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Multiple stock identification approaches of anglerfish (*Lophius piscatorius* and *L. budegassa*) in western and southern European waters

A.C. Fariña[1], R. Duarte[2], J. Landa[3], I. Quincoces[4], and J.A. Sánchez[5]

[1]*A.ntonio Celso Fariña: Instituto Español de Oceanografía, Muelle de Ánimas s/n , 15001 A Coruña, Spain [tel: +34 981 205362, fax: +34 981 229077, e-mail: celso.farina@co.ieo.es].*
[2]*R. Duarte: Instituto Nacional de Investigação Agrária e das Pescas INIAP-IPIMAR, Av. Brasilia, 1449-006 Lisboa, Portugal, [tel: + 351 21 302 7000, fax: +351 21 301 5948, e-mail: rduarte@ipimar.pt].*
[3]*J. Landa: Instituto Español de Oceanografía, Promontorio de San Martín s/n, 39004 Santander, Spain [tel: +34 942 291070, fax: +34 942 275072, e-mail: jorge.landa@st.ieo.es].*
[4]*I. Quincoces: Txatxarramendi ugartea z/g, 48395 Sukarrieta, Bizkaia, Basque Country, Spain [tel: +34 94 6029400, fax:+34 94 6870006, e-mail: iquincoces@suk.azti.es].*
[5]*J.A. Sánchez: Universidad de Oviedo, Julián Clavería s/n, 33071 Oviedo[tel: +34 985 103889, fax: +34 985 103534, e-mail: jafsp@correo.uniovi.es].*

ABSTRACT

Anglerfish, *Lophius piscatorius* and *L. budegassa*, are valuable commercial species distributed throughout the Northeast Atlantic and Mediterranean. This paper examines multiple information (genetic, morphometric, meristic, growth and mark-recapture) on stock structure of anglerfish species from western and southern European waters, to evaluate the suitability of current stocks units (northern and southern). For both anglerfish species, genetic structure showed significant heterogeneity observed mainly between geographically close population in *L. piscatorius*. More than 98% of the total genetic variation is attributable to differences within populations in both species, which suggest high gene flow within populations. On the other hand, the proportion of total genetic variation between stocks was relatively small. Morphometric characters allowed an acceptable discrimination of samples from certain areas, but not from stocks as a whole. Differences in the growth parameters between stocks was significant, although uncertainties in the ageing criteria diminished the utility of this approach. Tag-recapture experiments proved displacements of anglerfish going through the boundaries of the two stocks.

Combined data from genetic, morphometric, growth and tag-recapture studies provide indications that the current geographic separation between the northern and southern stocks is not supported and suggest the existence of discreteness populations along the study area. Given the repercussion of the results in the assessment and management of the two species in the area, more research to improve our understanding of anglerfish stock structure is required.

Keywords: Anglerfish, *Lophius piscatorius*, *Lophius budegassa*, stock identification, genetic, morphometric, growth, mark-recapture, Northeast Atlantic

1. INTRODUCTION

Two species of anglerfish (*Lophius piscatorius* and *L. budegassa*) are found in the Northeast Atlantic and the Mediterranean, however *L. budegassa* has a more southerly distribution than *L. piscatorius*. Both species have high commercial interest and can be distinguished primarily by the colour of the peritoneum, white (*L. piscatorius*) or black (*L. budegassa*). Anglerfish live on the sea bottom with large individuals being found in deep waters. Over the western and southern European waters (ICES Divisions VIIc-h, VIIIa-d and IXa) and southwestern Mediterranean, both species are caught as by-catches in bottom trawl mixed fisheries and as target species using gillnets. Average annual landings (1999-2003) from these areas were 28091 t (19,947 t of *L. piscatorius* and 8,144 t of *L. budegassa*).

For most of the 20th century, the references that have been published on anglerfish mainly cover the basic aspects of morphology, anatomy and habits, due to the curious appearance of the fish. Since 1990, the studies on European anglerfish species has risen substantially, their interest being focussed on biological characteristics relevant to stock assessment such as growth (Duarte *et al.*, 1997; Landa *et al.*, 2001), reproduction (Afonso-Dias and Hislop, 1996; Quincoces *et al.*, 1998a,b,c; Duarte *et al.*, 2001) and feeding (Azevedo, 1996; Pereda and Olaso, 1990; Silva *et al.*, 1997; Velasco *et al.*, 1996). However, basic aspects on reproductive cycle, larval biology and growth still remain largely to understand.

Populations of anglerfish from western and southern European waters were considered to be different stocks, not because of biological features but due to agreements on boundaries and considering the Cape Breton Canyon as a possible geographical barrier. The so-called northern and southern anglerfish stocks are distributed in ICES divisions VIIb-k-VIIIab, and VIIIc-IXa respectively. In this area, anglerfish are managed by a TAC and Quota system and the state of exploitation of both stocks and species is assessed yearly (ICES, 2004). Further knowledge on stock identity, geographical distribution patterns, reproduction and growth is still needed to reduce uncertainties in future stock assessments.

Many diverse characteristics and methods have been used to analyse stock structure in exploited species (Begg and Waldman, 1999), but the use of heritable genetic markers provided a universal and frequently abundant array of markers. Although molecular genetic procedures must provide the foundation for the identification of stocks, the good results obtained in freshwater species, do not always coincide with its informative value in marine species. Even when molecular genetic information is available, the analysis of tag returns, life-history variability, morphometric and meristic variability, parasite distribution, etc. is still necessary for discerning population structure. These characteristics reflect different evolutionary processes and time-scales, as well as informative ecological or environmental conditions (Begg and Waldman, 1999; Begg *et al.*, 1999), which often limited their use. A holistic approach to fish stock identification is highly desirable owing to the limitations and conditions associated with any particular method in marine fishes.

Concerning European anglerfish, only Crozier (1987; 1988) analysed the stock structure of these species from the Irish Sea and West Scotland using protein-coding loci. Grant and Leslie (1993) used allozyme phylogenia and the geologic and oceanographic history

of the Atlantic Ocean to construct a historical bio-geographical hypothesis for the species of *Lophius* known in the world. Morphological features for stock discrimination were studied in *L. vomerinus* but not in European anglerfish. Mark-recapture experiments in anglerfish have not been used before for stock discrimination, although previous experiments were carried out to validate growth interpretation (Pereda and Landa, 1997).

The present paper aimed to examine the stock structure identification of western and southern European anglerfish (*Lophius piscatorius* and *L. budegassa*) populations - northern and southern anglerfish stocks-, including information on a Mediterranean area. A multi-approach were used: 1) microsatellite DNA markers 2) morphometric and meristic traits, 3) ageing and growth parameters, and 4) mark-recapture. The results and signals from these approaches are discussed in relation to the current boundaries of both stocks.

2. MATERIALS AND METHODS

Sample collection
Samples on anglerfish, *L. budegassa* and *L. piscatorius,* of restricted length range of 40-47 cm and 30-37 cm respectively, were collected during 2000 and 2001 from different regions of the study geographic range, from West Ireland to Mediterranean [ICES divisions VIIcj, VIIgh, VIIIab, VIIIc (East and West) and IXa (North and South), and SW Mediterranean Sea]. Samples were indistinctly taking on research surveys, on board commercial vessels, buying fish from landings, but in all cases information of the fishing area was always available. Sampling was aimed at obtaining representation of each area (about 50 individuals) for genetic and morphometric characterization (Table 1).

2.1. Genetic

DNA extraction and Microsatellite analysis
A small amount of muscle (approx. 10 g) was removed from the anterior base of the insertion of the pelvic fins and stored in 100% ethanol at 4º C prior to DNA extraction. A genomic library was constructed and 8 specific sequences (single locus microsatellite named *Lb-OVI-A146**, *Lb-OVI-A152**, *Lb-OVI-A160**, *Lb-OVI-B20**, *Lb-OVI-C16**, *Lb-OVI-C26**, *Lb-OVI-C30** y *Lb-OVI-D11**) were amplified by PCR and used to assay genetic variation among samples of *L. piscatorius* and *L. budegassa*.

Data analysis
Levels of genetic variation were based on mean number of alleles per locus, expected, and observed heterozygosity and were calculated using the programme BIOSYS (Swofford and Selander, 1989). Allelic frequency differences between pair of samples and among all samples were analysed by the Fisher exact test.

