Chapter 10

Stock structure analysis and species identification

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Abstract: There is a particular lack of data for exploited squid on fundamental aspects of taxonomy, genetic population structure, locations and patterns of spawning and migrations, and factors affecting population dynamics. This review considers currently available information on species identity and stock structure in the genus *Illex* (order Teuthoidea), using predominantly ecological, parasitological, molecular genetic and morphometric data. Molecular genetic and ecological (distribution and abundance of life-history stages) approaches have so far been most useful at the population level, though species identity has been aided also by studies on squid morphology and parasite distributions. The majority of relevant population studies have been undertaken on *Illex argentinus* in the southwest Atlantic, where data strongly suggest the presence of two or more cryptic species. Indeed, cryptic speciation is evident among other ommastrephids (e.g. *Martialia hyadesi*) and loliginids (e.g. *Nototodarus sloani*, *Loligo edulis*). Despite the extensive migrations of these animals, gene flow appears to be sufficiently restricted to allow the accumulation of genetic and presumably life-history differentiation likely to affect recruitment dynamics. Thus, the feeding groups captured by most fisheries may not necessarily represent interbreeding units. There is an urgent need to integrate molecular, morphological and demographic studies to review teuthoid systematics. *Illex* typically display low levels of genetic variability (P, proportion of polymorphic loci ($P_{(O,95)}$) = 0.172–0.280; H_L mean observed heterozygosity per locus = 0.000–0.011), which together with the characteristic population differentiation at the allozyme level, indicates a particular susceptibility to over-exploitation of weakly represented stocks. Microgeographic differentiation in allozymes on the scale of tens of km has occasionally been observed in *I. argentinus*, perhaps representing movements of squid schools. Ecological studies based on larval and pre-spawning adult distributions support the notion of a complex stock structure in the southwest Atlantic. Several spawning groups based on water depth and season of maximal spawning activity have been proposed, though the genetic integrity of such groups remains unclear. Among other teuthoids, both population homogeneity (indicating freely interbreeding groups) and heterogeneity (indicating some restriction to gene flow) has been disclosed, indicating a complex relationship between dispersal capacity, gene flow and population differentiation in these animals.

1 Introduction

In recent decades the Cephalopoda, including cuttlefish, octopus and squid, have come to form one of the major invertebrate fisheries in the world (Rathjen and Voss 1987). Recent landings were well in excess of 2 000 000 t in 1987 and 1988, the last years for which official statistics are available (Roper and Rathjen 1991). Indeed, the world catch doubled between 1970 and 1980 (Roper *et al.* 1984), and the geographic areas where commercial fisheries operate expanded (Rathjen and Voss 1987). Despite the rapid growth in cephalopod landings, Voss (1983) estimated that the potential for such a fishery on the continental shelf alone is estimated at between 7 and 10 million t – "the largest source of harvestable but under-exploited protein in the oceans". The rapid rise in demand for cephalopod resources has, however, not been met by a commensurate increase in our understanding of their population biology.

The short life spans and unpredictable and extensive fluctuations in population size characterizing many cephalopod life histories (Amaratunga 1987, Calow 1987), together with the increased efficiency of capture techniques (Rathjen 1991), have placed severe pressure on cephalopod resources. Unlike the wealth of

information generated from plankton samples on the distribution and abundance of fish eggs and larvae, few cephalopod eggs have been obtained, and young stages are typically sparse. Indeed, the egg and juvenile stages are the least known of all stages in the cephalopod life cycle, especially in pelagic squid (Boyle 1990). Fundamental infonnation on spatial and temporal patterns of cephalopod recruitment is thus correspondingly fragmented.

Approximately 80 percent of the annual cephalopod catch (c. 1.8 million t, FAO *unpubl. data* 1990) comprise members of the order Teuthoidea, the squids. It is among both the myopsid (e.g. *Loligo* spp.) and oegopsid (e.g. *Illex* and *Todarodes*) squids that spectacular expansions in commercial operations have occurred, especially in the southwest Atlantic and northwest Pacific. The 1980s witnessed a massive surge in squid landings in the southwest Atlantic, starting with 31 000 t in 1980, and peaking at 743 189 t in 1987, of which 650 124 t were *Illex argentinus*, with smaller but significant catches of *Loligo gahi* (93 065 t) and *Martialia hyadesi* (22 000 t, 1986). In the northwest Pacific, *Todarodes pacificus* is the major target species, with catches since 1985 fluctuating between 128 000 and 243000 t. Indeed, substantial annual variations in catch statistics are common for all squid fisheries (Roper and Rathjen 1991), presumably representing the combined effects of natural and fishery-driven fluctuations in population size. It is noteworthy that even for *T. pacificus*, the best studied of commercial squid species, the cause for such interannual variation is unclear.

At the outset then, three primary features of squid fisheries are apparent:

a) a recent substantial increase in exploitation;
b) marked, and often unpredictable, fluctuations in population size; and
c) a poor understanding of fundamental aspects of their fisheries biology, most notably species and stock identification, spatial-temporal variability in spawning and recruitment, and patterns of juvenile and adult migration.

2 Concepts of species identification and stock structure analysis

2.1 Species identification and stock structure analysis: definitions

It is important to appreciate the role that species identification and stock structure analysis (SSA) play in the stock assessment and management of a fishery. Stock assessment involves the use of various statistical and mathematical calculations to make quantitative predictions about the reactions of exploited populations to alternative management options (Hilborn and Walters 1992). The data required to enable such predictions are based on such measures as fecundity, stock size, age structure and recruitment -all factors that should ideally be derived from observations on natural populations. In addition to species-specific differences in factors affecting recruitment, most species are fragmented into a number of distinct populations, either temporally or spatially, and it is therefore important to estimate the extent to which any identifiable "units" differ in any of the above measures. Thus, the extent to which a species exhibits heterogeneity in processes affecting distribution and abundance, whether environmental or genetic in origin, is critical to the formulation of meaningful estimates of mortality and recruitment, especially when the response to harvesting has to be predicted.

The species concept most useful to fisheries management, though not necessarily easiest to determine empirically, is the biological species concept of Mayr (1963): "a species is a group of interbreeding natural populations that is reproductively isolated from other such groups". Fundamental to Mayr's (1963) definition is that members of the same species form a reproductive community whereby individuals recognize and seek each other for reproduction, and as such share a common gene pool. Because complete reproductive isolation usually leads to the evolution of fundamental biological differences, many of which can affect rates of growth, reproduction and mortality, it is vital to determine the presence of such discrete entities in a fishery.

Problems of species identification –usually arise when populations comprise so-called" cryptic" or sibling species, which are morphologically similar or identical populations that are reproductively isolated. For example, *Loligo pealei* and *Loligo plei* are morphologically very similar and difficult to differentiate morphologically, but they are distinct at the allozyme level, differing completely at five out of nine enzymecoding loci (Garthwaite *et al.* 1989). Information on the abundance and distribution of young stages or larvae is often critical to aspects of stock assessment, and it is often such stages in the life cycle that are morphologically indistinct. Although molecular tools have revealed the existence of cryptic speciation among squid populations (e.g. Smith *et al.* 1981, Brierley *et al.* 1993a, Yeatman and Benzie 1993), taxonomic uncertainty remains a basic problem in teuthoid biology, including *Illex* (Thorpe *et al.* 1986, Carvalho *et al.* 1992), especially in view of the usually marked morphological plasticity among individuals of a species. Reliable taxonomic characters are scarce (Voss 1977, 1983), and within-species variability and lack of descriptions of juvenile stages are major obstacles to fishery management (Boyle 1990).

In addition to species-level systematics, intra-specific differentiation is of major concern to stock assessment. For the purposes of the present review, the population unit or "stock" will be defined as an intraspecific group of randomly mating individuals with temporal and spatial integrity (Ihssen *et al.* 1981), though the particular definition employed in fisheries biology will depend on the particular management objective (Carvalho and Hauser 1994). Although the above definition may resemble superficially that of the biological species, the degree of reproductive isolation in a stock is typically only partial, except of course where populations are entirely allopatric. In the latter case, distinct intra-specific stocks will exhibit markedly less genetic differentiation than among separate species (Thorpe 1983).

The extent to which individuals share a common gene pool will determine, through interactions with the environment, the extent of biological differentiation among stocks. Implicit in this definition of a stock are two central concepts:

a) the subdivision into local populations, that is, a definable stock structure; and
b) that differences, some of which may be genetic and occasionally adaptive (Carvalho 1993), exist between such populations.

The taxonomic status of the stock concept depends on the nature of the unit under study, and may refer to the species, sub-species, "race" or other identifiable unit (Carvalho and Hauser 1994). Irrespective of the degree of taxonomic divergence, it is critical that there are "indications of a significant degree of separation at spawning to support a biological basis for separate stocks" (Brown *et al.* 1987), which are absolute when distinct species are considered. Such separation may be spatially and/or temporally based. Since all natural populations display intra-specific variation for almost any measured character, the statistical properties of any groups should be examined to ensure that adequate sample sizes and sampling design are employed to detect differentiation. A stock should not be viewed as comprising a homogeneous group which is dissimilar to other such groups; rather it is a heterogeneous group of individuals that are characterized by exhibiting a specific range of variation, whether morphological, meristic, molecular or reproductive. Furthermore, sampling should take into account any small-scale differentiation due to habitat or behaviour, especially the influence of school integrity (Hurley 1978, Pitcher 1992).

The analysis of stock structure has four essential elements (Fig. 10.1):

a) the characterization of each sample or population in terms of phenotypic or genetic trait(s);
b) the statistical analysis of the data relative to the null hypothesis that all samples are drawn from a single, large randomly mating group, and therefore exhibit similar frequencies of the measured trait(s);
c) that some estimate be made of the degree of biological differentiation among recognized stocks; and
d) that such information be incorporated into models of stock assessment.

SSA starts by choosing an appropriate source of markers, and ends with the implementation of some management decision based on the options generated from stock assessment. Unfortunately, in the majority of fishery applications, including most of those concerned with squid, activity usually ceases after the second stage, and the process becomes *descriptive* rather than *functional*. Describing differences alone means little; it is the biological significance of any differentiation detected by markers that is of importance. From a fisheries perspective, SSA is incomplete unless the biological significance of stock structure is examined, and its implications in relation to population dynamics considered.

The data generated from SSA may be used either directly or indirectly (Fig. 10.1) to obtain biological information for stock assessment. Direct use includes the employment of detectable markers (e.g. coded wire tags, parasites, allele frequencies, blood pigments) to monitor the spatial and/or temporal distribution of stocks, and in stock composition analysis (Pella and Milner 1987). The latter does, however, require an ability to characterize separate stocks prior to mixing, such as when they arrive on spawning grounds (e.g. genetic stock identification [GSI] in Pacific salmon; Miller *et al.* 1983). Indirect use involves markers that identify groups which can then be characterized using measures such as age structure, fecundity, growth rate and recruitment. Comparisons among such groups can determine whether significant differentiation occurs, and whether or not it is necessary to take such data into account for stock assessment. Clearly, both approaches depend on the availability of suitable discriminatory markers; effective SSA thereby provides a mechanism for organizing biological diversity among populations into recognizable groups that can then be compared subsequently.

