer and Zaiser 1984, Hoffman et al. 1985, Aldenhoven 1986). Hoffman et al. (1985), for example, refer that nonreproductive individuals enjoy the benefit of faster

growth by spending more time per day in feeding than reproductively active individuals. Although the two alternative mechanisms to Ghiselin's size advantage model (the mortality-advantage model and the growth-rate-advantage model) had been previously proposed as favouring sex change evolution (e.g. Charnov 1982), Iwasa's innovation was in confirming them by modelling it in protogynous populations. When analysing the cost of reproduction under these models he pointed out that if either mortality or growth rate differ between sexes, natural selection may favour a sexual strategy in which each individual takes the less costly sex (with a lower mortality or higher growth rate) first, and then turns to the more costly sex, even with the size advantage of fertility equal between sexes.

Charnov (1993) proposed fundamental similarities – “life history invariants” – to be a major explanatory ingredient of life history evolution (De Jong 2005). It has been argued that the existence of these invariants implies “symmetry at deeper level of casual factors” molding life-history evolution (Charnov 1993, in De Jong 2005). Potentially, such invariants, along with other dimensionless ratios that can be derived from them, could underpin the form and shape of the tradeoffs that constrain life-history evolution (Nee et al. 2005). Life history invariants therefore, generalize a life history model over a wide range of animal sizes, leading to an understanding of universal life history strategies, and its existence is a major argument for one general theory of life history evolution, rather than a theory as a set of recipes for how to make species-specific models (De Jong 2005).

Life history invariants are canonically identified from a log-log plot of two life history traits involved in a dimensionless ratio being the slope of the plot expected to be equal 1 (De Jong 2005). Considering two life history traits, (*a*) and (*b*), inquiring whether their dimensionless ratio a/b is a life history invariant would imply that: if their ratio is constant (*c*), a log – log plot with $\ln(b)$ on the x axis and $\ln(a)$ on the y axis would show points on a line defined by $\ln(a) = \ln(c) + \ln(b)$, with a slope of 1 and intercept $\ln(c)$. The line is the regression line and the intercept can be used to estimate the life history invariant, (*c*). Such log-log plot of two traits involved in a life history invariant leads to a slope of 1 with all variation in the dependent variable on the y axis explained by the variable on the x axis (that is, $r^2 = 1$ for ideal invariant where r^2 of the regression is the proportion of the variation in the dependent variable (*a*) explained by the variation on the independent variable (*b*), De Jong 2005).

Many such log-log plots of traits that indicate potential life history invariants exist (De Jong 2005). Allsop and West (2003a) presented data on relative body size at sex change for animals ranging from a 2-mm shrimp to a 1.5 –m fish (De Jong 2005). The log-log plot of body size at sex change versus maximum body size showed a slope of 1.05, and all the points were near the regression line, with $r^2 = 0.98$. Although life invariant “relative body size at sex change” seemed perfectly present; Buston et al. (2004) threw a spanner in its research method. Commenting on Allsop and

West's data, these authors pointed out that random distribution of both total body size and size at sex change lead to identical properties in a log-log plot as a life history invariant: a slope of 1 and an r^2 of > 0.95 (De Jong 2005). Nevertheless evidence that previous analyses may have given a false picture and created an illusion of invariants that do not necessarily exist was presented by Nee et al. (2005). Nee et al (2005) described the general rationale of how slopes of 1 at high r^2 arise in log-log plots, independent of the distributions of the traits (De Jong 2005). The pointed culprit was a variable on the y axis that is a fraction of the x- variable: The plot is of $y=cx$, with $c<1$ (De Jong 2005). In a log-log plot of cx versus x , a slope of 1 follows automatically. The above argument applies to a distinct number proposed life-history invariants, where one variable is a simple fraction of the other; for example, size at sex change divided by the maximum body size, or the body size at maturity divided by the maximum asymptotic body size (Nee et a. 2005).

Allsop and West (2003a) collected data on this question and interpreted their log-log plot as showing a life history invariant indicating that a fundamental similarity exists among all animals in the fitness components leading to sex change (De Jong 2005). De Jong (2005) stressed that, looking at specific cases of sex change does not strengthen that impression. The author stated that in both the clown fish and the blue banded goby, an individual's sex is determined by its rank in the social hierarchy. Among clown fish, the largest fish of the group is female, the second largest fish is male, and lower ranking group members are queuing for their turn to reproduce (Buston 2003 in De Jong 2005). Among blue banded gobies, the fish at the top of the hierarchy is male and below him are several breeding females; the group has no non-breeding adults (Rogers et al 2005, in De Jong 2005). In both fish species, the second ranking member in the hierarchy changes sex and starts growing in size when the top brass exits (Buston 2003; Rodgers et al 2005 in De Jong 2005). De Jong (2005) suggested that interesting biology is to identify what determines differences such as those between these two fish species.

Given the clear invalidity of using the regression analysis just described, a major unresolved problem is how we should search for invariants (Nee et al. 2005). One approach that has been suggested is to continue using such analyses, but to compare the r^2 generated by an explicit null model, with that generated by a the actual data (Buston et al. 2004; Gardner et al 2005; Cipriani and Collins 2005; in Nee et al, 2005; and De Jong 2005). In those cases invariance has been accepted if the real data produce an unusually high r^2 (Nee et al. 2005). Nee et al. (2005) suggested that the best way forward in addressing the existence and importance of invariants is to develop procedures to compare the relative variation in the proposed invariant across species to variation in other scale-free, but not necessarily invariant, measures. If proposed invariants can be expressed in terms of other scale-free measurements, randomization procedures can be used to determine whether the observed values of invariant are a particular constrained subset of those that are obtained when the scale-free measurements are allowed to vary independently (Nee et al. 2005).

Application of optimality theory to the evolution of life histories nevertheless has been broadly successful in predicting the conditions favouring sex change, the type of change, and the timing of such changes (Charnov 1982, Hardy 2002, in Collin 2006). Because detailed demographic and breeding data are difficult and time-consuming to obtain, detailed studies of these predictions have usually been limited to single model species (e.g., bluehead wrasse, *Thalassoma bifasciatum*: Warner and Hoffman 1980; Warner 1987; Schultz and Warner 1989, 1990; Warner and Swearer 1991; Warner and Schultz 1992; Peterson et al. 2001; peppermint shrimp, *Lysmata wurdemanni*: Bauer and Holt 1998; Lin and Zhang 2001; Bauer 2002a,b, 2005; Baldwin and Bauer 2003; Baeza and Bauer 2004, in Collin 2006). Recent theoretical developments have used simplifying assumptions to make general predictions that were applied across disparate taxa which allowed comparative studies to use compilations of published data from distantly related species to test generalizations about patterns of sex change (see Allsop and West 2003a, 2004 in Collin 2006). When strong support for predicted patterns was found, the simplifying assumptions of these studies appear to be justified. Collin (2006) stressed that because these studies have tended to combine a haphazard assortment of disparate taxa with few species drawn from a single genus or family, it is difficult to determine if the commonly observed high levels of variation and deviations from expectations are due to a general failure of the theory, or if there are differences among clades that independently conform to theoretical expectations.

Muñoz and Warner (2003) proposed the “Expected Reproductive Success Threshold” (ERST) model of sex change, and tested some of its predictions using field manipulations of the parrotfish *Sparisoma radians* (Muñoz and Warner 2004). Munday et al. (2006) in their review of sex changing models, recognised it as part of the possible explanations for anomalies not predicted by the traditional size advantage model of sex change stressing that cues arising from local social conditions can shape an individual’s decision to change sex or not, or even to reverse a previous sex change. In its simplest form, the ERST model proposes that in some social systems, an individual can assess its future prospects as a male and as a female, and change sex or not depending on the relative value of those prospects (Warner and Muñoz 2008). Warner and Muñoz (2008) emphasized that the assessment of future prospects is easier to make in social systems where a male mates regularly with the same group of females. A decision to change sex or not by a female will be affected by her fecundity relative to the aggregate fecundity of the other females, and by the probability that some proportion of fertilizations by the male will be lost to interfering males (Warner and Muñoz 2008). The authors suggested that species with a large range of fecundity among females and a high rate of interference from other males in mating should be those in which some large females might forgo the opportunity to change sex.

Clifton and Rogers (2008) criticized the ERST and suggested that an alternative formulation relating fecundity to size reduces the estimated fecundity of large females (Warner and Muñoz

2008). According to Warner and Muñoz (2008) they claim that the fecundity differences and losses to sperm competition in *S. radians* would be insufficient to lead a large female to elect not to change sex upon the loss of a local dominant male and argue that other factors, as differential mortality must be involved.

Warner and Muñoz (2008) defended their position by arguing that a number of key features associated with differential habitat utilization by *S. radians* were not considered by Clifton and Rogers (2008) since previous work was conducted exclusively on sea grass populations. On the other hand they stressed that the original ERST model (Muñoz and Warner 2003) dealt only with a comparison of instantaneous reproductive rates expected for female versus male function, and was intended to demonstrate the potential importance of size-fecundity skew and losses of fertilization to other males as factors that could influence the decision to change sex. Besides, they emphasized that a full decision model should include other elements that affect future prospects, such as differential growth or mortality had already been indicated by them (Muñoz and Warner 2003). Therefore, Warner and Muñoz (2008) rejected the notion that it is unimportant to consider a female's fecundity relative to the fecundity of her potential mates as a male; and the potential losses of fertilizations to other males as suggested by Clifton and Rogers (2008). Nevertheless they were in accordance with the inclusion in models of sex change of vital rates data (such as differential growth and mortality) but stressed the idea that others had made that in the past (see Iwasa 1991; St Mary 1997).

Warner and Muñoz (2008) stated that rather than allowing a mix of general and locally specific responses to shape sex-change decisions, Clifton and Rogers (2008) proposed a return to models in which all responses are based on population-wide distributions of conditions affecting sex change. They suggested that some assessable terms in the model would be more accurately represented by local conditions rather than population averages, as suggested by the ERST model and others. Therefore, a combination of sophisticated modelling, comprehensive field observations, and well-designed experiments (e.g. Collin 1995; Buston 2003; Wittenrich & Munday 2005) will reveal more about the dynamics of sex change than will a return to purely demographic models (Warner and Muñoz 2008). In fact, Munday et al. (2006) had already stressed that a dynamic approach that incorporates local social circumstances has made great strides in explaining the details of sex change.

3.3.2. Why are hermaphrodites not ubiquitous?

To explain why there are so many species with fixed sex expression the costs accompanying sex change must be pondered. If as previously referred, pressure to be a hermaphrodite can assume such different patterns, which evolutionary constraints prevented those species from becoming

hermaphrodites? Ohno (1967) suggested that vertebrates higher than fishes are not hermaphrodites because of the development of a strong genetic sex-determining mechanism. In any group of animals, sequential hermaphroditism may develop only when the individual carries the genetic capacity to function both as female and as a male, with all the concomitant anatomical, physiological and behavioural details (Warner 1975). In addition, the individual must have the capacity to shift from one sex to another after differentiation has occurred. Such an achievement may only have evolved occasionally in some groups or may have proved too costly in terms of energy output or time lost from reproduction during the change itself. This seems to be the case of the vertebrates higher than fishes.

4. Sex determination and change in molluscs

Sex change, or sequential hermaphroditism, is phylogenetically widespread but uncommon in both the plant and animal kingdoms (reviewed in Policansky 1982). In molluscs, despite its theoretical advantages over gonochorism is not common (Heller 1993) and when it occurs is almost exclusively protandric (except in the bivalve superfamily Galeomatacea where some cases of protogyny are mentioned (see Oldfield 1961, Fretter and Graham 1964). There is evidence of sex change in most groups of invertebrates (see Ghiselin 1987) indicating independent lines of evolution and in molluscs it has been reported among gastropods and bivalves being more common among prosobranchs (Heller 1993). Nevertheless, alternating sexuality was reported in many oyster species (Coe 1943, Sastry 1979, Mackie 1984), where individuals change sex back and forth several to many times. Because sex change has evolved independently under many disparate circumstances (Ghiselin 1987), life-history theory has sought to determine the conditions for sex change to be favoured over simultaneous hermaphroditism and dioecy (e.g. Ghiselin 1969).

The sex change phenomenon has received particular attention in prosobranch gastropods of the genus *Crepidula*. However, despite a strong theoretical framework and many testable models (e.g. Charnov 1982) earlier research just speculated on the advantages of sex change for specific groups and provided little real understanding. As previously alluded a commonly invoked explanation of sex change is the size (age) advantage hypothesis (e.g. Ghiselin 1969, Charnov 1982, Warner 1988). An alternative hypothesis is that sex change is independent of size and acts to correct locally skewed sex ratio (the sampling error hypothesis in Ghiselin 1969). The former is well accepted to explain protandry in limpets (Ghiselin 1969, 1987; Warner 1975, 1978, 1988; Hoagland 1978; Wright 1988; Collin 1995). Charnov's (1982) review of sex allocation theory presents quantitative models of the optimal size at sex change based on the size-advantage hypothesis. According to him the size at sex change maximizing an individual's life time reproductive output is the size at which the number of offspring an individual could produce by acting as the second sex, exceeds the one produced by acting as the first sex. This is true in many molluscs where female fecundity is related to large body size, but male fecundity is related to mobility and therefore often with small size (Hoagland 1978). Thus, to maximize its lifetime reproductive success, an individual should begin life as the sex with the least increase of reproductive output with size and subsequently change to the other sex. Genetic and environmental determination on the timing for this sex shift is outlined by several authors (e.g. Orton 1927b; Coe 1935, 1953; Bacci 1951, 1955; Ghiselin 1969; Hoagland 1978; Collin 1995; Warner et al. 1996; Hobbday and Riser 1998).

In this section the most revealing work on sequential hermaphroditism will be reviewed with emphasis on the Calyptraeidae, Patellidae and Lottiidae gastropod families. The proximal

(ecological) and evolutionary (adaptive) questions raised from those studies will be integrated in the final discussion.

4.1 The research in *Crepidula* spp.: factors influencing its sex change

Environmentally-mediated sex determination (or labile sex determination) in the Calyptraeidae was demonstrated early last the century. Many authors (e.g. Gould 1917a,b; Ishiki 1936; Coe 1944) reported members of the genus *Crepidula* which are influenced physiologically and behaviourally by conspecifics. In *Crepidula fornicata* and *Crepidula plana*, the protandrous males are not only attracted to females but attain the male phase earlier and remain as males longer when in contact with females (Coe 1953). Hoagland (1978), who worked extensively on comparative life histories, has suggested that within the genus *Crepidula*, sex change via environmental determination is correlated with the mode of larval development and dispersal, and with substratum constraints. She stated that species with planktonic larval development and that are rarely substratum-limited exhibit labile sex determination and have socially influenced sex ratios. Their gregarious behaviour and female-induced delay of sex change appear to be mediated by pheromones, while species lacking planktonic larvae also lack gregarious behaviour, and sex change and sex ratio are independent of influence by other members of the species (Hoagland 1978). She came to this conclusion by comparing reproductive and sexual characteristics, group behaviour and ecological data of four species of *Crepidula* (*C. fornicata*, *C. onyx*, *C. plana* and *C. convexa*). All are protandrous, low in mobility and have two different ways of larval development and dispersal. In this genus some species disperse as young adults after metamorphosis within a brooded egg sac (e.g. *C. convexa* and *C. norrisiarum*), while others release planktonic larvae (e.g. *C. fornicata*, *C. onyx* and *C. plana*). Hoagland (1978) hypothesised that complementing these different reproductive and dispersal mechanisms this genus might have evolved distinct mechanisms of sex change and sexual behaviour. Several experiments were set up in the laboratory and in the field to quantify species-level differences in sexual behaviour such as gregariousness, degree of mobility of young males and permanence of mating pairs. Size and timing of sex change were addressed by comparing isolated and crowded conditions responses. Some points of her paper are presented next.

When describing species life-history she referred that *C. fornicata* and *C. onyx* have planktotrophic larval development preceded by a period of brooded development and form large cluster or stacks of individuals. *C. plana* has a similar larval development but lives inside empty shells or under stones and never forms stacks although several males might live attached to one female. These three species have sedentary females capable of limited movements but young juveniles and males with some mobility on hard substratum. *C. convexa* forms temporary male-female pairs, but not clusters and broods its young through metamorphosis. It is the smallest and most motile of the four (Hoagland 1978). From the experimental work the author concluded that *C. fornicata* and *C.*

onyx have the highest degree of gregariousness while *C. convexa* is the least gregarious. When testing whether environmental determination of sex change could indirectly affect the sex ratio of a population relative to its density Hoagland (1978) found differences between species. Sexuality of an individual *C. convexa* was not influenced by the biotic environment (density and sex ratio) since regardless the distinct tested conditions the final sex ratio was close to 0.5 and most individuals become female. In *C. fornicata* higher percentage of males were found in several dense natural populations and laboratory experiments which suggested a somehow correlation. Indeed in this species, size and age of mated individuals were invariably greater, showing that mated males delay sex change reflecting interaction among individuals as reported by many other authors (e.g. Gould 1917a, 1952; Coe 1938a).

With respect to the relationship between population density and size at sex change Hoagland (1978) found that for *C. convexa* size at sex change is inversely related to population and female density. So, dense crowded populations such as those living on *Zostera* sp. matured at small size. The same inverse correlation was exhibited by *C. onyx* and *C. plana*. In *C. fornicata* the general trend is for size at sex change to increase with population density (Hoagland 1978).

The small size at sex change attained by *C. convexa* snails under crowded conditions might have occurred by slower growth due to competition for food or physical contact among individuals although timing of sex change was not affected (Hoagland 1978). She suggested that in *C. convexa* timing of sex change is genetically programmed since revealed a lack of biotic influence. This would result in a sex ratio determined by age structure and genetic composition of a population rather than by group interaction (Hoagland 1978). The behaviour and sexuality of *Crepidula fornicata* are highly integrated among individuals of a population and individuals influence the settling behaviour and maturation rates of others (Hoagland 1978). Indeed, size and age at sex change were variable among *C. fornicata* populations studied. In non-colonizing populations, both size and age at sex change tended to increase with population density while data for new settlements where few females are present provided evidence for the occurrence of sex change at a very small size and young age despite the high density. On the other hand, an established population with only moderate density but a high proportion of females had a greater size at sex change than could be predicted by total density alone (Hoagland 1978). Hence, the density of females was implied on the delay of timing for sex change in this species supporting the female pheromone hypothesis.

From this Hoagland (1978) concluded that the sexual behaviour patterns of *C. convexa* and *C. fornicata* were representative of patterns in non-planktonic and planktonic developers, respectively, of the family Calyptraeidae. She found support to its conclusions in Coe's work (1953) with other species (*C. norrisiarum*, *C. adunca* and *C. williamsi*) which corresponded to *C. convexa* in mode of larval development and dispersal. Coe (1953) reported that in these species mating is temporary,

males leave females at the time of their sex change thereby precluding stack formation and protandrous sexual development is not environmentally regulated (but see Warner et al. 1996 and Hobday and Riser 1998).

The data available for *C. onyx* indicated that it is similar to *C. fornicata* in essential features of labile sexuality and gregariousness as well as in reproductive and dispersal modes and ability to form stacks. *C. plana*, in turn, substantiated Hoagland's hypothesis that the mode of reproduction, type of dispersal and degree of substratum limitation interact to determine the optimal sexual behaviour and population structure. It is substratum limited for it lives underneath objects or inside them, does not form stacks and with respect to gregariousness it is intermediate between *C. fornicata* and *C. convexa*. In reproduction and dispersal *C. plana* is like *C. fornicata*, it possess labile sex determination (Gould 1952) and the presence of females delays sex change in males (Coe 1953).

Thus, Hoagland (1978) classified *C. fornicata*, *C. onyx* and *C. plana* as having environmentally determined sex change (which she correlated among other features with planktonic larval development) while *C. convexa* was included in the non-environmental sex determination group.

Although protandry in slipper-limpets of the genus *Crepidula* is a common textbook example of sex change (Barnes et al. 1988, Kozloff 1990, Ruppert and Barnes 1994) it had not been studied within the framework of the sex allocation theory (Charnov 1982) until Collin's (1995) approach. The work was performed in *C. fornicata* which is the ideal species to investigate sex change (Collin 1995): these sedentary animals form semi-permanent stacks in which smaller younger males attach to the shells of larger females. Only the bottom-most male changes sex (Collin 1995), which results in a number of males on top of one to several females. As copulation occurs within a stack those can be viewed as independent mating groups among which the sex ratio, number and size of individuals vary (Collin 1995). Sex change is strongly influenced by an individual association with conspecifics (see Hoagland 1978). Indeed, when females are removed from stacks, single males change sex immediately, while only the bottom-most male of a group changes (Coe 1938a). Females also affect sexual differentiation of juveniles: in the absence of females newly settled juveniles sometimes differentiate directly into females, while in the presence of females they always differentiate as males before becoming female (see Hoagland 1978). Although Hoagland (1978) had suggested that individuals bypassing the functional male phase were explained by environmental influence rather than genetic determinism, Coe (1935, 1938ab) claimed that some individuals of *Crepidula* species are "true males" incapable of change sex.

Based upon this knowledge Collin (1995) developed and tested three models of the optimal size at sex change for *C. fornicata* that combined among-stack variation and the relationship between size and reproductive output. The models differed in how male reproductive output was related to male size and number. One examined the total reproductive success summed for all males from a given

stack ignoring the partitioning of fertilizations among males on a stack. The other examined individual reproductive success and assumed that male mating success is independent of size. The third was similar but assumed that male mating success scales with size. All three models predicted that sex change should occur at smaller sizes than observed in the field. Delaying sex change is expected if a loss of immediate reproductive output, replaced for greater reproductive output in life, increases lifetime fitness (Charnov 1982, Iwasa 1991). Such delays might increase the fitness of an individual animal because sacrificing immediate reproductive output might allow it to grow faster and reach a larger size before becoming female (Collin 1995). So, Collin (1995) reasoned that those results suggested advantages of remaining male resulting from factors not considered on the models such as differences between male and female growth. Differences in growth rates as Iwasa (1991) suggested can result in selection for sex change even in the absence of sex-specific relationships between size and reproductive output. Thus, being sure that her models omitted several pertinent factors to sex change (as the accurate relationship between male size and reproductive output, potential costs of sex change and seasonality of reproduction), Collin (1995) concluded that quantitative models with simplistic assumptions about growth, mortality, and reproductive output may not adequately reflect the tradeoffs involved in sex change. Hence, efforts must be made to include relevant data with as much precision as possible. Collin (1995) also recommended that, as for sedentary animals with environmentally mediated sex determination the optimal size at sex change is probably determined by local conditions in patches. Modelling it should focus on stimuli from that immediate environment rather than from factors that reflect distant conditions.