Total genetic variation (H_T) was evaluated by a hierarchical gene diversity analysis (Nei, 1973) using NEGST program (Chakraborty *et al*., 1982). Pair-wise population distances $(\delta\mu)^2$ (Goldstein *et al*., 1995) were calculated using the RSTCALC package (Goodman, 1997) and used to construct population trees using the neighbour-joining algorithm (Saitou and Nei, 1987) available in the PHYLIP 3.5c. Genetic relationships among samples were also examined using Principal Co-ordinates Analysis (PcoA;

Gower, 1966) of the $(\delta\mu)^2$ ´s genetic distance matrix.

Finally, for testing whether or not an individual was unlikely to be derived from a specific population an assignments tests (GENECLASS v 1.0; Cornuet *et al*., 1999), based on multi-locus genotypes, were used to assess the most likely population of origin.

2.2. Morphometric and meristic

The morphometric and meristic analysis was based on a total of 160 *L. piscatorius* and 298 *L. budegassa*. Morphometric data were obtained by taking a digitized image of each specimen, with the landmarks highlighted in each picture. Landmarks were mainly based on cephalic spines, due to the variable contour and flexible skin of these species. Other morphometric studies in the *Lophius* genera also used cephalic spines as landmarks (Leslie and Grant, 1990). With the digitised image of each specimen, the coordinates of each landmark was obtained and the distances between variables and all measurements were standardized to the overall mean length. A total of 29 and 36 morphometric variables were finally adopted for *L. piscatorius* and *L. budegassa* respectively. Data for the meristic analysis were obtained by counting the fin rays of the first and second dorsal fins, left and right pectoral fins and the anal fin.

Discriminant linear functions were fitted to both types of data and an agglomerative hierarchical clustering was performed for the morphometric data.

2.3. Growth parameters

Samples, ageing and growth parameters
Illicia of anglerfish from northern and southern stocks were collected within the framework of various international projects during 1996-2000. *Illicia* sections were used to ageing and estimate the von Bertalanffy growth parameters. The analysis was done compiling the period as one synthetic "biological year". The criteria for age interpretation established in Workshops on Anglerfish Growth (Dupouy, 1997; Anon., 1999) were followed.

The growth pattern of both species were done following two analyses: a) calculation of the von Bertalanffy growth curve by means of a non-linear regression fixing the L_∞ to the length of the larger fish recorded in each stock for the period analysed, and b) perform the same analysis but estimating the L_∞ by the function used.

Growth comparison approaches
Two statistical methods were applied with the aim of investigating the possible differences in the growth pattern between stocks. The first method tests the differences of the growth rate (k) (Wang and Milton, 2000) for each stock taking in account the asymptotic standard error of the estimations (Francis, 1996; Francis and Horn, 1997). The second method splits the von Bertalanffy growth function into n-1 regression lines (were n is the number of ages in the analysis) and performs a comparison of the paired slopes of each stock (He and Stewart, 2002) .

2.4. Mark-recapture

A total of 1464 anglerfish (800 *L. piscatorius*, 563 *L. budegassa* and 101 *Lophius* sp.) were tagged in the study fishing areas between 2000 and 2002. In previous experiments, 582 anglerfish (298 *L. piscatorius*, 284 *L. budegassa*) had been tagged, and these data were incorporated into this study (Figure 1). Size ranges of anglerfish tagged were 15-104 cm in *L. piscatorius* and 6-88 cm in *L. budegassa*. Anglerfish were captured by commercial fishing using gillnets and trawl, and during bottom research surveys. From catches, uninjured and alive anglerfish were measured, tagged and released. One external tags, spaghetti T-bar type (4 cm long, yellow or red), was inserted into the musculature just between the two dorsal fins. A tetracycline dose was also injected in the fish ventral cavity just forward of the anus. Data on fishing and location of released were recorded. The tagging programme and rewards was internationally advertised.

Movement patterns were examined by plotting the distance (measured by the direct route between the release and recapture localities), direction and times at liberty of recaptures.

3. RESULTS

3.1 Genetic

3.1.1. Differences between species

The number of the alleles for the eight microsatellite loci and the allelic size range for each species of *Lophius* is summarized in Table 2. In general, *L. piscatorius* showed more number of alleles (142) than *L. budegassa* (116).

The allelic distribution allowed distinguish between both species, mainly using *Lb-OVI-A152**, *Lb-OVI-A160**, *Lb-OVI-B20**, *Lb-OVI-C16** and *Lb-OVI-C26** loci. The percentage of assignment and rejection (1% criterion) was 100% and 0, respectively for *L. budegassa* and 98.78% and 0, respectively for *L. piscatorius*.

3.1.2. Pattern of genetic variation within *Lophius piscatorius* samples

The total number of alleles ranged from 9 to 45 (Table 2). Almost the 25% percentage of all alleles were private for a single sample, and all at a frequency below 0.054. Eleven of the 54 test (20.4%) yielded a significant departure from Hardy-Weinberg expected genotype frequency, all of them due to heterozygote deficiency but after multiple probability test by locus and population four significant deviation from HW equilibrium remain significant. The mean number of allele per locus varied from 8.13 (IXa-S) to 12.25 (VIIcj). The mean observed and expected heterozygosity ranged between 0.543 (VIIgh) and 0.672 (VIIIabd) and between 0.598 (VIIgh) and 0.711 (VIIIabd), repectively.

Five (62.5%) of the eight polymorphic loci (*Lb-OVI-A160**, *Lb-OVI-B20**, *Lb-OVI-C16*, *Lb-OVI-C26* and *Lb-OVI-D11**) showed significant differences in allele frequencies among all the samples, overall heterogeneity being highly significant (p=0.0001). Twelve (80%) out of the 15 gene frequencies comparisons between pair of samples were significant. Analyses of θ_{ST} revealed no evidence of population genetic substructure when estimated over all loci. Nevertheless, ρ_{ST} estimate showed higher population sub-structuring than θ_{ST} value. No significant correlation was found between

θ_{ST} and ρ_{ST} estimates (r= 0,278; p=0.25). Partitioning of genetic variation showed that more than 98% of the overall variation was found within samples. Estimates of $\delta\mu^2$ pair-wise genetic distance ranged between 0.0019 (VIIgh-VIIIc-E) and 0.1963 (VIIIabd-IXa-S), being 0.076 ± 0.019 the average. The pattern of clustering for this estimate using a neighbour-joining method clearly showed that there was no relationship between genetic and geography distance, which appeared to be supported by the PCoA (Figures 2 and 3).

The assignment tests showed that a rather low number of individuals were assigned correctly to its original sample. The percentage of assignment ranged between 14.3% (VIIcj) to 36.4% (IXa-S). The highest percentage of rejection was found in VIIcj (12.2%) and the lowest in VIIgh (4%).

3.1.3. Pattern of genetic variation within *Lophius budegassa* samples

The total number of alleles ranged from 8 to 36 (Table 2). Only a few private alleles were observed (12), and all at a frequency below 0.046. Seven of the 64 test (11%) yielded a significant departure from Hardy-Weinberg expected genotype frequency, five of them due to heterozygote deficiency, but after multiple probability test by locus and population only three significant deviation from HW equilibrium remain significant. The mean number of allele per locus varied from 8.38 (VIIIc-E) to 10.63 (IXa-S and MB). The mean observed and expected heterozygosity ranged between 0.677 (MB) and 0.735 (IXa-S) and between 0.674 (IXa-S) and 0.731 (VIIIc-W), respectively.

Three (37.5%) of the eight polymorphic loci (*Lb-OVI-A160**, *Lb-OVI-C30**, and *Lb-OVI-D11**) showed significant differences in allele frequencies among all the samples, overall heterogeneity being highly significant (p=0.0001). Ten (35.7%) out of the 28 gene frequencies comparisons between pair of samples were significant. Analyses of θ_{ST} and ρ_{ST} estimates among the eight samples revealed no evidence of population genetic structure with either measure when estimated over all loci. Significant correlation was found between θ_{ST} and ρ_{ST} estimates (r= -0,81; p=0,007). Partitioning of genetic variation showed that more than 98% of the overall variation was found within samples. Estimates of $\delta\mu^2$ pair-wise genetic distance ranged between 0.011 (VIIgh-VIIIab-d) and 0.0782 (VIIcj-IXa-S), being 0.032 ± 0.0036 the average. The pattern of clustering for this estimate using a neighbour-joining method clearly showed that there was no relationship between genetic and geography distance, which appeared to be supported by the PCoA (Figures 4 and 5).

The assignment tests showed that a rather low number of individuals were assigned correctly to its original sample. The percentage of assignment ranged between 3.8% (VIIIc-W) to 28.3% (IXa-S). The highest percentage of rejection was found in VIIIc-E with 37.5% and the lowest in VIIgh with 4.4%.