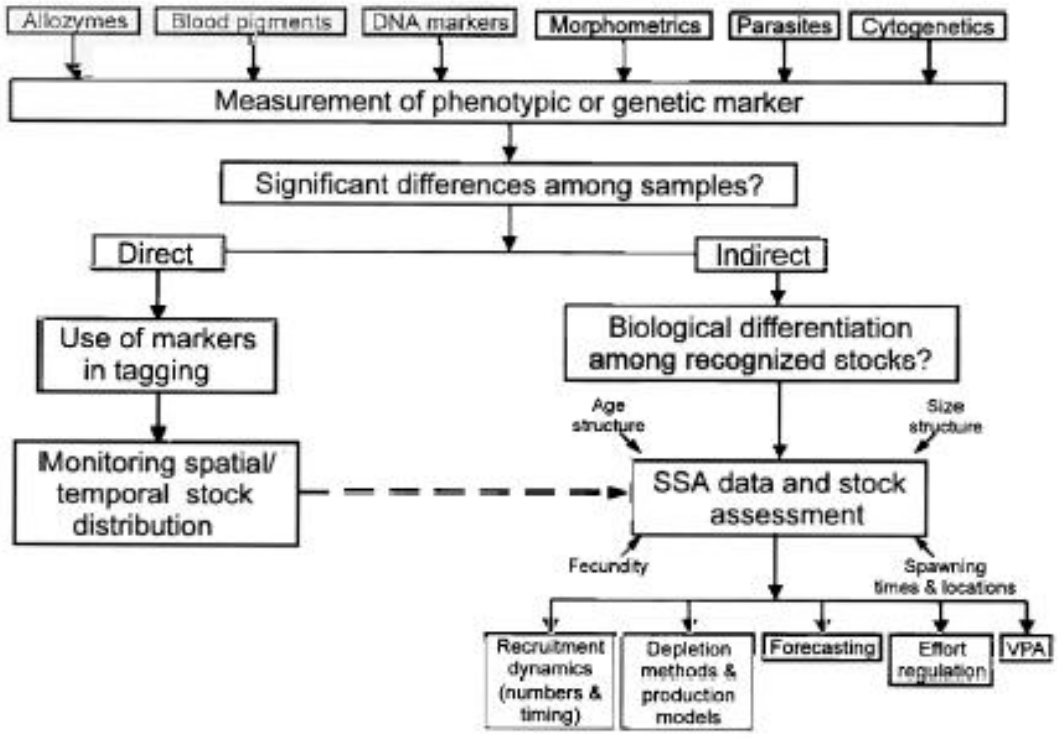

Figure 10.1. The process of stock structure analysis (SSA) in fisheries; a variety of markers are available to examine differentiation among population samples. Such markers can be used either directly in tagging to monitor stock dynamics, or indirectly as markers in the assessment of differentiation at other biological levels. A vital component of SSA is the incorporation of data into stock assessment and subsequent management of the exploited resource. VPA = virtual population analysis

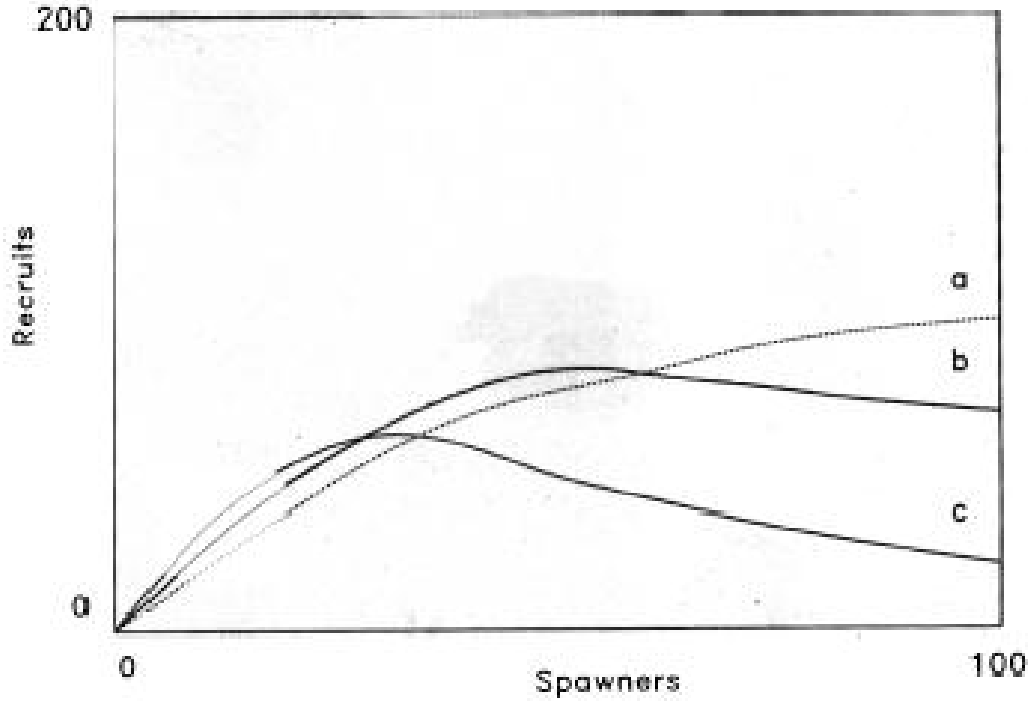

Figure 10.2. Stock recruitment relationships for an exploited species that consists of spatially separated stocks when all stocks are present (a), and when less productive stocks have been eliminated by over-exploitation (b and c); stock separation, detectable by SSA, thus means that stock-recruitment curves are not necessarily fixed in time (modified after Hilborn and Walter 1992).

2.2 Stock structure and recruitment dynamics: The importance of time scale

Within the context of recruitment dynamics and stock structure, it is important to point out that the implications of management decisions can be viewed on two time scales. The first, and most commonly considered, is the short-term consequence in terms of the effects of harvesting (stock-recruitment data; e.g. Lange and Sissenwine 1983) on stock size, usually associated with the calculation of an escapement rate (Beddington *et al.* 1990) designed, at least in principle, to maintain sustainable yields. Such an approach is concerned essentially with quantitative changes in populations. For example, if stocks differed in their relative strengths (Larkin 1981), an exploited species with a discrete stock structure (that is, composed of several stocks) would exhibit a markedly different stock-recruitment relationship compared with that of a single stock situation (Fig. 10.2). If a stock is strong, it may be harvested at a high intensity (e.g. 80–90 percent) and still leave sufficient breeding individuals to maintain the population at a sustainable level. However, for a numerically depleted stock, maximum harvest may only permit an intensity of perhaps 10–20 percent. Thus in a mixed fishery, none can be harvested at an optimal level; either the weaker ones are over-exploited or the stronger under-exploited. Over-harvesting of less productive stocks may reduce the overall number of recruits or, ultimately, cause destruction of the stock.

Because the ideal measure of spawning stock, the number of eggs, is rarely obtainable, estimates are often made in species with a life span >1 year by multiplying the number of individuals at a particular age by the average fecundity of individuals of that age, and summing over ages. Persistent and marked heterogeneity in age structure or fecundity among putative stocks will profoundly affect recruitment dynamics. The problem then is to decide when such heterogeneity falls into sufficiently discrete classes to warrant a multi-stock approach (Hilborn and Walters 1992).

The second, and much less often considered aspect of stock structure, is related to qualitative changes in populations, that is alterations in genetic structure. Because genetic considerations are equated with evolutionary change, the time scale involved is often considered, wrongly, to be irrelevant to resource management. Evolutionary change in populations may take place in a matter of decades (Gharrett and Thomason 1987, Vuorinen *et al.* 1991, Magurran *et al.* 1992) or even less (Reznick *et al.* 1990), depending on such factors as population size, levels of genetic variability for ecologically significant traits, and the intensity of selection pressures. The critical point here is that because the parameters affecting recruitment are determined by the interaction between genotype and environment, progressive changes in gene frequencies may modify life-history traits, especially where the nature of harvesting is intense and/or selective (Turner 1977, Smith *et al.* 1991, Carvalho and Hauser 1992). Perpetuation of a resource presumably implies perpetuation of similar morphological and life-history features over time, rather than induction of changes in such features as fecundity, age at maturity or overall body size. In addition to minimizing genetic and phenotypic change, there is value also in conserving genetic variability due to the theoretical positive correlation between genetic variation and ability to adapt to natural or man-made changes in the environment (Fisher 1930, Nelson and Soulé 1987, Carvalho 1993).

The notion that exploited species constitute a renewable resource is a dangerous one, and is true only in part. The loss of a locally adapted population or an endemic species clearly cannot be reversed, and a decrease in genetic variability within a population can, in the absence of migration, be compensated only through mutations on an evolutionary time scale. By failing to distinguish between reversible and irreversible genetic changes in fisheries management, dramatic species changes have sometimes occurred (Ribbink 1987, Witte *et al.* 1992). More common consequences, however, include a reduced adaptation to localized conditions (Hindar etal. 1991, Carvalho 1993) and permanent changes in life-history characters (Ricker 1972).

SSA can play a role in both monitoring genetic changes and in conserving genetic diversity (Carvalho and Hauser 1994). Using appropriate methodology such as the analysis of size frequencies (e.g. Rodhouse 1991), morphometrics (e.g. Borges 1990), fecundity differentials (e.g. Tingley *et al.* 1992) or allozyme frequencies (e.g. Carvalho and Pitcher 1989), it is possible to determine whether significant differences exist among samples separated in time, though genetic and phenotypic change may occur independently. Where possible, it is necessary first to estimate the range of fluctuation in any measured trait(s) in order to distinguish annual variation from directional change that may be due to environmental factors such as exploitation.

The extent of stock separation can be determined directly using stock identification procedures. If a population comprises freely interbreeding, genetically homogeneous groups, certain areas in which concentrated fishing occurs would be restocked from unflshed areas. If, however, more than one stock exists, and they differ either in their abundance or susceptibility to exploitation (Hilborn and Walters 1992, Fig. 10.2), one or more stocks may crash and fail to recover, thus eliminating part of the gene pool. There may then be a corresponding reduction in overall levels of genetic variability-considerations that are particularly important in those species showing either marked population differentiation or low levels of genetic variability. Both such features are typical of at least some exploited squid populations, including *Illex* (Thorpe *et al.* 1986, Carvalho *et al.* 1992).

SSA may thereby reveal information about both quantitative and qualitative changes in populations. Although there has been a general reluctance to accept the importance of the latter in fisheries biology, primarily because of the immediate desire to minimize the chances of stock collapse, studies on stock structure provide a mechanism for combining data on population dynamics with changes in genetic structure. Management options should incorporate data from both aspects in order to ensure perpetuation of a resource, since perpetuation involves genetic transmission across generations, where there are opportunities for shortterm genetic and evolutionary change.

2.3 Methods of species and stock identification

Several methods have been employed in species identification and SSA including the use of ecological studies, tagging, distribution of parasites, physiological and behavioural aspects, morphometrics and meristics, calcified structures, cytogenetics, immunogenetics, blood pigments, allozyme electrophoresis and nucleic acid analysis (Ihssen *et al.* 1981, Kumpf *et al.* 1987, Ryman and Utter 1987, Ovenden 1990, Carvalho and Hauser 1994, Ward and Grewe 1994). Given the diversir;y of approaches available, it is recommended that data from more than a single source be used, and the results compared, especially where the range of intraspecific variation is marked. Criteria considered in choosing the appropriate techniques include adequate provision of clear markers, speed of processing, range and control of intra-specific variation, running costs and equipment requirements (Kumpf *et al.* 1987).

In the present review, attention will focus on the application of ecological (e.g. life history, size structure, spawning and migration patterns), parasitological, molecular genetic (allozyrne electrophoresis) and morphometric studies to species identification and SSA in *Illex* and other teuthoids. Detailed information on practical aspects of data collection are given elsewhere including ecological (Patterson 1988, Nigmatullin 1989, Rodhouse and Hatfield 1990a), parasitological (Nigrnatullin and Shukhgalter 1990, Bower and Margolis 1991), molecular (Richardson *et al.* 1986, Carvalho and Hauser 1995), and morphometric (Kashiwada and Recksiek 1978) aspects. Molecular techniques have become particularly popular in recent years (Ryman and Utter 1987, Carvalho and Pitcher 1994), offering a variety of protein and nucleic acid markers that differ in discriminatory powers.

3 Management of squid fisheries

3.1 Aspects of squid biology in relation to stock structure

Despite the application of fin-fish stock-assessment models to squid populations, there are fundamental differences between the population biology of most commercially exploited fish and squid species (Saville 1987). Such differences are relevant here because they influence the choice of methods employed in SSA, the interpretation of data generated, and their incorporation into stock-assessment models.

3.1.1 Taxonomic difficulties

When formulating realistic parameters of growth, recruitment and mortality, it is critical that reproductively isolated units are recognized unambiguously to examine the respective contributions of withinand between-species differentiation. If several squid species are included in a sample, the variance in any measures may not only lead to false predictions regarding such things as escapement rates, but also ignore differences in the relative production of distinct species (Fig. 10.2). Extreme sexual dimorphism, morphological plasticity and the production of juveniles lacking species-specific diagnostic features (Voss 1983) serve to confound attempts to describe squid species in simple terms. Furthermore, because of a lack of detailed morphometric studies on a population basis, there is a marked lack of sub-specific taxa, thus ignoring cases of incipient speciation (Augustyn and Grant 1988, Brierley 1992).

Among *I. argentinus* in the southwest Atlantic, for example, there is strong molecular evidence indicating the existence of more than a single species (Thorpe *et al.* 1986, Carvalho *et al.* 1992) that is presently not incorporated into stock-assessment models. Similar cryptic speciation is apparent in other squid, including *Notodarus* (Smith *et al.* 1981), *Martialia* (Brierley *et al.* 1993a) and *Loligo* (Yeatman and Benzie 1993). The overall effect of such confusion has been to cloud the taxonomic significance of biological differentiation among nominal squid species.

3.1.2 Short life spans

The generally semelparous life cycle of extant squid (cf. Harman *et al.* 1989), in which individuals grow rapidly, become reproductively mature, spawn and then die, has profound implications both on the rate of evolutionary change and on susceptibility to harvesting. In fish populations, the norm is for individuals to live and breed for several years, thus dampening any demographic response to exploitation. In most species of squid, however, there is no competition between successive cohorts, and each annual bout of recruitment is dependent mainly upon the size and nature of spawning individuals remaining from the previous years. The stock exploited by the fishery is thus usually composed of recruits only. Furthermore, the fishery in one year relates to the fishery in subsequent years through the stock-recruitment relationship, however weak this may be. The management aim must therefore be to enable sufficient spawners to escape the fishery at the end of the season to allow sufficient recruitment in the subsequent year. Under such circumstances of semelparity, especially in the face of sudden reductions in population size, the response to directional selection may be rapid, emphasizing the importance of monitoring harvesting activities carefully. Modelling the effects of fishery selection has shown that harvesting may indeed impose artificial selection on size at maturity in *Illex* (*see Chapter 12*).