In line with the size-advantage model (Ghiselin 1969) is the idea that the advantages of being a male or female should depend on the size of the individual relative to others in the local mating group (Wright 1988). For example, it may profit even a small individual to function as a female if it is the largest in the group; conversely, a large individual could benefit by staying a male if there are yet larger females available to inseminate. This idea was investigated by Warner et al. (1996) in a combination of field collections and laboratory experiments which revealed that sex change in *Crepidula norrisiarum* is strongly affected by the size of the individual and the composition of the local group. This species occurs in discrete groups on the shells of individual *Norrisia norrisia* an herbivorous snail from the intertidal and subtidal areas of North America. It feeds in plankton, mating takes place on the host snail through direct copulation (Coe 1953) and young are brooded through complete development in encapsulated eggs attached to the substratum and disperse by crawling away from the host (Coe 1949, Hoagland 1977). The laboratory experiments were set in order to overcome the fact that several factors such as dispersal, delay or acceleration of sex change and differential growth, interact in complex ways obscuring the causes of the size-structure of natural occurring groups (Sadovy and Shapiro 1987, Collin 1995). Since the largest solitary male found was about 16 mm in length they assumed that individuals not influenced by social conditions tended to change sex when that length was reached. Large males were used to determine the

circumstances under which social conditions delayed sex change. Each individual of the both types (large-delay and small-not delay) was placed into the following treatments: 1-alone, 2-with relatively smaller males and no larger individuals, 3-with a larger female and no other individual, 4-with five smaller empty limpet shells glued to the host shell to test whether the presence of live individuals was necessary to induce sex change. In accordance with the predictions, males changed sex when they became the largest member of a group regardless of their initial size. Individuals changed sex faster in the presence of smaller males than when alone which was proposed as a response to the potential for competition for dominance status among the larger individuals in the local population. As Warner et al. (1996) reasoned that if being a large female conveys high fitness, and if females can suppress sex change of smaller individuals, then a rapid conversion to female may be favoured. This was also suggested as the reason why smaller males changed sex faster than larger ones for example in colonizing groups of smaller individuals. Warner et al. (1996) reported sex-change in individuals that had significantly higher growth rates (see Iwasa 1991) and comparisons among groups (non-sex-changing males in groups v.s. non-sex-changing solitary males and sex-changed solitary males v.s. sex-changed males in groups of smaller individuals) suggested that growth differences are conditional and not simply due to the presence of feeding competition from larger individuals. Hence, they concluded that in this species rapid growth was almost invariably associated with sex change. As possible adaptive reasons for remaining small as a male they referred the ability to emigrate if the local social group conveys low fitness which might be facilitated been small (Charnov 1982). On the other hand if a male opted to stay in a local group, remaining small was postulated still being adaptive. Since male fitness depends on the fecundity of its mates, and female fecundity depends on the size, males may actually profit more by increasing the growth rates of their mates than by growing themselves (Warner et al. 1996).

Work with *Crepidula* (Hoagland 1978, Charnov 1982) divided the species in two groups: Large species (e.g. *C. fornicata* and *C. onyx*) that have planktonic larvae, exist in stacks and have strong social control of sex change. Small species which brood their young (e.g. *C. convexa* and *C. adunca*), do not form permanent stacks and sex change is relative insensitive to social context. *C. norrisiarum* does not clearly fall into either group yet it shows strong social control of sex change (Warner et al. 1996). It is relatively large in size, does not have planktonic larvae and does not form stacks, albeit individuals tend to remain associated in small groups because of the limited substratum on the host. This led Warner et al. (1996) to question Hoagland (1978) conclusions. As Wright (1988) suggested a highly mobile adult stage would reduce the variation in local demography and consequently reduce the selective advantage of socially mediated sex change; this may be the case of the smaller non-stacking species of *Crepidula* (Warner et al. 1996). Hence, they suggested that in gastropods the presence of relatively permanent small local mating populations is the critical factor associated with social control of sex change, rather than mode of larval development and dispersal. In this view, determining the boundaries of those local mating

groups would be a first step to a better understanding of sex change modulation especially in the patellids.

An alternative to increasing fitness via sex change in *C. norrisiarum* was explored by Hobday and Riser (1998): he proposed the increase in fitness due to individuals changing its social situation. Being aware that *C. norrisiarum* can change its social condition through movement (e.g. Warner et al. 1996), experiments were made to assess the ability of this species to move as a function of size and sex. They observed that *C. norrisiarum* of all sizes and both sexes have some ability to reattach to an host if separated, but only males move on and between hosts. Most of those movements resulted in a increase in reproductive potential (Hobday and Riser 1998). Thus, they concluded that if the immediate cost of changing sex is large in terms of time or energy, the choice to move may represent a reduction in terms of time or energy. This is true until those individuals change sex which is inevitable (Hobday and Riser 1998). Immediate benefits from moving occur when males find an available female in the proximity. Indeed moving males were implicated in several successful fertilizations in *C. fornicata* (Gaffney and McGee 1992 in Hobday and Riser 1998). Transference of *C. norrisiarum* was enhanced in larger hosts, which are older and have longer periods of time to contact other *N. norrisi*. A female *C. norrisiarum* alone on a *N. norrisi* was the most likely social situation to attract a male while males were least likely to move when stacked on a female (Hobday and Riser 1998). In their report Hobday and Riser (1998) stressed the potential problem of using the size and sex of solitary individuals to determine the size at sex change in the absence of social influences (see Warner et al. 1996). As solitary males may have recently moved from conspecifics or had companion males just left, they suggested that much of the variance in the size at sex change may be attributable to the influence of “ghost of *Crepidula* past”. This was pointed out as an outstanding feature to include in models of sex change which consider the size at sex change in varying social conditions, often assumed to be static (e.g. Collin 1995). Hence, they strongly contested the use of “social-free size at sex change” as obtained from field data (see Warner et al. 1996) and recommended the rearing of single individuals in guaranteed isolation.

Recently, Collin (2006) used data from 27 populations of 19 species of calyptraeid gastropods to evaluate three predictions of sex change theory and compare the patterns observed in this monophyletic family to those of a compilation of animals from the literature (Allsop and West 2003a, 2004). The tested predictions were (Collin 2006):

- Sex ratios are biased toward the first sex;
- Size at sex change/maximum size is less variable than expected at random; and
- Intraspecific variation in size at sex change is associated with social environment, such that species with more variation in the social environments to which different individuals

are exposed show more variation in size at sex change than species in which all individuals are exposed to similar social environments.

Having her sample as a model, Collin (2006) also evaluated some of underlying assumptions of the theoretical framework associated with the research mentioned above. Some aspects of her approach are presented next.

At the evolutionary stable size at sex change the adult sex ratio (r ; males/total) is given by the equation (eq. 4 of Charnov and Bull 1989, in Collin 2006):

$$r/(1 - r) = [F/f(t)]/[M/m(t)]$$

Where F is average female fertility, M is average male fertility, $f(t)$ is the fertility of a female at the size of sex change, and $m(t)$ is the fertility of a male at the size of sex change.

If it is assumed that both male and female fitness increase with size (as is often the case in protandry) then $M/m(t) < 1 < F/f(t)$ (eq. 5 of Charnov and Bull 1989, in Collin 2006). As a result $r/(1 - r) > 1$ and therefore sex ratio is expected to be greater than 0.5. For protogyny sex ratio is predicted to be less than 0.5 (Collin 2006). Data from protogynous coral reef fishes show more species and populations with female-biased than male-biased sex ratios, i.e. biased toward the first sex (Charnov 1993, in Collin 2006). In fact a recent comparative analysis of published studies of various marine animals demonstrated that, despite a wide range of sex ratios, protogynous species are generally female biased and that protandrous species are generally male biased (Allsop and West 2004, in Collin 2006).

The life-history invariant approach (Charnov 1993) that has been applied to the study of sex-changing animals (Charnov 1993; Charnov and Skúladóttir 2000; Allsop and West 2003a,b) was also the focus of Collin (2006) attention. Being aware of the controversies underlying the dimensionless approach (see Allsop and West 2003a,b; De Jong 2005; Nee et al. 2005; Munday 2006) on the search for constant relationships between traits that might shed light on underlying organizing principles of evolution, Collin (2006) enquired for the following dimensionless life-history invariants predictions (Charnov and Skúladóttir 2000, in Collin 2006):

$$(\text{size at sex change})/(\text{maximum size}) = \text{constant}$$

$$(\text{age at maturity})/(\text{age at sex change}) = \text{constant}$$

Predictions of sex ratio and life-history invariants are derived using a number of basic simplifying assumptions (Collin 2006). For example, they assume a stable age distribution and equivalent mortality rates for males and females (Charnov 1982, 1986; in Collin 2006). Life-history invariant

theory makes other more complicated assumptions about demographics (e.g., k/M , δ , and ∞M are invariant, where k = von Bertalanffy growth coefficient, M = instantaneous mortality rate, ∞ = age at maturity, and δ = exponential scaling coefficient of male reproductive success with size; Charnov and Skúladóttir 2000, in Collin 2006). The author pointed out that for most groups of sex-changing animals data are not available to test these assumptions since most studies often make other assumptions for which data may more often be available (Collin 2006). According to Collin (2006) the most common assumption is that the individuals sampled represent a single population and that there is a single optimal size at sex change across that population. However sex changing species often have populations with structured mating groups: labrid fishes often form harems, anemone fish live in breeding pairs, and calyptraeid gastropods often form clusters or stacks (Collin 2006). The author stressed that a large sample of individuals, as would be collected to assess population sex ratio, probably represents a pool of several groups. In species with labile sex change, where animals change sex in response to cues from conspecifics, a population sample that includes several groups would be expected to show considerable within-population variation in size at sex change (Collin 2006). In fact as mentioned previously such variation in size at sex change is common among calyptraeid gastropods (see Collin 1995, 2000).

As in latter research (see Charnov 1993; Charnov and Skúladóttir 2000; Allsop and West 2003a,b) the size at which 50% of the animals were female was considered to be the size at sex change (L_{50}) and maximum size was the size of the largest individual in the samples collected. Previous studies seeking to find the invariant relationship between size at sex change and maximum size (L_{50}/L_{max}) have relied on a regression approach (Collin 2006). If the slope of the regression of $\ln(L_{50})$ on $\ln(L_{max})$ is equal to one, the ratio has been considered invariant because the relationship between the two variables is linear (Charnov 1993; Allsop and West 2003a,b). However, the same relationship is expected from random data for data bounded by Y , X , and therefore the recovery of a slope of one is not informative (Cipriani and Collin 2005, in Collin 2006). Cipriani and Collin (2005) suggested that variables with an underlying linear relationship would be expected to produce a regression with a higher r^2 value than random data and that the r^2 rather than the slope of the regression could be used to determine if there is an underlying relationship. This approach may be sensitive to the choice of the null model (Buston et al. 2004), and the regression model varies significantly when the range of the Y -values is smaller than the range in X or when the ranges only partially overlap (Cipriani and Collin 2005, in Collin 2006). To overcome these problems they suggested comparing the variance in the observed L_{50}/L_{max} to the variance of bootstrap datasets generated from the observed values of L_{50} and L_{max} (Cipriani and Collin 2005). Collin (2006) used three different approaches to generate null expectations of the variation in L_{50}/L_{max} .

In order to determine if intraspecific variation in size at sex change is associated with intraspecific variation in social environment Collin (2006) used a correlation approach. The standardized size overlap between males and females was used as a proxy for variation in size at sex change and was calculated for each species as the difference in size between the largest male and smallest female divided by the average size. Variation in social environment was assessed by four different measures because it is unknown how calyptraeids assess their social situation (Collin 2006). The author emphasized that if the stack size is important, then the average number of snails in a stack, the range in stack size, and the standard deviation in stack size, would all reflect different aspects of the social environment.

Because shared evolutionary history may cause close relatives to be more similar than expected, it has become a common practice to search for such phylogenetic effects by using methods such as independent contrasts (Collin 2006). Collin (2006) looked for phylogenetic effects by conducting a phylogenetic generalized least squares analysis with “Continuous” (Pagel 1997, 1999) and using a likelihood ratio test to test for a significant phylogenetic correlation (λ) and by examining the data to determine how the within-species variation compares to variation among species.

From the overall analysis Collin (2006) found that:

- There were no statistically significant phylogenetic correlations for sex ratio, L_{\max} , L_{50} , L_{50}/L_{\max} , or stacking behavior, hence there was little evidence to justify the use of phylogenetically corrected data, and all subsequent analyses treated each species as an independent datapoint.
- Eight populations (seven species including two populations of *C. fornicata*) had a statistically significant male (first sex) bias, and six populations (four species including three populations of *C. dilatata*) had significant female (second sex) bias; examination of all populations or a single population per species showed that sex ratios across calyptraeids are not significantly more biased toward the first sex than expected at random; the range and extremes of observed sex ratios (0.05–0.91) was greater for these calyptraeids than for the 40 species of various protandrous fish and invertebrates included in Allsop and West (2004) review of sex-changing animals (0.11–0.89)
- Logistic regression across all populations showed that sex ratio was significantly associated with proportion stacked ($p < 0.0001$), skew in the size distribution ($p < 0.0001$), mode of development ($p < 0.0001$), L_{50}/L_{\max} ($p < 0.0001$), but not with maximum body size ($p > 0.1$); skew in the size distribution and L_{50}/L_{\max} had the largest effect.; when only a single population for each species was included, sex ratio was significantly associated with proportion stacked, skew in the size distribution, mode of development, L_{50}/L_{\max} and maximum body size ($p < 0.001$).

- Comparisons of the observed data with randomly generated data and the bootstrap datasets made from randomizations of the observed L_{50} and L_{max} values show that the mean of L_{50}/L_{max} for calyptraeids does not differ from the random expectations, but the standard deviation of these values is significantly smaller than expected. In fact, there is a predicted significant difference in L_{50}/L_{max} between protandrous and protogynous species, with protandrous species changing sex at a smaller size relative to their maximum size (protandrous $L_{50}/L_{max} = 0.61$, protogynous $L_{50}/L_{max} = 0.78$, t -test, $p = 0.0001$; see Gardner et al. 2005 for details). There are numerous values of L_{50}/L_{max} for which the confidence intervals do not overlap and in the cases of *C. atrasolea*, *C. dilatata*, and *C. fornicata* the values for different populations are significantly different. Collin (2006) found support for the idea that L_{50}/L_{max} varies within and between calyptraeid species.
- Variation in size at sex change as measured by size overlap between males and females was correlated with social structure. All four variables reflecting intraspecific variation in social experience showed a significant association with this measure of variation in size at sex change when examined independently across all populations (proportion stacked: $r^2 = 0.23$, $p < 0.05$; range in stack size: $r^2 = 0.43$, $p < 0.0005$; average number in a stack: $r^2 = 0.21$, $p < 0.05$; standard deviation of stack size: $r^2 = 0.42$, $p < 0.001$). Multiple regression with stepwise removal of non significant factors showed that only range in stack size had a significant effect in combination with the other factors. When only a single population was included for each species, range in stack size and standard deviation in stack size were the only factors that were significant alone (range in stack size: $r^2 = 0.36$, $p < 0.01$; standard deviation of stack size: $r^2 = 0.42$, $p < 0.05$) and range in stack size was the only significant factor in the multiple regression. Analyses treating stacked and unstacked individuals separately showed that there was often (nine of 20 populations) a significant effect of stacking on sex change. All populations with significant differences showed a larger size at sex change in stacked than in solitary individual animals.

Confronting her findings with the underlying theory, Collin (2006) found that:

- The considerable, unpredicted variation in sex ratio and L_{50}/L_{max} in samples from a single taxon suggested that calyptraeids violate the basic assumptions of sex allocation theory. The theoretical derivation of L_{50}/L_{max} as an invariant relies on the assumptions that k/M , δ , and $\propto M$ are constant across the species examined. The large variation in L_{50}/L_{max} among calyptraeids suggested that at least one of these three values is not constant across species but no real data were available at the time of the study to test this idea. The significant differences in L_{50}/L_{max} between different populations of the same species of calyptraeids suggested that these values may not even be constant across different populations of the same species.

- The prediction that sex ratio should be biased toward the first sex is based on minimal assumptions and is not altered by size-specific mortality or growth rates (Charnov and Bull 1989). The main underlying assumption is that both male and female fertility increases with size (Charnov and Bull 1989). Data on egg production for several species of calyptreids make it clear that female fertility increases with size (e.g., Collin 1995, 2000; Chaparro et al. 1999; Chaparro and Flores 2002). A male-biased sex ratio, consistent with the idea that both sexes have increasing fertility with size, was observed for several calyptreids. The female-biased sex ratios observed for other species imply that male fertility at the size of sex change is less than average male fitness. Therefore, it seemed likely to the author that male fertility decreases with size in these species. Such a decrease could be explained by the difference in mobility between large and small males that has been suggested for some species (Warner et al. 1996). However, the sex ratio data reported indicated that there are differences in the shape of the male fertility curves, providing indirect evidence that one of the basic assumptions of the original theoretical derivation of L_{50}/L_{max} as a life-history invariant is violated. Whatever the mechanism, Collin (2006) stated that the shape of the relationship between male fertility and size probably differs between species, increasing in some and decreasing in others. The predictions of first-sex-biased sex ratios could also be altered by high mortality during the transition phase. This is unlikely to explain the lack of fit for calyptreids, as laboratory studies have not found different mortality rates in transitional animals (Collin et al. 2005).
- The field-collected data presented by Collin (2006) was consistent with previous experimental evidence that showed that associations with conspecifics affect the size at sex change in several calyptreid species (Gould 1917b, 1919, 1952; Coe 1938b 1948b, 1953; Collin 1995, 2000; Warner et al. 1996; Collin et al. 2005). Collin (2006) found out that stacked individuals change sex at larger sizes than solitary animals of the same species, and that sex ratios often differ for stacked and unstacked animals. There was also a correlation between the amount of stacking and variation in size at sex change, with up to 40% of the interspecific variation in variation in the size at sex change being explained by range in stack size. All of these lines of evidence supported the idea that animals in the field experience difference social environments and alter the size at sex change in response to their interactions with conspecifics. Collin (2006) reinforced the idea that population wide analyses may obscure interesting patterns and that analysis on the level of aggregations or stacks would be the best direction for future research on sex change.

Dupon et al. (2006) presented revealing genetic results based on paternity analyses using five microsatellite loci, undertaken in 239 larvae sampled from a set of 18 brooding in *Crepidula fornicata* stacked females sampled in three sites in Brittany, France. Given the genotypes of offspring, their known mothers and the candidate fathers, the paternity was assigned by Dupont et al. (2006) to the most likely father, i.e. the individual with the highest log-likelihood ratio (LOD)

(Meagher 1986) and being the difference between the LOD score of the most likely father and that of the second most likely father statistically significant. Due to the possibility for change of sex in the time interval between copulation and sampling, all the mature individuals of the stack (i.e. males, females and individual in sexual transition), including the mother, were considered by Dupont et al. (2006) on account of the possibility for self-fertilization (as hypothesized by Orton 1950).

The expected male-biased sex ratio in protandrous species (Allsop & West 2004) including *Crepidula fornicata* (Hoagland 1978; Collin 1995) was only observed in Rozegat (Dupont et al. 2006). The authors suggested that the relatively higher number of females in Roscanvel is thus likely to be due to a low recruitment in this population, as revealed by the demographic analysis: the smallest size-class observed in the two other populations was absent in Roscanvel.

Dupont et al. (2006) also observed some variation of size at sex change (L_{50}) across populations. Between Rozegat and Keraliou, this difference was congruent with the difference of mean size of males (5.3 ± 2.1 and 5.7 ± 2.1 cm, respectively), but Roscanvel showed the lower size at sex change together with the higher mean size of males (7.9 ± 2.40 cm) possibly due to low recruitment (and hence lack of small (young) males) in the population and to the strong overlap of male and female size distributions (Dupont et al., 2006).

Of 200 larvae unambiguously assigned, 183 (91.5%) were assigned to an individual belonging to the maternal stack (Dupont et al., 2006). The authors found this pattern in the three Breton populations studied, confirming previous expectations based on observations of copulatory behaviour (Hoagland 1978) and ruled out the hypothesis that mobile males might obtain most of the fertilizations by crawling among stacks (Coe 1936, 1938a; Wilczynski 1955; cited in Dupont et al., 2006). According to Dupont et al. (2006) the true percentage of external fathers might be even lower as the possibility of having lost some 'candidate fathers' from the stack since copulation (for instance during the sampling, i.e. dredging effects) couldn't be excluded.

Seven percent of the larvae were assigned to four individuals located in a side position (out of 37 identified fathers) and three of these were old, i.e. large; one large male, one female and one individual in sexual transition (Dupont et al., 2006). The authors concluded that mobile males in *Crepidula fornicata*, if any, are thus exceptions and mating between individuals within a stack is the rule.

Dupont et al., (2006) stressed the importance of gregariousness in the reproductive success of *C. fornicata* highlighted by their study. Adult aggregations, which occur in numerous gastropod species (reviewed in Baur 1998), may enhance reproductive success in species with internal fertilization and low mobility like *C. fornicata* by enhancing the probability for individuals to meet

and realize mating (Baur 1998, cited in Dupont et al. 2006). In addition, age segregation of sex allows reproduction between different age groups, a pattern favouring outbreeding as well as temporal genetic homogeneity, and this aptitude to find a mate, in such a species with low mobility in the adult phase, is exemplified by a tricking pattern observed within stacks: a significant contribution to paternity by individuals collected as transitional individuals and females in the three study populations (Dupont et al. 2006). These authors referred “bisexuality of the assigned fathers” and “sperm storage by the studied mothers” as possible explanations for such results.