3.2. Morphometric and meristic

Results from the morphometric and meristic analysis are described in more detail in Duarte *et al.*, 2004 (ICES, 2004, EE:20). Basically, morphometric results indicated segregation between the analysed areas for both species. *L. piscatorius* from VIIcj, VIIIabd and VIIIc was clearly segregated by the discriminant functions, and the total correct reallocation was of 89 %. The results for *L. budegassa* showed a segregation of

area IXa from the other areas, and a latitudinal gradient. The total correct reallocation was of 76 % considering all areas.

Meristic results were less determinants due to the uniformity of the meristic characters along the study area, and the meristic analysis showed lower segregation among areas for both species.

3.3. Ageing and growth parameters

3.3.1 *Lophius piscatorius*

Age length keys 1996-2000 for northern and southern anglerfish *L. piscatorius* were elaborated from 2820 and 2294 *illicia* readings respectively. Age length key was more homogeneous in southern stock in terms of size range and number of samples per length class.

The growth rates estimated for the northern stock were k = 0.09 and k = 0.07 using the method of fixing the L_∞, and L_∞ estimated by the regression respectively. Both methods explain similar variability of the model (r^2 = 0.94 in both cases). Von Bertalanffy growth curves primarily differ for ages older than 15 years. In the case of the southern stock the growth rates were k = 0.05 and k = 0.04 with the two methods respectively (r^2= 0.95 in both cases). Differences between methods appear in ages older than 20 years.

The results of the test for differences in the growth rate between stocks are presented in Table 3. This test shows significant differences between the growth rates between stocks independently of the method used. Growth curves between stocks showed larger differences in older ages (Figure 6). Inter-annual growth coefficients of northern and southern anglerfish stocks were different in age intervals 1-2, 3-4, 7-8 an 15-16 (Table 4).

3.3.2 *Lophius budegassa*

Age Length Keys for northern and southern stock of *L. budegassa* were elaborated from 4160 and 3135 *illicia* readings respectively.

The growth rates estimated for the northern stock were k = 0.10 using the method of fixing the L_∞, and k = 0.07 for L_∞ estimated by the regression. Both methods explain similar variability of the model (r^2 = 0.96). Von Bertalanffy growth curves primarily differ for ages older than 15 years. In the case of the southern stock the growth rates were k = 0.10 and k = 0.06 with the two methods respectively (r^2= 0.88). Differences between methods appear in ages older than 15 years.

The results of the test for differences in the growth rate and the comparison between stocks are presented in Tables 5 and 6. Differences between stocks were found in growth curves in the whole length range (Figure 7).

3.4. Mark-recapture

Over 2046 anglerfish tagged (1098 *L. piscatorius*, 847 *L. budegassa*, 101 *Lophius* sp.) in western and southern European waters, rates of recoveries were 4.6 % and 1.8 % for *L. piscatorius* and *L. budegassa* respectively. Recaptured fish had been at liberty from 1 to 665 days. The 73 % of the recoveries were during the first month at liberty.

Movements of tagged *L. piscatorius* were limited, with the 85 % of recaptures being less than 50 km (in a straight line) from the respective release site (Figura 8). The larger movement observed for a recapture was 290 km. Larger displacements between northern and southern stocks boundary were corroborated in 3 recoveries (from ICES div. VIIIc to VIIIa, 290 km and 15 months at liberty, from VIIIb to VIIIc, 123 km and 15 months at liberty, and from VIIIc to VIIIb, 109 km and 3 months at liberty). Larger movements within stock were also verified in 2 recaptures (12 months at liberty), with displacements of 120 and 230 km to shallower waters of the east (div. VIIIa) and northeast (VIIe) of where they had been tagged (VIIIa)

In relation to *L. budegassa* tagged, 77% of recaptured moved less than 50 km from their release sites. Displacements between stocks boundaries was verified in 1 recapture, moving 156 km from ICES div. VIIIc to VIIIb (7 months at liberty) (Figure 9).

The distances that anglerfish moved were correlated with their times at liberty (Figure 10) in both species ($r = 0.824$, $n = 49$, $P < 0.001$ in *L. piscatorius*; $r = 0.597$, $n = 18$, $P < 0.01$ in *L. budegassa*).

4. DISCUSSION

The numerous definitions of stock, differing in their emphasis on the degree of homogeneity within populations, the importance of reproductive isolation and the relevance to exploitation. In addition, there are interactions with political, social and economical factors (Carvalho and Pitcher, 1995). The stock concept, throughout its numerous definitions, is the population-base for fisheries management purposes, although the considerations of the stock structure information for implementation of assessment and management are scarce. Related to anglerfish, ICES considers for assessment purposes two stocks in the western and southern European waters, with the boundaries between these stocks established in the South of the Bay of Biscay. Our study shows different signals on heterogeneity of population structure of anglerfish in the area, suggesting the actual stock limits are no biologically supported. A revisitation of the stock separation would be larger implications in the current anglerfish fisheries management.

A large array of molecular genetic markers are available to describe population structure either proteins (usually enzymes or more specifically allozymes, which are allelic forms at a single enzyme locus) or DNA (mitochondrial or nuclear segments). The choice of an appropriate marker to use for particular applications is a difficult task and depends on the level of detectable genetic variation detected for that marker in a specific species (Abaunza *et al.*, 2003).

From the different techniques available for the identification of marine fish populations, microsatellites are more powerful than another genetics molecular markers due their higher level of variation (Hauser and Ward, 1998, Cagigas *et al*., 1999). Microsatellite data are currently scarce for marine fish, although they are more likely to reveal significant differentiation among closely related populations and are currently being

used to studies of stock structure in other species (Ward *et al.*, 1999).

The genetic structure of anglerfish showed significant heterogeneity in gene frequencies among samples from different areas (observed mainly between geographically close populations in *L. piscatorius*) suggesting discreteness populations along the distribution range in western and southern European waters. Our results indicated significant genetic differences among populations within both species (more than 98% of the total genetic variation), attributable to gene flow among populations, although the proportion of total genetic variation between stocks is relatively small for both species (0.21% *L. budegassa*, 0.35% *L. piscatorius*), which would suggest that the that the current geographic separation between the so-called northern and southern stocks is not genetically supported. The lack of genetic differentiation is most likely due to the effect of gene flow, which prevent genetic divergence arising among populations by either drift or selection.

The low genetic distance observed between the Mediterranean sample and the rest of the samples from *L. budegassa,* appeared to be contrary to that observed in other marine species. Genetic discontinuities between the Atlantic and Mediterranean populations have been reported or suspected in other marine species with different molecular markers (Roldán *et al*., 1998; Lundy *et al*., 1999; Naciri *et al*., 1999). Nevertheless, the transition zone appeared to be located in different places, depending on the species. The Mediterranean sample from *L. budegassa* was caught in southeast Spain. It could be possible that transition zone, if exits, in *L. budegassa* was located eastwards in the Mediterranean Sea. Therefore, further investigation of the population structure of *Lophius* species should include more extended sampling areas especially along the Mediterranean basin and the Atlantic waters adjacent to the Strait of Gibraltar.

The degree of genetic differentiation for both anglerfish species (θ: 0.0018; 0.0066 for *L. budegassa* and *L. piscatorius*, respectively) appeared to be lower than other marine species (*Merluccius merluccius*, 0.026, Lundy *et al*., 1999; *Gadus morhua*, 0.0084, Ruzzante *et al*., 1998; *Dicentrarchus labrax*, 0.023, Naciri *et al*., 1999; *Clupea arengus*, 0.024, Shaw *et al*., 1999). Marine fishes in general tend to show little genetic differentiation between geographic stocks because the absence of physical barriers allows gene flow among samples.

Contrary to the genetic results, *L. piscatorius* and *L. budegassa* from different large areas can be discriminate based in morphometric characters up to 89% and 76 % respectively. Similar incongruence between genetic and morphometric stock structure was found in southern African anglerfish (Leslie and Grant, 1990). *L. piscatorius* showed high segregation between three analysed areas that corresponds in part to the distribution of northern (VIIcj, VIIIabd) and southern (VIIIc) anglerfish stocks. The low number of samples from specific areas have prevented to extent the analysis to other areas such area IXa that be also part of the southern stock. *L. budegassa* showed a high segregation of the division IXa (southern stock) to the other areas and also a latitudinal gradient with some overlap amongn areas. Differences in morphometric characters may be driven by the environmental conditions between the geographical areas and not related to segregation of specific genes (Begg and Waldman, 1999). Individual flow between areas may occur in the case of anglerfish populations, but the dominance of sedentary adults would determine larger environmental influences on their morphological growth (Leslie and Grant, 1990).