The fragility of gene pools in species with an annual life cycle, compared with those that survive and reproduce between years, is exacerbated in the case of *I. argentinus* because of the already apparently low levels of genetic diversity and marked population differentiation (Thorpe *et al.* 1986, Carvalho *et al.* 1992).

3.1.3 Spawning and recruitment

Realistic estimates of recruitment -the number of juveniles entering the fisheries each year as a result of the last spawning season –depend upon the availability of data on population size, age and fecundity. In a species with an essentially annual life span, a constant and rather high recruitment is necessary to maintain stocks against fishing and natural mortality. Recruitment in squid fisheries is determined by analysing the length frequency modes in the catch, and noting the numbers and timing of young squid entering the fishery (Beddington *et al.* 1990, Rosenberg *et al.* 1990, Hatfield and Rodhouse 1991). In a fishery conducted by jigging, as in many *Illex* catches (Rathjen 1991), the size of the recruits caught is determined by the catch characteristics of the commercial jigs: small animals are unable to take the jig. Similarly, in a trawl fishery, the size of the animals is determined by the mesh size. Thus the size range of squid available for the recruitment determination is limited. In fin-fish fisheries, the recruitment is determined, in many cases, long before the young appear in the catch, and thus there is a forecast capability. Squid eggs, larvae and early juveniles are rarely taken in sufficient quantities to have a predictive value (Mangold 1987, Boyle 1990). Significant obstacles will remain in providing stock early recruitment forecasts until information on the location and timing of spawning, and depth levels sought by the hatchlings and early juveniles, is more widely available. The majority of information on spawning time, with the exception of work by Nigmatullin (1989), is based on capture of mature females or back calculation of hatching date, and furthermore, there are few records of whether eggs are contained within the oviduct, which is the only sure indication of mature, ready-to- spawn females.

3.1.4 Growth and influence of temperature

Forsythe and Hanlon (1989) pointed out the inappropriateness of using fin-fish stock models for squid, where cephalopods do not grow asymptotically. Further, temperature is generally not critical in assessing the potential productivity of fish resources, since slight thermal changes do not significantly affect growth rates. Virtually the opposite is true for squid where, except for food availability, possibly no other factor has a stronger influence on growth than temperature. Growth at different temperatures can result in squid of markedly different size and life span, thus introducing a high degree of variability in growth-related parameters on both a seasonal and regional basis.

3.1.5 Migrations

Although many aquatic species undergo extensive feeding and reproductive migrations, the locations and timing of animal movements and aggregations are in many cases well documented (e.g. McCleave *et al.* 1984). In the case of most squid, however, there is a marked scarcity of such data (Coelho 1985), and one of the fundamental problems of SSA is estimating the origin and composition of stocks that comprise a fishery. For example, in *I. argentinus* from the Falkland (Malvinas) Islands fishery (Beddington *et al.* 1990), juveniles arrive from the main winter spawning in the northern section of the shelf in the austral spring, presumably having been carried from spawning grounds in the Brazil Current. They move southward and off-shore, and during summer they move over the shelf to the main feeding grounds, and are harvested by the fishery. Subsequently in the autumn they move north towards the spawning grounds, where, after reproducing, they presumably die. Uncertainties in the extent of temporal or spatial separation in spawning, and subsequent juvenile and adult migrations, greatly complicate attempts to monitor within- and between-season variability in recruitment.

3.1.6 Schooling

Although schooling behaviour in squid is well described (Clarke 1966, Hurley 1978, Mather and O'Dor 1984), its significance in terms of population structure and impact on harvesting is virtually unknown. A schooling species may be more vulnerable to over-fishing because the CPUE (catch-per-unit-effort) of vessels equipped with location-seeking gear does not decrease with declining stock size as assumed by conventional assessment methods (Pitcher 1989). For schools easily located by fishing vessels, as in the case of both jigging and trawling, catchability increases with decreasing stock size, leading to unstable depensatory mortality and risk of stock collapse. Models suggest that even a small over-estimate of optimal fishing effort could produce a rapid population decline.

In addition to the effect that schooling may have on catchability, the degree of genetic relatedness among individuals within a school may impart a scale of structure relevant to SSA (Carvalho *et al.* 1992). Large genetically distinct schools of squid may not only lead to micro geographic genetic differentiation, but also increase the likelihood of mixing demographically distinct units in a catch (Arkhipkin 1993), as well as overexploiting weakly represented assemblages.

In conclusion, a combination of uncertainty and potential for rapid demographic and evolutionary changes in natural squid populations render them particularly vulnerable to artificial selection and over-exploitation. There is thus an urgent need to develop a soundly based taxonomy, both at the species and population level.

3.2 Major *Illex* fisheries

Four closely related species from the genus Iliex have been described in the western Atlantic: *I. argentinus* in the Southern Hemisphere, and *I. coindetii, I. oxygonius* and *I. illecebrosus* in the Northern (Roper *et al.* 1969). The major areas for Iliex fisheries are the northwest (*I. illecebrosus*) and southwest Atlantic (*I. argentinus*), though smaller catches of I. coindettii are caught in the northeast and central west Atlantic (Caddy 1983, Wysokinski 1986, Csirke 1987, see other chapters in this volume). Catches of *I. argentinus* have increased markedly in recent years (Roper and Rathjen 1991) and copstitute a major international resource. Information on stock and species identification is, however, far from complete.

The remainder of this review will focus on the inter- and intra-specific status of *Illex* populations, which by virtue of the opportunities and motivation for data collection, are exclusively concerned with exploited populations. It is difficult to identify and compare squid populations exposed to different harvesting pressures because so little information is available regarding the extent of geographic migrations, and subsequent stock composition at any specific location.

4 Species and stock identification of *Illex*

4.1 Species identity

There are no data known to the authors that specifically address the question of cryptic speciation in the genus Iliex, other than samples of *I. argentinus* collected from the southwest Atlantic (Thorpe *et al.* 1986, Carvalho *et al.* 1992). Studies on the major *I. illecebrosus* fisheries (e.g. Amaratunga 1981, O'Dor 1983, Rathjen and Voss 1987) provide no suggestion that more than a single species is present in samples collected over a wide geographic area from the western central to the northwest Atlantic. It must be stated, however, that no published genetic studies have been undertaken on these populations, and there are only limited data available on regional variation in morphological differentiation (Amaratunga 1981, O'Dor 1983).

There are strong indications, however, based on allozymic data (Thorpe *et al.* 1986, Carvalho *et al.* 1992), that the nominal species of short-finned squid, *I. argentinus*, comprises more than a single species within the southwest Atlantic. *I. argentinus* occurs over the Patagonian Shelf and slope from about 30°S to 46°S at depths of 50–1000 m. Within this area it attains sexual maturity within one year (Rodhouse and Hatfield 1990a, Hatfield *et al.* 1992, Rodhouse and Hatfield 1992), and is harvested predominantly by jiggers, though trawling is also employed. Animals are caught from three major grounds: 42°S, ~45°–46°S, and within 150 nautical miles of the Falkland (Malvinas) Islands. The principal fishery and highest landings are, however, in the area around the Falkland (Malvinas) Islands, especially since the establishment of the Interim Conservation Zone (FICZ) in 1987 (Anon 1989). Indications from age and growth studies (Rodhouse and Hatfield 1990a) support the existence of a single stock in the two southern-most grounds, though electrophoretic studies suggest a more complex stock structure and species composition.

Thorpe *et al.* (1986) examined enzyme polymorphism at 29 putative enzyme-coding loci in six samples of *I. argentinus* collected north of the Falkland (Malvinas) Islands from approximately 45°S to 51°S (Fig. 10.3). Fixed allelic differences (where a particular allele is exclusive to a particular group), presumably representing substitutions at the scored loci, were observed at six loci, as summarized by Nei's (1972) genetic identities (Table 10.1). The probability of such a high proportion of consistently discrete fIXed differences, if genetically based, being found among samples representing conspecific populations is small (Ayala 1983, Thorpe 1983). Mean I values for conspecific populations are typically between 0.9 and 1.0, with only 2 percent falling below 0.90. The high identities of samples C, F and E (I = 1.0; Fig. 10.3; Table 10.1) indicate that they most likely comprise a single species, though the I value of 0.864 between samples C, E, F and D suggests that the latter may be a distinct species. It is difficult to comment on data for samples A and B since their poor state of preservation yielded somewhat weak and unclear enzyme phenotypes, though Thorpe *et al.* (1986) did suggest that sample B may represent a third sibling species.

It is not possible, based on these data alone, to conclude that several cryptic species of *I. argentinus* exist in these waters. Two of the enzyme loci screened, α- and β-esterases, are sometimes unreliable as taxonomic indicators (Richardson *et al.* 1986), and it is vital that molecular data be supplemented by studies at other biological levels, for example whether or not differentiation exists in hard and soft body parts (Borges 1990), spawning patterns (Nigmatullin 1989), and age and fecundity distributions (Hatfield and Rodhouse 1991). Clearly, further work was required.

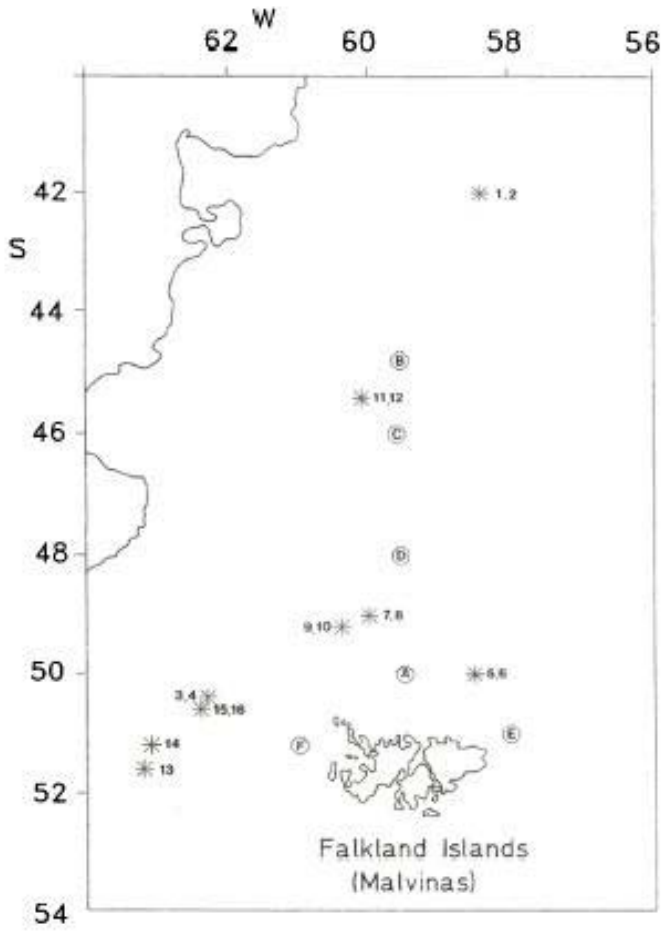

Figure 10.3. Sample locations of *Illex argentinus* used in allozyme electrophoresis by Thorpe *et al.* (1986) (A-F) and Carvalho *et al.* (1992) (1–16); all samples were collected by commercial vessels (jigging) between May 1985 – May 1986 (A-F) and February – May 1990 (1–16).

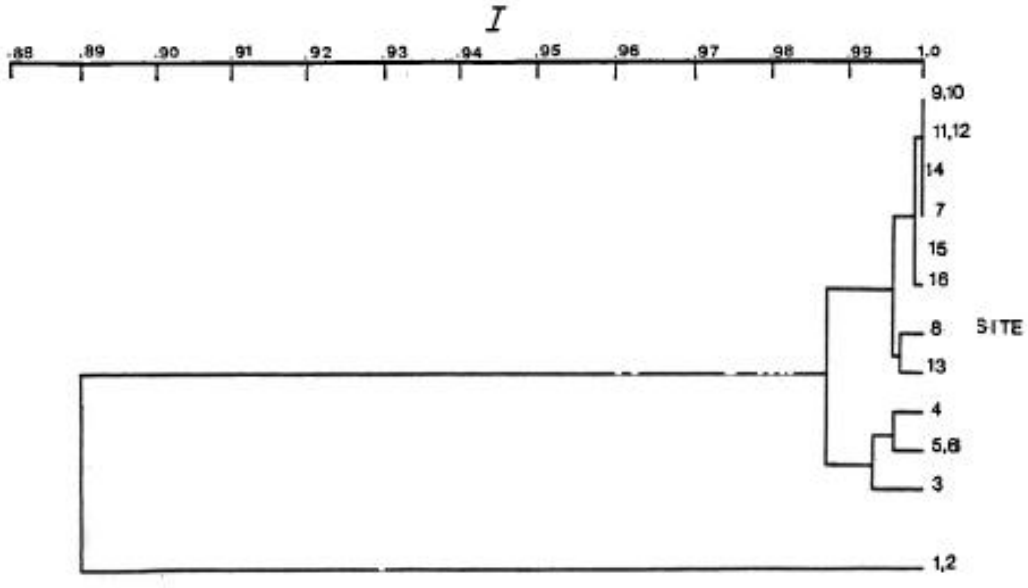

Figure 10.4. Mean genetic identities (*I*) between *I. argentinus* samples examined electrophoretically by Carvalho *et al.* (1992); values are based on 59 alleles at 25 enzyme-coding loci, of which 19 were monomorphic (UPGMA cluster analysis). Numbers refer to sample localities, as given in Fig. 10.3.