Dupont et al. (2006) considered the former hypothesis (i.e. occurrence of transitory simultaneous hermaphrodites) unlikely based on the following arguments:

- None of the assigned fathers that were females at time of collection exhibited a penis and thus none of them could have been a functional male.
- Although functional hermaphroditism was once suggested to occur exceptionally (Coe 1938b), bisexual individuals have never been documented by histology studies (e.g. see Le Gall 1980; Martin 1985).
- The sequential changes in morphology and anatomy during sex reversal in *C. fornicata* prevent bisexuality (Orton 1909; Coe 1938b; Chipperfield 1951; Martin 1985); for example, the first step is the cytolysis of the spermatogenic tissues; also the distal part of the gonoduct can develop into a prominent uterus with folded walls, into which a number of seminal receptacles opens, only when the penis degenerates, allowing the width of the gonoduct to increase and the inner walls to become folded longitudinally (Orton 1909; Chipperfield 1951).

However evidence has been documented that support the hypothesis of sperm storage, (e.g. Gaffney and McGee 1992) such as histological observations (Martin 1985, J. Richard personal observation; cited in Dupont et a. 2006) and experimental data where Hoagland (1978) asserted that ‘females can store sperm for at least one year’ according to one of her previous study (Hoagland 1975). In their study, Dupont et al. (2006) noted that one of the study mothers also produced a second set of broods after the first hatching indicating that sperm was stored for at least one month.

The authors stressed that even if a rare event of bisexuality was occurring in the study populations, this could not explain the large proportion of assigned fathers (32%, 13% and 35%, respectively, in the Roscanvel, Keraliou and Rozegat populations) that were females at time of collection.

Dupont et al. (2006) concluded that sperm storage was the most likely explanation for the observed results. That some fathers were brooding females at time of collection demonstrated that an individual can effectively reproduce as both a male and a female over a relatively short time interval

and contribute as both father and mother to larvae of a given cohort (Dupont et al. 2006). These authors emphasized that sexual transformation in *Crepidula fornicata* lasts 61 days (Coe 1938b); a much longer time than in many protogynous fish species (Reavis and Grober 1999; Sunobe et al. 2005) but similar to other protandrous species of calyptraeids (Warner et al. 1996; Collin et al. 2005). Egg production lasts 14 days, whereas hatching occurs in a minimum of 21 days (Chipperfield 1951) and consequently, individuals that were both females with eggs and assigned fathers had been males and transmitted sperm at least 54 days before (Dupont et al. 2006).

An outstanding result presented by Dupont et al. (2006) was the level of multiple paternity, which was observed in 14 out of 18 broods. On average, two to three fathers, with a maximum of five fathers, were identified in subsamples of only 11–16 larvae per brood (Dupont et al. 2006). Although investigations of paternity among gastropods have been largely restricted to pulmonates (e.g. Baur 1998) with few studies within marine prosobranchs (Gaffney and McGee 1992; Paterson et al. 2001), multiple paternity has been reported in several gastropod families, suggesting that multiple copulations and fertilizations by different males are common (Dupont et al. 2006). In terms of population effective size, multiple paternity coupled with sex reversal is an advantageous breeding tactic: the increased number of reproducing males and the sex-ratio adjustment both enhance the effective population size (Sugg and Chesser 1994; Martinez et al. 2000 cited in Dupont et al. 2006).

As a result of multiple copulations, sperm from different males may compete to fertilize a single brood (Parker 1970, cited in Dupont et al. 2006) and the occurrence of sperm storage increases the probability of biased paternity. Dupont et al. (2006) stressed that as a result of multiple paternity and sperm storage, male–male competition might indeed occur, and that being the case, paternity is frequently determined by the relative number of competing sperm present from rival males but also by sperm quality (i.e. sperm size, viability and mobility; review in Snook 2005). Indeed Dupont et al. (2006) found a significant size difference between fathers and nonfathers in the three *C. fornicata* populations, and there was still a size difference when comparing only males, suggesting that the most successful males were the largest, a pattern expected in protandrous species exhibiting a sex ratio biased towards the first sex where male fertility is predicted to increase with size (Charnov and Bull 1989).

The study by Munoz and Warner (2003) of protogynous fish showed that social conditions indicative of sperm competition may cause sex reversal to be deferred because intense competition can substantially lower the expected reproductive success of males (Dupont et al. 2006). Dupont et al. (2006) suggested that since sperm competition might influence the timing of sex change, it is likely that population characteristics such as sex ratio and optimal size at sex change vary with the intensity of sperm competition. They defended that their study showed that sex ratio, demographic structure and mating patterns varied across the three study populations,

and that Rozegat displayed the highest incidence of external assigned paternity, the that highest frequency of multiple paternities together with the largest male-biased sex ratio, the largest size at sex change and the lowest proportion of brooding females. In this population, sperm competition should increase as the number of eggs available to fertilize decrease and under the assumption that the intense male–male competition causes sex change to be delayed, Dupont et al. (2006) were not surprised to observe a large optimal size at sex change and a sex ratio largely male-biased in Rozegat population. Conversely, they documented the lowest levels of multiple paternity and external paternity in Roscanvel, for which the smaller size at sex change and a sex ratio close to 1:1 were also noticed.

The fact that mating patterns and gender allocation patterns varied in concert across sites suggests that multiple paternities, reflecting perhaps differential sperm competition intensity, might enhance sex reversal in *C. fornicata* (Dupont et al. 2006). Besides that fact, the benefits of multiple paternity, sex change and social structure combined, had been previously referred as maintaining large genetic diversity as well as large effective size over time in *C. fornicata* populations (Dupont *et al.* 2003, cited in Dupont et al. 2006). On the other hand, Dupont et al. (2006) stressed that the occurrence of male–male competition influencing sex change in protandrous gregarious species might also explain why species-forming mating groups have more variation in size at sex change within a population than solitary species do as suggested by Collin (2006).

4.2 The research in the Patellidae and the Lottidae

Since the early work of Orton (1920, 1928, 1946) and colleagues (Dodd 1956, Orton et al. 1956, Orton and Southward 1961) and cytological work by Bacci (1952, 1965), it has been known that some species of the genus *Patella* are protandrous hermaphrodites. *Patella vulgata* is the sex changer species most widely studied in Europe but some data exist for sex change in *P. caerulea* (Montatenti 1958, Bacci 1965) in the Mediterranean and *Cymbula oculus* (Branch 1974a) in South Africa. In any of the cases knowledge of sex change patterns comes mainly from the interpretation of gender-age/size data from field collections. Indeed, the preponderance of males in smaller size classes and females in the larger ones suggests that other species may also be protandric hermaphrodites. However, it does not prove the existence of protandry. Change of sex ratio with age due to females having faster growth, and/or male's earlier maturation and/or higher mortality rate may produce then same effect (Thompson 1979, Branch 1981). Thompson (1979) noted that *P. ulyssiponensis* in this sense has the appearance of a protandrous hermaphrodite but stressed the idea that conclusive proof of sex change requires that oogenesis be observed in animals known to be or to have been male. Individuals that appear to be functionally male and female simultaneously have been recorded in a number of species including all three British *Patella* spp. (Dodd 1956), but were considered to be unrelated to a change of sex (Branch 1981). In *P. caerulea*

the change from male to female gonad occurs during the resting period (Bacci 1947, Pellegrini 1948). In fact one major difference in the breeding cycle of *P. depressa* compared with the sex changer *P. vulgata* is the absence of a resting period (Orton and Southward 1961). If its absence obstructs *P. depressa* of being a sex changer species is still unknown.

Few reports are known, in this genus of sex change phenomenon being addressed using the experimental approach of the contemporary intertidal ecology. This might have to do with the difficulty of following sex change through an individual's life. Unlike in the genus *Crepidula* there are no visible external sexual characters and removing an individual from the substratum has often great influence on its survival. So, determining the sex in these limpets usually implies killing the animals. To overcome this, Wright and Lindberg (1979) presented a non-fatal method of sex determination for patellacean gastropods. It requires the extraction of a gonad sample by the use of a syringe with a hypodermic needle. The method was developed in *Lottia gigantea* and as critical aspects of the technique they pointed possible damages in other organs and the removal of limpets from the rock which would affect their survival. The application of this method in the *Patella* spp. would allow the design of experiments testing specific possible cues of sex change. However, before its utilization previous studies of its influence upon the biology of the species should be done in order to minimize possible artefacts.

Le Quesne and Hawkins (2006) claimed to have made direct observations of protandrous sex change in *Patella vulgata* following the method of Wright and Lindberg (1979), applied with some the modifications such as the use of sea water as a carrier medium for the small amount of gonadal material collected. In October 2002, 4515 *P. vulgata* were collected from Lynmouth beach (U.K.) measured to the nearest mm and sexed by the standard dissection method (Orton 1928 cited in Le Quesne and Hawkins 2006) in order to determine the likely size-range over which sex change might be observed (Le Quesne and Hawkins 2006). In October 2003, 200 limpets covering the size-range over which sex change was thought likely to occur (15-25mm) were measured to the nearest mm and marked with small plastic numbered discs attached with epoxy resin and their sex determined by taking a biopsy of the gonad with a hypodermic needle inserted into the gonad through the posterior mantle (Le Quesne and Hawkins 2006).

Le Quesne and Hawkins (2006) tested their method by sexing 30 individuals with hypodermic biopsies, and subsequently verifying the sex by the traditional dissection method (Orton 1928 cited in Le Quesne and Hawkins 2006) and referred that the sex matched for all test individuals.

In October 2004 only 37 of the initially 200 marked limpets were found. Le Quesne and Hawkins (2006) suggested that the low number collected could be the result of increased mortality due to the biopsy procedure and tag loss, in addition to natural mortality. From the 37 found limpets, 9 of the 31 limpets (29%) that had initially been male were female at the time of the second

observation, and 1 of the 8 limpets that had initially been female was male at the time of the second observation. The remaining 22 male and 7 female limpets were the same sex at both observations. Le Quesne and Hawkins (2006) concluded from this that *P. vulgata* can undergo a male to female sex change and can be considered a protandrous hermaphrodite and that the single observation of a female becoming male could be the first observation of two-way sex change in the superfamily Patellidae. Although the authors had acknowledged that further research was required to confirm their results the fact that the *in situ* gonadal observations were not confirmed microscopically is in my point of view one of the feebleness of their study. The standard dissection method (Orton 1928 cited in Le Quesne and Hawkins 2006) they used based on colour and gonad texture and the direct observation of the collected gonad tissues with the hypodermic syringe are not 100% accurate and often microscopic observations of gonad material are necessary to clear out uncertainties. Therefore in future studies the application of such methods combined with microscopically confirmation of gonad tissues will be a plus to the progress of the knowledge of sex change phenomenon in *P. vulgata*.

The occurrence of sex change in a giant endemic limpet, *Patella kermadecensis*, from the Kermadec Islands was reported by Creese et al. (1990) who collected individuals three times of the year during 1984 and 1985 at Raoul Island in the Kermadecs group. Creese et al. (1990) found that small limpets were predominantly male, while amongst larger ones about half were female. The presence of hermaphroditism in *P. kermadecensis* was confirmed by microscopic examination of gonad material from over 50 limpets, which revealed a high percentage of individuals with both male and female gametes in their gonads (Creese et al. 1990).

Branch (1981) acknowledged two forms of hermaphrodites in *Cymbula oculus*: transitional and mosaic. The first type had morphologically uniform gonads which were predominantly male, but which had small oocytes scattered throughout and arising from the germinal epithelium. Eventually, all sperm were either shed or reabsorbed, and the limpet became entirely female (Creese et al. 1990). This situation has been reported for *Cymbula oculus* (Branch 1974a) and *P. caerulea* (Bacci 1947). Creese et al. (1990) found a high incidence of this form of hermaphrodite gonad in *Patella kermadecensis* and interpreted it as an intermediate stage in the transformation of a male gonad into a female one (sequential hermaphroditism). Mosaic hermaphrodites, however, contained some patches of gonad that were functional female and some that were functional male (i.e., simultaneous hermaphrodites). This form of hermaphroditism has a small, sporadic incidence in many *Patella* spp. from South Africa (Branch 1981), England (Dodd 1956) and elsewhere, but this is not necessarily related to sex change (Branch 1981). Creese et al. (1990) found only one mosaic hermaphrodite in the samples of *P. kermadecensis*, but argued that this was almost certainly associated with sex change in this case: a transitional gonad in which resorption of sperm had not occurred for some reason and a hermaphroditic condition had been retained. Since the transitional hermaphrodites in *P. kermadecensis* were found at three different times of the year, Creese et al.

(1990) reasoned that sex change was unlikely to be closely linked with spawning. Being that the case, it would represent a different pattern of sex-change from three other well-documented patellid limpets (Branch 1981): in *P. vulgata* the transition takes place in the resting phase between spawnings so that transitional hermaphroditic gonads are never encountered, and in *Cymbula oculus* and *P. caerulea* transitional gonads are only encountered after spawning (Creese et al. 1990).

In line with the size advantage model (Ghiselin 1969, 1974), Creese et al. (1990) postulated that protandric sex change in *P. kermadecensis* is a phenomenon related to a change in habitat from the backs of large shells (limpets less than approximately 50 mm) to bedrock (larger limpets). In fact, *P. kermadecensis* can attain sizes about 155 mm, therefore the relative reproductive advantage for large females is likely to be particularly pronounced. On the other hand, it may be disadvantageous to be a small female because as previously argued it is energetically more expensive to produce eggs than sperm (Brach 1981, Hughes 1986; in Creese et al. 1990). In a situation where young *P. kermadecensis* at the time of sexual maturity (25 to 40 mm) may have limited supply of energy for reproduction by virtue of the limited grazing surface available on adult shells, production of sperm would be favoured (Creese et al. 1990). Creese et al. (1990) stressed that by rapidly and inexpensively developing a male gonad, young individual *P. kermadecensis* could participate in a non-trivial way in at least some spawning activity before making the probably hazardous transition from adult shell to rock surface.

When comparing the pattern of sex change in *P. kermadecensis* with that from other species of limpet, Creese et al. (1990) suggested that a reproductive system consisting of gonochoristic males and sequential hermaphrodites may be particularly advantageous for a large, slow-growing species that has a very restricted geographical range. Although recognizing the need for more sampling, the authors indicated that like *L. gigantea* (Lindberg and Wright 1985, Wright 1989) protandric sex change in *P. kermadecensis* may be at least partly controlled by environmental factors, being unlikely to be just a function of growth, as the sizes of the hermaphroditic limpets varied considerably; between 31 and 143 mm (Creese et al. 1990).

Besides the genus *Patella* and *Cymbula*, sex change is also known to occur in the Lottidae. Indeed the method previously referred enabled Wright (1989) to investigate the effects of intraspecific density and agonistic interactions on the sex change in *L. gigantea*. This species is a large intertidal gastropod, with planktonic larval phases, whose adults graze microalgae from bare rock surfaces, and defend feeding territories from competing herbivores (Galbraith 1965, Stimson 1970). Territory acquisition is accompanied by a marked change in the individual behavioural strategy from an exploitative competitor that flees from conspecific contact (Wright 1989) to an interference competitor, which aggressively defends its territory against other limpets (Stimson 1970, 1973; Wright 1982). Sex change in this species was reported by Wright and Lindberg (1982)

and Lindberg and Wright (1985). The latter suggested that sex change in *L. gigantea* is facilitated by territory acquiring within a two years delay. Indeed several teleost fishes that exhibit both sex change and a territorial ecology, territorial acquisition is closely associated with sex change (e.g. Fishelson 1970, Robertson 1972, Fricke and Fricke 1977, Charnov 1982). Knowing this Wright (1989) attempted to confirm the existence of environmentally mediated sex change in *L. gigantea* and evaluate the relative importance of density *versus* agonistic interaction as ecological cues to sex change. In order to do this he experimentally manipulated the environment of individual males and observed their gender after one year. Initially the experiment was set to vary simultaneously dominance status and intraspecific density. Dominance status was controlled by containing each limpet in a ring with either a larger ringmate or a smaller one. Density was manipulated by containing the two limpets in rings of two different sizes that approximated the natural range of effective densities extremes. Nevertheless, after six months the experiment continued with only the ring size treatment because the author had realised that within three months the smaller limpet of many pairs was found outside the ring presumably due to the territorial effects of the large one. With his one-year field experiment Wright (1989) concluded that limpets in the larger rings (which mimicked low density), were more likely to change sex than those in the small rings (which mimicked high density). In addition, relatively large initial pre-experimental size appeared to enhance the probability of sex change. In an effort to fortify density and initial size effects discovery, gender-age distributions of populations covering a range of intraspecific densities were determined for small populations. Wright (1989) observed that females appeared at the youngest age in the lowest density, as predicted by the density effect discovered in the ring size experiment. However, he mentioned that female age distribution did not rise monotonically with increased intraspecific density. So, some other influence appeared to be acting in addition to intraspecific density and Wright (1989) suggested female variation in the level of aggression, and hence territorial status, as a possibility to be investigated. When estimating the possible additional influence on sex change of dominance status the author observed that more females performed territorial behaviour than males. In contrast evasive behaviour was shown almost exclusively by males. So, Wright (1989) concluded that dominant territorial status might be a good cue for the initiation of sex change since it correlates with an individual's size and therefore egg-producing capacity relative to its neighbours. Pooling his results with those of two previous studies (Wright and Lindberg 1982, Lindberg and Wright 1985) confirmed that sex change in *L. gigantea* is enhanced by low density. This enhancement was observed among the largest members of a local population in the first year of each experiment, while among the smaller members it was delayed until the second or third year which was consistent with the conclusions of Wright and Lindberg (1982).

4.3 Proximal questions on sex change

4.3.1 Genetic versus environmental control

One of the most important questions in understanding the adaptative value of a given pattern of sex allocation is the elucidation of its ecological correlates (Wright 1989). For that it is important to distinguish whether allocation is strictly genetically determined, or whether environmentally mediated phenotypic plasticity “smears” the genetic signal. Coe (1935, 1938ab) claimed that some individuals of every *Crepidula* species are true males incapable of sex change and Hoagland (1978) observed individuals of *C. fornicata* in natural populations and in laboratory experiments that bypassed the male phase. The term “unbalanced hermaphrodite” was used by Bacci (1951, 1955) to describe protandrous animals in which the age at sex change varies genetically which included the genus *Crepidula* and *Patella*.

Apart from this, phenotypically plastic environmentally mediated sex change has been known in gastropod molluscs since early in the century (e.g. Gould 1919, Hoagland 1978, Breyer 1980) and general interest in the phenomenon has arisen as a result of work on sex allocation theory (e.g. Warner et al. 1975, Leigh et al. 1976, Charnov and Bull 1977, Charnov 1982, Policansky 1982, Collin 1995, Warner et al. 1996, Hobday and Riser 1998). It allows an individual of a sex-changing species to enhance the adaptative value of its sex change by responding to local changes on its environment even if the genetic constitution of local individuals reflects a much broader gene pool from a larger range of environments. This is particularly helpful for planktonically dispersed species whose adults are constrained, either by dispersion (i.e., very patchy distributions) or a lack of mobility, to live in small persistent groups as many molluscan species (Ghiselin 1969, Charnov and Bull 1977, Hoagland 1978, Charnov 1982; cited in Wright 1989).

The experiments reported by Wright (1989) confirmed the hypothesis (Lindberg and Wright 1985) that sex change in *Lottia gigantea* can be environmentally mediated. Most accounts of sex change in other patellacean (e.g. Branch 1974b, Webber 1977, Russell-Hunter 1979, Fretter 1984) emphasized the role of polymorphic genetic control of sex change to explain the large overlap in sizes between male and female limpets (Orton 1927b, Bacci 1947, 1965, Montalenti and Bacci 1951). In this view, there would be several genetically determined ages of sex change in a population, including a nonchanging pure-male/female genotype. A pure-male genotype hypothesis (Orton 1928, Orton et al. 1956) predict that relatively older males would consist to a large degree of the pure-male genotype, since most individuals with the sex change genotype would have previously left the male ranks by changing to female (Wright 1989). Thus it would predict the rates of sex change among old males to be relatively lower than that of the intermediate-aged. This was opposite to the pattern found by Wright (1989) where the initially larger males had the highest probability of changing sex. Wright (1989) proposed a less extreme genetic polymorphism in the

age of sex change in *L. gigantea* than had been suggested for other species (e.g. Orton 1927b; Bacci 1947, 1965; Montalenti and Bacci 1951). He stressed that environmental variability might be a more parsimonious explanation of the large overlap in size between males and females rather than genetic determination. However, the latter might support the variation in age of sex change within relatively homogeneous isolated habitats (Wright 1989). Indeed the existence of such a genetically based variation in age of sex change has been proved conclusively for the polychaete *Ophyotrocha puerilis* (Bacci 1978; cited in Wright 1989). Collin (1995) suggested three possible reasons for the variation in size at sex change commonly seen as the overlap of male and female sizes in field studies of several species (e.g Wright 1989, Soong and Chen 1991, Sewell 1994), which are:

- a. Animals may change sex based on cues like age, which may not correlate exactly with size.
- b. Individual genetic variation could cause scatter around the optimal size at sex change. However she stressed that the large degree of variation found in size at sex change when compared to variation in other major life histories traits weaken this hypothesis.
- c. Size at sex change may be environmentally determined by factors that vary spatially within a patchy environment. This was also pointed out by Collin (1995) as the most important explanation for the co-occurrence of males and females over a wide size range in sedentary animals living in a heterogeneous environment such as benthic molluscs. Differences among patches in size distributions, growth rates, mortality rates, or mating opportunities may lead to among patch variation in size at sex change. Combining data from many patches would then result in the apparent overlap of male and female size (Collin 1995).

Further studies, complementing experimental work in the environmental determination of sex, still need to be done investigating the genetic basis for age ranges of sex change and to look for possible pure-male/female genotypes.