Growth parameters vary spatio-temporaly within and between stocks. They lack of long-term stability, and their use as indicators of stocks structure may be relative. The comparison between growth parameters seems not to be a good method to investigate the stock structure of European anglerfish, given the current state of ageing studies, where estimations from otoliths and *illicia* were not always in agreement (Wright *et al*., 2002; Woodroffe *et al*., 2003). Differences in the fish availability (specially larger fish) for both stocks and in the ageing criteria (Anon. 1999), particularly in the definition of the 1-age group, seem to be the mayor reasons for the statistical differences found in the growth pattern between stocks on both species.

Differences in sampling times and gear types between the phases of study may result in a lack of homogeneity in the origin of the samples used to, and this was not taking into account. In this study, the pooled samples of different years and areas for each stock may produce poor differences in growth rates and parameters to indicate large differences between stocks.

The results from the information set on tagging show that the overall recovery indexes are similar to those estimated for other species in which tag-recovery results are considered successful. The recovery index varied depending on the tagging area, highlighting the high index in Subdivision VIIIc-West (8.5% in *L. piscatorius* and 8.6% in *L. budegassa*) and the high values in Subdivision VIIIc-East (5.2% and 2.6% respectively) and Division VIIIb (5.0% and 2.3% respectively). This variation of recoveries by area probably has a direct relationship with the fishing gear used and the interest of the fishermen for communicating the recoveries.

The displacements observed from mark-recapture experiments point to some degree of migratory behaviour of this species, which contrasts with their supposed poor adaptation to swimming. Also, they indicate interactions between the components of both stocks and serve to question the biological basis of the currently considered stocks.

Tagging data combined with the temporal variation of commercial catches in along the boundary between both stocks (VIIIb-South and VIIIc-East) suggest that, at least, part of adult *L. piscatorius* move from VIIIb-South to VIIIc-East during the 1st quarter along the slope (between 300 and 1000 m depth) and continue to shallow waters, where they appear in greater abundance during the 2nd quarter. It is suggested that they follow the same route but in the opposite direction in the following months, probably related with the spawning cycle.

Future research must aim to extent the approaches (parasites, life history characteristics, otolith microstructure, etc.) already applied to other fishes (Begg & Waldman, 1999), and achieve the integration of the whole set of methods used in a multiple stock identification approach to maximise the likelihood of correctly defining stocks over the complete distribution area of anglerfish in the Northeast Atlantic and Mediterranean.

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Table 1. Number of sampled anglerfish for the genetic and morphometric studies.

	Genetic		Morphometric	
Area	*L. budegassa*	*L. piscatorius*	*L. budegassa*	*L. piscatorius*
VIIcj	49	45	49	47
VIIgh	45	50	-	-
VIIIabd	54	47	53	46
VIIIc-E	24	53	24	53
VIIIc-W	26	-	26	-
IXa-N	25	25	23	-
IXa-S	46	22	54	11
W Mediterranean	77	3	69	3

Table 2. Characteristics of 11 *L. budegassa* microsatellite loci: allelic size range and number of alleles in *L. budegassa* and *L. piscatorius*.

		Lophius budegassa		*Lophius piscatorius*	
Locus	Repeat motif	Allelic size range (bp)	Nº of alleles	Allelic size range (bp)	Nº of alleles
*OVI-Lb-A146**	$(CA)_{15}$	139-167	11	139-197	25
*OVI-Lb-A152**	$(CA)_{12}CCCCCAGA(CA)_9$	91-115	10	81-113	11
*OVI-Lb-A160**	$(CA)_{14}$	106-115	9	110-181	17
*OVI-Lb-B20**	$(CT)_{16}CC(CT)_4$	143-199	18	143-189	13
*OVI-Lb-C16**	$(TAGA)_4TGGATAGA(TGGA)_{10}$	159-207	8	159-203	9
*OVI-Lb-C26**	$(CCAT)_5CAT(CCGT)_2(CCAT)_{10}$	171-207	10	139-207	11
*OVI-Lb-C30**	$(CA)_{16}$	115-151	14	113-149	11
*OVI-Lb-D11**	$(GATA)_{35}(GATC)_2(GATA)_2$	168-340	36	160-408	45
*OVI-Lb-A178a**	$(AG)_{17}$	--	--	--	--
*OVI-Lb-A194**	$(CA)_{15}CCTA(CA)_2ACTA(CA)_3$	--	--	--	--
*OVI-Lb-D1**	$(GATA)_9GGTA(GATA)_{18}$	--	--	--	--

Table 3. Comparison of k between stocks of *L. piscatorius*.

Method	Stock	k	SD	n	t-value	p
Linf=136	VIIIabd+VII	0.087	0.0262	2820		
					63.98	0.00
Linf=186	Ixa+VIIIc	0.051	0.0129	2294		

Method	Stock	k	SD	n	t-value	p
	VIIIabd+VII	0.065	0.1091	2820		
Linf estimated					8.44	0.00
	Ixa+VIIIc	0.043	0.0682	2294		

Table 4. Comparison of the interannual growth coefficient between stocks of *L. piscatorius.*

	Interannual growth coefficient			
Age interval	VIIIabd+VII	IXa+VIIIc	f-ratio	p
1-2	**3.95**	**8.33**	**16.12**	**0.00**
2-3	7.47	6.80	0.70	0.40
3-4	**8.60**	**5.46**	**19.91**	**0.00**
4-5	7.94	6.96	2.24	0.13
5-6	7.40	6.60	1.50	0.22
6-7	7.07	6.70	0.31	0.58
7-8	**7.61**	**5.98**	**5.22**	**0.02**
8-9	6.46	6.78	0.15	0.70
9-10	5.85	7.16	1.51	0.22
10-11	6.44	4.48	2.43	0.12
11-12	3.81	5.03	0.62	0.43
12-13	4.35	6.95	2.01	0.16
13-14	0.71	4.25	3.44	0.06
14-15	1.64	2.94	0.38	0.54
15-16	**-1.84**	**3.21**	**4.14**	**0.04**
16-17	8.70	4.15	2.48	0.12
17-18	0.86	2.06	0.12	0.73
18-19	2.04	2.24	0.00	0.96
19-20	1.40	3.39	0.15	0.7

Table 5. Comparison of k between stocks of *L. budegassa*.

Method	Stock	k	SD	n	t-value	p
Linf=97	VIIIabd+VII	0.096	0.022	4160		
					-7.68	0.00
Linf=97	IXa+VIIIc	0.101	0.042	3135		

Method	Stock	k	SD	n	t-value	p
	VIIIabd+VII	0.069	0.078	4160		
Linf estimated					3.51	0.00
	Ixa+VIIIc	0.059	0.160	3135		

Table 6. Comparison of interannual growth coefficient between stocks of *L. budegassa*.

	Interannual growth coefficient			
Age interval	VIIIabd+VII	IXa+VIIIc	f-ratio	p
1-2	**1.95**	**6.35**	**25.89**	**0.00**
2-3	**2.71**	**4.73**	**11.37**	**0.00**
3-4	4.93	5.29	0.66	0.42
4-5	**5.55**	**4.72**	**4.57**	**0.03**
5-6	5.65	5.28	0.76	0.38
6-7	5.34	5.58	0.23	0.63
7-8	**5.13**	**6.69**	**8.87**	**0.00**
8-9	5.43	5.59	0.08	0.77
9-10	4.95	4.83	0.04	0.84
10-11	4.44	3.25	4.01	0.05
11-12	3.50	3.36	0.05	0.83
12-13	3.24	3.28	0.00	0.96
13-14	3.85	4.31	0.30	0.58
14-15	2.61	2.06	0.31	0.58
15-16	1.75	2.50	0.30	0.59
16-17	1.58	2.08	0.16	0.69
17-18	1.43	0.68	0.40	0.53
18-19	1.94	3.15	0.60	0.44
19-20	0.71	-1.53	0.88	0.36