Table 10.1. Mean genetic identities (*I*) of *Illex argentinus* samples collected by Thorpe *et al.* (1986) based on 29 enzyme-coding loci; sample locations are shown on Fig. 10.3.

	SAMPLES				
	A	B	C	D	E
B	0.91	-	-	-	-
C	0.91	0.91	-	-	-
D	0.95	0.95	0.86	-	-
E	0.91	0.91	1.00	0.86	-
F	0.91	0.91	1.00	0.86	1.00

A more extensive allozymic survey of genetic differentiation in *I. argentinus* from Patagonian waters (Carvalho *et al.* 1992) was undertaken on 16 samples (N = 588 animals) collected by commercial vessels between 42°06'–51°17'S (Fig. 10.3). Marked genetic differentiation between samples was observed, though the scale of divergence was in most cases within that expected for conspecific populations (Fig. 10.4). Samples collected from the northerly limits of the sampled area (42°06' S, 58°09' W) did, however, display a much higher degree of genetic differentiation (Nei's I = 0.878–0.904), suggesting the existence of a hitherto unrecognized subspecies or species of *Illex*. It was not possible to obtain interpretable variation at the α- and β-esterases, though variation at the other loci screened by Thorpe *et al.* (1986) were examined. Those animals collected at 42°S were characterized both by fixed differences at two loci (*ADA*, 3.5.4.4; *MDH-I*, 1.1.1.37), though these were not the same loci displaying substitutions in the earlier study by Thorpe *et al.* (1986), and by distinct allele frequencies at some other loci. Morphometric analysis of soft body parts from each of the sixteen samples (Carvalho *et al.* 1990) detected significant differentiation among some samples, but without any correspondence to geographic separation.

Although the above genetic studies differ in the extent of proposed cryptic speciation in *I. argentinus* from the southwest Atlantic, it is evident that these squid may comprise more than one biological species. Both allozyme studies reveal clearly a pattern of marked genetic differentiation, with several allelic substitutions at a level that normally indicates complete reproductive isolation. The existence of sibling species, and their unknown contribution to respective fisheries, would complicate markedly the construction of stock-assessment models and provision of subsequent fishery advice (Beddington *et al.* 1990, Rosenberg *et al.* 1990), especially, as is likely, if part of the observed variance in reproductive, size and age-related parameters was species specific.

There is therefore a need to undertake intensive genetic studies in combination with detailed morphological analysis of taxonomic characters such as chromatophores, gladius and beaks. By linking such work with reproductive and demographic variation, it would be possible to examine whether any identifiable assemblages differ in their life histories and patterns of recruitment. Allozyme electrophoresis has proven increasingly valuable for delimiting sibling species of other squid including ommastrephids (Smith *et al.* 1981, Brierley *et al.* 1993a) and loliginids (Yeatman and Benzie 1993).

Levels of genetic differentiation have been examined in samples of the nominate squid species, *Martialia hyadesi*, obtained from regions of the Patagonian Shelf and Antarctic Polar Frontal Zone over 1000 km apart (Brierley *et al.* 1993a). One of the samples from the Patagonian Shelf exhibited fixed allelic differences at 16 of 39 enzyme coding loci (mean I = 0.51), despite apparent morphological uniformity. Such marked genetic differentiation indicates complete reproductive isolation (Ayala 1983) indicative of congeneric species (Thorpe 1983). Preliminary morphological studies nevertheless failed to separate samples on morphological grounds, though only soft body parts were examined. Since there is no described morphologically similar squid species within the subfamily Todarodinae, it seems likely that *M. hyadesi* contains at least two sibling species.

Smith *et al.* (1981) used differences in allozyrnes, comparative morphology of hectocotylized ventral arms in mature males, and the prevalence of parasites to delimit two species of arrow squid in New Zealand waters: *Nototodarus sloani* found in southern waters, and *N. gouldi* in more northerly waters around New Zealand and the southern waters of Australia. Additional morphological characters were examined subsequently (Smith *et al.* 1987) including club length index and the number and rows of suckers on the first right arm, demonstrating the valuable role that genetic studies can play in delimiting assemblages for later comparison at other biological levels. The two species are now managed separately, providing the only clear example of species identification in squid that has led to a direct change in fishing policy and that combines evidence from several sources.

The taxonomic status of *Loligo vulgaris* and *Loligo reynaudii* found off the southern coast of Africa and northwest Africa respectively (Porebski 1970) has for some time been unclear; there appear to be few morphological differences between the two forms. Morphological (morphometrics and meristics) and allozyrne comparisons between the nominal species (Augustyn and Grant 1988) revealed that differences were subspecific (mean Nei's D = 0.030; outgroup comparison with *Loligo opalescens*, D = 0.686) rather than specific in nature, and *L. reynaudii* was demoted to subspecific standing with *L. vulgaris*. It was suggested that geographical isolation between the two taxa was caused probably by an environmental barrier of cold, oxygen-deficient water off the west coast of southern Africa.

Electrophoretic and morphological analysis of loliginids from northern Australia (Yeatman and Benzie 1993) revealed three distinct taxa: *Loligo chiensis*, which was distinguished from other taxa by between 4 to 6 fixed allelic substitutions, and differentiation in gladius width index, hectocotylus length index and sucker dentition; and the other two taxa, possibly representing previously described morphs of *L. edulis*, were separated mainly by allozyme differences, though multivariate morphometric analysis differentiated most mature specimens. *Loligo edulis* in northern Australia therefore most likely consists of two cryptic species, which await full description. Given the extensive distribution of *L. edulis*, further crypsis within this nominate species is likely, though other species of *Loligo* appear to maintain effective panmixia (homogeneous allele frequencies, indicative of random mating) across extensive geographic areas (Carvalho and Pitcher 1989, Brierley 1992).

The above taxonomic studies demonstrate that crypsis is present in both ommastrephid and loliginid squid, and underlines four important points:

a) that taken alone, the typically plastic morphology of teuthoids is an unreliable indicator of taxonomic affinities;
b) that despite the apparent extensive migrations of these animals, gene flow may still be restricted; the distances travelled *per se* are relatively unimportant, it is the fidelity with which spawning groups return to breeding grounds that is critical;
c) the feeding groups that are usually captured by a fishery may not necessarily represent interbreeding units, hence the population heterogeneity detected; and
d) the urgent need to integrate molecular, morphological and demographic studies to undertake a review of teuthoid systematics.

4.2 Genetic diversity

It is a dictum of evolutionary genetics that genetic diversity is necessary for adaptive evolutionary change (Fisher 1930). It follows that genetic diversity endows a species with an ability to adapt to natural or

Table 10.2. Levels of genetic diversity in squid species, compared with the average for marine invertebrates; levels of mean observed heterozygosity per locus (H_L) proportion of polymorphic loci ($P_{O.95}$) calculated from original allele frequency data, and number of loci screened (*N* loc)

Species	H_L	$P_{0.95}$	*N*loc	Reference
Loligo bleekeri	.03	0.89	23	Suzuki *et al.* (1993)
Loligo chinensis	.007	0	11	Yeatman and Benzie (1993)
Loligo forbesi	.005	.069	33	Brierley (1992)
Loligo forbesi		.100	33	Brierley *et al.*, (1993b)
Loligo gahi	.069	.273	21	Carvalho and Loney (1989)
Loligo opalescens	.037	.100	30	Augustyn and Grant (1988)
Loligo pealei	.006	.050	19	Garthwaite *et al.* (1989)
Loligo plei	.00	0	9	Garthwaite *et al.* (1989)
Loligo vulgaris vulgaris	.011	.060	30	Augustyn and Grant (1988)
Loligo vulgaris reynaudii	.03	.060	30	Augustyn and Grant (1988)
Loliguncula brevis	.00	0	9	Garthwaite *et al.* (1989)
PhotoLoligo edulis		.209[a]	43	Natsukari *et al.* (1986)
Illex argentinus	.011	.172	29	Thorpe *et al.* (1986)
Illex argentinus	.011	.280	25	Carvalho *et al.* (1992)
Illex illecebrosus		0	14	Romero and Amaratunga (1981)
Martialia sr. 1	.013	.128	39	Brierley *et al.* (1993a)
Martialia sr. 2	.003	0	39	Brierley *et al.* (1993a)
Berryteuthis magister	.131	.348	23	Katugin (1993)
Marine invertebrates	.147	.587		Nevo (1978)

[a] Allele frequencies not provided, therefore level of polymorphism uncertain
Definitions given in text

man-made changes in the environment as determined by interactions between breeding system, population size, gene flow and selection pressures (Carvalho 1993). Thus, levels of genetic diversity are an important component of population structure, especially since it both affects our ability to detect stock separation (Pella and Milner 1987) and is significantly affected by it (Nelson and Soulé 1987).

Estimates of genetic diversity based on allozymic data, that is the proportion of polymorphic loci (*P*) and mean observed heterozygosity per locus (H_L) (Ferguson 1980), provide a universal means of comparison across taxa. It is usual to present heterozygosity estimates as "expected heterozygosity" (H_{exp}) based on Hardy-Weinberg expectations (Ferguson 1980). However, in the case of *Illex*, where cryptic species may exist, fixed allelic differences will inflate values (expected H_L for *I. argentinus* = 0.071, Carvalho *et al.* 1992). Expected values not only have little evolutionary significance since presumably such populations would be reproductively isolated, but H_{exp} estimates would also fail to emphasize the low heterozygosity detected within samples. Indeed, most estimates of genetic diversity in squid are presented as measures of observed heterozygosity (Brierley *et al.* 1993a). Hereafter, all H_L values refer to mean observed heterozygosity.

One notable feature of genetic diversity estimates in *Illex* is their generally low levels (Table 10.2), with $P_{(0.95)}$ ranging from 0.172–0.280, and H_L displaying values of 0.00–0.011 (Romero and Amaratunga 1981, Thorpe *et al.* 1986, Carvalho *et al.* 1992). Nevo (1978) found an average proportion of polymorphic loci ($P_{O.95}$) among 27 invertebrate species (excluding insects) of 0.399 (S.E. 0.275) and an average H_L of 0.100 (S.E. 0.074), though marine invertebrates typically exhibited higher variability (P = 0.587 (S.E. 0.084); H_L = 0.147 [S.E. 0.019]). The typically low levels of polymorphism in squid (<0.02) are unusual, and generally

occur in populations which have undergone severe reductions in population size (e.g. Selander and Kaufman 1973, Bonnell and Selander 1974, O'Brien *et al.* 1987), a feature seldom observed across any major invertebrate group (Ward *et al.* 1992).

Higher levels of genetic diversity ($P_{(0.95)} = 0.348$, $H_L = 0.131$; Table 10.2) have, however, been revealed in the squid *Berryteuthis magister* (Katugin 1993). This is the first species of the family Gonatidae to be examined genetically, and demonstrates that some squid may harbour levels of diversity more typical of other marine invertebrates. It is thus important to extend the taxonomic range of genetic studies.

The evidently low level of genetic diversity in *Illex* may be related to:

a) population bottlenecks and founder events;
b) other evolutionary factors;
c) recent population expansion; or
d) limitations in the molecular techniques.

Populations undergoing severe bottlenecks with rapid and persistent reductions in population size may lose rare alleles through genetic drift, though simulations suggest that for significant genetic erosion to occur, effective population size (N_e, numbers of reproductive individuals) would have to fall below approximately one hundred, with slow subsequent rates of population growth (Carvalho and Hauser 1995). It is noteworthy, however, that in heavily exploited populations, Ne may be an order of magnitude smaller than the census number of individuals (Nelson and Soulé 1987): it is not the number of squid counted that is important, but rather the proportion that contribute to the gene pool. Although severe fishing pressure could bring about rapid population declines, especially in a semelparous species, it is difficult in such highly mobile species to conceive of drastic reductions, especially where high growth rates are so typical (Forsythe and Van Heukelem 1987).

