

## Exceptional abundance of the snake pipefish (*Entelurus aequoreus*) in the north-eastern Atlantic Ocean

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Pipefish (Syngnathidae) have occurred with unprecedented frequency in Continuous Plankton Recorder (CPR) samples to the west of the British Isles from 2003 to 2005. Identification by mtDNA sequencing established that they were snake pipefish, *Entelurus aequoreus*. The geographical range of the records were from the outer continental shelf of the Celtic Sea and north-west of Ireland to the mid-Atlantic Ridge between 40° and 57°N, with the greatest abundance near the shelf edge and adjacent oceanic waters south of Ireland and west of Brittany. There were records in every month from February to November but most were in late spring and summer. A proposed mechanism for the increase in abundance of the species is that recent climate change has had beneficial impacts on the reproduction of adults and the survival of larvae and juveniles.

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### Introduction

The CPR survey (Warner and Hays, 1994) has been sampling the waters to the west of the British Isles continuously since 1947. During most of that period pipefish (Syngnathidae), mostly in the size range 5-15mm but some up to 70mm occurred occasionally, mainly over deep water (Coombs, 1980). Although the specimens were not identified to species their distribution was consistent with at least the majority of specimens being the most oceanic of the north-east Atlantic species, *Entelurus aequoreus*, the snake pipefish or ocean pipefish. From 2003 onwards, pipefish have occurred frequently in the samples. Although specialist analysis of the fish taken in the CPR has not been carried out routinely since the late 1970s, the pipefish specimens are conspicuous in the samples and it is improbable that previous occurrence of significant numbers would have been overlooked. Damage to fish when caught by the CPR can result in difficulties in identification, so molecular methods were used to specifically identify a sub-sample of specimens. The unprecedented abundance of *E. aequoreus* is supported by large catches of the species, identified by Ingvar Byrkjedal using meristics and genetics, in the region of the mid-Atlantic Ridge during the MARECO cruise in 2004 (R. Serrão Santos Pers. Comm.).

Here we present the geographical and seasonal distributions of the recent pipefish records in the CPR survey. This has been reported by Kirby *et al.* (In Press) who proposed that the

increase in numbers has been due to the effects of increasing temperature on reproductive biology. Here we also consider relationships with other environmental variables.

## Methods

### CPR sampling and identification of fish

Plankton samples in the north-eastern Atlantic have been collected by the Continuous Plankton Recorder (CPR) north and west of Ireland since late 1947 and further south from 1954 (Richardson *et al.* 2006). The CPR is towed behind merchant ships on regular routes. Seawater enters the CPR through a square front aperture 1.61 cm<sup>2</sup> and phytoplankton and zooplankton are retained on a moving band of silk gauze that is slowly wound into a tank of formalin (Batten *et al.*, 2003). In the laboratory the gauze is cut into sections (a CPR sample), each representing the plankton from 3 m<sup>3</sup> of water taken during 10 nautical miles (18 km) of tow, and up to 450 taxa are identified and counted (Richardson *et al.*, 2006). In most cases only alternate samples are routinely analysed.

Analysis of fish in the samples was carried out by specialists (G.T.D. Henderson, V. Bainbridge, G.A. Cooper, P.J.B. Hart, S.H. Coombs, R.K. Pipe, C.A. Mitchell, N.C. Halliday and C.A. Fosh). However this has not been part of the regular procedure of the survey for >25 years. Data for the period 1948 to 1972, including records from the alternate samples not used in the routine analysis, have been extracted from the hard copy records and entered into a database. These records have been searched for records of Syngnathidae. Since 2002 specimens of pipefish have been noted during routine analysis of the samples.