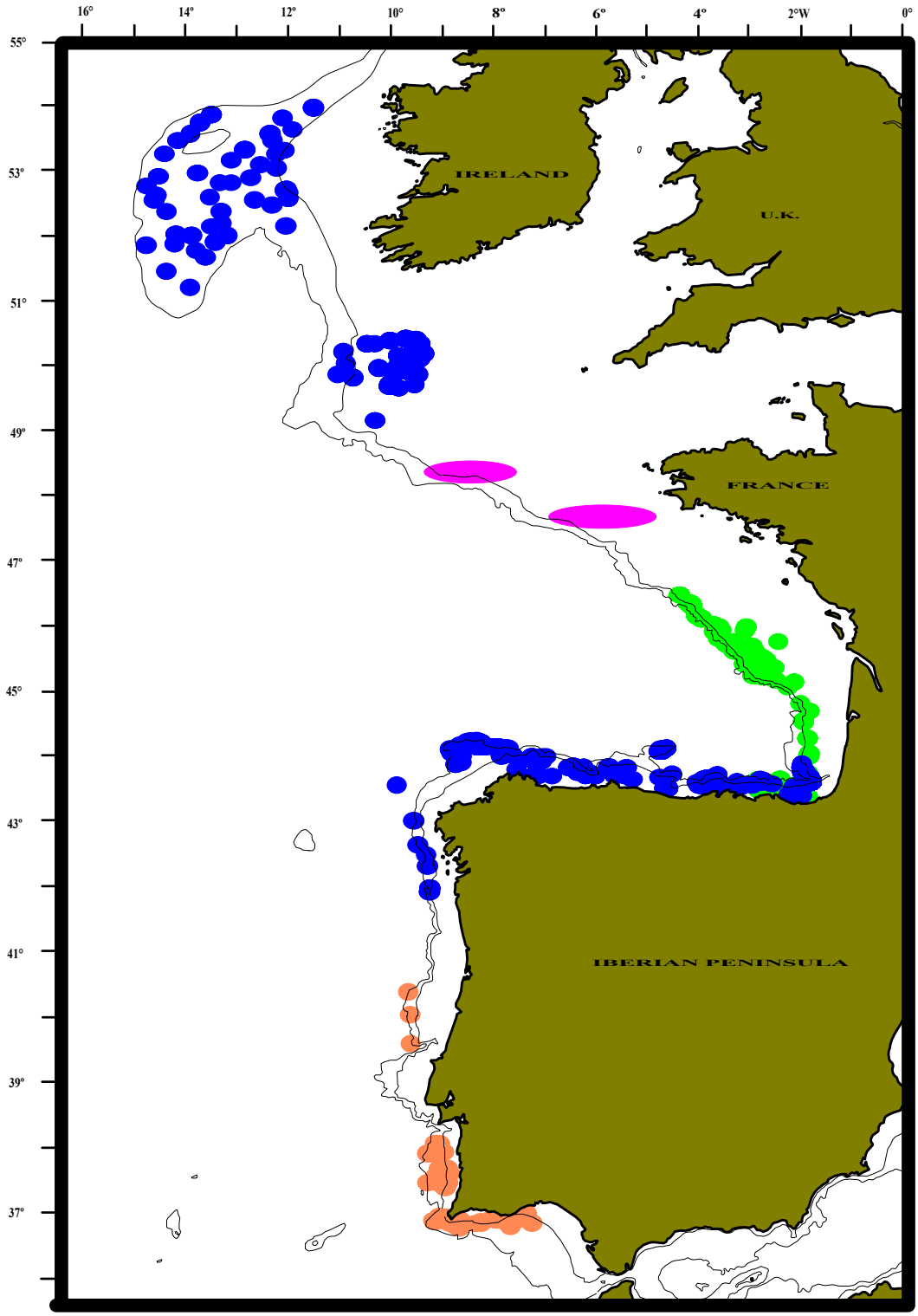

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Figure 1. Tagging locations of anglerfish, 1995 - 2002.

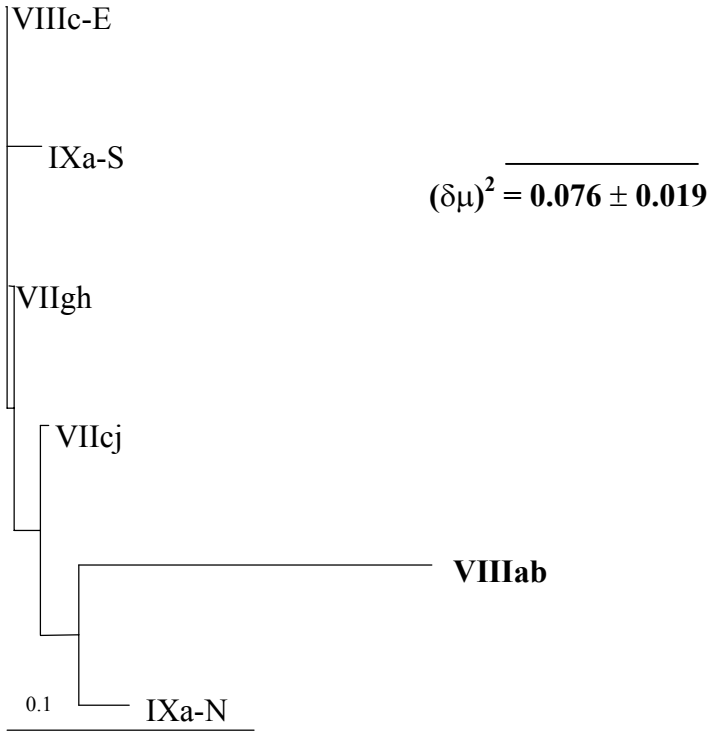

Figure 2. Unrooted neighbour-joining tree from $(\delta\mu)^2$ distances illustrating relationships among six *L. piscatorius* samples. Codes refer to populations identified in Table 1.

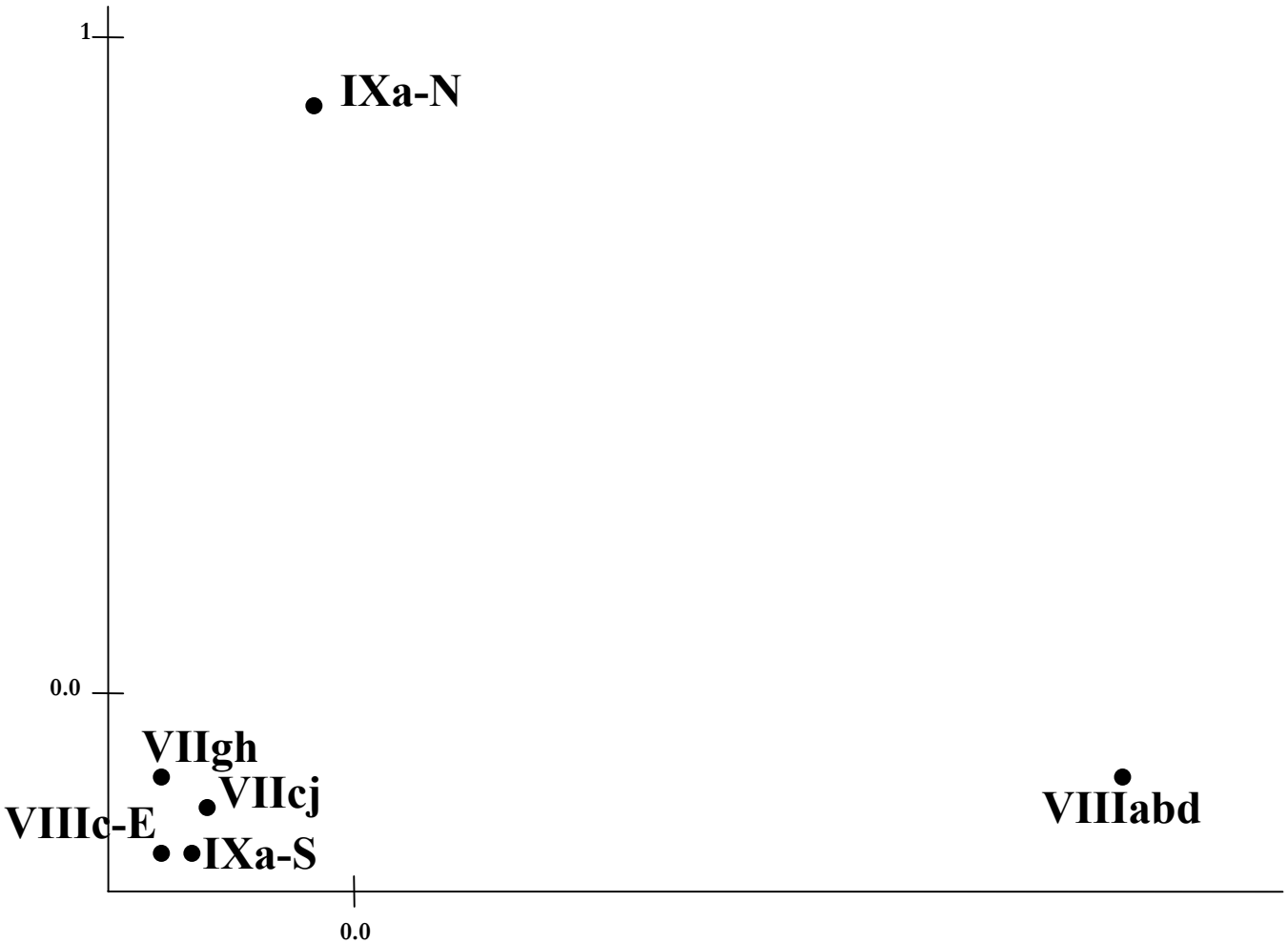

Figure 3. Principal Co-ordinates Analyses (PCoA) of *Lophius piscatorius* populations based on $(\delta\mu)^2$ index of genetic distance. The populations have been projected onto the first two co-ordinates axes, which accounted for 85.74% of the total variation. (Coordinate 1: 71.64%; Coordinate 2: 14.1%)