In *I. argentinus* however, where several stocks or species may exist, it is possible, at least in principle (Hilborn and Walters 1992), for weakly represented, yet distinct groups, to become readily over-exploited, especially if they exhibit spatial integrity and remain unrecognized. Mass removal through intense harvesting may result in an irreversible loss of part of the gene pool -an event more likely to occur in schooling animals such as *Illex* (Pitcher 1992). Indeed, an absence of overlapping year classes, taken together with the population heterogeneity typical of *I. argentinus* and other teuthoids, will render localized population bottlenecks more likely (Nelson and Soulé 1987). There has, however, been no evidence of any severe crashes in *I. argentinus* populations since the Falkland (Malvinas) fishery began in the early 1980s, though the fishing season was closed early in 1990 owing to a rapid and atypical reduction in CPUE (J. Barton, Falkland Islands Government Fisheries Department *unpubl. data*).

Historical events and ancestry have been proposed as causes for depressed levels of genetic diversity in some marine animals, especially in those from polar waters where heterozygosities are typically low (Nevo *et al.* 1984, Fevolden *et al.* 1989, Patarnello *et al.* 1990). However, not all data fits such a pattern; the Patagonian squid, *L. gahi*, for example, exhibits moderate levels of genetic variability (Carvalho and Loney 1989) and yet inhabits geographic areas similar to *I. argentinus* (Table 10.2).

Populations with persistently small population sizes may exhibit low levels of genetic variability (Soulé 1976). It is possible that the large extant populations of squid represent relatively recent expansions, and that genetic diversity has not yet reached the new, increased, equilibrium levels. Relief from predation by diminishing stocks of marine mammals and large predatory fish may have contributed to contemporary squid productivity (Rodhouse 1988), though such ideas are difficult to reconcile with the higher diversity found in some squid (Table 10.2).

A final possibility is that allozyme electrophoresis has failed to reveal "hidden" genetic variability, though there is no *a priori* reason to expect this proportion to be higher in squid than in other invertebrates. Further, such an explanation appears unlikely in view of the apparent generality of similarly low levels in other squid (Table 10.2). The overall number of species and populations examined electrophoretically remains small. More extensive screening is necessary to examine the range in genetic diversity estimates, together with the use of more sensitive nucleic acid analyses.

In summary, the low levels of diversity detected in *I. argentinus* and some other teuthoids is likely to be real, and may derive from a combination of stochastic and historical events. Whatever the reason, it is imperative that management practices do not cause a decline to still lower levels, underlining the importance of accurate delineation of cryptic species and distinct stocks.

4.3 Stock structure analysis

4.3.1 Ecological data

Several sources of ecological information have been used to examine stock structure in squid, including length and age structure analysis (e.g. Lange and Sissenwine 1983, Rodhouse and Hatfield 1990b, Rodhouse 1991), maturity indices (e.g. Rodhouse *et al.* 1988, Nigmatullin 1989, Brunetti 1990), and the distribution of juvenile stages (e.g. Brunetti 1990, Brunetti and Ivanovic 1992). There has been a particular focus on population variability in spawning, in particular the degree of temporal and spatial integrity of identifiable groups, a necessary condition for reproductive isolation. If assemblages with 'clearly defined and distinct patterns of spawning could be recognized, it would then be possible to examine differentiation at both the genetic (molecular markers) and phenotypic (growth, fecundity, age structure) levels, thus providing estimates for forecasting variance in recruitment. There have been particular efforts to characterize separate spawning aggregations of *I. argentinus* in the southwest Atlantic (Nigmatullin 1989, Brunetti 1990, Brunetti and Ivanovic 1992, Nigmatullin, *unpubl. data*), though unfortunately, these data have not yet been integrated with genetic studies (see section 4.3.3).

We will concentrate here on the use of ecological observations to describe stock structure of *I. argentinus* in waters off the Patagonian Shelf (Nigmatullin 1986, Tsygankov 1986, Nigmatullin *unpubl. data*) based on the distribution of seasonal and spatially defined aggregations. The approach has been to monitor the occurrence of various maturity stages (Lipinski 1979), including spawning individuals and larvae in time and space, and to reconstruct seasonal patterns of spawning and recruitment. Based on such observations, it has been possible to propose the general locations of spawning grounds. Much of the work was undertaken between 1984 and 1986, mainly in sea areas 40°–53°S, 35°–45°W. Animals were collected and compared on the basis of water depth ("shelf" [shallow waters, up to 500m] and "slope" [deeper waters, >500 m]) and the time of maximal spawning activity (autumn, winter, spring, summer).

Spawning of *I. argentinus* continues throughout the year, but is at a maximum during the austral winter (end of May to August). Nigmatullin (1986, 1989) summarizes the findings by concluding that four seasonal groups exist based on timing of spawning, growth rate, and distribution of larval stages. The "autumn" group exhibits maximal spawning between March and the beginning of May, and occurs predominantly in shelf waters between 200 and 400 m. It has, however, been less well studied than other putative groups.

The "summer" group spends its entire life cycle in shelf waters, with spawning between December and early February, though possibly during November and March as well (Fig. 10.5). Adult males range in size from 14 to 22 cm, and females from 15 to 25 cm. Feeding and pre-spawning migrations are unclear, but it appears likely on the basis of larval distribution, that spawning grounds are distributed across a wide geographic area, extending south to 46°S.

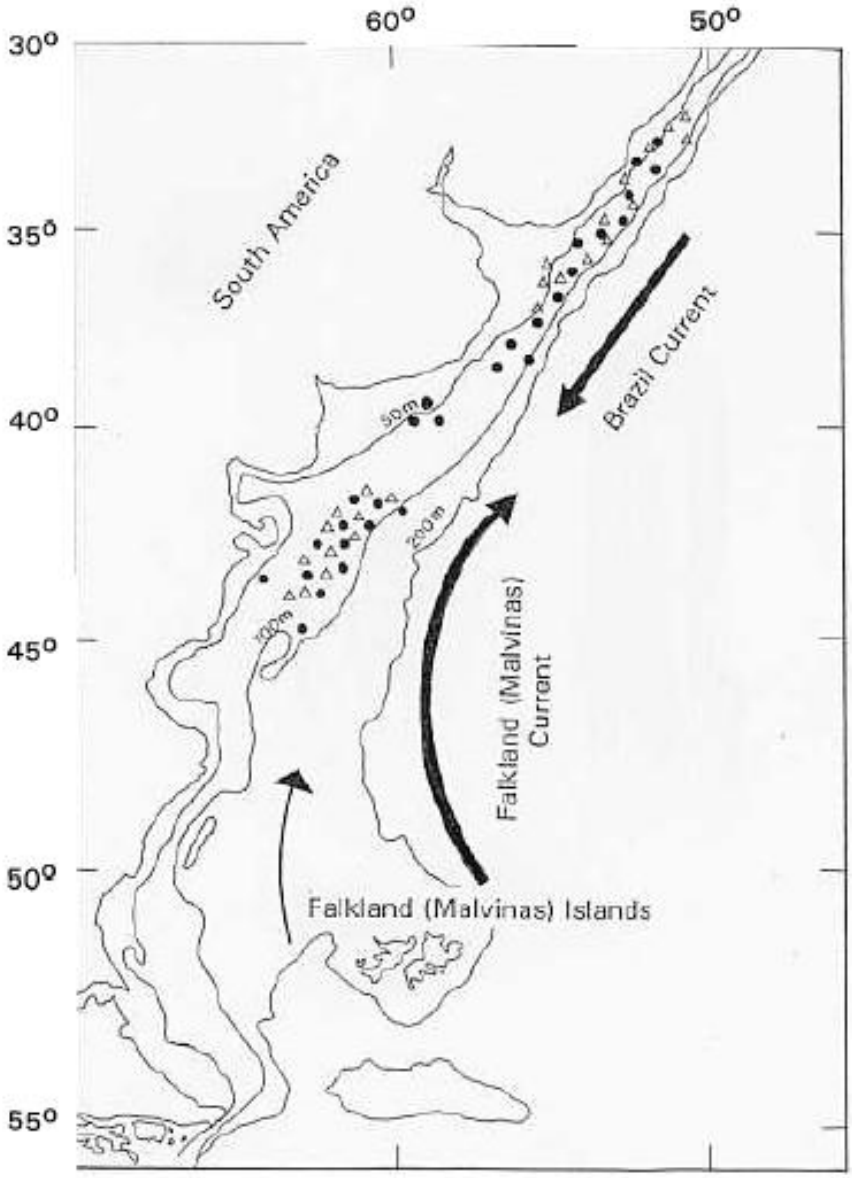

Figure 10.5. Ecological observations on putative summer-spawning shelf group of *I. argentinus*; feeding and pre-spawning migrations are not fully documented, and are therefore not included. Data was collected during cruises between 1984 and 1986. ●.= larvae; Δ = putative spawning grounds

I. argentinus belonging to the "winter" grouping have been subdivided into winter-spawning shelf and slope groups. The winter shelf group occurs in the outer shelf in depths up to 250–350 m north of 30°–40°S (Fig. 10.6). Spawning occurs typically during May to August with a peak in June-July, mainly within the Patagonian Shelf from 30° to 37°S. As the temperature of the shelf waters increases in spring, juveniles move southward. During the late summer, individuals forage in the southern areas, until maturation from late February to March, when adults begin their pre-spawning migrations along the outer-shelf region northward to the spawning sites. The mantle length of adult males is 18–26 cm, of females 22–32 cm.

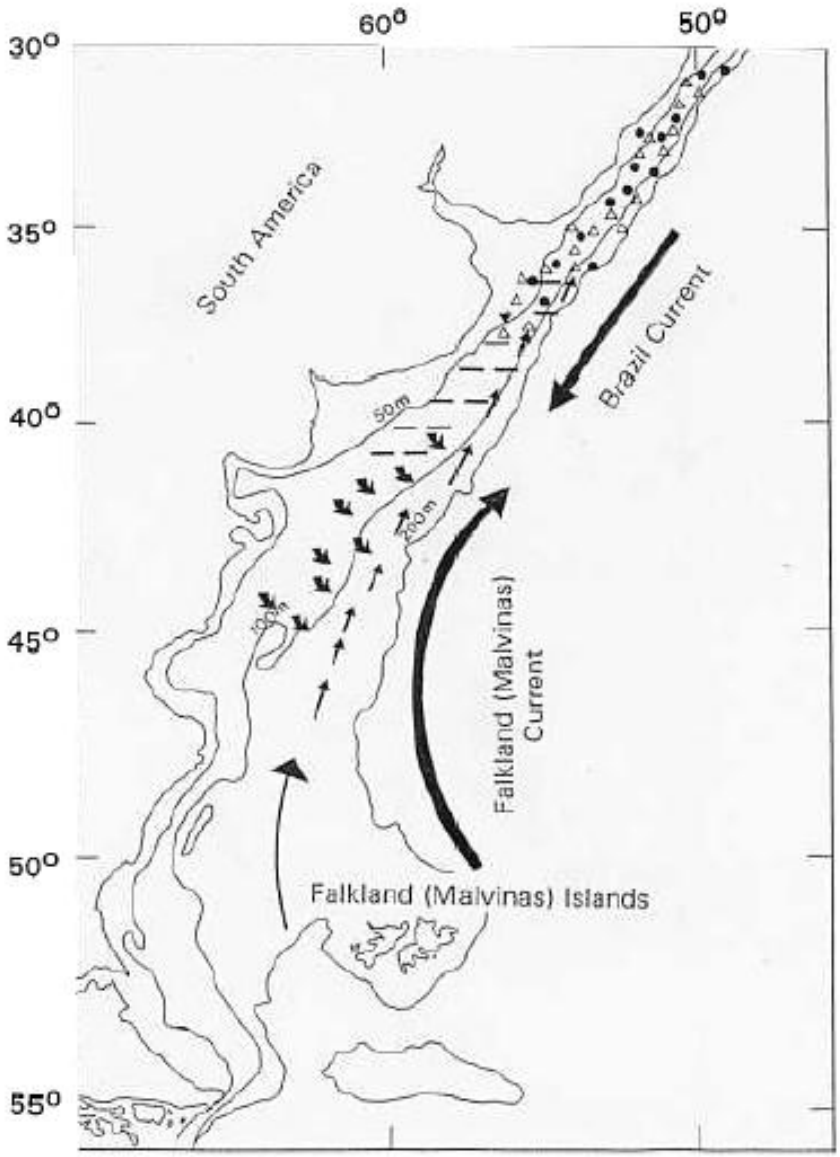

Figure 10.6. Ecological observations on putative winter-spawning shelf group of *I. argentinus*,
●.= larvae, -= juveniles, Δ = putative spawning grounds, ➡ = feeding migration,
← = pre-spawning migration

The winter-spawning slope group is characterized by pre-spawning and spawning individuals at depths of 500–1200 m, usually with spawning during June to September, with a peak in July–August, somewhat later than the analogous shelf group (Fig. 10.7). The spawning grounds probably lie on the continental slope between 28°–30°S and 40°S, while the feeding grounds are much wider and extend into shelf waters up to 53°–54°S and the oceanic area eastward to 54°W. Such observations underline the probable mixing of such groups for much of the life cycle during extensive foraging migrations, during which it is likely that genetically heterogeneous populations exist (see sections 4.3.1, 4.3.3).