Wright (1989) demonstrated that reduced density in *Lottia gigantea* can accelerate sex change. Intraspecific density was proposed as been used by individuals to assess local mortality rate, and a highly valuable cue for the proper timing of sex change. As low density implies high mortality and a reduced number of individuals of the second sex (females), anticipating sex change would grant reproductive advantages to those able of that performance. The author suggested as possible proximal signals of density "sounded" by limpets, the frequency of contact, movement area availability, food intake, gut distension, growth rate and pheromonal information. The latter would involve water-borne chemicals or substances laid on the substratum as limpets move. In fact some mesogastropod limpets of the genus *Crepidula* appear to assess local sex ratio via water-borne pheromones and gauge the proper age of sex change accordingly (Gould 1919, 1952; Coe 1953;

Hoagland 1978). Nonetheless, a pheromone hypothesis is weakened by the results of Wright's (1989) ring-size experiment. He observed that the sex change frequencies in the two ring sizes were enormously different despite sex-ratio equivalence: only males within the rings and equivalent sex ratio outside the rings. This led him to conclude that the density effect could only be supplemented by a sex-ratio pheromone hypothesis. Nevertheless as he stressed the high-energy intertidal area in which *L. gigantea* lives would probably dilute and smear pheromonal signals. Indeed the early experiments in calyptraeid species established that the removal of larger female neighbours induced sex change but the masculinizing effect of associated females although did not require contact, was only obtained when individuals were separated by millimetres in relatively still water (e.g. Gould 1952). Hence, substances imbedded in the mucus of limpets as they move over the substratum might provide another kind of chemical cue for local sex ratios (Wright 1989). Coe (1938a, 1948a) reported starved animals that tended to remain male and Hoagland (1978) referred insufficient stored energy to accomplish the remodelling required to change from male to female as a possible explanation. If higher densities prevent food intake this might be one reason for sex change to be restrained.

Evidence for the environmental regulation of sex change via perception of mortality was revealed several times in *Crepidula* spp. (e.g. Gould 1952, Coe 1953, Hoagland 1978) when removing the largest animals led the younger to change sex earlier (Wright 1988).

4.3.2 The physiological pathway to sex change

Once a sex changer individual had assessed all the cues determining its sex change (whatever they might be) all he has to do is reorganize its reproductive apparatus with the concomitant anatomical and physiological changes. Advances in sex change physiology were mainly achieved by French researchers who removed the undifferentiated juvenile gonad and cultured it in the presence of various hormonal factors, usually derived from one or more pairs of ganglia from the central nervous system (reviewed in LeGall and Streiff 1975, Josse and Geraerts 1983).

In *Crepidula* sp. the cerebral ganglia apparently acts as the central mediator of sex change, sending out a masculinizing hormone to the gonads directly, and to the penis and other accessory sexual organs indirectly via the pedal ganglia. When this signal ceases, the gonads develop oogonia, which can differentiate in the absence of any hormonal factors, and the pedal ganglia stops their secretion of penis-growth hormone (Wright 1988).

The mechanism appears to be different in the patellacean *Patella vulgata* which has very simple reproductive morphology as it is a broadcast spawner. Neither sperms nor eggs can differentiate in an untreated gonad; both require the presence of a common mitogenic factor from the cerebral ganglia (Choquet 1965, 1967, 1971). The sex change switch is apparently not central, but actually

lies in the spermatogonia and oogonia themselves, the latter of which appear to change their sensitivity to the cerebral mitogenic factor as the animal ages. Thus, the mitogenic factor causes the spermatogonia to differentiate and multiply initially, but has no effect on oogonia until they reach a "threshold of responsiveness" later in the life of the animal. After this threshold has been reached, proliferating oogonia exert an inhibitory effect on the spermatogonia, such that sperm production in ensuing years becomes replaced by eggs production. Choquet (1971) has assumed that the sensitivity of the oogonia is ontogenetically set, but hormonal or metabolic modulation of the switch *in vivo* is certainly a possibility (Wright 1988). The demonstration, in the field of a profound environmental influence on the sex change in *Lottia gigantea* (Wright 1989), confirmed that the modulation hypothesis can also be applied to the Patellogastropoda order. Indeed, since field experiments suggested food supply as a possible proximal cue of sex change, experimental manipulations of the metabolic conditions of the gonads *in vitro* might reveal a proximal influence on the sex change schedule (Wright 1988).

Thus, there is a fundamental difference in the site of control of sex change in *Crepidula* spp. and *Patella vulgata* (LeGall and Streiff 1975) which summed to their distinct life-histories and ecology certainly reinforce the idea of an also different causality of sex change in these genera and respective families.

4.4 Evolutionary and adaptative questions about sex change

A successful search for proximal cues inducing sex change does not necessary answer the question of the adaptative value of sex change; neither does it elucidate how protandry evolved. Which potential cues (e.g. food supply, social status, age, growth, size, territory acquisition) may be useful to the individual will depend on how tightly coupled they are to the real evolutionary driving force as well as how easily they can be sensed.

Ghiselin (1969) first proposed his size-advantage model as one explanation of the evolution of protandry in the Mollusca as well as in other phyla. This model when extended by other authors (Warner 1975, Charnov 1982) predicted that when the expected reproductive success (RS, or number of viable offspring produced) of males is less sensitive to size or age than is the RS of females, a protandric mutant would have a reproductive advantage. Ghiselin (1969) further noted that most molluscan protandric species are patchly distributed over space and/or have limited adult mobility. This would tend to select for protandry because males would have limited opportunity for mating (Hoagland 1978, Ghiselin 1969). Another elaboration of the size-advantage hypothesis (Orton 1927a, Tanter 1958, Russell-Hunter 1979, Sastry 1979) stems from the fact that egg and sperm productions have different metabolic requirements. If more energy is required to produce eggs than sperm, then a protandrist which funneled the energy savings of its period as a male into survival or growth, and later took advantage of these savings by changing to female, would have

an advantage over a gonochorist. Iwasa (1991) combined this idea on two alternative models for sex change evolution among animals: the mortality-rate-advantage model and the growth-rate-advantage model. Nevertheless, differential growth was not found in the Patelloidea (Branch 1974b, Daly 1975) and contradictory observations were made in the Calyptraeidae. Although faster growing females were reported for *Crepidula plana* (Gould 1952) and *Crepidula norrisiarum* (Warner et al. 1996), Collin (1995) found higher growth rates in *Crepidula fornicata* males relative to females. Regarding to this, metabolic versions of the size-advantage hypothesis seem to have scant support.

Ghiselin (1969) proposed the sampling error version of his gene-dispersal model as another hypothesis to explain sex change. It states that if individuals of a gonochoric species typically spend most of their lives in small groups such that random variation in gender can significantly skew local sex ratio, then a mutant conditional sex changer that could assess the local sex ratio and choose the locally rare sex (by changing sex or not) would have an advantage. The observation that there are almost no known cases of protogyny (except in the bivalve superfamily Galeomatacea, see Oldfield 1961, Fretter and Graham 1964) among the molluscan sex changers is an indirect evidence against the sampling error hypothesis, which predicts an equal advantage for either kind of sex change (Wright 1988). Nevertheless, the alternating sexuality reported for some oyster species (e.g. Coe 1943, Mackie 1984) allows the maintenance of an open question. Furthermore, the size-advantage and sampling error effects are not mutually exclusive and, for example, may reinforce each other in species whose adults are restricted to small associations as in *Crepidula fornicata* (Wright 1988).

Any hypothesis of the adaptive advantages of a particular sex allocation pattern must make assumptions about the dynamics of fertilization (Wright 1989). Such knowledge is almost nonexistent for patellacean limpets. The absence of copulatory organs suggest that both eggs and sperm are spawned into the sea (Fisher 1904) but sporadic observations suggest that gamete release may be much more precise (Ankel 1936, Von Meden 1945, Picken 1980) and interanimal proximity and relative gamete output may be of great importance (Pennington 1984). Supposing that in a typical gonochoric patellacean with the 1:1 sex ratio, spawning is similar to copulation in ways that might imply the pairing of individuals at some level, then a small male will gain the benefit from proximity to larger individuals, half of which are expected to be female. The fertility of a given small limpet as a male will be related to the fecundity of the neighbouring females, a percentage of which the small male will inseminate. Since the neighbouring females are much larger, and can thus produce many more eggs than a smaller limpet, the small male that fertilizes those eggs will attain greater reproductive success than if he were female. However, if a limpet becomes larger than all his nearby neighbours, his fertility as a male will not reach his potential fertility as an egg producer and he might profit by changing sex. Nevertheless, if animals cannot perceive and respond to the others presence, random local sex ratio variations could swamp out these effects unless the increase in potential gamete output with increasing size is very great (Wright 1989).

Given that a limpet evolved protandry what might be the adaptative value of adjusting the age of sex change to intraspecific density? Once it had been demonstrated that individuals of *Lottia gigantea* in low density situations changed sex more readily than those in high densities, Wright (1989) outlined that estimation of sex ratio by intraspecific density resorted to pheromones, as it occurs in *C. fornicata* (Gould 1952), is ecologically too risky due the turbulent habitat of this species. Hence, he suggested that the connection between density and sex ratio is accessed via mortality. A sex changing population undergoing heavy mortality will have a predominance of the first gender and any individual that can anticipate its age of sex change will be advantaged. Thus low density implies high mortality, a paucity of the second sex and an adaptative edge to individuals that can change sex earlier. High density implies low mortality, a build-up of the second sex and an advantage of delayed age of sex change (Wright 1989). Release from dominance was also pointed as possibly enhancing sex change, i.e. territory acquisition favouring reduction on the age of sex change. He proposed that territorial status as an accurate cue of any given limpet's gamete volume relative to that of its neighbours. Many agonistic losses are a good indicator of much larger, and therefore more fecund neighbours, and thus greater fertility being a male. An extended period of agonistic victories on the other hand would indicate neighbours with a lower egg carrying capacity, circumstances under which greater fertility could be attained by changing to female. This hypothesis implies that non-territorial protandric patellaceans would be less able to accurately assess local conditions and, therefore, should present less variation in the age of sex change (Wright 1989). If this is true for the non-territorial *Cymbula oculus* (Branch 1974ab, 1975b), contradictory data in the quasiterritorial *Patella vulgata* (Ballantine 1961, Charnov 1982, Funke 1968), and the absence of accurate data in the age of sex change in the territorial *Scutellastra longicosta*, *Scutellastra tabularis* and *Patella caerulea* (Bacci 1947) the latter of which social behaviour is unreported, leave the question open to further research.

A common assumption for the evolution of protandry is the pairing of individuals during fertilization (Ghiselin 1969, Warner 1975, Hoagland 1978, Charnov 1982). Although pairing is evident in the many *Crepidula* species, as mentioned above it is still unknown whether it is important during fertilization on patellacean limpets. The possibility of mass spawning in a turbulent environment raises the spectre of close-to-perfect sperm competition (Parker 1970, 1984, Smith 1984) as males shed gametes that are rapidly mixed in the turbulent water column over them. This would result in males gaining fertility in proportion to gamete output exactly as do females, and would imply no differential gain to females of large size. If such fertilization dynamics were found to occur within the patellaceans a very different hypothesis to explain the evolution of protandry would be required, such as a metabolic hypothesis involving the size-specific cost of producing sperm versus eggs (Tranter 1958; Russell-Hunter 1979, Russel-Hunter and McMahon 1975, 1976; Sastry 1979; in Wright 1989).

In summary, this debate about the possible pathway for the evolution of protandry in the Patellacea depends on there being some level of pairing during spawning. Protandry with a sex change at a single age would evolve in species whose size and shape allowed an exponential increase in gamete output with increasing size, an increase that would rarely be swamped out by local variation in sex ratio (Wright 1989). To the extent that local density reflected mortality rate, limpets that used density as a sex change cue might have evolved and those with a territorial ecology would even further fine-tune the optimal timing of sex change to local conditions. Intraspecific density might be assessed through intraspecific contact frequency or food abundance, the latter of which could be coded as growth. In turn, this view also raises the additional (not mutually exclusive) possibility that other protandrous limpet species, with neither a linkage between density and mortality, nor a territorial system with which to measure the relative sizes of neighbours, might be unable to assess important environmental changes that impinge on the proper age of sex change. However, different regulation systems might be revealed by further research since many available data are questionable.

Given that most bivalves and gastropod are gonochoric why there are not many more protandric species since a patchy relative sessile way of life is common in these two classes? And, why some genera present both dioecious and sex-changing species?

Many of the proposed reasons lay on the context of sex change in the animal and plant kingdoms (Charnov 1982, Polikansky 1982). When applied to molluscs these include phylogenetic constraints and evolutionary history that do not allow protandry even when it seems to give an advantage. However, if it had occurred even in species of the same genus that contain the two types of sexuality, nothing in their present ecology suggests which specific constraints and evolutionary history consummated it. Indeed the cost of changing sex might be too great to some species than others. Wright (1988) emphasised that the knowledge about ecological dynamics of fertilization and paternity in the molluscs is not enough to link a sessile habit with male reproductive success as been less sensitive to size than that of females. Since this has been a common practice in several proposed models (Ghiselin 1969, Warner 1975, Charnov 1982, Iwasa 1991) the author pointed out the significance of elucidating the ecology of fertilization among sex changers and closely related dioecious species in the understanding of sex change evolution and sex allocation theory research.

From those species that change sex which should evolve environmentally mediated sex change?

Since the ecology of some species results in substantial variation in local demographic conditions, those are the species that should show environmentally mediated sex change (see Hoagland 1978, Bull 1981, Charnov 1982, Charnov and Bull 1977). Hoagland (1978) noted that local age structure of populations of *Crepidula* that recruited from a planktonic larval pool were inherently more variable than that of populations whose recruits come from a benthic larval pool. She found, as

expected, that species with planktonic larvae showed a marked tendency to respond to experimentally induced changes in demography, while species with benthic larvae were much less sensitive to it (but see Warner et al. 1996). Consistent with these predictions *Epitonium tinctum* (Breyer 1980) and *Lottia gigantea* (Lindberg and Wright 1985, Wright 1989) have been shown to change sex in response to environmental change. Both species live in persistent small constrained groups, and recruitment is from a planktonic larval pool. Nonetheless, demographic local conditions were inferred in the timing of sex change in *Crepidula norrisium* (Warner et al. 1996, Hobday and Riser 1998) which does not have planktonic larvae. On the other hand *Cymbula oculus* which has planktonic larval recruitment, shows a narrowly constrained sex change (Branch 1974a) which implies, but not proves, relatively low environmental sensibility to the age of sex change (Wright 1988). Wright (1988) suggested that behavioural differences between *Cymbula oculus* and *L. gigantea*, such as in intensity of migration, could explain the absence of variation in the timing of sex change in the former species: possibly dispersal of adults in *Cymbula oculus* greatly dampen local demographic variation preventing its influence.

This reinforces the idea that the adaptative value of sex change in any species cannot be fully understood without some idea of the proximate factors that affect its sex change and the rules that govern when an animal changes sex (Wright 1989, Collin 1995).

5. Recent insights on the sex change strategies in animals

Munday et al. (2006) reviewed empirical advances up to their paper publication, on the understanding of why and when sex change occurs. They presented other documented examples related to the diversity of sex-changing animals strategies to increase their reproductive success than those previously recognized and mentioned in this dissertation. Munday et al. (2006) stated that some individuals change sex early, others change sex late, some individuals change sex more than once, and others do not change sex at all. They have also shown that variation in the timing of sex change among species, populations and individuals can often be traced back to differences in the structure of the local mating group (Munday et al. 2006). In line with the previous research presented here the authors concluded that their findings contrasted those of Allsop and West (2003a) and Gardner et al. (2005) in the sense of the latest proposed that sex change occurs at the same relative size across species. Munday et al. (2006) strongly stressed the idea, already mentioned by other authors, that understanding the scale at which individuals gather information on reproductive value (RV) is crucial to our understanding of the selective advantage of sex change. The main trends identified by these authors are presented next.

5.1 The mating system and variation in the timing of sex change

Empirical evidence suggests that the mating system, or mating group structure, influences the relative timing of sex change between species (Munday et al. 2006). The authors mentioned Collin (2006) results presented previously, where large amount of the variation in size at sex change among 19 species of the calyptraeids was associated with variation in mating group structure. This indicated that variation in social structure can indeed drive differences in the timing of sex change between species (Munday et al. 2006).

5.2 Between-population variation in the timing of sex change

The size advantage model predicts that sex change will occur earlier in populations with slower growth rates and/or higher mortality rates (Warner 1988, in Munday et al. 2006). This pattern has been documented in a range of protogynous fishes (Cowen 1990, Buxton 1993, Gust 2004) and protandrous shrimp and fish species (Taylor et al. 2000, Charnov and Hannah 2002) and further analyses are uncovering the mechanisms responsible for this variation (Munday et al. 2006). The mean age at sex change and demographic rates co-vary in genetically connected populations of fishes (Gust et al. 2002, Gust 2004) and shrimp (Charnov and Hannah 2002), indicating that the timing of sex change in these populations is a flexible response by individuals to local conditions. This conclusion is supported by the relatively rapid changes (within a few years and less than one

generation) in the age or size at sex change in some fish populations following a change in the mortality rate (Hawkins and Roberts 2003, in Munday et al. 2006). Nevertheless, evidences have been found that suggest that other species have a relatively fixed size at sex change. In at least one protandrous shrimp (Bergström 1997), one protandrous limpet (Branch and Odendaal 2003) and some large protogynous fishes (Petersen and Warner 2002) there was no variation in size at sex change despite considerable shortterm variation in age structure and mortality rates within and between populations (Munday et al. 2006).

5.3 No sex change

In harem species, the largest female in a group usually changes sex following the disappearance of the dominant male (Warner 1988, 1984; Ross 1990; Kuwamura and Nakashima 1998) been able to increase its reproductive value by spawning with all the remaining females (Munday et al. 2006). Some females even change harems to advance their position in the size hierarchy, and in doing so increase their reproductive value because they end up monopolizing a harem sooner (Sakai et al 2001, in Munday et al. 2006). Similarly, in species that exhibit resource defence polygyny, a large female will usually change sex following the disappearance of a territorial male (Warner and Swearer 1991, in Munday et al. 2006). Territorial males can spawn with 50 females per day (Warner and Hoffman 1980), so the advantage to change sex when a dominant position becomes available can be significant (Munday et al. 2006). Nevertheless, not always the largest female changes sex following the disappearance of a dominant male though it appears non-adaptive, because the largest female could spawn with all the remaining females if she did change sex. A recent modification of the size advantage model (Muñoz and Warner 2003) demonstrated that the largest female in a group would not increase her reproductive value by changing sex if the combined fecundity of the other females in the group is less than her current fecundity, and/or if sperm competition was intense (Munday et al. 2006). Under these circumstances, the larger individual would do better to remain female and one of the smaller females might gain the most by changing sex (Munday et al. 2006). These predictions were borne out in a manipulative experiment with the bucktooth parrotfish *Sparisoma radians*, where the largest female usually declined to change sex following the removal of the dominant male, and it was one of the smaller females that became male (Muñoz and Warner 2003, in Munday et al. 2006).

5.4 Early and late sex change

There are reports that females sometimes change sex at a size where they appear to have little chance of breeding as a male (early sex change) and thus experience no immediate gain in reproductive success (Moyer and Zaiser 1984, Hoffman et al. 1985; in Munday et al. 2006). As

previously refereed, early sex change might be a viable alternative strategy if non-reproductives have decreased mortality rates or increased growth rates (Iwasa 1991), so that a small decrease in current reproductive success results in a much larger gain in reproductive value (Munday et al. 2006). In fact, early sex change in the spotlight parrotfish *Sparisoma viride* appears to be favoured because non-reproductive males grow faster than do territorial males (van Rooij et al. 1995) and do not appear to suffer markedly higher mortality rates (van Rooij and Videler 1997).

5.5 Repetitive sex change

The occurrence of repetitive sex change in some invertebrates has been recognised since the 80's (Charnov 1982, Polikansky 1982) but most of the knowledge related to its occurrence in vertebrates comes from studies later in the 90's and at the beginning of this century. From those studies prompted an increasing list of fish species in which multiple sex reversals can occur (see Kuwamura and Nakashima 1998, Sakai et al. 2003, Liu and Sadovy 2004, Wittenrich and Munday 2005) and by the recognition of ecological conditions that favour repetitive sex change (Nakashima et al. 1995, Munday 2002). The polychaete worm *Ophryotrocha puerilis* forms breeding pairs: in each pair, the largest individual functions as a female, enabling individuals to take advantage of the female size–fecundity relationship (Munday et al. 2006). Males grow more quickly than do females and reciprocal sex change occurs when the male becomes larger than the female (Munday et al. 2006). Monogamous coral gobies also exhibit repetitive sex change, but the benefit of this strategy is not related to the relative size of individuals in the pair, and reciprocal sex change has not been observed. In coral gobies, when one of the breeding pair dies (or the host coral dies) the survivor might move to find a new partner (Munday et al. 2006). Searching for a new mate is, however, risky and when new partnerships form, one individual will change sex if the new partners are the same sex (Nakashima et al. 1995, Munday 2002; in Munday et al. 2006). Thus the advantage of bidirectional sex change is that an individual maximizes its reproductive value by searching as little as possible for a new mate (Nakashima et al. 1995, Munday 2002; in Munday et al. 2006).

These different strategies can be unified by the principle that individuals change sex when it increases their reproductive value (Munday et al. 2006). The breeding tactics (male, female or non-breeder) adopted by individuals often appear to be adaptive responses to their own social – ecological context and variation in these conditions results in significant differences in the timing of sex change within and between species (Munday et al. 2006).