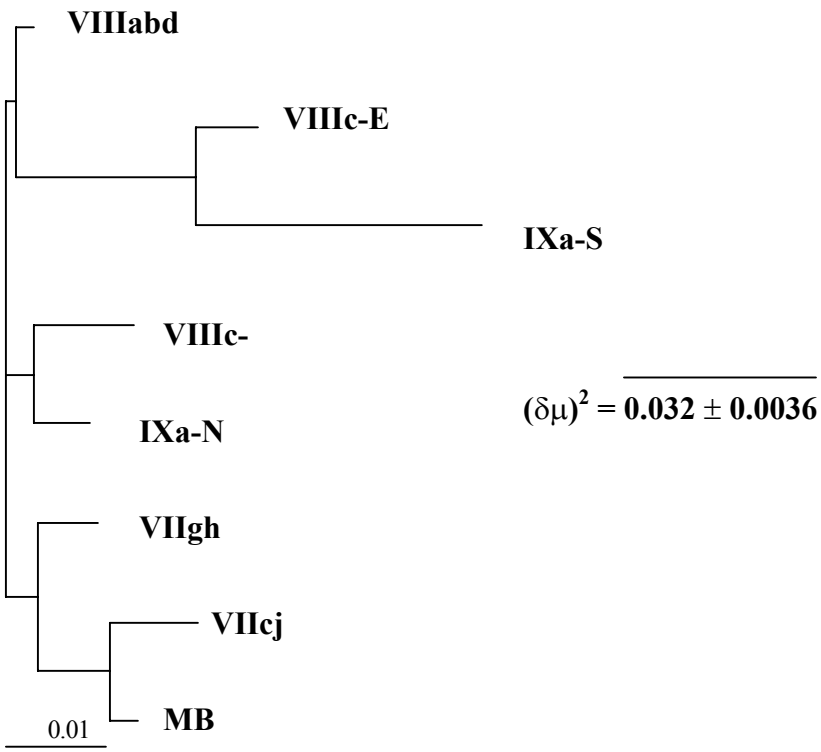

Figure 4. Unrooted neighbour-joining tree from $(\delta\mu)^2$ distances illustrating relationships among eight *L. budegassa* samples. Codes refer to populations identified in Table 1.

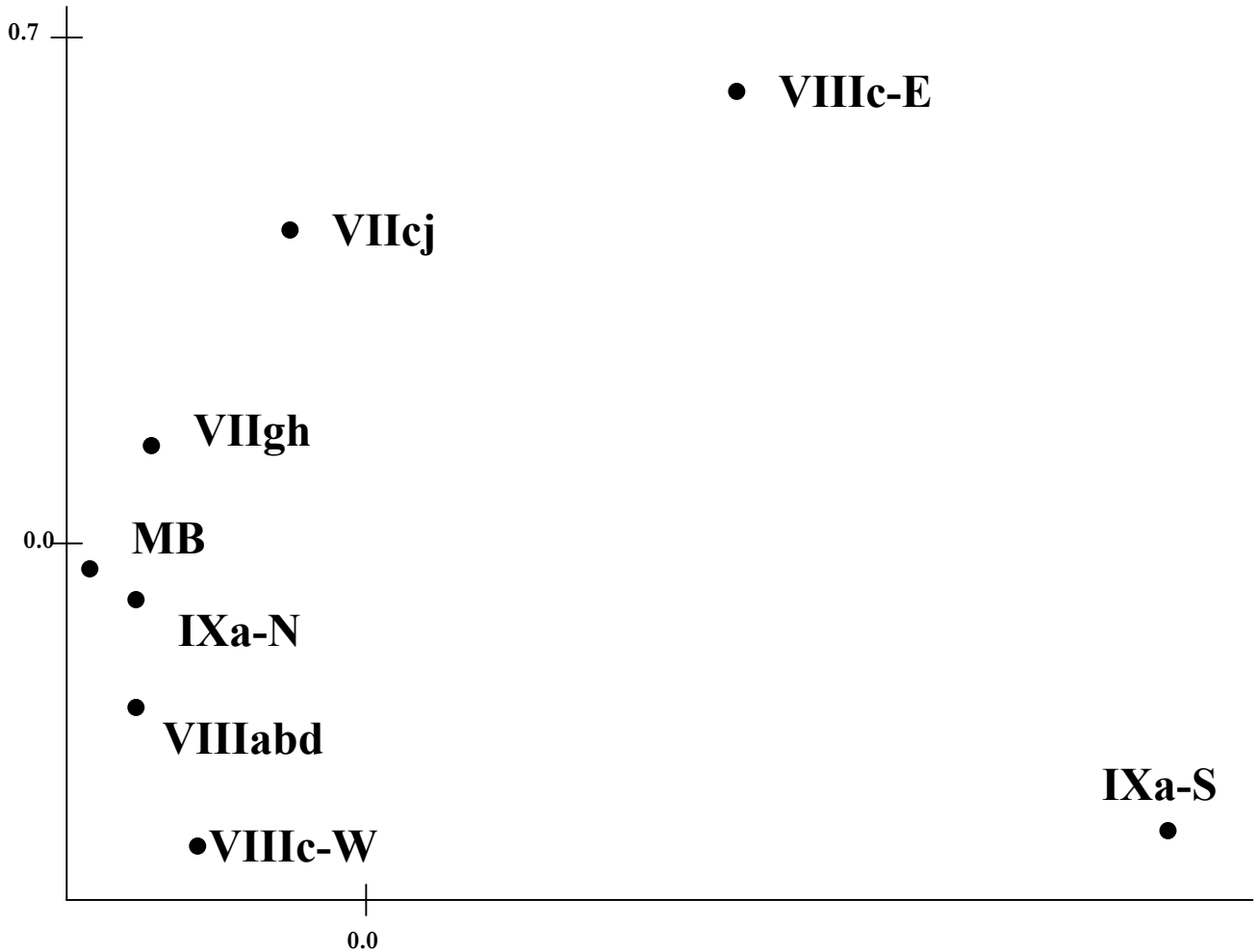

Figure 5. Principal Co-ordinates Analyses (PCoA) of *Lophius budegassa* populations based on $(\delta\mu)^2$ index of genetic distance. The populations have been projected onto the first two co-ordinates axes, which accounted for 60.3% of the total variation. (Coordinate 1: 44.6%; Coordinate 2: 15.5%)