Information on the spring-spawning group is scanty and contradictory. Their abundance is low, and it is likely that they do not constitute a separate group, but represent a delayed portion of the winter-spawning group.

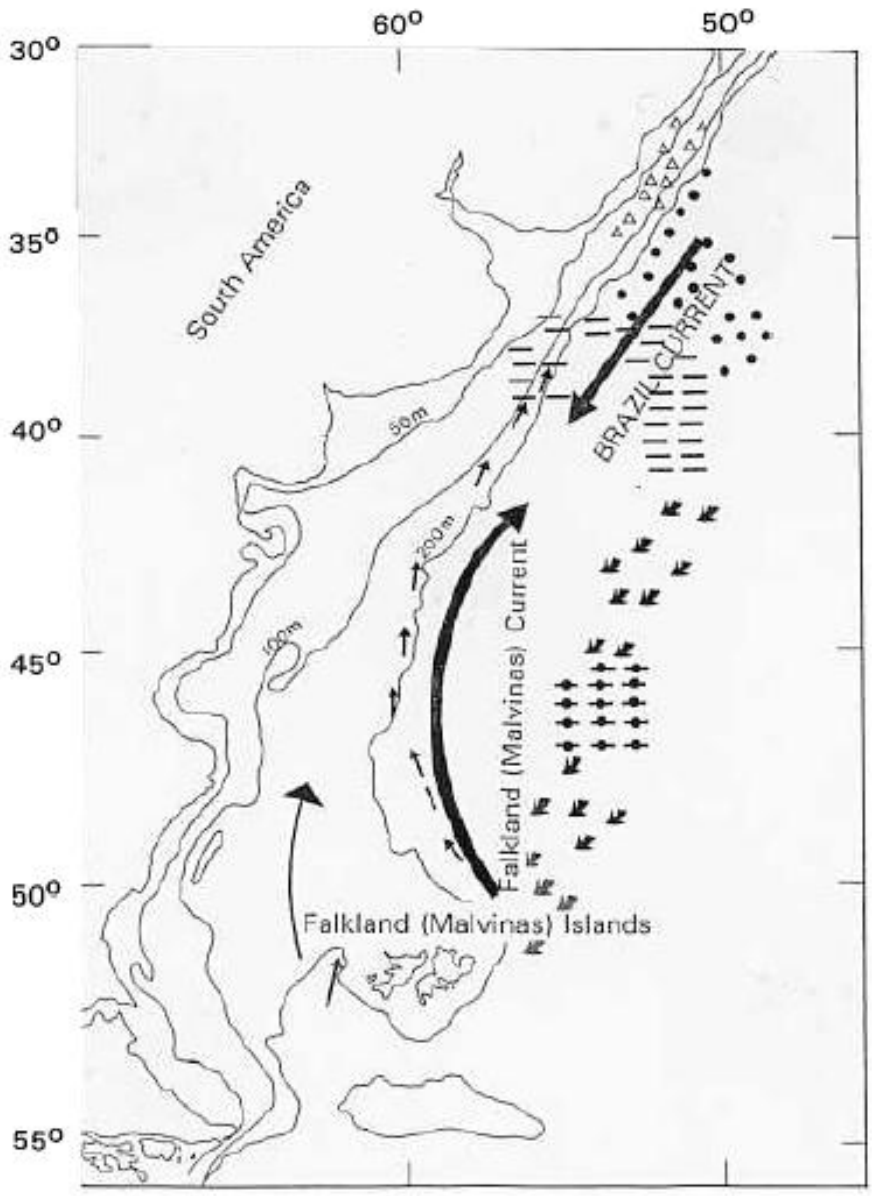

Figure 10.7. Ecological observations on putative winter-spawning slope group of *I. argentinus*, .● = larvae, – = juveniles (1-5 cm mantle length), • = young squid (5–12 cm mantle length), Δ = putative spawning grounds, ➡ = feeding migration, ← = pre-spawning migration

Although the defInitive extent of spawning grounds is unclear based on ecological data, it is evident that spawning individuals and larvae occur across a wide geographic area, providing marked overlap, at least spatially, among putative groups. It is clear also from Figs. 10.5–10.7 that wide-ranging migrations occur within *Illex*, greatly complicating attempts to determine both the source of recruits to the fishery, and description of stock composition on feeding grounds.

It is critical in terms of defining population structure to determine the taxonomic status of such putative groups. Nigmatullin (1989) proposes two alternative hypotheses to explain the seasonal and spatial patterns of juveniles and spawning adults: the single and multi-stock population models.

I. argentinus in the southwest Atlantic may represent a single interbreeding population with extended and overlapping spawning periods that merely reflect life-history plasticity related to depth selection of different maturity stages; such a model would support a population structure based on a single dominant stock. Alternatively, the seasonal groups may be reproductively isolated to varying degrees, resulting in phenotypically and genetically distinct stocks or assemblages. There is some evidence for both hypotheses based on data from statoliths, growth studies, morphometrics and preliminary electrophoretic data.

In favour of the single-stock model is the observation that on occasions, individuals apparently from more than one putative group coexist, making it possible for such assemblages, at least in principle, to maintain genetic continuity. The extensive range in terms of time to reach maturity (280–370 days) could provide a basis for such overlap in reproductive activity.

In support of the multi-stock model, there is some suggestion of allozymic differences among representative autumn- and winter-spawning groups (Nigmatullin 1989), but these data are preliminary. If putative spawning grounds could be located, and there was some degree of temporal separation of reproduction, there would be a good chance of characterising assemblages that were genetically distinct, in a way similar to genetic stock identification in Pacific salmon (Miller *et al.* 1983). Further suggestions of discrete differences among spawning groups come from morphometric studies (Nigmatullin 1986), where spawning individuals belonging to the summer group display generally distinct body shapes similar to *I. oxygonius*, yet exhibiting hectocotylized arms similar to *I. argentinus*. Nigmatullin (1986) suggests that the summer-spawning group may represent a distinct species, similar to *I. oxygonius*, though further complementary morphometric and genetic studies are required.

In addition to the above studies, the distribution of early life stages of *I. argentinus* during winter spawning (August–September) in waters off the Argentinean and Uruguayan shelf (between 35°S and 45°S and west of 50°W) have been examined (Brunetti and Ivanovic 1992). A few rhynchoteuthion larvae were found in subtropical waters of the Brazil Current, next to the Brazil-Falkland (Malvinas) confluence, and in frontal zone shelf water, but always at temperatures above 14°C. Large numbers of juveniles were found inshore <200 m), with highest densities between 38°S and 43°S. The exact position of the winter-spawning ground, thought to be off the shelf, was not identified because no mating or spent individuals were found, though data suggest that spawning may occur in the Falkland (Malvinas) Current north of the Falkland (Malvinas) Islands. Egg masses would then be transported north up to 37°–39°S, where their embryonic development would be accelerated in the subtropical waters of the Brazil Current. The Brazil Current may playa dominant role in carrying the larvae from the northern part of the outer shelf (34°–36°S) to the intermediate shelf.

It is difficult at the present time to detennine the taxonomic status of the putative spawning groups described by Nigmatullin (1987, 1989, *unpubl. data*), though it is clear that there are distinct differences in the distribution of spawning adults and juveniles, both in time and space. Such observations, taken together with the genetic studies so far carried out on *I. argentinus* in the southwest Atlantic (Thorpe *et al.* 1986, Carvalho *et al.* 1992), are a strong indication of the existence of several distinct stocks and possible sibling species. The extensive migrations of such distinct assemblages also complicate methods for stock assessment (Arkhipkin 1993). For example, in the Falkland (Malvinas) fisheries, the commonly applied Leslie-DeLury forecast model (Beddington *et al.* 1990, Rosenberg *et al.* 1990, Basson *et al.* 1996) assumes no significant immigration or emigration, and an insignificant level of natural mortality. The extensive movement of distinct stocks through a fishery complicates the CPUE statistics where it is difficult to distinguish between a reduction in CPUE due to harvesting, and that associated with the emigration of several schools. Indeed, the occasional microgeographic differentiation detected at single fishing sites (Carvalho *et al.* 1992) supports the notion of a migration of genetically discrete assemblages, even on a local scale.

4.3.2 Parasitological markers

The presence of parasites in fish hosts has sometimes provided useful markers for elucidation of stock identity and migration patterns (Kumpf *et al.* 1987, Lester 1990). In squid, parasites have been employed in SSA somewhat less, though there have been extensive studies on several helminth species in *I. argentinus* from the southwest Atlantic (Nigmatullin and Shukhgalter 1990, Nigmatullin *unpubl. data*). Examination of the distribution of 15 helminth species among putative spawning groups of *I. argentinus* within the area 45°–47°S (Nigmatullin 1986) revealed wide host specificity, rendering them ineffective markers for characterizing populations. In contrast, however, larvae of the cestode *Pelichibothrium speciosum* were found only in the summer-spawning groups, indicating their origins in inner shelf waters.

On a larger geographic scale, metacercariae of trematodes belonging to the Didymozoidae family were specific to *I. argentinus* from subtropic waters, where they are common, but were absent in adult squid from shelf and slope waters between 42°S and 52°S, at least during the period of study 1984–1990. Metcercariae were recorded, however, in juveniles in the region of 39°–46°S, 43°–53°W in 1988 and 1989, ranging in their incidence of infestation from 1–5 percent to 30–80 percent. The presence of infected juveniles in southerly populations supports the notion of their northerly migration from subtropical waters. It appears likely that the lower temperatures of subantarctic waters result in parasite mortality, restricting infection in southerly distributed juveniles.

In *I. argentinus* at least, parasitic markers have so far provided some support for the integrity of surnrnerspawning *I. argentinus,* as well as data on the source of recruits on an oceanic scale. More promising data concerning the use of helminth parasites in stock identification of squid have been found in the flying squid, *Ommastrephes bartrami* (Bower and Margolis 1991). Although there were some difficulties in identifying helminth species, major differences were found in their distribution among squid collected from northeast and northwest regions of the Pacific Ocean. For example, *Nybelinia surmenicola* was absent from squid in the northeastern region, but common in the Pacific northwest (infection rate of 40 percent). Because this helminth is a tissue parasite, once acquired, it likely survives throughout the squid's life, supporting the view that flying squid in the eastern and western range in the North Pacific do not intermingle. Further, by using *N. surmenicola* as a stock marker, it might be possible to determine whether such usually allopatric populations intermix or remain segregated on their spawning grounds. Clearly, such data can be of great value in elucidating stock integrity, especially if used in conjunction with genetic markers (Smith *et al.* 1981). A major obstacle, however, is the complex taxonomy of parasitic larval stages, though molecular markers may themselves help to resolve such problems.

4.3.3 Molecular data

Differences in allozyme frequencies among samples separated in time or space can be used to estimate the extent of population differentiation, from which inferences can be made regarding the degree of stock separation. The fit of genotype (electromorph) frequencies to that expected for a large, randomly mating population, the so-called Hardy-Weinberg test (Ferguson 1980), which estimates deviations from equilibrium using a chi-squared or *F*-statistic (Brown 1970., Richardson *et al.* 1986), can provide additional information on whether or not individuals are drawn from the same gene pool. In addition to the description of population structure, stable allele frequencies or fixed allelic differences can be used to identify particular stocks. Reliable estimates of allele frequencies, which reflect stable differences among stocks that mix differentially throughout their life cycle, can provide a valuable data base for measuring the proportions of distinct stocks in mixed populations (lhssen *et al.* 1981, Pella and Milner 1987). The use of molecular data in genetic marking of discrete assemblages of squid has, however, been mainly restricted to species identification (see section 4.1). Efforts at the population level have concentrated on estimating the degree of genetic differentiation among spatially or temporally distinct aggregations.

Four molecular studies have been undertaken on population differentiation in *Illex*: one on *I. illecebrosus* samples from the Scotian Shelf (Romero and Arnaratunga 1981) and three on *I. argentinus* from the southwest Atlantic (Thorpe *et al.* 1986, Nigmatullin 1989, Carvalho *et al.* 1992). The preliminary allozyme study on *I. illecebrosus* examined 21 enzyme loci but only four were chosen for routine study. The results revealed exceptionally low levels of genetic diversity (H_L = 0.005), which unfortunately precluded any meaningful geographic comparison, though there was no indication of fixed allelic differences among the four samples examined.

The studies of Thorpe *et al.* (1986) (Fig. 10.3) detected marked differentiation among samples of *I. argentinus* in the southwest Atlantic, as summarized by Nei's (1972) mean genetic identities (Table 10.1). Unfortunately it is not possible to comment on genotypic structure or allele frequency divergence because allele or genotype frequencies were not provided. Nevertheless, the important finding was that *I. argentinus* populations from the sampled areas were probably composed not only of several genetically distinct stocks, but possibly also of two or more sibling species. Some combination of factors related to spawning behaviour and migration patterns appeared to be restricting gene flow between spatially distinct assemblages. Associated with probable stock separation were exceptionally low levels of heterozygosity (H_L = 0.00–0.001), which further supports the existence of reproductively isolated units (Richardson *et al.* 1986).