6. Proximal cues and variation in the timing of sex change: snapshot of present and future insights

Empirical studies have revealed surprising variation in the timing of sex change within and between species (Munday et al. 2006). That individuals generally change sex when it increases their RV is a certain, but what cues do they use to assess the RV of being male or female, and how does this influence variation in the timing of sex change (Munday et al. 2006)? Munday et al. (2006) referred experiments that have confirmed that timing of sex change is sensitive to:

- Immediate social environment, some of which were detailed presented in previous sections, in limpets (Warner et al. 1996, Collin 2005), snails (Chen et al. 2004), shrimp (Baeza and Bauer 2004) and many species of fish (Warner 1984, Shapiro 1987, Ross 1990, Kuwamura and Nakashima 1998)
- Size of an individual relative to others in the social group (Warner and Swearer 1991, Warner et al. 1996, Buston 2003)
- Sex ratio of the social group (Shapiro 1984, Munday 2002, Collin et al. 2005)
- Local density (Wright 1989, Lutensky 1994)

If each of these factors can provide reliable information on the probable success of an individual as one sex or the other, selection could have favoured simple decision rules for sex change, based on a single cue such as relative size, sex ratio, or density, if one cue is a reliable predictor of sex specific RV (Munday et al. 2006). For example, anemonefish have highly predictable social hierarchies, where changing sex to female is advantageous when a male becomes the largest individual in a social group (Munday et al. 2006). On the other hand the authors mention that selection could also have favoured a more complex decision algorithm based on multiple cues, if several factors have a major influence on sex-specific RV. In the basslet *Pseudanthias squamipinnis*, the largest individual adopts male sex in small groups, but as group size grows (and sex ratio becomes female biased) additional individuals might benefit from becoming male where relative size and sex ratio act as cues to sex change (Shapiro 1984, cited in Munday et al. 2006) Munday et al. (2006) stressed that as the social situations that individuals encounter become more varied, selection might favour more complex algorithms governing the timing of sex change, and variation in the cues that individuals use to make decisions about sex change would influence variation in the timing of sex change, within and among populations.

Munday et al. (2006) predicted that the greatest variation should occur in populations where the size structure, sex ratio, or density of local social groups varies greatly, since the simplest algorithms about when to change sex can generate significant variation in the timing of sex change if there is variation in the structure of local social groups. Elucidating the proximal mechanisms that

control sex change is important because these mechanisms indicate the scale at which individuals are assessing RV (Munday et al. 2006). In many species, the timing of sex change varies at the scale of local social groups, and this is the scale at which we should examine the selective advantage of sex change; whilst some other species appear to have a relatively fixed size at sex change, and in these species the advantage of sex change could be examined at the population level (Munday et al. 2006). Being aware that other species are likely to exhibit interactions between genetic and environmental sex determination, Munday et al. (2006) indicated that their study would provide a deeper understanding of the multiple scales over which factors influencing RV can operate. The authors reinforced the idea that understanding the benefits of sex change to individuals is the key to understanding its adaptive significance and that it will require detailed information about sex-specific fecundity, growth, mortality and movement patterns at the individual level. Munday et al. (2006) suggested that the application of a multiplayer game theoretic approach tools (Alonzo 2002, cited in Munday et al. 2006) could be useful for modelling the advantage of sex change in species where this trait is under social control, and compare the RV of different breeding tactics for a range of different social and ecological conditions.

7. Effects of human exploitation upon gonochoric and sequential hermaphroditic prey - the role of marine protected areas

The effects of human foraging activities upon target populations have been enquired by comparing exploited and non-exploited populations been the latest assigned Marine Protected Areas (MPAs) or areas with restricted access. Lasiak (1993) described the temporal and spatial variations in the density, size composition and growth of exploited and non-exploited populations of the intertidal limpet *Cellana capensis* from the Transkei coast of southern Africa, between 1989 and 1991. The timing and intensity of recruitment varied between shores and amongst sites within shore, and recruitment success was relatively poor and, not even recorded at some of the protected sites until July 1991 (Lasiak 1993). Juvenile and adult limpets were both significantly more abundant at the exploited than at the protected sites although limpets at the exploited sites tended to be much smaller than those found at the protected sites (Lasiak 1993). Lasiak (1993) suggested that the gradual decline in density of limpets observed at the protected sites reflected poor recruitment as well as senescence of older individuals. The absence of significant differences in growth rate amongst shores suggested that differences in the intensity of competitive interactions may have been offset by the higher microalgal production at the exploited shore and that upshore migration of larger individuals may counter the intense competition experienced at mid-tidal levels on the exploited shore (Lasiak 1993). Differences in the rate of recruitment could also account for some of the observed differences in population characteristics (Lasiak 1993). The author concluded that both of these explanations could be viable alternatives to an earlier proposal which attributed differences solely to the effects of size-selective predation by man.

Kido and Murray (2003) studied *Lottia gigantea* population structures, growth rates, and gonadal production at 8 southern California sites, 4 of which were longstanding MPAs. The authors reported that greater mean sizes and higher frequencies of larger individuals occurred at sites with lowest human visitation and mean *L. gigantea* shell lengths were negatively correlated with the number of visitors and collectors. The presence of collectors, small mean shell lengths, and the absence of large limpets indicated that *L. gigantea* populations were affected by humans at the sites studied (Kido and Murray 2003). Kido and Murray (2003) stated that lower densities and higher frequencies of larger limpets were found in a subpopulation living on open-rock surfaces compared with a subpopulation occupying smaller, patch habitats within mussel beds. Limpets in patch habitats grew slower and produced less gonadal mass than limpets inhabiting open rocks, despite the availability of more microalgal food (Kido and Murray 2003). The authors concluded that given the age of larger limpets and the potential for collectors to remove larger individuals and shift populations towards smaller size structures, effective MPAs may take decades to reverse the effects of human impacts on *L. gigantea* populations in southern California.

Being aware of the spatial variation influence in the outcome of reproduction, competition and predation, and thereby affecting the viability of populations in MPAs, Lasiak (2006) set a nested sampling design to assess differences in density and biomass of seven species of patellid limpets at the scale of plots, sites and shores inside and outside a MPA on the southeast coast of South Africa. The author found that at the scale of plots, significant variation was more common inside than outside the MPA and related that with possible differences in the general pattern of space occupancy and quality of habitats available to limpets inside and outside the MPA. Nevertheless, significant variation at the scale of sites was rare suggesting that either the processes contributing to variability at this scale counteract each other or that the sites were similar in terms of habitats and ecological processes (Lasiak 2006). At the scale of shores, significant variation was more common in densities than in biomass, but both occurred with equal frequency inside and outside the MPA, which was probably driven by a combination of recruitment and/or mortality (Lasiak 2006). All the five species studied exhibited greater spatial variability inside than outside the MPA, and Lasiak (2006) concluded that the spatial patterns observed did not appear to be linked with differences in the mobility, habitat requirements or susceptibility of those species to exploitation. Lasiak (2006) emphasized that the lack of consistent patterns suggested that each species probably responds in a different way to the ecological processes operating at these spatial scales.

Branch and Odendaal (2003) compared populations of the protandric limpet, *Cymbula oculus*, between two South African MPAs, Dwesa and Tsitsikamma, and four exploited sites, and between sites exposed to or sheltered from strong wave action. *Cymbula oculus* is particularly vulnerable to harvesting (Lasiak 1991, cited in Branch and Odendaal 2003). It has no subtidal refuge, and because it is a broadcast spawner, with the possibility of been affected by Allee effects, such as depression of fertilisation success once population density drops (Quinn et al. 1993, cited in Branch 2003). In particular, it is a protandric hermaphrodite (Branch, 1974), like several other limpets (Bacci 1947, Orton et al. 1956, Wright and Lindberg 1982, Creese et al. 1990). In common with fish that change sex (e.g. Buxton, 1993, Harmelin et al. 1995) this carries the risk that one sex will be selectively harvested because it is larger, thus distorting sex ratios (Branch and Odendaal 2003). It has a dispersive larval stage and questions therefore exist about its capacity to export larvae to adjacent areas (Branch and Odendaal 2003) and thus influence recruitment there, since at least for two other limpets, there is circumstantial evidence that dispersal occurs over relatively short distances (Shanks 1998), and that the availability of nearby adult stocks strongly influences recruitment (Hockey and Branch 1994). Limpets are strongly influenced by intensity of wave action, some thriving and others suffering from high wave action (e.g. Denny et al. 1985, Bustamante et al. 1995, cited in Branch and Odendaal 2003). *Cymbula oculus* is known to be relatively mobile and has a low tenacity, both features suggesting it will be more successful in areas with relatively low wave action (Branch and Marsh 1978, cited in Branch and Odendaal 2003). Branch and Odendaal (2003) reported that compared with harvested sites, limpets in MPAs were 30–50% larger, adult densities 75% greater and biomass 30–90% greater (Branch and Odendaal 2003). Growth rate and

age-at-maturity were unaffected by harvesting, but survivorship was 10-fold higher inside MPAs, reproductive output a staggering 80-fold higher and the female: male ratio was 0.58:1 inside the MPAs, but 0.11:1 at harvested sites (Branch and Odendaal 2003). Conversely, recruitments were three times higher in harvested than protected areas, and inversely correlated with adult density (Branch and Odendaal 2003). Limpets at sheltered sites were 65% larger, biomass 80% greater, female proportions 40% higher, survivorship 25% greater and growth 33% greater, leading Branch and Odendaal (2003) to conclude that wave action had negative effects of similar magnitude to harvesting and it was not surprising that recruitment was 45% greater at wave-exposed sites. The authors stated that outside MPAs harvesting is decimating *Cymbula oculus* populations and that the effects reported were detected only inside the MPAs, being masked by harvesting elsewhere. Branch and Odendaal (2003) concluded that the impacts of harvesting and wave action could never have been detected without the existence of MPAs, emphasizing their importance for baseline studies as well as for protection tools used for coastal management.

Hawkins and Roberts (2003) studied parrotfish (Scaridae) assemblages on coral reefs in relation to fishing pressure around six Caribbean islands. Parrotfish are protogynous hermaphrodites with two distinct colour phases; the initial phase is predominantly female, and the terminal phase exclusive to sexually mature males (Hawkins and Roberts 2003). The authors ranked islands for their fishing pressure using published studies, personal observations, plus information gained from fisheries departments and marine park personnel, having identified population exploitation levels that ranged from virtually none to extremely high levels. In St Lucia parrotfish assemblages were also compared between fishing grounds and fully protected marine reserves, from 1995, six months prior to establishment, to 2001 (Hawkins and Roberts 2003).

Within each country, Hawkins and Roberts (2003) performed replicate counts of the number and size of all parrotfish species within, or passing through the counting area and calculated biomass for seven species from these data. The authors found that biomass of the two largest species, *Sparisoma viride* and *Scarus vetula*, was greatest in islands with low fishing pressure and by contrast, smaller species constituted an increasing proportion of the total parrotfish assemblage as fishing pressure increased. The average size of all species except *Sc. vetula* tended to decrease with increasing fishing pressure and percentages of fish that were terminal phase males showed order of magnitude declines with increasing fishing pressure for *Sp. viride* and *Sc. vetula* (Hawkins and Roberts 2003). Terminal males of these species were absent from counts in Jamaica and Dominica (with the highest exploitation levels) suggesting that persistence of these populations may depend on recruitment from distant sources. Following reserve implementation in St Lucia, all species, except uncommon *Sp. chrysopteron*, increased in mean biomass (Hawkins and Roberts 2003). Hawkins and Roberts (2003) reported that in six years the total biomass for all species combined, increased to become nearly four times as high in reserves, almost twice as high in

fishing grounds, and that mean size of five species increased significantly in both reserves and fishing grounds reinforcing the importance of establishing MPAs.

Hockey and Branch (1994) suggested biogeographic patterns to be used to site marine reserves and proposed a middle/edge arrangement of 'biodiversity reserves', linked to biogeographical regions. They argued that such reserves would achieve conservation of both representativeness and high diversity areas, and could be complemented by a second tier of reserves with the specific purpose of improving local yields of exploited species. The sizes of biodiversity reserves should be determined by local habitat heterogeneity and second-tier reserves should be designed to maximize their benefit to adjacent areas while minimizing their size (Hockey and Branch 1994).

Recently, Frid and Paramor (2008) proposed incorporating ecological functioning into the designation and management of marine protected areas. Marine protected areas are generally designed and managed on the basis of the presence and extent of specific habitat types or the habitats of important species (Frid and Paramor 2008). However, it has become clear that in addition to including these 'structural' elements of marine systems, management strategies should incorporate a consideration of the functional aspects of the ecosystems (Frid and Paramor 2008). The authors stated that due to success of biological traits analysis (BTA) to describe ecological functioning in marine benthic systems (eg. see Frid et al. 2000; Bremner et al. 2003, 2005, 2006) it represents the best tool currently available for quantifying ecological functioning and two expert workshops led to the agreement on 10-key ecological functions delivered by marine benthic communities (e.g. propagule supply/export; adult immigration/emigration and productivity). BTA focuses on the behaviour and attributes of biological entities that contribute to the maintenance of ecosystem processes and differs from previous trait-based approaches (e.g. trophic or functional groups (Pearson and Rosenberg 1986, Snelgrove and Butman 1994, Grall and Glemarec 1997, Whitlatch et al. 1997, Clarke and Warwick 1998, Dauwe et al. 1998, Telesh et al. 1999, Herrando-Pérez and Frid 2001) as it utilises a wider range of information on organism (Frid and Paramor 2008). In order to apply BTA two things are required, a knowledge of what are the important functions to include in consideration and secondly what traits can be used to index those functions in the biota (Frid and Paramor 2008). Twenty-four biological traits (such as maximum size, fecundity and food type) were also identified at the workshops as indices of those ten functions (Frid and Paramor 2008). In order to demonstrate the practical utility of the approach, Frid and Paramor (2008) applied BTA using those traits to a dataset covering benthos from within and around the proposed Eddystone Special Area of Conservation (SW England). The authors stated that their case study demonstrated that with the type of data normally available from conservation assessment type surveys, and knowledge of the relevant biological traits, it is possible to use a consideration of ecological functioning to set boundaries for the MPA and to set the site management objectives.

Marine systems are dynamic and, ecologically, open meaning that ecological processes extend across physical habitat boundaries and the health of the biological assemblage in a habitat may be dependent on processes occurring elsewhere (Frid and Paramor 2008). The approach developed and demonstrated by them addressed these issues by explicit consideration of biological diversity and ecological functioning using BTA. In addition to its use for describing ecological functioning across a potential Special Area of Conservation (SAC) site, the ability of the approach to identify whether communities in similar habitats but different geographic locations function in the same way (Bremner et al., 2006), means it can provide information on differences or similarities in functioning between sites proposed for inclusion in SAC series (Frid and Paramor 2008). This information will be useful in the process of identifying sites for protection, because two sites with similar Annex I Council Directive 92/43/EEC habitat types (see EC, 1992) may not necessarily function in the same way (Frid and Paramor 2008).

Along with delimiting MPA boundaries, monitoring programmes should be designed to allow changes in functioning, space or time, to be detected with a high degree of confidence (Frid and Paramor 2008). This will aid managers in determining the effects of natural change and/or human activities in MPAs and BTA allows the links between function delivery and the taxa responsible to be explicitly linked (Frid and Paramor 2008). The authors stated that it is impossible to manage the marine ecosystem and it is equally impossible to manage ecological functioning of the system, however, by being able to link functions back to taxa, our knowledge of the vulnerability of specific taxa to various human activities would allow management schemes to be advanced providing explicit consideration for, and protection of ecosystem functioning.

References

- Aldenhoven J.M., 1986. Different reproductive strategies in a sex-changing coral reef fish *Centropyge bicolor* (Pomacanthidae). *Aust. J. Freshwater Res.*, 37: 353-360.
- Allsop D.J. and West S.A., 2003a. Changing sex at the same relative body size. *Nature*, 425:783–784.
- Allsop D.J. and West S.A., 2003b. Constant relative age and size at sex change for sequentially hermaphroditic fish. *J. Evol. Biol.*, 16(5):921–929.
- Allsop D.J. and West S.A., 2004. Sex ratio evolution in sex changing animals. *Evolution*, 58:1019–1027.
- Alonzo S.H., 2002. State-dependent habitat selection games between predators and prey: the importance of behavioral interactions and expected lifetime reproductive success. *Evol. Ecol. Res.*, 4: 759–778.
- Andersen S.H., 1991. Norsminde. A “kokkenmødding” with Late Mesolithic and Early Neolithic occupation. *Journal of Danish Archaeology*, 8: 13-40.
- Andersen S.H., 1993. Bjørnsholm. A stratified Køkkenmødding on the central Limfjord, north Jutland. *Journal of Danish Archaeology*, 10: 59-96.
- Andersen S.H. and Johansen E., 1987. Ertebølle revisited. *Journal of Danish Archaeology*, 10: 59-96.
- Ankel W.E., 1936. Prosobranchia. *Tierwelt N. U. Ostsee*, 29:1-240.
- Armisen R. and Galagas F., 1987. Properties and uses of agar. In *Production and Utilization of Products from the Commercial Seaweeds*. (Mchugh D.J. ed.). FAO, Rome, FAO Fish. Tech. Pap., 288:1-57.
- Artz J.W., 1964. Intersexuality in fishes. In *Intersexuality in Vertebrates Including Man*. (Armstrong C.N. and Marshall A.J., eds.). Academic Press, London, pp. 145-232.
- Bacci G., 1947. L'inversione del sesso ed il ciclo stagionale della gonade in *Patella coerulaea* L. *Publicazioni della Stazione Zoologica di Napoli*, 38:1-17.
- Bacci G., 1951. L'ermafroditismo di *Calyptraea chinensis* L. e di altri Calyptraeidae. *Publicazioni della Stazione Zoologica di Napoli*, 23:66-90.
- Bacci G., 1952. Diverso comportamento sessuale delle *Ophryotrocha puerilis* di Napoli e di Plymouth. *Boll. Soc. It. Biol. Sper.*, 28:1293.
- Bacci G., 1955. La variabilità dei genotipi sessuali negli animali ermafroditi. *Publicazioni della Stazione Zoologica di Napoli*, 26:110-137.
- Bacci G., 1965. *Sex determination*. International Series of Monographs in Pure and Applied Biology. Division: Zoology, 26.(Kerkut G.A., ed.). Pergamon Press, Oxford.
- Bacci G., 1978. Genetics of sex determination in *Ophryotrocha* (Annelida, Polychaeta). In *Marine organisms: genetics, ecology, and evolution* (Battaglia B. and Beardmore J. eds.). Plenum Press, New York.

- Baeza J.A. and Bauer R.T., 2004. Experimental test of socially mediated sex change in a protandric simultaneous hermaphrodite, the marine shrimp *Lysmata wurdemanni* (Caridea: Hippolytidae). *Behav. Ecol. Sociobiol.*, 55(6):544–550.
- Bailey G.N. and Craighead A.S., 2003. Late Pleistocene and Holocene coastal Palaeoeconomies: a reconsideration of the molluscan evidence from northern Spain. *Geoarchaeology: An International Journal*, 18 (2): 175-204.
- Baldwin A.P. and Bauer R.T., 2003. Growth, survivorship, lifespan, and sex change in the hermaphroditic shrimp *Lysmata wurdemanni* (Decapoda: Caridea: Hippolytidae). *Mar. Biol.*, 143(1):157–166.
- Ballantine W.J., 1961. The population dynamics of *Patella vulgata* and other limpets. Ph.D. Thesis. Queen Mary College. London University.
- Barnes R.S.K., Calow P. and Olive P.J.W., 1988. *The invertebrates, a new synthesis*. Blackwell Scientific Publications, Oxford.
- Bauer R.T. 2002a. Tests of hypotheses on the adaptive value of an extended male phase in the hermaphroditic shrimp *Lysmata wurdemanni* (Caridea: Hippolytidae). *Biol. Bull.*, 203(3):347–357.
- Bauer R.T. 2002b. Reproductive ecology of a protandric simultaneous hermaphrodite, the shrimp *Lysmata wurdemanni* (Decapoda: Caridea: Hippolytidae). *J. Crust. Biol.*, 22(4):742–749.
- Bauer R.T. 2005. Cost of maleness on brood production in the shrimp *Lysmata wurdemanni* (Decapoda: Caridea: Hippolytidae), a protandric simultaneous hermaphrodite. *J. Mar. Biol. Ass. U.K.*, 85:101–106.
- Bauer R.T. and Holt G.J., 1998. Simultaneous hermaphroditism in the marine shrimp *Lysmata wurdemanni* (Caridea: Hippolytidae): an undescribed sexual system in the decapod Crustacea. *Mar. Biol.*, 132(2):223–235.
- Baur B., 1998. Sperm competition in molluscs. In: *Sperm Competition and Sexual Selection* (eds Birkhead T.R., Moeller A.P.), pp. 255– 305. Academic Press, London.
- Baxter J.M., 1982. Population dynamics of *Patella vulgata* in Orkney. *Netherlands Journal of Sea Research*, 16:96-104.
- Baxter J.M., 1983. Allometric relationships of *Patella vulgata* shell characters at three adjacent sites sandwich Bay in Orkney. *Journal of Natural History*, 17:743-755.
- Begon M., Harper J.L. and Townsend C.R., 1996. Conservation and biodiversity. In *Ecology: Individuals, Populations and Communities* (3rd edn.). Blackwell Science, Oxford., pp. 913-952.
- Bell G. and Koufonpanou V., 1986. The cost of reproduction. *Oxford Surv. Evol. Biol.*, 3:83-131.
- Bell G., 1982. *The Masterpiece of Nature: The evolution and genetics of Sexuality*. University of California Press, Berkeley.
- Bender E.A., Case T.J. and Gilfin M.E., 1984. Perturbation experiments in community ecology: theory and practice. *Ecology*, 65: 1-13.