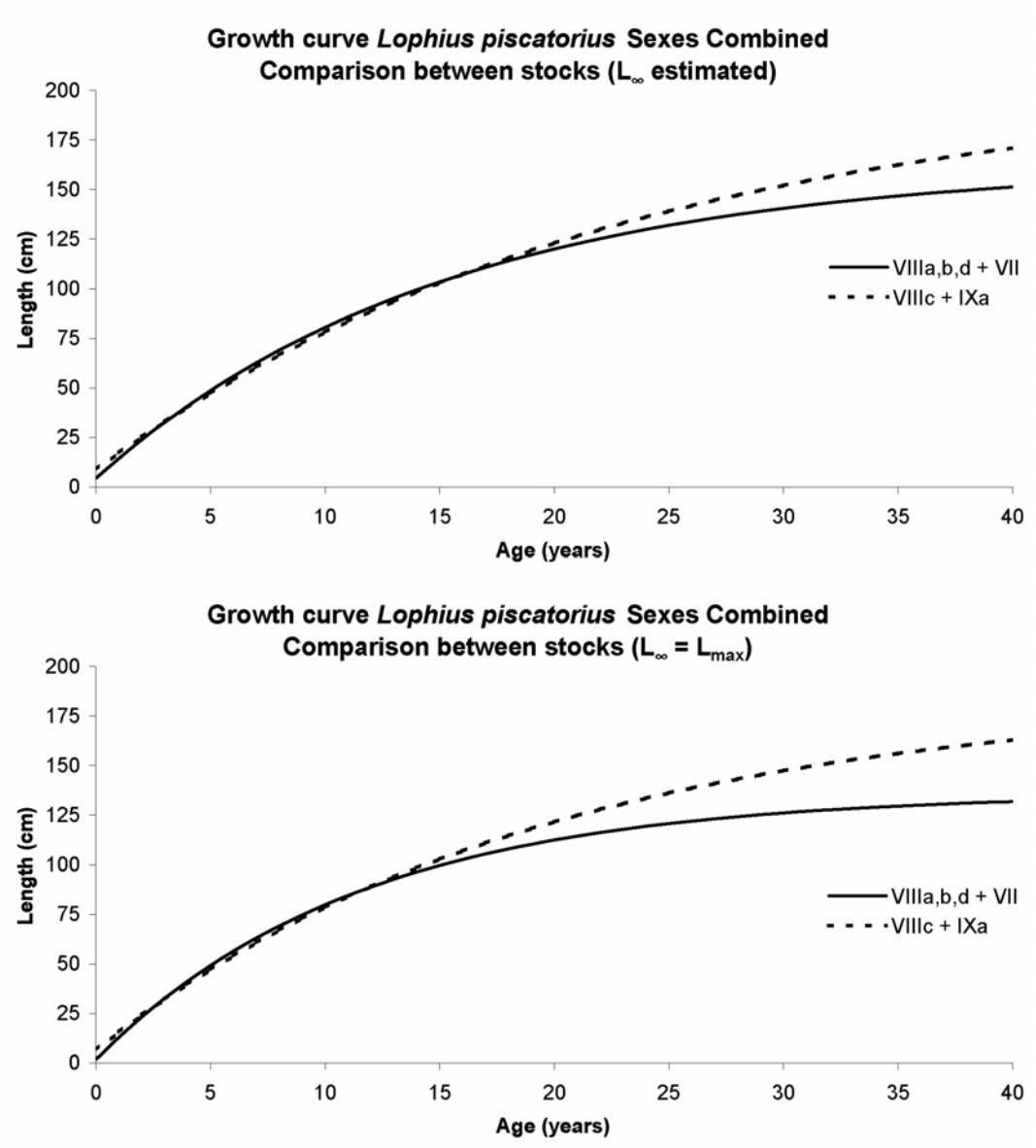

Figure 6. Growth curves for *L. piscatorius*. Comparison between northern and southern stocks.

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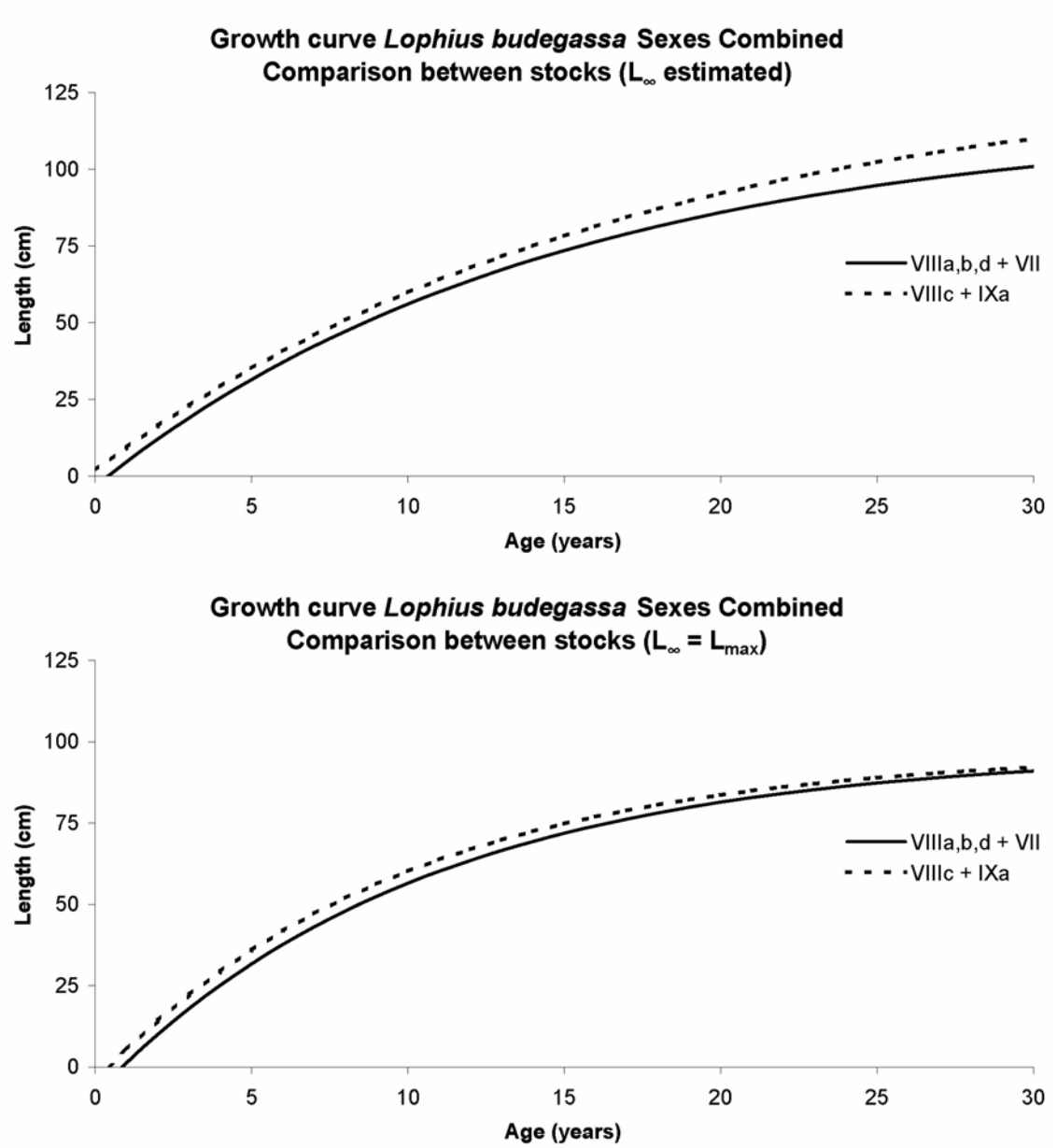

Figure 7. Growth curves for *L. budegassa*. Comparison between northern and southern stocks