Similarly marked population differentiation was observed among samples of *I. argentinus* collected from the southwest Atlantic during the 1990 *Illex* Falkland (Malvinas) Islands fishing season (Fig. 10.3; Carvalho *et al.* 1990, 1992). Significant allele frequency divergence among samples, as summarized by Nei's mean genetic distances (*D*) and identities (*I*), which ranged from 0.00–0.13 and 1.00–0.88 respectively, indicated. marked divergence (Fig. 10.4). There was, however, no detectable relationship between genetic distance and geographic separation, with the exception of a discrete sample from more northerly waters (42°S; Fig. 10.3). Significant deviations from Hardy-Weinberg equilibrium due to heterozygote deficiencies were detected at most polymorphic loci, arising from several possible factors, including non-random mating, the Wahlund effect (Richardson *et al.* 1986) or inbreeding. In view of the apparent vagility and typically high abundance of *Illex*, it is unlikely that inbreeding would be a major contributor, though N_e (see section 4.2) on the spawning grounds may be appreciably smaller than fishery census estimates based on feeding groups. The effects of non-random mating due to typically unbalanced sex ratios (Carvalho *et al.* 1990), and the pooling of genetically differentiated assemblages (Wahlund effect) may be primary causes of the observed genotypic distributions. The absence of heterozygotes between alternative alleles indeed supports partial reproductive isolation among samples.

Not only was genetic divergence detected among locations, but some evidence was also found for microgeographic differentiation of allele frequencies among replicates taken at two of the nine sites examined. Replicates were obtained by collecting animals at approximately 6–10 h apart by jigs operating on the same vessel. In addition to allele frequency differences, allelic substitution was observed between some replicates, suggesting strongly that schooling may impart a scale of structure relevant to SSA. It is not possible to identify the absolute scale of such differentiation, which requires information on the speed and directionality of school movement during the sampling interval between replicates, but it is, however, probably in the order of tens of km. It thus appears that distinct stocks may co-exist on the feeding grounds.

In ommastrephid squid, large egg "balloons" containing up to 100 000 eggs are released and may drift in currents for several hundred km (*see Chapter 5*). Such structures become inhabited by plankton, and provide food and a stable habitat for hatchlings (O'Dor 1983), which are retained in close proximity until they become compulsive schoolers. Even if hatchlings disperse, the relative isolation of separate egg balloons may still result in the formation of large numbers of sibs or half-sibs. Such social structure would be expected to impart significant microgeographic genetic differentiation, especially if schools from distinct populations coexist on feeding grounds.

The existence of discrete assemblages migrating throughout a fishery was provided by data documenting temporal and spatial variation in the age and abundance of *Illex argentinus* in the southwest Atlantic (Arkhipkin 1993). Several waves of abundance (CPUE) were detected that could be explained either by changes in stock accessibility, or by the appearance of new waves of migrating squid. Data which compared the age-length structure of each wave in several fishery regions (52°S, 46°S, 42°S) supported the notion of successive groups of migrating squid passing through the region. Due to the high migratory speed of squid (23.2–28.9 km d^{-1}), schools apparently passed quickly through an area, detectable as short pulses of increased CPUE. Such findings confonn with predictions based on the observed microgeographic genetic heterogeneity.

Small-scale patchiness in genetic structure not only has implications on sampling design for stock structure studies, where single samples may be unrepresentative, but re-emphasizes the importance of monitoring the migration and composition of catches in a fishery. If, for example, such patchiness was due to the movement of genetically discrete schools within a fishery, the probability of removing rare genotypes through predation (natural or due to fishing) is greater than if genotypes were more uniformly distributed. The overall effects on genetic diversity would depend on such factors as the distribution and intensity of fishing effort (Tingley *et al.* 1992), allele frequency differences, and the size and movement of schools. These preliminary observations highlight the importance that spatial scale may play in SSA, underlining the need to determine more fully both the incidence of small-scale structuring in schooling squid and the underlying behavioural basis of such aggregations.

The final study undertaken on *I. argentinus* using allozymes was carried out from 1984 to 1988, mainly in sea areas 40°–53°S: 35°–45°W (summarized by Nigmatullin 1989, Nigmatullin *unpubl. data*), though on available evidence it is unclear precisely where samples were collected. Animals were collected and compared on the basis of water depth ("shelf" and "slope") and seasonal spawning group ("autumn", March–early May; "winter", end of May–early August), and examined using four "variable" enzymes. Allele frequency differences were detected between these shelf and slope populations, though esterases obtained from the buccal cone provided the most discrete differences which persisted between years. *Illex argentinus* obtained from the shallower shelf waters (autumn) were characterized by low frequencies of a "fast allele" if = 0.09–0.3), with deep-water animals (winter) typically displaying higher incidences if = 0.4–0.5). Nigmatullin (1989 *unpubl. data*) concludes, in combination with other evidence (see section 4.3.1), that at least two isolated stocks of *I. argentinus* exist in these waters: shelf autumn- and slope winter-spawning groups. In the absence of additional sampling and electrophoretic details it is difficult to say more than that Nigmatullin's (1989) studies support the existence of geographic and temporal structuring of *I. argentinus* stocks. Indeed, a biologically meaningful separation of stocks would require at least some restriction to gene flow, such as provided by the temporally distinct spawning groups proposed (Nigmatullin 1989), though allozyme frequency differences can become generated through other stochastic or selective forces (Richardson *et al.* 1986).

To conclude, molecular studies to date on *I. argentinus* indicate a highly fragmented gene pool, detectable as distinct stocks, subspecies or sibling species. Fixed allelic differences of the magnitude disclosed argue for significant biological differentiation at levels likely to affect patterns of recruitment and mortality, and demonstrate the operation of spatial and/or temporal separation of spawning. How representative are these findings among teuthoids in general?

There is no clear-cut taxonomic distinction in the genetic population structure of ommastrephid and loliginid squid, though studies on the former, where sufficient data are available, have always shown marked genetic divergence among populations, with occasional allelic substitution (Thorpe *et al.* 1986, Carvalho et ai. 1992, Brierley *et al.* 1993a). Both homogeneous allele frequencies indicative of panmixia (Christofferson *et al.* 1978, Carvalho and Pitcher 1989, Brierley 1992) and heterogeneous allele frequencies indicative of significant population differentiation (Ally and Keck 1978, Garthwaite *et al.* 1989, Brierley *et al.* 1993b, Yeatman and Benzie 1993) have been detected in loliginids.

In addition to extensive allelic substitution among samples of *Martialia hyadesi* (Brierley *et al.* 1993a), marked population differentiation and unbalanced genotype frequencies with heterozygote deficiencies were observed. In agreement with findings for *I. argentinus*, *M. hyadesi* appears unable to maintain panmixia across its geographic range. The occurrence of both temporal and spatial structuring indicated sympatry of reproductively isolated stocks.

It might be expected generally that deep-water, highly mobile oegopsid squids, such as *Illex* and *Martialia*, would have greater powers of dispersal based on the production of large, diffuse, neutrally buoyant egg masses, than coastal myopsid squid, such as *Loligo*, which lay compact egg masses on the sea floor. Evidently, it is the degree of relatedness of prospective mates arriving on the spawning grounds that is critical in giving rise to, and presumably maintaining stock separation, not dispersal of eggs and juveniles. Although it would be premature to generalize based on relatively limited data, it is tempting to suggest that some aspect of the patterns and timing of reproductive migrations in the Oegopsida renders them likely to display population differentiation. The respective contributions of intra- and inter-specific variation to the population heterogeneity detected is, however, unclear.

In contrast to findings in the Ommastrephidae, *Berryteuthis magister* (family Gonatidae), from the North Pacific, exhibits largely homogeneous allele frequencies among sites, with significant population heterogeneity at only one locus (Katugin 1993). Furthermore, genotype frequencies were all in equilibrium. These genetic data are in stark contrast to findings so far published on oegopsid squid, highlighting the diversity of patterns present in different families.

Loliginids show both apparent panmixia and population heterogeneity. Allele frequencies in 19 samples of the Patagonian squid, *L. gahi*, collected from Falkland (Malvinas) waters between March 1987 and April 1988 were examined at 21 enzyme-coding loci (Carvalho and Pitcher 1989). Analysis of polymorphism at six loci ($P_{0.95}$) showed no evidence of stock separation, and the frequency distribution of genotypic classes fitted almost exclusively to Hardy-Weinberg expectations for a randomly interbreeding population. Nei's mean genetic distances and identities between samples ranged from 0.000–0.002 and 0.997–1.00 respectively, supporting the contention of panmixia in *L. gahi*, at least over the ~ 600 km examined. Although two distinct spawning periods of *L. gahi* separated by several months are observed in Falkland (Malvinas) waters (Patterson 1988, Hatfield and Rodhouse 1991), no evidence for restricted gene flow was detected; variable growth rates and prolonged spawning of males (Rodhouse *unpubl. data*) could provide sufficient opportunity for interbreeding and subsequent genetic homogeneity. It is likely that in addition to their locomotory powers, pelagic squids utilize ocean currents to achieve fast growth and extensive distribution during a short life span (Coelho 1985), so enhancing gene exchange. Presumably similar opportunities of vagility pertain to oegopsid squid; differences in fidelity to distinct spawning grounds may playa significant role.

Similar population homogeneity was detected among two spawning groups of *Loligo forbesi* from waters around the Isle of Man, UK (Brierley *et al.* 1993b), despite the existence of two discrete size classes at certain times of the year. The effect that geographic separation can have on gene flow was, however, demonstrated by significant population heterogeneity among samples of *L. forbesi* collected from locations along the European Atlantic continental shelf, Rockall, the Faroes and Azores (Brierley 1992, Brierley *et al.* 1993b). Significant differences in allele frequency at five out of 33 enzyme loci, and Nei's mean *I* values of <0.9, indicated that squid from the Azores and European continental shelf should perhaps be regarded as subspecies.

Population heterogeneity has also been detected in *L. opalescens* (Ally and Keck 1978), though data were preliminary only. Collections of *L. pealei* from the Atlantic seaboard between Cape Hatteras and Georges Bank differed significantly in both allele and genotype frequencies at a single locus (PGM, 2.7.5.1; Garthwaite *et al.* 1989), the only one found to be sufficiently polymorphic for a rpeaningful test. The authors suggested that *L. pealei* in the northwest Atlantic is comprised of at least three "populations", though given its migratory and highly mobile nature, it is unlikely that the spatial arrangement of populations is constant.

Among myopsid representatives, therefore, both population differentiation and panmixia have been revealed through molecular studies. It does appear that where oceanic distances of separation are involved, divergence is generally, although not universally evident. There is, however, no evidence for genetic patchiness on the fine scale observed for *I. argentinus*, though the scale and intensity of sampling is not comparable among studies.

Several additional molecular markers are now available for species and stock identification (Park and Moran 1994), including the use of restriction fragment length polymorphisms and polymerase chain reaction (PCR)-based restriction analysis of nucleic (Wirgin and Maceda 1991, Martin *et al.* 1992) and mitochondrial DNA (Ferris and Berg 1987, Knox and Verspoor 1991, Cronin *et al.* 1993), nucleic acid sequencing (Turner *et al.* 1991) and single-locus probes of minisatellite DNA sequences (Taggart and Ferguson 1990). These nucleic acid methods have yet to be applied to squid but have particular potential in SSA due to their usually greater sensitivity compared to allozymes.

The contribution of molecular data to our current understanding of population structure in squid can be summarized thus:

a) both loliginid and ommastrephid squid show significant genetic divergence, usually on geographic scales, though occasionally with microgeographic structuring;
b) extreme population heterogeneity is typical of ommastrephid squid, indicating a complex stock structure;
c) distinct spawning periods or discrete size class variation within a population should not be taken as evidence of genetic separation;
d) as in other marine invertebrates, a complex relationship exists between dispersal capacity and population structure (Ward 1989): a highly mobile life style does not necessarily lead to panmixia; and
e) the combination of low genetic diversity and marked population divergence is particularly risky if populations are exploited.

Such characteristics may increase the probability of stock collapse where a group(s) is only weakly represented, with the consequence of a further decline in genetic variability; adequate SSA as defined in Fig. 10.1 becomes imperative.

4.3.4 Morphometrics

Anatomical characters have traditionally been used in fisheries biology to describe geographic variation in a wide variety of exploited species (Ihssen *et al.* 1981) including squid (e.g. Kashiwada and Recksiek 1978, Kristensen 1982, Augustyn and Grant 1988, Borges 1990). Unlike molecular markers, phenotypic variation in body parts is influenced markedly by environmental factors, and the genetic component of such variance is uncertain. Furthermore, differences between workers recording the measurements may add significantly to variance between samples (G. Pierce, University of Aberdeen, unpubt. data), and there is no general consensus regarding the most appropriate methods of multivariate statistical analyses. Despite these drawbacks, characters such as body shape and size, tentacle and club dimensions continue to be widely examined among squid populations; they are generally easy and cheap to measure. Most applications among teuthoids, however, have been concerned with resolving taxonomic ambiguities (see section 4.3.1), perhaps an indication of the extreme morphological plasticity evident in soft body parts due to environmental and sexual dimorphism.