- Bergström B.I., 1997. Do pandalid shrimp have environmental sex determination? *Mar. Biol.*, 128: 39–407.
- Berstein C. and Berstein H., 1991. *Ageing, Sex and DNA Repair*. Academic Press, San Diego, C.A.
- Bierzychudek P., 1987. Patterns in plant parthenogenesis. In *The Evolution of Sex and Its Consequences* (Stearns S.C., ed.). Birkhauser Verlag, Basel, pp.197-217.
- Blake R.W., 1979. Exploitation of a natural population of *Arenicola maritima* (L) from the north-east coast of England. *J. Appl. Ecol.*, 16: 663-670.
- Blanchette C.A., 1996. Seasonal patterns of disturbance influence recruitment of the sea palm *Postelsia palmaeiformis*. *J. Exp. Mar. Biol. Ecol.*, 197(1):1-14.
- Bock W.J., 1959. Preadaptation and multiple evolutionary pathways. *Evolution*, 13:194-211.
- Bohn J., 1859. *C. R. Acad. Sci. Paris*, 148:868-870.
- Bowman R.S., 1981. The morphology of *Patella* spp. Juveniles in Britain, and some phylogenetic inferences. *J. Mar. Biol. Ass. U.K.*, 61:647-666.
- Bowman R.S., 1985. The biology of the limpet *Patella vulagta* L. in the British Isles: spawning time as a factor determining recruitment success. In *The ecology of Rocky Coasts*. (Moore P.G. and Seed R. eds.). Hodder and Shoughton, London, pp. 97-166.
- Branch G.M., 1974a. The ecology of *Patella* Linnaeus from the Cape peninsula, South Africa. 2. Reproductive cycles. *Trans. R. Soc. S. Afr.*, 41:111-160.
- Branch G.M., 1974b. The ecology of *Patella* Linnaeus from the Cape peninsula, South Africa. 3. Growth rates. *Trans. R. Soc. S. Afr.*, 41:161-193.
- Branch G.M., 1975a. Notes on the ecology of *Patella concolor* and *Cellana capensis*, and the effects of human consumption on the limpet populations. *Zool. Afr.*, 10(1): 75-85.
- Branch G.M., 1975b. Mechanisms reducing intraspecific competition in *Patella* spp: migration, differentiation, and territorial behaviour. *J. Anim. Ecol.*, 44:575-600.
- Branch G.M., 1976. Interspecific competition experienced by South African *Patella* species. *J. Anim. Ecol.*, 45:507-530.
- Branch G.M., 1981. The biology of limpets: physical factors, energy flow and ecological interactions. *Oceanogr. Mar. Biol. A. Rev.*, 19: 235-380.
- Branch G.M., 1984. Competition between marine organisms: Ecological and evolutionary implications. *Oceanogr. Mar. Biol. A. Rev.*, 22: 429-593.
- Branch G.M. and Marsh A.C., 1978. Tenacity and shell shape in six *Patella* species: adaptive features. *Journal of Experimental Marine Biology and Ecology*, 34: 111–130.
- Branch G.M. and Odendaal F., 2003. The effects of marine protected areas on the population dynamics of a South African limpet, *Cymbula oculus*, relative to the influence of wave action. *Biol. Conserv.*, 114: 255–269.
- Branch G.M, Eekhout S. and Bosman A.L., 1990. Short-term effects of the 1988 Orange River floods on the intertidal rocky-shore communities of the open coast. *Trans. R. Soc. S. Afr.*, 47(3):331-354.

- Brawley S.H. and Johnson L.E., 1991. Survival of furoid embryos in the intertidal zone depends upon developmental stage and microhabitat. *Journal of Phycology*, 27:179-186.
- Breen P.A. and Mann K.H., 1976. Changing lobster abundance and the destruction of kelp beds by sea urchins. *Mar. Biol.*, 34:137-142.
- Bremner J., Frid C. L. J. and Rogers S. I., 2005. Biological traits of the North Sea benthos: does fishing affect benthic ecosystem function? *American Fisheries Society Symposium*, 41: 477–489.
- Bremner J., Rogers S. I. and Frid C. L. J., 2003. Assessing functional diversity in marine benthic ecosystems: a comparison of approaches. *Marine Ecology Progress Series*, 254: 11–25.
- Bremner J., Rogers S. I. and Frid C. L. J., 2006. Matching biological traits to environmental conditions in marine benthic ecosystems. *Journal of Marine Systems*, 60: 302–316.
- Breyer A., 1980. Life history of *Epitonium tinctum* (Prosobranchia: Mesogastropoda). *A. Rep. West. Soc. Malacologists*, 12:9.
- Buchanan W.F., 1988. *Shellfish in Prehistoric Diet: Elands Bay, S. W. Cape Coast, South Africa*. British Archaeological Reports, Oxford.
- Buchanan W.F., Parkington J.E., Robey T.S. and Vogel J.C., 1984. Shellfish, subsistence and settlement: some western Cape Holocene observations, in: M. Hall, G. Avery, D.M. Avery, M.L. Wilson, A.J.B. Humphreys (Eds.), *Frontiers: Southern African Archaeology Today*, British Archaeological Reports, Oxford, pp. 121–130.
- Buchanan W.F., Hall S.L., Henderson J., Olivier A., Pettigrew J.M., Parkington J.E. and Robertshaw P.T., 1978. Coastal shell middens in the Paternoster area, southwestern Cape. *South African Archaeological Bulletin*, 33: 89–93.
- Bull J.J., 1981. Sex-ratio evolution when fitness varies. *Heredity*, 46:9-26.
- Bull J.J. and Charnov E.L., 1988. How fundamental are Fisherian sex ratios? *Oxford Surv. Evol. Biol.*, 5:97-135.
- Bustamante R.H. and Castilla J.C., 1987. The shell fishery in Chile: an analysis of 26 years of landings (1960-1985). *Biologia pesq.*, 16:79-97.
- Bustamante, R.H. and Castilla, J.C., 1990. Impact of human exploitation on populations of the intertidal southern bull-kelp *Durvillaea antarctica* (Phaeophyta, Durvilleales) in central Chile. *Biological Conservation*, 52(3):205-220.
- Bustamante R.H., Branch G.M. and Eekhout S., 1995. Maintenance of an exceptional intertidal grazer biomass in South Africa: subsidy by kelps. *Ecology*, 76: 2314–2329.
- Buston P.M., 2003. Size and growth modification in clownfish. *Nature*, 424: 145–146
- Buston P. M., Munday P. L., and Warner R. R., 2004. Evolutionary biology: sex change and relative body size in animals. *Nature*, 428, 8 April 2004.
- Buxton C.D., 1993. Life-history changes in exploited reef fishes on the east coast of South Africa. *Environmental Biology of Fishes*, 36: 47– 63.

- Castilla J.C., 1986. Sigue existiendo la necesidad de establecer parques y reservas maritimas en Chile? *Ambiente y Desarrollo*, 2:53-63.
- Castilla J.C. and Bustamante R.H., 1989. Human exclusion from rocky intertidal of Las-Cruces, central Chile - effects on *Durvillaea antarctica* (Phaeophyta, Durvilleales). *Marine Ecology*, 50(3):203-214.
- Castilla J.C. and Duran L.R., 1985. Human exclusion from the rocky intertidal zone of central Chile - the effects on *Concholepas concholepas* (Gastropoda). *Oikos*, 45(3):391-399.
- Caswell H., 1978. Predator-mediated coexistence: a nonequilibrium model. *Am. Nat.* 112:127-154.
- Catterall C.P. and Pointer I.R., 1987. The potential impact of human gathering on shellfish populations, with reference to some NE Australia intertidal flats. *Oikos*, 50:14-122.
- Chaparro O.R. and Flores M.L., 2002. Reproductive output of *Crepidula fecunda* females: distribution of energy in the production of gametes and capsular walls. *New Zealand J. Mar. Freshwater Res.*, 36:661–673.
- Chaparro O.R., Oyarzun R.F, Vergara A.M. and Thompson R.J., 1999. Energy investment in nurse eggs and egg capsules in *Crepidula dilatata* Lamarck (Gastropoda, Calyptraeidae) and its influence on the hatching size of the juvenile. *J. Exp. Biol. Ecol.*, 232:261–274.
- Charlesworth B., 1989. The evolution of sex and recombination. *Trends Ecol. Evol.*, 4:264-267.
- Charlesworth B., 1978. The population genetics of anisogamy. *J. Theor. Biol.*, 73:347-357.
- Charnov E.L., 1982. *The Theory of Sex Allocation*. Princeton University Press, Princeton.
- Charnov E.L., 1986. Size advantage may not always favour sex change. *J. Theor. Biol.*, 199:283-285.
- Charnov E. L., 1993. *Life history Invariants: Some Explorations of Symmetry in Evolutionary Ecology*. Oxford Univ. Press., Oxford.
- Charnov E.L. and Bull J.J., 1977. When is sex environmental determined? *Nature (Lond.)*, 266:828-830.
- Charnov E.L. and Bull J.J., 1989. Non-Fisherian sex ratios with sex change and environmental sex determination. *Nature*, 338:148–150.
- Charnov E.L. and Hannah R.W., 2002. Shrimp adjust their sex ratio to fluctuating age distributions. *Evol. Ecol. Res.*, 4: 239–246.
- Charnov E.L. and Skúladóttir U., 2000. Dimensionless invariants for the optimal size (age) of sex change. *Evol. Ecol. Res.*, 2: 1067–1071.
- Chelazzi G., Santini G., Parpagnoli D. and Della Santina P., 1994. Coupling motographic and sonographic recording to assess foraging behaviour of *Patella vulgata*. *Journal of Molluscan Studies*, 56:595-600.
- Chen M-H., Yang Y-W., and Soong K., 2004. Host effect on size structure and timing of sex change in the coral-inhabiting snail *Coralliophila violacea*. *Mar. Biol.*, 144: 287–293.
- Chipperfield P.N.J., 1951. The breeding of *Crepidula fornicata* in the river Blackwater, Essex. *Journal of the Marine Biological Association of the United Kingdom*, 30: 49–71.

- Choquet M., 1965. Recherche en culture organotypique sur la spermatogenese chez *Patella vulgata* L., role des ganglions cerebroides et des tentacules. *C. R. Acad. Sci. Paris*, 261:4521-4524.
- Choquet M., 1967. Gametogenese in vitro au cours du cycle annuel chez *Patella vulgate* L. en phase male. *C. R. Acad. Sci. Paris*, 265:333-335.
- Choquet M., 1968. Croissance et longevite de *Patella vulgata* L. (Gasteropode: Prosobranche) dans le Boulonnais. *Cah. Biol. Mar.*, 9:449-468.
- Choquet M., 1971. Etude du cycle biologique et de l'inversion du sex chez *Patella vulgata* L. (Mollusque Gasteropode Prosobranche). *Gen. Comp. Endocrinol.*, 16: 59-73.
- Cipriani R. and Collin R., 2005. Life-history invariants with bounded variables: Artifacts of an incorrect null hypothesis? *J. Evol. Biol.*, 18:1613–1618.
- Clarke K. R. and Warwick R. M., 1998. Quantifying structural redundancy in ecological communities. *Oecologia*, 113: 278–289.
- Clifton K. E. and Rogers L., 2008. Sex-specific mortality explains nonsex-change by large female *Sparisoma radians*. *Animal Behaviour*, 75: 1-10.
- Coe W.R., 1935. Sexual phases in *Crepidula*. *J. Exp. Zool.*, 72: 455-477.
- Coe W.R., 1936. Sexual phases in *Crepidula*. *Journal of Experimental Zoology*, 72: 455–477.
- Coe W.R., 1938a. Conditions influencing change of sex in molluscs of the genus *Crepidula*. *J. Exp. Zool.*, 77: 401-424.
- Coe W.R., 1938b. Influence of association on the sexual phases of gastropods having protandric consecutive sexuality. *Biological Bulletin*, 75:274-285.
- Coe W.R., 1943. Sexual differentiation in molluscs. I. Pelecypods. *Q. Rev. Biol.*, 154-164.
- Coe W.R., 1944. Sexual differentiation in molluscs. II. Gastropods, Amphineurans, Scaphopods, and Cephalopods. *Q. Rev. Biol.*, 19: 85-97.
- Coe W.R., 1948a. Variations in the expression of sexuality in the normally protandric gastropod *Crepidula plana* Say. *J. Exp. Zool.*, 108: 155-169.
- Coe W.R., 1948b. Nutrition and sexuality in protandric gastropods of the genus *Crepidula*. *J. Exp. Zool.*, 108:155–169.
- Coe W.R., 1949. Divergent methods of development in morphologically similar species of prosobranch gastropods. *J. Morph.*, 13:383-400.
- Coe W.R., 1953. Influences of association, isolation, and nutrition on the sexuality of snails of the genus *Crepidula*. *J. Exp. Zool.*, 122:5-19.
- Collin R., 1995. sex, size and position: a test of models predicting size at sex change in the protandrous gastropod *Crepidula fornicata*. *Am. Nat.*, 146(6):815-831.
- Collin R., 2000. Sex change, reproduction and development of *Crepidula adunca* and *C. lingulata* (Gastropoda: Calyptraeidae). *Veliger*, 43:24–33.
- Collin R., 2006. Sex ratio, life history invariants, and patterns of sex change in a family of protandrous gastropods. *Evolution*, 60(4):735-745.

- Collin R., McLellan M., Gruber K. and Bailey-Jourdain C., 2005. Effects of conspecific associations on size at sex change in three species of calyptraeid gastropods. *Marine Ecology Progress Series*, 293:89–97.
- Connell J.H., 1961. The influence of intra-specific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. *Ecology*, 42:710-723.
- Connell J.H., 1978. Diversity in tropical rain forests and coral reefs. *Science*, 199:1302-1310.
- Cook A., Bamford O.S., Freeman J.D.B. and Teideman D.J., 1969. A study of the homing habit of the limpet. *Animal Behaviour*, 17:330-339.
- Cowen R.K., 1990. Sex change and life history patterns of the labrid, *Semicossyphus pulcher*, across an environmental gradient. *Copeia*, 1990: 787–795.
- Creese R.G., 1980. An analysis of distribution and abundance of populations of the high-shore limpet, *Notoacmaea petterdi* (Tenison-Woods). *Oecologia*, 45:252-260.
- Creese R.G., Schiel D.R. and Kingsford M.J., 1990. Sex change in a giant endemic limpet, *Patella kermadecensis*, from the Kermadec Islands. *Marine Biology*, 104: 419–426.
- Cunliffe B. and Hawkins S.J., 1988. *The shell midden deposits in Mout Batten Plymouth - A Prehistoric and Roman Port* (Cunliffe B. ed.). Oxford University Committee for Archaeology, Monograph 26, pp. 35-38.
- Daly G.P., 1975. *Am. Malacol. Union. Inc. Bull.*, 1975: 69.
- Darwin C., 1876. *The Effects of cross and self fertilization in the vegetable kingdom*. John Murray, London.
- Dauwe B., Herman P. M. J. and Heip C. H. R., 1998. Community structure and bioturbation potential of macrofauna at four North Sea stations with contrasting food supply. *Marine Ecology-Progress Series*, 173: 67–83.
- Davies P.S., 1967. Physiological ecology of *Patella*. II. Effect of environmental acclimation on the metabolic rate. *J. Mar. Biol. Ass. U.K.*, 47:61-74.
- Davis J.R.A., 1885. The habits of limpets. *Nature (Lond.)*, 31: 200-201.
- Davis J.R.A., 1895. The habits of limpets. *Nature (Lond.)*, 51:511-512.
- Dayton P.K., 1971. Competition, disturbance and community organization: The provision and subsequent utilization of space in a rocky intertidal community. *Ecol. Monogr.*, 41:357-389.
- Dayton P.K., 1975. Experimental evaluation of ecological dominance in a rocky intertidal algal community. *Ecol. Monogr.*, 45:137-159.
- De Jong G., 2005. Is invariance across animal species just an illusion? *Science* 309:1193–1195.
- Della Santina P., Santini G. and Chelazzi G., 1995. Factors Affecting variability of foraging excursions in a population of the limpet *Patella vulgata* (Mollusca, Gastrodopa). *Mar. Biol.*, 122:265-270.
- Denny M.N., Daniel T.L. and Koehl M.A.R., 1985. Mechanical limits to size in wave-swept organisms. *Ecological Monographs*, 55: 69– 102.

- Dodd J.M., 1956. Studies on the biology of limpets. III. Hermaphroditism in the three British species of *Patella*. *J. Mar. Biol. Ass. U.K.*, 35:327-340.
- Dupont L. and Viard F., 2003. Isolation and characterization of highly polymorphic microsatellite markers from the marine invasive species *Crepidula fornicata* (Gastropoda: Calyptraeidae). *Molecular Ecology Notes*, 3: 498–500.
- Dupont L., Richard J., Paulet Y-M., Thouzeau G. and Viard F., 2006. Gregariousness and protandry promote reproductive insurance in the invasive gastropod *Crepidula fornicata*: evidence from assignment of larval paternity. *Molecular Ecology*, 15:3009-3021
- Duran L.R. and Castilla J.C., 1989. Variation and persistence of the middle rocky intertidal community of central Chile, with and without human harvesting. *Mar. Biol.*, 103(4): 555-562.
- Duran L.R. and Oliva D., 1987. Intensity of human predation on rocky shores at Las-Cruces in central Chile. *Environmental Conservation*, 14(2):143-149.
- EC, 1992. COUNCIL DIRECTIVE 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. European Commission, Brussels.
- Eekhout S., Raubenheimer C.M., Branch G.M., Bosman A.L. and Bergh M.O., 1992. A holistic approach to the exploitation of intertidal stocks – limpets as a case study. *South African Journal of Marine Science*, 12: 1017-1029.
- Evans M.R. and Williams G.A., 1991. Time partitioning of foraging in the limpet *Patella vulgata*. *J. Anim. Ecol.*, 60: 563-575.
- Evans R.G., 1947a. The intertidal ecology of selected localities in the Plymouth neighbourhood. *J. Mar. Biol. Ass. U.K.*, 27:173-218.
- Evans R.G., 1947b. The intertidal ecology of Cardigan Bay. *J. Ecol.*, 34:273-309.
- Feldman M.W., Christiansen F.B. and Brooks L.D., 1980. Evolution of recombination in a constant environment. *Proc. Natl. Acad. Sci. USA*, 77: 4838-4841.
- Fischer-Piette E., 1941. Croissance, taille maximale et longevité possible de quelques animaux intertidaux en fonction du milieu. *Annls. Inst. Oceanogr. (Monaco)*, 21:1-28.
- Fish J.D. and Fish S., 1989. *A student guide to the seashore*. Unwin Hyman, London.
- Fishelson L., 1970. Protogynous sex reversal in the fish *Anthias squamipinnis* (Teleostei, Anthiidae) regulated by the presence or absence of a male fish. *Nature (Lond.)*, 227:90-91.
- Fisher R.A., 1930. *The Genetical Theory of Natural selection*. Clarendon Press, Oxford.
- Fisher W.K., 1904. The anatomy of *Lottia gigantea* Gray. *Zool. Jb. (Abt. Anat. Ont. Tiere)*, 20:1-66.
- Fletcher W.J., 1984a. Variability in the reproductive effort of the limpet, *Cellana tramoserica*. *Oecologia*, 61:259-264.
- Fletcher W.J., 1984b. Intraspecific variation in the population dynamics and growth of the limpet *Cellana tramoserica*. *Oecologia*, 63:110-121.
- Frank P.W., 1965. The biodemography of an intertidal snail population. *Ecology*, 46: 831-844.
- Frank S.A., 1990. Sex Allocation Theory for birds and mammals. *Annu. Rev. Ecol. Syst.*, 21:13-55.

- Fretter V., 1984. Prosobranch gastropods. In *The Mollusca 7: Reproduction* (Wilbur K.M. ed.). Academic Press, New York.
- Fretter V. and Graham A., 1964. *Physiology of Mollusca* (Wilbur K.M. and Yonde C.M. eds.). Academic Press, pp.127-164.
- Fretter V. and Graham A., 1976. The prosobranch molluscs of Britain and Denmark. Part I. Pleurotomariacea, Fissurellacea and Patellacea. *Journal of Molluscan Studies (supplement)*, 1:1-33.
- Fricke H. and Fricke S., 1977. Monogamy and sex change by aggressive dominance in coral reef fish. *Nature (Lond.)*, 266:830-832.
- Frid C. L. J. and Paramor O. A. L., 2008. Incorporating ecological functioning into the designation and management of marine protected areas. *Hydrobiologia*, 606: 69–79.
- Frid C. L. J., Rogers S. I., Nicholson M., Ellis J. R. and Freeman S., 2000. Using biological characteristics to develop new indices of ecosystem health. 88th Annual ICES Science Conference; Mini-symposium on defining the role of ICES in supporting biodiversity conservation ICES CM Mini:02. ICES, Copenhagen.
- Funke W., 1968. Heimfindevermogen and Ortstreue bei *Patella* L. (Gastropoda, Prosobranchia). *Oecologia (Berl.)*, 2:19-142.
- Gadgil M., 1972. Male dimorphism as a consequence of sexual selection. *Am. Nat.*, 106:574-581.
- Gaffney P.M. and McGee B., 1992. Multiple paternity in *Crepidula fornicata* (Linnaeus). *Veliger*, 35(1):12-15.
- Gaines S.D. and Lubchenco J., 1982. A unified approach to marine plant-herbivore interactions. II. Biogeography. *Ann. Rev. Ecol. Syst.*, 13:111-138.
- Galbraith R., 1965. Homing behaviour in the limpets *Acmaea digitalis* and *Lottia gigantea*. *Am. Midl. Nat.*, 74:245-246.
- Gardner A., Allsop D.J., Charnov E.L. and West S.A., 2005. A dimensionless invariant for relative size at sex change in animals: explanation and implications. *American Naturalist*, 165(5):551–566.
- Ghiselin M.T., 1969. The evolution of hermaphroditism among animals. *Q. Rev. Biol.*, 44:189-208.
- Ghiselin M.T., 1974. *The Economy of Nature and the Evolution of Sex*. University of California Press, Berkeley.
- Ghiselin M.T., 1987. Evolutionary aspects of marine invertebrate reproduction. In *Reproduction of marine invertebrates* (Giese A.C., Pearse J.S. and Pearse V.B. eds.) Vol 9. Blackwell Scientific, Palo Alto, California, pp.609-665 .
- Glesener R.R. and Tilman D., 1978. Sexuality and the components of environmental uncertainty: Clues from the geographic parthenogenesis in terrestrial animals. *Am. Nat.*, 112:659-673.
- Gould H.N., 1917a. Studies on sex in the hermaphrodite mollusc *Crepidula plana*. I. History of sexual cycle. *J. Exp. Zool.*, 23:1-68.