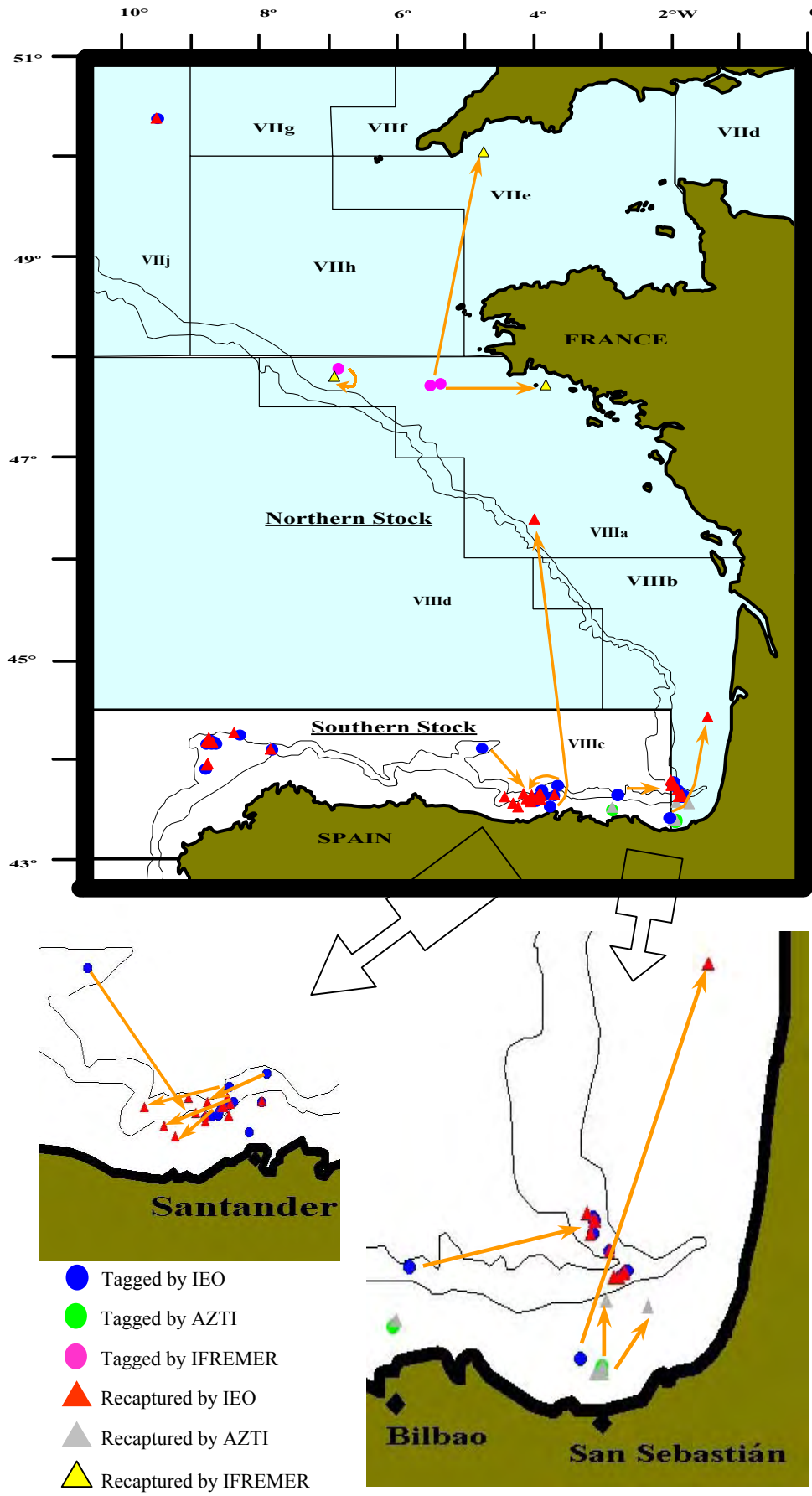

Figure 8. Movements of *L. piscatorius* from tag-recapture data, 1995-2002.

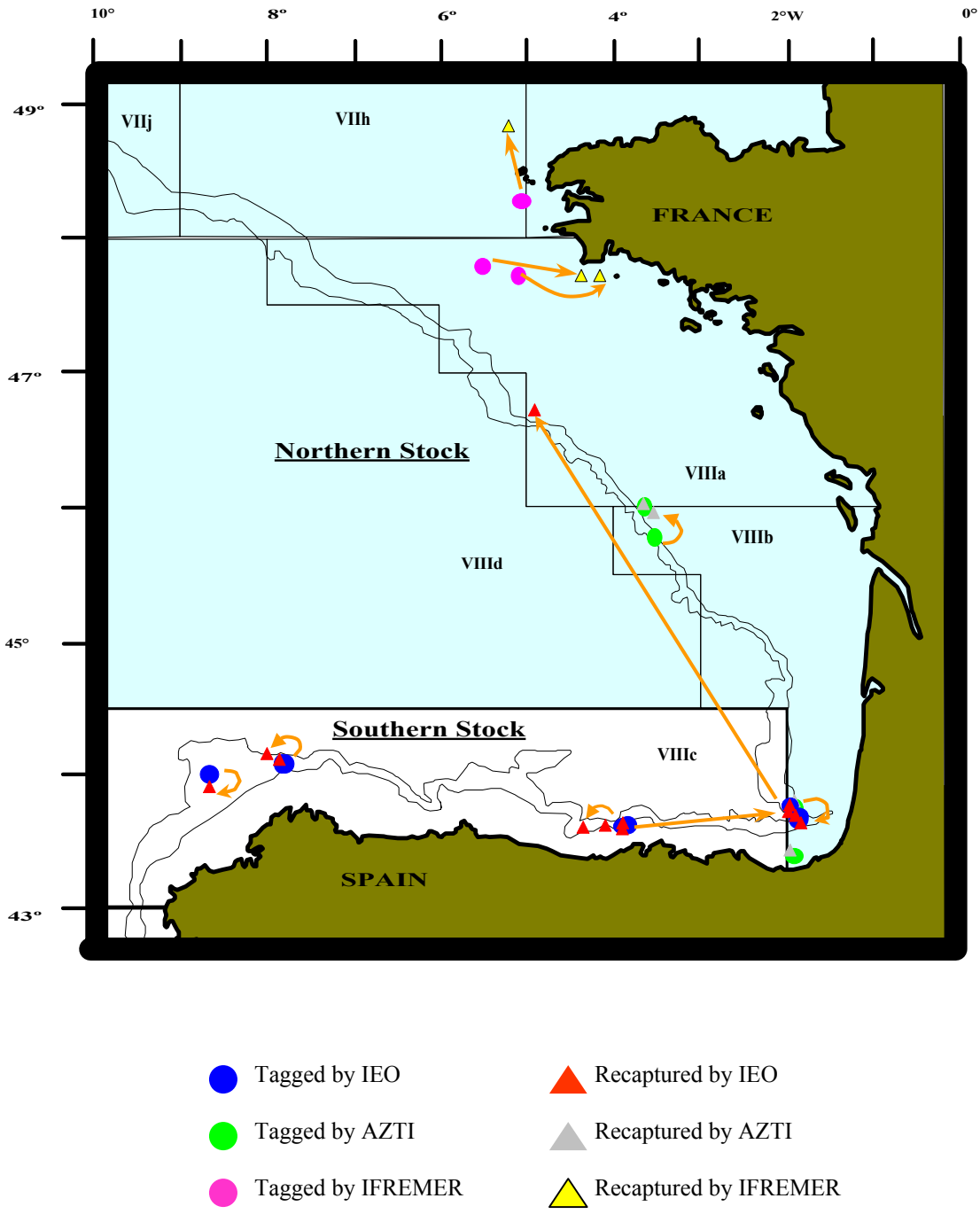

Figure 9. Movements of *L. budegassa* from tag-recapture data, 1995-2002.

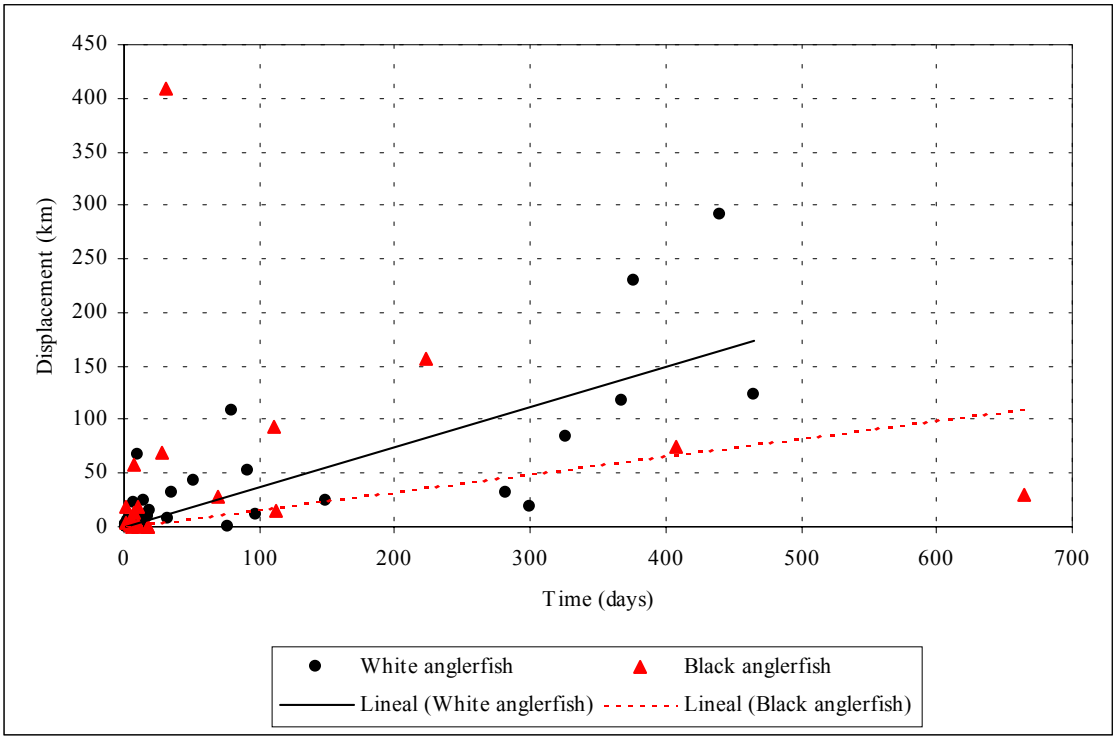

Figure 10. Movements an times at liberty of recaptured anglerfish (*L. piscatorius* and *L. budegassa*), from tag-recapture data 1995-2002.