In teuthoids, geographic variation in measures such as mantle length, fin length and width, club size and form of hectocotylized arms (Kashiwada and Recksiek 1978, Smith *et al.* 1981), as well as hard structures including gladius, beaks and statoliths (Borges 1990) are used to estimate population differentiation. The most extensive study on *Illex* includes work carried out by Nigmatullin and co-workers (Tsygankov 1986,

Nigmatullin 1986, 1989, *unpubl. data*) on populations of *I. argentinu*s in the southwest Atlantic (see section 4.3.1 for locations). Variability in various body parts was examined (e.g. width and thickness of mantle, length and width of fin and head, arm lengths, tentacles, diameter of tentacle suckers), as well as dimensions and morphology of hectocotylus, radula morphology and structure of the spermatophore complex.

The various measures were compared between putative spawning groups of *I. argentinus* (see section 4.3.1), including the so-called autumn, winter, spring and summer groups. Although there is some evidence of stock integrity among these assemblages, morphological indicators were generally non-discriminatory. Most characters exhibited overlapping variances among seasonal groups, though there was some suggestion of a significant size difference in spermatophore glands: individuals from the summer-spawning group had, on average, longer spermatophore glands (*c*. 1.5 times) than the autumn- and winter-spawning groups. Such differences are most likely related to distinct growth conditions between the various assemblages, and as such, may not be consistent markers. Nigmatullin concludes that morphologically based methods of stock identification, at least of soft body parts, have limited value in *I. argentinus*.

Morphometric analysis of *I. argentinus* samples used simultaneously in allozyme studies (Carvalho *et al.* 1992; Fig. 10.3) revealed differentiation between certain sites, replicates and sexes (Carvalho *et al.* 1990). Comparisons were made among groups collected at approximately the same time to reduce the confounding influence of temporal variation. As previously found in studies on *L. gahi* (Carvalho and Pitcher 1989), there was no consistent correspondence between morphometric and genetic data; morphological discrimination was evident between apparently genetically homogeneous samples, and vice versa. For example, electrophoretically distinct samples (sites 1 and 2; Fig. 10.3) failed to show significant morphological differentiation of soft body parts, though such characters did exhibit typically high variance (Carvalho *et al.* 1990). Significant sexual dimorphism in morphological characters, and highly unbalanced sex ratios, underline the importance of analysing each sex separately. Morphological differentiation and distinctive sex ratios of some replicates support the existence of small-scale structure in these ommastrephid squid, possibly related to schooling behaviour. There is ample evidence of size-sorting among schooling species (Pitcher and Parrish 1993), including squid (see Fig. 5.10).

Statoliths from *I. argentinus* (n = 682) in the southwest Atlantic have also been used as markers in SSA (Brunetti and Ivanovic 1991). Two putative stocks were compared, the summer-spawning stock (see section 4.3.1) and an assemblage referred to as the "Bonaerensis northpatagonic stock". Comparison of characters such as total length, dome length, rostrum length, dome width and wing width, revealed significant differences between sexes within putative stocks, and between stocks while comparing the same sex. Recent studies on *I. argentinus* from fishery regions within and adjacent to the exclusive economic zone of Argentina (Arkhipkin 1993) also indicated distinct and consistent differences in statolith structure (colour, clarity and abundance of growth rings) between oceanic and shelf populations.

The value of statoliths as estimators of growth rate and age structure in squid populations is well established (Hurley *et al.* 1985, Lipinski 1986, Rodhouse and Hatfield 1990b), and data provided (Brunetti and Ivanovic 1991, Arkhipkin 1993) indicate their potential utility as markers for stock identification. Statolith-specific differentiation among samples lends credence to the existence of isolation among spawning groups of *I. argentinus* in the southwest Atlantic (see section 4.3.1).

Morphometric data on *Illex* are limited, and suffer from a dissimilarity in the methods of recording and analysis, together with insufficient information on allometric changes during the life cycle. A study on hard structures in the ommastrephid squid *Todarodes* sagittatus (Borges 1990) indicates that characters such as the gladius and beaks, especially the upper beak, may be more effective discriminatory tools than conventional soft body parts.

5 Impact of species identification and stock structure on recruitment dynamics: Future directions

Despite the available information on species identification and SSA in *Illex* and other squid, studies are largely fragmented and poorly integrated. The tendency for marked population heterogeneity, and possible cryptic speciation among *Illex* as revealed by genetic studies thus far, together with the generally low levels of genetic variability, necessitates a greater investment of resources in species description and SSA. Indeed, there is a general need to examine more comprehensively the population structure of commercially exploited squid species, especially in view of the conflicting patterns in population structure so far revealed. What is most needed, however, is not only a more detailed description of species identity and stock structure in major *Illex* fisheries, but also a concentrated effort on certain fundamental aspects of squid biology, in particular taxonomy, aspects of life history including patterns and distributions of spawning individuals and juveniles, and a scrutiny of the extent and significance of migrations in population dynamics. Below we make several suggestions regarding techniques and topics that may contribute usefully not only to our understanding of recruitment dynamics in *Illex*, but also to teuthoid biology in general.

5.1 Taxonomic studies

In view of the significant differences in growth rates, reproduction and mortality that sometimes occur among populations of a single species, it is risky to attempt stock assessment where cryptic speciation is evident. Cryptic speciation appears characteristic not only of the few I.argentinus populations so far studied at the molecular (see section 4.3.3) and ecological (see section 4.3.1) levels, but is apparent also in other ommastrephid and loliginid squid. Both in terms of forecasting the impact of harvesting and natural mortality on recruitment, and in terms of species conservation, it is important that detailed taxonomic studies be undertaken. Such work must integrate classical data on such factors as morphometrics and the structure of hard body parts and chromatophores, together with direct estimates of reproductive isolation using molecular genetic markers. It would then be possible to begin describing aspects of life history that are characteristic of distinct species, with the intention of adjusting exploitation in relation to species-specific recruitment dynamics.

5.2 Integration of data from several sources

As emphasized previously, adequate SSA requires the integration of data using more than a single investigative tool. It is particularly desirable that molecular genetic studies are supported by measures of differentiation at other biological levels. It is not the existence of molecular differences among stocks that is relevant, except in genetic tagging, but whether the unique molecules act as markers for differentiation likely to affect recruitment dynamics. Of particular value would be the application of genetic tools to previously defined assemblages such as the putative spawning groups of *I. argentinus* described by Nigmatullin (1986, 1989).

5.3 Identification of spawning grounds

In contrast to many commercially exploited fishes, there is a particular lack of information among squid species regarding the distribution of spawning individuals and juveniles. It is therefore difficult to assess the likely source of recruits to a fishery and their possible degree of interbreeding. In all ommastrephid squid fisheries, the catch is usually comprised of individuals on their feeding grounds, where a mixture of genetically distinct assemblages may exist that separate at spawning. The degree of genetic continuity among populations will affect the extent and rate of accumulation of biological differences, and is thus fundamental to an understanding of the origin and significance of population heterogeneity.

Studies are required that document, both spatially and over several years, the distribution of ripe individuals, especially females, and early juveniles. For example, the studies of Brunetti (1990) on the distribution of rhynchoteuthion larvae of *I. argentinus* made it possible to suggest not only the probable source of recruits ("summer" spawning group, Nigmatullin, 1989), but also by using information on developmental rates, direction of ocean currents, the size of larvae and the probable location of spawning grounds. Direct genetic comparison of larvae collected from temporally and spatially segregated spawning areas would provide a valuable estimate of reproductive isolation and begin to provide some understanding of the origins of population heterogeneity.

5.4 Advanced molecular techniques

In addition to the availability of allozyme electrophoresis, there is now a wide range of sensitive nucleic acid techniques, such as mitochondrial and nuclear DNA markers, that can be ued to describe population structure, (Carvalho and Pitcher 1994). With several techniques to choose from, it is important to match a particular type of molecular marker with the nature of information required. For example, if data are required on the breeding structure of a species, then allozyme electrophoresis remains the most valuable approach because it provides direct information on the distribution of genotypes in accordance with the Hardy-Weinberg paradigm. If, however, a "genetic tagging" tool is needed that uniquely characterizes specific stocks, then more sensitive methods such as mitochondrial DNA analyses (Ovenden 1990) or single-locus fingerprinting probes (Taggart and Ferguson 1990) may be more appropriate. Provided it was possible to identify individuals as belonging unequivocally to a particular stock or stocks, then once this was done it would be possible to determine the contribution of each stock to a mixed fishery.

The application of advanced DNA techniques to fisheries, and to population biology in general, has been facilitated by the development of the polymerase chain reaction (PCR) (Park and Moran 1994), which allows rapid visualisation of DNA polymorphisms by using minute quantities of DNA and non-invasive sample collection. The ability to amplify certain DNA sequences up to a million-fold, using either specific or universal primers, obviates the need for Southern blotting and complex, often time-consuming methods of visualizing DNA. Using PCR it is possible to stain DNA directly using ethidium bromide, increasing markedly the speed of sample analysis. Judicious use of such technology, together with an expansion of allozyme studies, should facilitate our description of stock separation and migration.

5.5 Combination of statolith studies and genetics

Advances in the use of statolith growth rings for ageing squid and deriving growth data (Rodhouse and Hatfield 1990a) present new opportunities for research. It is now possible to assign age more accurately than was previously possible with highly variable and environmentally sensitive morphological characters. The temporal separation of certain spawning groups (Patterson 1988, Carvalho and Pitcher 1989, Nigmatullin 1989, *unpubl. data*) is based on observations on the occurrence of juveniles and mature adults, usually with some degree of overlap between such assemblages. The opportunity to link genetic identity from molecular techniques with the ageing of individuals using statoliths would enable a more accurate assessment of when individuals hatched. The utility of such an approach would, however, depend markedly on the extent of temporal separation among putative groups, and the influence of environmental factors on growth rate (Villanueva 1992). It may be possible not only to examine genetic differentiation of animals belonging to distinct groups in relation to putative spawning groups, but also to identify probable sources of recruits on the basis of developmental rates and the speed and directionality of major ocean currents (Coehlo 1985). Furthermore, morphological differences between statoliths from squid of different origins (Arkhipkin 1993) may serve as useful markers to facilitate genetic comparison.

5.6 Extent and patterns of squid migrations

It is important when formulating estimates of recruitment and mortality in a fishery to assess the extent of squid movements on and off the fishing grounds. Such information is of relevance both as a measure of the relative contributions of distinct stocks to a mixed fishery and when apportioning changes in CPUE to either harvesting or migration; it is information critical to the calculation of appropriate forecast models (Beddington *et al.* 1990). Detailed studies are necessary which monitor the movement of different life-history stages in conjunction with the distribution of ocean currents.

5.7 The genetic integrity of schools

In addition to macrogeographic studies on stock structure, there is a need to examine fine-scale differentiation in *Illex* at the level of the school. It appears from preliminary studies (see section 4.3.3) that schooling may sometimes impart a scale of structure relevant both to stock assessment and to sampling design. Genetic analysis of individuals collected at short time intervals from several vessels simultaneously so as to examine within- and between-school genotype distributions, would indicate the extent of microgeographic differentiation. Such structuring would be particularly significant if schools differed significantly in size, fecundity or other life-history characters as well as genotype distribution.

6 Concluding remarks

The origin of species and stock differentiation is, to a large degree, dependent upon mating patterns and the degree of reproductive isolation. It is thus not surprising that a generally poor understanding of fundamental aspects of the reproductive biology of teuthoids has led to a correspondingly poor account of species and sub-specific taxonomy. Although *Illex* is among the world's most important economic marine resources, its species and populations have been poorly described. Probable cryptic speciation, marked genetic heterogeneity and low levels of genetic variability evident in the few populations so far studied underline the need to undertake comprehensive and integrated morphological, ecological and genetic studies on stock structure. *Illex* is rare among exploited species in that basic aspects of taxonomy and biology are either unclear or unknown, which taken together with the high variability in population size and susceptibility to harvesting (see section 3) renders it particularly fragile in the face of natural or fishery-imposed changes in the environment.

It is worthwhile stating that data on species identification and stock structure are useful in the context of management and conservation only if such information is fully incorporated into stock assessment (Fig. 10.1). For this to happen successfully, fishery managers must identify clear questions in order of priority, and field biologists and geneticists must communicate their findings in an unambiguous and relevant manner. It is not sufficient to state that "results are fundamental to the rational management of a fishery" and expect others to continue the process; an explanation of why specific results are important and how they can be used, is critical to ensuring their worthwhile contribution to fisheries management and species conservation.

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