- Gould H. N., 1917b. Studies on sex in the hermaphrodite mollusc *Crepidula plana*. II. Influence of the environment on sex. *J. Exp. Zool.*, 23:225-250.
- Gould H.N., 1919. Studies on sex in the hermaphrodite mollusc *Crepidula plana*. III. Transference of the male-producing stimulus through sea-water. *J. Exp. Zool.*, 29:113-120.
- Gould H.N., 1952. Studies on sex in the hermaphrodite mollusc *Crepidula plana*. IV. Internal and external factors influencing growth and sex development. *J. Exp. Zool.*, 119:93-163.
- Grall J. and Glemarec M., 1997. Assessment of benthic structure in the Bay of Brest. *Annales De L Institut Oceanographique*, 73: 7–16.
- Gust N., 2004. Variation in the population biology of protogynous coral reef fishes over tens of kilometres. *Can. J. Fish. Aquat. Sci.*, 61: 205–218
- Gust N., Choat J.H. and Ackerman J.L., 2002. Demographic plasticity in tropical reef fishes. *Mar. Biol.*, 140: 1039–1051.
- Halketta D., Harta T., Yatesb R., Volmanc T. P, Parkingtond J. E, Ortona J., Kleine R. G., Cruz-Uribef, K. and Averyd G., 2003. First excavation of intact Middle Stone Age layers at Ysterfontein, Western Cape Province, South Africa: implications for Middle Stone Age ecology. *Journal of Archaeological Science* 30: 955–971.
- Hamilton W.D., 1967. Extraordinary sex ratios. *Science*, 156:477-488.
- Hardy I. C. W., 2002. *Sex ratios: concepts and research methods*. Cambridge Univ. Press, Cambridge, U.K.
- Harmelin J.-G., Bachet F., García F., 1995. Mediterranean marine reserves: fish indices as tests of protection efficiency. *P S Z N I: Marine Ecology*, 16: 233–250.
- Harrington R.W., 1971. How ecological and genetic factors interact to determine when self-fertilizing hermaphrodites of *Rivulus marmoratus* change into functional secondary males with reappraisal to the modes of intersexuality among fishes. *Copeia*, 1971:389-432.
- Hartnoll R.G. and Hawkins S.J., 1985. Patchiness and fluctuation on moderately exposed rocky shores. *Ophelia*, 24:53-63.
- Hartnoll R.G. and Wright J.R., 1977. Foraging movements and homing in the limpet *Patella vulgata* L. *Animal Behaviour*, 25:806-810.
- Hatch D.J., 1977. The inter-relationships of three *Patella* spp. on the south coast of Britain. PhD. Thesis, University of Southampton.
- Haven S.B., 1973. Competition for food between the intertidal gastropods *Acmaea scraba* and *A. digitalis*. *Ecology*, 54: 143-151.
- Hawkins J. P. and Roberts C. M., 2003. Effects of fishing on sex-changing Caribbean parrotfishes. *Biological Conservation*, 115: 213–226.
- Hawkins S.J. and Hartnoll R.G., 1982. The influence of barnacle cover on the numbers, growth and behaviour of *Patella vulgata* on a vertical pier. *J. Mar. Biol. Ass. U.K.*, 62:855-867.
- Hawkins S.J. and Hartnoll R.G., 1983. Grazing of intertidal algae by marine invertebrates. *Oceanogr. Mar. Biol. Ann. Rev.*, 21:195-282.

- Hawkins S.J., 1979. Field studies on Manx rocky shore communities. PhD. Thesis, University of Liverpool.
- Hawkins S.J., Burnay L.P., Neto A.I., Tristão da Cunha R. and Frias A.F., 1990a. A description of the zonation patterns of molluscs and other biota on the south coast of Sao Miguel, Azores. *Acoreana Supl.*, 21-38.
- Hawkins S.J., Corte-Real H.B.S.M., Martins H.R., Santos R.S. and Martins A.F., 1990b. A note on the identity of *Patella* species in the Azores. *Acoreana Supl.*, 167-173.
- Heller J., 1993. Hermaphroditism in molluscs. *Biological Journal of the Linnean Society*, 48:19-42.
- Henshilwood C.S., Sealy J.C., Yates R.J., Cruz-Urbe K., Goldberg P., Grine F.E., Klein R.G., Poggenpoel C., Van Niekerk K.L. and Watts I., 2001. Blombos Cave, southern Cape, South Africa: Preliminary report on the 1992–1999 excavations of the Middle Stone Age levels. *Journal of Archaeological Science*, 28:421–448.
- Herrando-Pérez S. and Frid C. L. J., 2001. Recovery patterns of macrobenthos and sediment at a closed fly-ash dumpsite. *Sarsia*, 86:389 – 400.
- Herre E.A., 1985. Sex ratio adjustment in fig wasps. *Science*, 228:896-898.
- Herre E.A., 1987. Optimality, plasticity, and selective regime in pig wasp sex ratios. *Nature*, 329:627-629.
- Hill A.S. and Hawkins S.J., 1991. Seasonal and spatial variation of epilithic microalgal distribution and abundance and its ingestion by *Patella vulgata* on a moderately exposed rocky shore. *J. Mar. Biol. Ass. U.K.*, 1:403-423.
- Hoagland K.E., 1975. Reproductive strategies and evolution in the genus *Crepidula* (Gasteropoda: Calyptraeidae). p.H.D. Dissertation, Harvard University, Cambridge, Massachusetts.
- Hoagland K.E., 1977. Systematic review of fossil and recent *Crepidula* and discussion of evolution of the Calyptraeidae. *Malacologia*, 16:353-420.
- Hoagland K.E., 1978. Protandry and the evolution of environmentally mediated sex change: a study of the Mollusca. *Malacologia*, 17:365-391.
- Hobday A.J. and Riser K.L., 1998. The role of movement by *Crepidula norrisiarum* Williamson in altering reproductive potential. *J. Exp. Mar. Biol. Ecol.*, 225:139-154.
- Hockey P.A.R. and Bosman A.L., 1986. Man as an intertidal predator in Transkei - disturbance, community convergence and management of a natural food resource. *Oikos*, 46(1):3-14.
- Hockey P.A.R. and Branch G.M., 1994. Conserving marine biodiversity on the African coast: implications of a terrestrial perspective. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 4: 345– 362.
- Hockey P.A.R., Bosman A.L. and Siegfried W.R., 1988. Patterns and correlates of shellfish exploitation by coastal people in Transkei: an enigma of protein production. *J. Appl. Ecol.*, 25:353-363.
- Hoekstra R.F., 1987. The evolution of sexes. In *The Evolution of Sex and Its Consequences* (Stearns S.C. ed.). Birkhauser Verlag., Basel, pp. 59-91.

- Hoffman S.G., Schildhauer M.P. and Warner R.R., 1985. The costs of changing sex and the ontogeny of males under contest competition for mates. *Evolution*, 39:915-927.
- Hughes R.N., 1986. *A functional biology of marine gastropods*. Croom Helm., London.
- Hurst L.D., 1990. Parasite diversity and the evolution of diploidy, Multicellularity and anisogamy. *J. Theor. Biol.*, 144:429-433.
- Ishiki H., 1936. Sex changes in the slipper limpets, *Crepidula aculeata* and *Crepidula Walshi*. *Journal of Science, Hiroshima University*, series B, Division 1, 4:91-99.
- Iwasa Y., 1991. Sex change evolution and cost of reproduction. *Behavioral Ecology*, 2(1):56-68.
- Jerardino A., Castilla J.C., Ramirez J.M. and Hermosilla N., 1992. Early coastal subsistence patterns in Central Chile: a systematic study of the marine invertebrate fauna from the site of Curaumilla-1. *Latin. Am. Antiq.*, 3:43-62.
- Jones N.S., 1948. Observations and experiments on the biology of *Patella vulgata* at Port St. Mary, Isle of Man. *Proceedings and Transactions of the Liverpool Biological Society*, 56:60-77.
- Josse J. and Geraerts P.M., 1983. In *The Mollusca Physiology* (Salenddin A.S.M. and Wilbur K.M. eds.) Vol 4. Academic Press, pp.318-406.
- Kalmus H., 1932. Ueber die Erhaltungswert der Phanothypischen (Morphologischen) Anisogamie und die Entstehung der Erstern Geschlechtsunterschiede. *Biol. Zeutralbaltt*, 552:716-726.
- Kay E.A. and Madruger W.H., 1977. The biology of the Opihi (October 1975-October 1976). *Report of the Department of Planning and Economic Development, State of Hawaii*, 46 pp. (mineo).
- Kay E.A., Corpuz G.C. and Magruder W.H., 1982. Opihi. Their biology and culture. *Report of the Aquaculture Development Program, Departement of Land and Natural Resorces, State of Hawaii*, 98 pp. (mineo).
- Kido J. S. and Murray S. N., 2003. Variation in owl limpet *Lottia gigantea* population structures, growth rates, and gonadal production on southern California rocky shores. *Marine Ecology Progress Series*, 257: 111–124.
- Kingsford M.J., Underwood A.J. and Kennelly S.J., 1991. Humans as predators on rocky reefs in New South Wales, Australia. *Mar. Ecol. Prog. Ser.*, 72(1&2):1-14.
- Koike H., 1986. Prehistoric hunting pressure and palaeobiomass: an environmental reconstruction and archaeozoological analysis of a Jomon shellmound area. In: Akazawa, T., Aikens, C.M. (Eds.), Prehistoric HuntereGatherers in Japan: New Research Methods. *The University Museum Bulletin*, 27: 27-53 (University of Tokoyo).
- Kondrashov A.S., 1993. Classification of hypotheses on the advantage amphimixis. *J. Hered.*, 84:372-387.
- Kozloff E.N., 1990. *Invertebrates*. Saunders, New York.
- Krebs J.R. and Davies N.B. (ed.), 1997. *Behavioural Ecology. An Evolutionary Approach*. Fourth edition. Blackwell Scientific, Oxford.
- Krebs J.R. and Davies N.B., 1993. *An Introduction to Behavioural Ecology*. Third edition. Blackwell Scientific, Oxford.

- Kuwamura T. and Nakashima Y., 1998. New aspects of sex change among reef fishes: recent studies in Japan. *Env. Biol. Fish.*, 21: 125–135.
- Kyle R., Pearson B., Fielding P.J., Robertson W.D. and Birnie S.L., 1997. Subsistence shellfish harvesting in the Maputaland Marine Reserve in northern Kwazulu-Natal, South Africa: Rocky shore organisms. *Biol. Conserv.*, 82:183-192.
- Lasiak T. 1991. The susceptibility and/or resilience of rocky littoral molluscs to stock depletion by the indigenous coastal people of Transkei, Southern Africa. *Biol. Conserv.*, 56: 245-264.
- Lasiak T., 1992. Contemporary shell-gathering practices of indigenous coastal people in Transkei: Some implications for interpretation in the archaeological record. *S. Afr. J. Sci.*, 88:19-28.
- Lasiak T., 1993. Temporal and spatial variations in exploited and non-exploited populations of the intertidal limpet *Cellana capensis*. *J. Moll. Stud.*, 59: 295-307.
- Lasiak T., 1997. Temporal and spatial variations in the pattern of shoreline utilization in a region subject to subsistence exploitation. *Intern. J. Environ. Studies*, 52: 21-46.
- Lasiak T., 1998. Multivariate comparisons of rocky infratidal macrofaunal assemblages from replicate exploited and non-exploited localities on the Transkei coast of South Africa. *Mar. Ecol. Prog. Ser.*, 167: 15-23.
- Lasiak T., 1999. The putative impact of exploitation on rocky infratidal macrofaunal assemblages: a multiple-area comparison. *J. Mar. Biol. Ass. U.K.*, 79: 23-34.
- Lasiak T. 2003. Influence of taxonomic resolution, biological attributes and data transformations on the outcome of multivariate comparisons of rocky infratidal macrofaunal assemblages. *Mar. Ecol. Prog. Ser.*, 250: 29-34.
- Lasiak T, 2006. Spatial variation in density and biomass of patellid limpets inside and outside a marine protected area. *Journal of Molluscan Studies*, 72: 137–142.
- Lasiak T. and Dye A., 1989. The ecology of the brown mussel *Perna perna* in the Transkei, southern Africa: implications for the management of a traditional food source. *Biol. Conserv.*, 47:245-257.
- Lasiak T.A. and Field, J.G. 1995. Community-level attributes of exploited and non-exploited rocky infratidal macrofaunal assemblages in Transkei. *J. Exp. Mar. Biol. Ecol.*, 185: 33-53.
- Le Gall P., 1980. Etude expérimentale d'association en chaîne et de son influence sur la croissance et la sexualité chez la crépidule (*Crepidula fornicata* Linné 1758). PhD. Thesis, University of Caen, France.
- Le Gall S. and Streiff W., 1975. In *Intersexuality in the Animal Kingdom* (Reinboth R.ed.), Springer-Verlag.
- Le Quesne W.J.F. and Hawkins S.J., 2006. Direct observations of protandrous sex change in the patellid limpet *Patella vulgata*. *J. Mar. Biol. Ass. U.K.*, 86: 161-162.
- Leigh E.G., 1970. sex ratio and differential mortality between the sexes. *Am. Nat.*, 104:205-210.
- Leigh E.G. Jr., Charnov E.L. and Warner R.R., 1976. Sex ratio, sex change and natural selection. *Proc. Natn. Acad. Sci. U.S.A.*, 73:3656-3660.
- Levin D.A., 1975. Pest pressure and recombination systems in plants. *Am. Nat.*, 109: 437-451.

- Lewis J.R. and Bowman R.S., 1975. Local habitat induced variations in the population dynamics of *Patella vulgata* L. *J. Exp. Mar. Biol. Ecol.*, 17:165-203.
- Lin J. and Zhang D. 2001. Reproduction in a simultaneous hermaphroditic shrimp, *Lysmata wurdemanni*: Any two will do? *Mar. Bio.*, 139(6):1155–1158.
- Lindberg D.R. and Wright W.G., 1985. Patterns of sex change of the protandric patellacean limpet *Lottia gigantea* (Mollusca: Gastropoda). *Veliger*, 27:261-265.
- Little C., Williams G.A., Morrill D., Perrins J.M. and Stirling P., 1988. Foraging behaviour of *Patella vulgata* L. in an Irish sea-lough. *J. Exp. Mar. Biol. Ecol.*, 120:1-21.
- Liu M. and Sadovy Y., 2004. The influence of social factors on adult sex change and juvenile sexual differentiation in a diandric, protogynous epinepheline, *Cephalopholis boenak* (Pisces, Serranidae). *J. Zool. Soc. Lond.*, 264: 239–248.
- Lubchenco J., 1978. Plant species diversity in a marine intertidal community, importance of herbivore food preference and algal competitive abilities. *Am. nat.*, 112:23-39.
- Lubchenco J. and Gaines S.D., 1981. A unified approach to marine plant-herbivore interactions. I. Populations and communities. *Ann. Rev. Ecol. Syst.*, 12:405-437.
- Lui J.H., 1994. The ecology of the Hong Kong limpets *Cellana grata* (Gould 1859) and *Patelloida pygmaea* (Dunker 1860): distribution and populations dynamics. *Journal of Molluscan Studies*, 60:55-67.
- Lutensky M.M.F., 1994. Density-dependent protogynous sex change in territorial-haremic fishes: models and evidence. *Behav. Ecol.*, 5: 375–383.
- Mackie G.L., 1984. In *The Mollusca Reproduction* (Tompa A.S., Verdonk N.H. and Van Der Biggelaar J.A.M. eds.) Vol 7. Academic Press, pp. 351-418.
- Martin M-C., 1985. *Etude expérimentale de l'inversion sexuelle et de la morphogénèse génitale femelle chez un mollusque hermaphrodite protandre* *Crepidula fornicata*. PhD. Thesis, University of Caen, France.
- Martinez J.L., Moran P., Perez J., 2000. Multiple paternity increases effective size of southern Atlantic salmon populations. *Molecular Ecology*, 9: 293–298.
- Martins H.R., Santos R.S. and Hawkins S.J., 1987a. *Estudos sobre as lapas dos Açores. Exploração e Avaliação*. VII Semana das Pescas dos Açores. Secretaria Regional de Agricultura e Pescas, Direcção Regional das Pescas, pp. 175-182.
- Martins H.R., Santos R.S. and Hawkins S.J., 1987b. Exploitation of limpets (*Patella* spp.) in the Azores with a preliminary analysis of the stocks. ICES C.M. 1987/K53:18pp.
- Mckusic M. B. and Warren C.N., 1959. Introduction to San Clemente Island archaeology. *University of California Los Angeles Archaeological Survey, Annual Report 1958-1959*: 107-186.
- McLachlan A. and Lombard H.W., 1981. Growth and production in exploited and unexploited populations of a rocky shore gastropod, *Turbo sarmaticus*. *Veliger*, 23:221-119.
- McNeeley J.A., 1988. *Economics and Biological Diversity: Developing and using economic Incentives to conserve Biological Resources*. IUCN, Gland, Switzerland.

- Meagher T.R., 1986. Analysis of paternity within a natural population of *Chamaelirium luteum*. 1. Identification of most-likely male parents. *American Naturalist*, 128: 199–215.
- Meighan C.N., 1959. The Little harbor site, Catalina Island: An example of ecological interpretation in archaeology. *Am. Antiquity*, 24:383-405.
- Mellars P.A., 1978. Excavation and economic analysis of Mesolithic shell middens on the Island of Oronsay (Inner Hebrides). In: Mellars, P.A. (Ed.), *The Early Postglacial Settlement of Northern Europe*. Duckworth, London, pp. 371-396.
- Menezes G.M., 1991. Aspectos da biologia e ecologia de *Patella ulyssiponensis ulyssiponensis* e *Patella candei candei* (Mollusca, Gasteropoda, Patellidae) dos Acores. Potencial da sua exploracao. Relatorio de Estagio de Licenciatura, Faculdade de Ciencias de Lisboa, 134pp.
- Michod R.E. and Levin B.R. (eds.), 1988. *The Evolution of Sex: An Examination of Current Ideas*. Sinauer Associates, Sunderland, MA.
- Milner N., Barrett J. and Welsh J., 2007. Marine resource intensification in Viking Age Europe: the molluscan evidence from Quoygre, Orkney. *Journal of Archaeological Science* 34: 1461-1472.
- Minchington T.E. and Scheibling R.E., 1991. The influence of larval supply and settlement on the population structure of barnacles. *Ecology*, 72:1867-1879.
- Montalenti G., 1958. Perspectives of research on sex problems in marine organisms. In *Perspectives in Marine Biology* (Buzzati-Travesso A.A. ed.). University of California Press, Berkeley, pp.589-602.
- Montalenti G. and Bacci G., 1951. Osservazioni e ipotesi sulla determinazione del sesso negli ermaproditi. *Scientia genet.*, 4:5-12.
- Moreno C.A., 1986. Un resumen de las consecuencias ecologicas de la exclusion del hombre en la zona intermareal de Mehuin-Chile. *Estud. Oceanol.*, 5:59-66.
- Moreno C.A., Lucnecke K.M. and Lepez M.I., 1986. The response of an intertidal *C. concholepas* (Gasteropoda) population to protection from Man in Southern Chile and the effects on benthic sessile assemblages. *Oikos*, 42: 354-364.
- Moreno C.A., Sutherland J.P. and Jara F.H., 1984. Man as a predator in the intertidal zone of Southern Chile. *Oikos*, 42: 155-160.
- Moyer J.T., Zaizer M.J., 1984. Early sex change: a possible mating strategy of *Centropyge* angelfishes (Pisces: Pomacanthidae). *J. Ethol.*, 2:63-67.
- Muller H. J., 1964. The relation of recombination to mutational advance. *Mutat. Res.*, 1:2-9.
- Munday P.L., 2002. Bi-directional sex change: testing the growth-rate advantage model. *Behav. Ecol. Sociobiol.*, 52: 247–254.
- Munday P. L., Buston P. M. and Warner R. R., 2006. Diversity and flexibility of sex-change strategies in animals. *Trends in Ecology and Evolution*, 21: 89-95.

- Muñoz R. C. and Warner R. R., 2003. A new version of the size-advantage hypothesis for sex-change: incorporating sperm competition and size-fecundity skew. *American Naturalist*, 161: 749-761.
- Muñoz R. C. and Warner R. R., 2004. Testing a new version of the size-advantage hypothesis for sex change: sperm competition and size-skew effects in the bucktooth parrotfish, *Sparisoma radians*. *Behavioral Ecology*, 15:129-136.
- Nakashima Y., Kuwamura, T. and Yogo Y., 1995. Why be a both-ways sex changer? *Ethology*, 101: 301–307.
- Nee S., Colegrave N., West S.A., and Grafen A., 2005. The illusion of invariant quantities in life histories. *Science*, 309:1236–1239.
- Nikolski G.V., 1963. *The ecology of fishes*. Academic Press, London.
- Noe M.A., 1969. Biology of the red grouper *Epinephelus morio* (Valenciennes) from the eastern Gulf of Mexico. *Florida Det. Natur. Resource Lab. Professional Papers*, 10:1-95.
- Ohno S., 1967. *Sex Chromosomes and sex-linked genes*. Springer, Heidelberg.
- Oldfield E., 1961. The functional morphology of *Kellia suborbicularis* (Montagu), *Montacuta ferruginosa* (Montagu) and *M. substriata* (Montagu), (Mollusca, Lamellibranchiata). *Proc. Malacol. Soc. London*, 34:255-295.
- Oliva D. and Castilla J.C., 1986. The effect of human exclusion on the population-structure of key-hole limpets *Fissurella crassa* and *Fissurella limbata* on the coast of central Chile. *Pubblicazioni della Stazione Zoologica di Napoli I*, 7(3):201-217.
- Ortega S., 1987. The effect of human gathering on the size distribution of *Siphonaria gigas* (Mollusca, Pulmonata) on the Pacific coast of Costa Rica. *Veliger*, 29(3): 51-255.
- Orton J.H., 1909. On the occurrence of protandric hermaphroditism in the Mollusc *Crepidula fornicata*. *Proceedings of Royal Society of London B, Biological Sciences*, 81:468-484.
- Orton J.H., 1920. Sex phenomena in the common limpet (*Patella vulgata*). *Nature (Lond.)*, 104:373.
- Orton J.H., 1927a. Observations and experiments on sex-change in the European oyster, *Ostrea edulis*. *J. Mar. Biol. Ass. U.K.*, 14:967-1045.
- Orton J.H., 1927b. A note on the physiology of sex and sex determination. *J. Mar. Biol. Ass. U.K.*, 14:1047-1055.
- Orton J.H., 1928. Observations on *Patella vulgata*. I. Sex phenomena, breeding and shell growth. *J. Mar. Biol. Ass. U.K.*, 15:851-862.
- Orton J.H., 1929. Observations on *Patella vulgata*. Part III. Habitat and habits. *J. Mar. Biol. Ass. U.K.*, 16:277-288.
- Orton J.H., 1946. The biology of *Patella* in Britain. *Nature (Lond.)*, 158:173.
- Orton J.H., 1950. Recent breeding phenomena in the American lipper-limpet, *Crepidula fornicata*. *Nature*, 165: 433–434.
- Orton J.H. and Southward A.J., 1961. Studies on the biology of limpets. IV. The breeding of *Patella depressa* Pennant on the North Cornish Coast. *J. Mar. Biol. Ass. U.K.*, 41:653-662.

- Orton J.H., Southward A.J. and Dodd J.M., 1956. Studies on the biology of limpets. II. The breeding of *Patella vulgata* L. in Britain. *J. Mar. Biol. Ass. U.K.*, 35:149-176
- Pagel M., 1997. Inferring evolutionary processes from phylogenies. *Zool. Scripta*, 26:331–348.
- Pagel M., 1999. Inferring the historical patterns of biological evolution. *Nature*, 401:877–884.
- Paine R.T., 1966. Food web community and species diversity. *Am. Nat.*, 100:65-76.
- Paine R.T., 1971. A short term experimental investigation of resource partitioning in New Zealand rocky intertidal habitat. *Ecology*, 52:1096-1106.
- Paine R.T., 1974. Intertidal community structure: experimental studies on the relationship between a dominant competitor and its principal predator. *Oecologia (Berlin)*, 15:93-126.
- Paine R.T., 1977. Controlled manipulations in the marine intertidal zone, and their contributions to ecological theory. In *The changing scenes in natural sciences 1776-1976* (Goulden C.E. ed.). Academy Natural Sciences, Philadelphia (Spec. Publ.) 12, pp.245-270.
- Paine R.T., 1980. Food webs: linkages, interaction strength and community infrastructure. *J. Anim. Ecol.*, 49:667-685.
- Paine R.T., 1994. *Marine Rocky Shores and Community Ecology: An experimentalist's Perspective*. Ecology Institute, Nordbrunten, Germany.
- Paine R.T. and Levin S.A., 1981. Intertidal landscapes, disturbance and the dynamics of pattern. *Ecol. Monogr.*, 51:145-178.
- Paine R.T. and Vadas R.L., 1969. The effects of grazing by sea urchins, *Strongylocentrotus* sp., on benthic algal populations. *Limnology and Oceanography*, 14:710-719.
- Parker G.A., 1970. Sperm competition and its evolutionary consequences in the insects. *Biological Review*, 45: 525–567.
- Parker G.A., 1984. Sperm competition and the evolution of animal mating strategies. In *Sperm competition and the evolution of animal mating systems* (Smith R.L. ed.). Academic Press, Orlando, Florida.
- Parker G.A., Baker R.R. and Smith V.G.F., 1972. The origin and evolution of gamete dimorphism and the male-female phenomenon. *J. Theor. Biol.*, 36: 529-553.
- Pearson T. H. and Rosenberg R., 1986. Feast and famine: structuring factors in marine benthic communities. In: Gee, J. H. R. & P. S. Giller (eds), *Organization of Communities: Past and Present*. Blackwell Scientific: 373–398.
- Pellegrini G., 1948. *Boll. Zool.*, 15:115-121.
- Pennington J.T., 1984. The ecology of fertilization of echinoid eggs: the consequences of sperm dilution, adult aggregation, and synchronous spawning. *Am. Zool.*, 24:130A.
- Petersen C.W. and Warner R.R., 2002. The ecological context of reproductive behavior. In *Coral Reef Fishes: Dynamics and Diversity in a Complex Ecosystem* (Sale, P.F., ed.), pp. 103–118, Academic Press.

- Petersen C.W., Mazzoldy C., Zarella K.A. and Hale R.E., 2005. Fertilization mode, sperm characteristics, mate choice and parental care patterns in *Artedius* spp. (Cottidae). *Journal of Fish Biology*, 67:239–254.
- Peterson C. W., Warner R.R., Shapiro D.Y. and Marconato A., 2001. Components of fertilization success in the bluehead wrasse, *Thalassoma bifasciatum*. *Behav. Ecol.*, 12:237–245.
- Picken G.B., 1980. The distribution, growth and reproduction of the Antarctic limpet *Nacella (patinigera) concinna* (Strebel, 1908). *J. Exp. Mar. Biol. Ecol.*, 42:71-85.
- Policansky D., 1982. Sex change in plants and animals. *Ann. Rev. Ecol. Syst.*, 13:471-495.
- Pombo O.A. and Escofet A., 1996. Effect of exploitation on the limpet *Lottia gigantea*: A field study in Baja California (Mexico) and California (U.S.A.). *Pacific Science*, 50(4):393-403.
- Price M.V. and Waser N.M., 1982. Population structure, frequency-dependent selection, and the maintenance of sexual reproduction. *Evolution*, 36: 35-43.
- Quinn J.F., Wing, S.R. and Botsford, L.W., 1993. Harvest refugia in marine invertebrate fisheries: models and applications to the red sea urchin, *Strongylocentrotus franciscanus*. *American Zoologist*, 33: 537–550.
- Raffaelli D., 1979. The grazer-algae interaction in the intertidal zone on New Zealand rocky shores. *J. Exp. Mar. Biol. Ecol.*, 38:81-100.
- Raffaelli D. and Hawkins S.J., 1996. *Intertidal Ecology*. Chapman and Hall, London.
- Reavis R.H., Grober M.S., 1999. An integrative approach to sex change: social, behavioural and neurochemical changes in *Lythrypnus dalli* (Pisces). *Acta Ethologica*, 2: 51–60.
- Robertson D.R., 1972. Social control of sex reversal in a coral reef fish. *Science (N. Y.)*, 177:1007-1009.
- Roche J., Texier J.-P., Découverte des restes humains dans un niveau atérien supérieur de la grotte des Contrebandiers, à Témara (Maroc). *Comptes Rendus des Séances de l'Académie des Sciences, Paris Série D* 282: 45– 47.
- Ross R.M., 1990. The evolution of sex-change mechanisms in fishes. *Env. Biol. Fish.*, 29: 81–93.
- Roubet F.-E., 1969. Le niveau atérien dans le stratigraphie côtière à l'Ouest d'Alger. *Palaeoecology of Africa*, 4:124–129.
- Ruppert E.E. and Barnes R.D., 1994. *Invertebrate Zoology*. 6th ed. Saunders, Fort Worth, Texas.
- Russel-Hunter W.D., 1979. *A life of invertebrates*. MacMillan, New York.
- Russell-Hunter W.D. and McMahon R.F., 1975. An anomalous sex-ratio in the sublittoral marine snail *Lacuna vincta* Turton, from near Woods Hols. *Nautilus*, 89:14-16.
- Russell-Hunter W.D. and McMahon R.F., 1976. Evidence for functional protandry in a fresh-water basommatophoran limpet, *Laevapex fuscus*. *Trans. Am. Microsc. Soc.*, 95: 174-182.
- Russell N.J., Bonsall C. and Sutherland D.G., 1995. The exploitation of marine molluscs in the Mesolithic of western Scotland: evidence from Ulva Cave, Inner Hebrides. In: Fischer, A. (Ed.), *Man and the Sea in the Mesolithic*. Oxbow books, Oxford, pp. 273-288.

- Sadovy Y. and Shapiro D.Y., 1987. Criteria for the diagnosis of hermaphroditism in fishes. *Copeia*, 1987:136-156.
- Sakai Y., Masanori K. and Tetsuo K., 2001. Effect of changing harem on timing of sex change in female cleaner fish *Labroides dimidiatus*. *Anim. Behav.*, 62: 251–257.
- Sakai Y., Kenji K., Tetsuo K., Yasuhiro N. and Yukiko M., 2003. Sexually dichromatic protogynous angelfish *Centropyge ferrugata* (Pomacanthidae) males can change back to females. *Zool. Sci.*, 20: 627–633.
- Santos, R.S., Martins, H.R. and Hawkins, S.J., 1989. *Relatório de estudos sobre o estado das populações de lapas do Arquipélago dos Açores e da Ilha da Madeira*. Departamento de Oceanografia e Pescas. Universidade dos Açores, Horta. 34p.
- Santos, R.S., Hawkins, S.J., Monteiro L. R., Alves, M. and Isidro, E.J., 1995. Marine research, resources and conservation in the Azores. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 5:311-354.
- Sastry A.N., 1979. *Reproduction of Marine Invertebrates* (Giese A.C. and Pearse J.S. eds.) Vol 5. Academic Press.
- Schultz E.T. and Warner R.R., 1989. Phenotypic plasticity in life-history traits of female *Thalassoma bifasciatum* (Pisces: Labridae). 1. Manipulations of social structure in tests for adaptive shifts of life-history allocations. *Evolution*, 43:1497–1506.
- Schultz E.T. and Warner R.R., 1990. Phenotypic plasticity in life-history traits of female *Thalassoma bifasciatum* (Pisces: Labridae). II. Correlation of fecundity and growth rate in comparative studies. *Environ. Biol. Fish*, 30:333–344.
- Schweitzer F.R., 1979. Excavations at Die Kelders, Cape Province, South Africa: the Holocene deposits. *Annals of the South African Museum*, 78:101–233.
- Scudo F.M., 1967. The adaptative value of sexual dimorfism. I. Anisogamy. *Evolution*, 21:285-291.
- Sewell, M.A., 1994. Small size, brooding, and protandry in the apodid sea cucumber *Leptossynapta clarki*. *Biological Bulletin (Woods Hols)*, 187:112-123.
- Shanks A.L., 1998. Apparent oceanographic triggers to the spawning of the limpet *Lottia digitalis* (Rathke). *Journal of Experimental. Marine Biology and Ecology*, 222: 31–41.
- Shapiro D.Y., 1984. Sex reversal and sociodemographic processes in coral reef fishes. In *Fish Reproduction: Strategies and Tactics* (Potts, G.W. and Wootton, R.J., eds), pp. 103–118, Academic Press.
- Siegfried W.R., Hockey P.A.R. and Crowe A.A., 1985. Exploitation and conservation of brown mussel stocks by coastal people of Transkei. *Environmental Conservation*, 12(4):303-307.
- Simenstad C.A., Estes J.A. and Kenyon K.W., 1978. Aleuts, sea otters and alternative stable state communities. *Science*, 200:403-411.
- Smith J.M., 1978. *The Evolution of Sex*. Cambridge Press, Cambridge.
- Smith J. M., 1982. *Evolution and the Theory of Games*. Cambridge University Press, Cambridge.
- Smith R.L. (ed.), 1984. *Sperm competition and the evolution of animal mating systems*. Academic press, Orlando, Florida.

- Snelgrove P. V. R. and Butman C. A., 1994. Animal sediment relationships revisited—cause versus effect. *Oceanography and Marine Biology*, 32: 111–177.
- Snook R.R., 2005. Sperm in competition: not playing by the numbers. *Trends in Ecology and Evolution*, 20: 46–53.
- Soong K. and Chen J.L., 1991. Population structure and sex-change in the coral-inhabiting snail *Coralliophila violacea* at Hsiao-Liuchiu, Taiwan. *Marine Biology*, 111:81-86.
- Sousa W.P., 1979a. Disturbance in marine intertidal boulder fields: the nonequilibrium maintenance of species diversity. *Ecology*, 60: 1225-1239.
- Sousa W. P., 1979b. Experimental investigations of disturbance and ecological succession in a rocky intertidal community. *Ecol. Monogr.*, 49:227-254.
- Sousa W.P., 1984. The role of disturbance in natural communities. *Ann. Rev. Ecol. Syst.*, 15:353-391.
- Southward A.J. and Dodd J.M., 1956. Studies on the biology of limpets. I. The late J.H. Orton's work on *Patella*. *J. Mar. Biol. Ass. U.K.*, 35:145-147.
- St Mary C. M., 1997. Sequential patterns of sex allocation in simultaneous hermaphrodites: do we need models that specifically incorporate this complexity? *American Naturalist*, 150: 73-97.
- Stanley N., 1987. Products and uses of carrageenans. In *Production and Utilization of Products from Commercial Seaweeds*. (McHugh D.J. ed.), FAO, Rome, FAO Fish. Tech. Pap., 288:116-146.
- Stearns S.C. (ed.), 1987. *The Evolution of Sex and Its Consequences*. Birkhauser Verlag, Basel.
- Steneck R.S., 1982. A limpet-coraline alga association: adaptations and defences between a selective herbivore and its prey. *Ecology*, 63:(2):507-522.
- Steneck R.S., 1991. Plant-herbivore coevolution; a reappraisal from the marine realm and its fossil record. In *Plant-Animal Interactions in the Marine Benthos*. (John D.M., Hawkins S.J. and Price J.H. eds.). Oxford, Clarendon press, for the Systematics Association, pp.477-491.
- Stimson J.S., 1970. Territorial behaviour of the owl limpet *Lottia gigantea*. *Ecology*, 51:113-118.
- Stimson J.S., 1973. The role of the territory in the ecology of the owl limpet *Lottia gigantea* (Gray). *Ecology*, 54:1020-1030.
- Stiner M.C., 1994. *Honor Among Thieves: A Zooarchaeological Study of Neandertal Ecology*. Princeton University Press, Princeton.
- Strathmann R., 1974. The spread of sibling larvae of sedentary marine invertebrates. *Am. Nat.*, 108: 29-44.
- Sugg D.W. and Chesser R.K., 1994. Effective population sizes with multiple paternity. *Genetics*, 137: 1147–1155.
- Sunobe T., Nakamura M., Kobayashi Y., Kobayashi T. and Nagahama Y., 2005. Immunoreactivity in the gobiid fish *Trimma okinawae* during bidirectional sex change. *Ichthyological Research*, 52: 27–32.
- Sutherland J.P., 1970. Dynamics of high and low populations of limpet *Acmaea scraba* (Gould). *Ecol. Monogr.*, 40:169-188.

- Sutherland J.P., 1972. Energetics of high and low populations of the limpet *Acmaea scabra* (Gould). *Ecology*, 53:430-437.
- Swadling, P.A., 1976. Changes induced by human predation in prehistoric shellfish populations. *Mankind*, 10: 156-162.
- Taylor R.G., Whittington J. A., Grier H. J. and Crabtree R. E., 2000. Age, growth, maturation, and protandric sex reversal in common snook, *Centropomus undecimalis*, from the east and west coasts of South Florida. *Fish. Bull.*, 98: 612–625
- Telesh I. V., Alimov A. F., Golubkov S. M., Nikulina V. N. and Panov V. E., 1999. Response of aquatic communities to anthropogenic stress: a comparative study of Neva Bay and the eastern Gulf of Finland. *Hydrobiologia*, 393: 95–105.
- Thompson G.B., 1979. Distribution and population dynamics of the limpet *Patella ulyssiponensis* in Bantry Bay. *J. Exp. Mar. Biol. Ecol.*, 40:115-135.
- Thompson G.B., 1980. Distribution and population dynamics of the limpet *Patella vulgata* in Bantry Bay Southwest Ireland. *J. Exp. Mar. Biol. Ecol.*, 45:173-218.
- Thompson J.D. and Brunet J., 1990. Hypotheses for the evolution of dioecy in seed plants. *Trends Ecol. Evol.*, 5:11-16.
- Tranter D.J., 1958. Reproduction in Australian Pearl Oysters (Lamellibranchia). III. *Pinctada albina* (Lamarck): Breeding Season and Sexuality. *Aust. J. Mar. Freshwater Res.*, 9:191-216.
- Underwood A.J., 1976. Food competition between age classes in the intertidal Neritacean *Nerita atramentosa* Reeve (Gastropoda: Prosobranchia). *J. Exp. Mar. Biol. Ecol.*, 23:145-154.
- Underwood A.J., 1979. The ecology of intertidal gastropods. *Adv. Mar. Biol.*, 16:11-210.
- van Rooij, J.M. and Videler, J.J. (1997) Mortality estimates from repeated visual censuses of a parrotfish (*Sparisoma viride*) population: demographic implications. *Mar. Biol.* 128, 385–396.
- van Rooij J.M., Bruggemann J. H., Videler J. J. and Breeman A. M., 1995. Plastic growth of the herbivorous reef fish *Sparisoma viride*: field evidence for a trade-off between growth and reproduction. *Mar. Ecol. Prog. Ser.*, 122: 93–105.
- Vermeij G.J., 1972. Intraspecific shore-level size gradients in intertidal molluscs. *Ecology*, 53:57-85.
- Voight E., 1973. Stone Age molluscan utilization at Klasies River mouth caves. *S. Afr. Sci.*, 69(10):306-309.
- Volman T.P., 1978. Early archaeological evidence for shellfish collecting. *Science*, 201:911-913.
- von Medem F.G., 1945. Untersuchungen über die Ei- and Sperma-wirkstoffe bei marinen Mollusken. *Zool. Jb. (Abt. Anat. Ont. Tiere)*, 61:1-44.
- Waechter J.D.A., 1964. The excavation of Gorham's Cave, Gibraltar, 1951–54. *Bulletin of the Institute of Archaeology*, 4:189–213.
- Warner R.R., 1975. The adaptative significance of sequential hermaphroditism in animals. *Am. Nat.*, 109:61-82.

- Warner R.R., 1978. The evolution of Hermaphroditism and unisexuality in the aquatic and terrestrial vertebrates. In *Contrast in behaviour* (Reese W.S., Lighter F.L., eds.). New York, pp. 77-102.
- Warner R.R., 1984. Mating behavior and hermaphroditism in coral reef fishes. *Am. Sci.*, 72: 128–136.
- Warner R.R., 1987. Female choice of sites versus mates in a coral reef fish *Thalassoma bifasciatum*. *Animal Behaviour*, 35:1470–1478.
- Warner R.R., 1988. Sex change and the size-advantage model. *Trends Ecol. Evol.*, 3:133-136.
- Warner R.R. and Hoffman S.G., 1980. Local population size as a determinant of mating system and sexual competition in two tropical marine fishes (*Thalassoma* spp.). *Evolution*, 34:508-518.
- Warner R.R. and Muñoz R C., 2008. Needed: a dynamic approach to understand sex change *Animal Behaviour*, 75: 11-14.
- Warner R.R. and Schultz E.T., 1992. Sexual selection and male characteristics in the bluehead wrasse, *Thalassoma bifasciatum*: mating site acquisition, mating site defense and female choice. *Evolution*, 46:1421–1442.
- Warner R.R. and Swearer. S.E., 1991. Social control of sex change in the bluehead wrasse *Thalassoma bifasciatum* (Pisces: Labridae). *Biol. Bull.*, 181:199–204.
- Warner R.R., Fisch D.L. and Standish J.D., 1996. Social control of sex in the shelf limpet, *Crepidula norrisiarum*: size-specific responses to local group composition. *J. Exp. Mar. Biol. Ecol.*, 204:155-167.
- Warner R.R., Robertson D.R. and Leigh E.G., 1975. Sex change and sexual selection. *Science*, 190:633-638.
- Webber H.H., 1977. Gastropoda: Prosobranchia. In *Reproduction of Marine Invertebrates* (Giese A.C. and Pearse J.S. eds.) vol 4. Academic Press, New York.
- Well S.M., 1981. International Trade in ornamental shells. *Proc. 4th Int. Coral Reef Symp.*, Manila 1, pp.323-330.
- Wenner A.M., 1972. Sex ratio as a function of size in marine crustacea. *Am. Nat.*, 106:321-350.
- Werren J.H., 1980. Sex ratio adaptations to local mate competition in a parasitic wasp. *Science*, 208:1157-1159.
- Whitlatch R. B., Hines A. H., Thrush S. F., Hewitt J. E. and Cummings V., 1997. Benthic faunal responses to variations in patch density and patch size of a suspension-feeding bivalve. *Journal of Experimental Marine Biology and Ecology*, 216: 171–189.
- Wilczynski J.Z., 1955. On sex behaviour and sex determination in *Crepidula fornicata*. *Biological Bulletin*, 109: 353–354.
- Williams G.C., 1975. *Sex and Evolution*. Princeton University Press, Princeton.
- Wittenrich M.L. and Munday P.L., 2005. Bi-directional sex change in coral reef fishes from the family Pseudochromidae: an experimental evaluation. *Zoological Science*, 22: 797-803.

- Wood E. and Wells S.M., 1988. *The Marine Curio Trade: Conservation Issues*. Marine Conservation Society, Ross-on-Wye, Herefordshire, UK.
- Workman C., 1983. Comparisons of energy partitioning in contrasting age-structured populations of the limpet *Patella vulgata* L. *J. Exp. Mar. Biol. Ecol.*, 68:81-103.
- Wright S., 1938. Size of population and breeding structure in relation to evolution. *Science*, 87: 430-431.
- Wright W.G., 1982. Ritualized behaviour in a Territorial limpet. *J. Exp. Mar. Biol. Ecol.*, 60: 245-251.
- Wright W.G., 1988. Sex change in the Mollusca. *Trends Ecol. Evol.*, 3:137-140.
- Wright W.G., 1989. Intraspecific density mediates sex-change in territorial patellacean *Lottia gigantea*. *Marine Biology*, 100:353-364.
- Wright W.G. and Lindberg D.R., 1979. A non-fatal method of sex determination for patellacean gastropods. *J. Mar. Biol. Ass. U.K.*, 59:803.
- Wright W.G. and Lindberg D.R., 1982. Direct observations of sex change in the patellacean *Lottia gigantea*. *J. Mar. Biol. Ass. UK*, 62:737-738.
- Yates R.J., 1998. A Phase 2 archaeological investigation of site 1 on Farm 1062 (previously 967), Paternoster, Western Cape, South African Museum, Cape Town.
- Yesner D.R., 1984. Population pressure in coastal environments: an archaeological test. *World Archaeol.*, 16(1): 108-127.
- Yonge C.M., 1949. *The sea shore*. Bloomsbury Books, London.