### Genetic identification of juvenile pipefish in CPR samples

Larval and juvenile pipefish taken in 2005 were removed from the CPR samples listed in Table 1 and a small piece (approximately 1mm) of tissue was placed into 180 µl of chelex solution (Instagene Matrix, Biorad) together with 6 µl of 1M DTT, 4 µl of proteinase-K (10 mg/ml) and 10 µl of 10% SDS and incubated at 55°C for 4 hr. Each sample was then vortexed briefly and centrifuged at 12,000 g for 15 sec. Samples were then heated at 105°C for 10 min in a dry-block heater, vortexed for 10 sec and centrifuged at 12,000g for 3 min. The supernatant was then transferred to a Micropure-EZ centrifugal filter device (CFD) (Millipore Corp.) inserted into a Microcon YM-30 CFD (Millipore Corp.) and centrifuged at 14,000 g for 8 min. After discarding the Micropure-EZ CFD, the sample retained by the YM-30 was washed three times with 200 µl of sterile water; the first two washes were centrifuged at 14,000 g for 8 min and the final wash was centrifuged at 14,000 g for 5 min. The DNA retained by the YM-30 was then recovered according to manufacturer's instructions. All centrifugation steps were performed at room temperature (22°C). A partial 3' mtDNA 16S rDNA gene sequence was amplified from 1 µl of DNA template by PCR using 10 pmol each of the 'universal' mtDNA 16S rDNA gene primers, 16sar-L and 16sbr-H (3) and *Taq* DNA polymerase. Purified PCR products were sequenced commercially (MWG-Biotech). The putative identities of juvenile pipefish sampled in 2005 from samples listed in Table 1 (note that these are samples not used for routine CPR analysis and so these locations are in addition to the records in Fig.1) were confirmed as *E. aequoreus* by comparison of their 16S rDNA to other 16S rDNA sequences in GenBank.

## Results

Henderson (1961) made no mention of Syngnathidae in the CPR samples taken between 1948 and 1956 but re-examination of the data records revealed 11 specimens were recorded in 9

samples during this period. Coombs (1980) describing results from 1958 to 1972, plotted occurrences in 12 1° latitude x 2° longitude rectangles. These were based on 17 specimens recorded in 16 samples. The largest number found in any one year between 1948 and 1972 was 5 specimens in 1953 (Table 2). These records were mainly in deep water to west of the British Isles and occurred between March and October, mostly in March and April.

The geographical and seasonal distributions of records in samples analysed in the routine survey between 2002 and July 2005 are shown in Figure 1. The only sample in which pipefish were noted in 2002 was over the Porcupine Bank in May. In 2003 records extended from the Celtic Sea continental shelf to the mid-Atlantic ridge between March and November, mainly south-west of Ireland. Numbers were higher between April and October 2004, the number taken in June (of that year exceeding the total for the period 1948-1972, but mainly in the same area. In 2005 there were several records north of 55°N.

## Discussion

It is known that pipefish have been exceptionally abundant in continental shelf and inshore waters as well as in the oceanic areas (<http://www.glaucus.org.uk/Pipefish.htm>; B. Mackenzie, Pers. Comm; S. Wanless, Pers. Comm., K. Hiscock Pers. Comm.) and some of this information is being prepared for publication. The abundance of pipefish and the paucity of sandeels in the North Sea have caused problems for seabirds trying to rear chicks. ([http://www.birdlife.org/news/features/2005/01/north\\_sea\\_seabirds.html](http://www.birdlife.org/news/features/2005/01/north_sea_seabirds.html)). The increase in pipefish abundance is clearly a widespread phenomenon of ecological significance.

Fish distributions in the north-eastern Atlantic have changed in response to climatic impacts (Brander *et al.*, 2003). Kirby *et al.* (In Press) have proposed that the increase in numbers of *Entelurus aequoreus* between the European continental shelf and the mid-Atlantic ridge may be due to the effects of increased temperature on reproduction and larval survival. Little direct evidence is available on *E. aequoreus* so known physiological effects on other Syngnathidae were used to support this proposition. They showed that sea surface temperatures in 2002 to 2005 were significantly higher than the mean for 1948-1972 and it is suggested that this rise in temperature has influenced the reproductive rate by shortening embryonic development time and may have shortened the vulnerable pelagic larval phase, thereby improving recruitment. *Entelurus* is not the only fish to have increased in the waters off the European shelf. According to the ICES data blue whiting (*Micromesistius poutassou*) have been abundant in recent years, increasing to a maximum in 2003 and have remained above the pre-1998 levels since then (Figure 2.) despite fisheries exceeding advised limits, (Anon. 2005).

Results from the CPR survey for the Phytoplankton Colour Index (PCI) and total copepods in three standard areas in the north eastern Atlantic are given in Figure 3. The PCI, a measure of chlorophyll, which is highly correlated with SEAWIFS measurements (Batten *et al.* 2003; Raitos *et al.* 2005), is increasing, indicating a higher standing crop (Leterme *et al.* 2006). However the numbers of copepods are declining (Edwards *et al.* 2006) indicating a decline in biomass of crustacean zooplankton. In the North Sea echinoderm larvae have become more numerous than copepods in the CPR samples (Kirby *et al.*, In Press) and the possibility that gelatinous zooplankton are becoming relatively more important in the oceanic areas needs to be investigated further. Substantial phenological changes have also occurred in the plankton (Edwards and Richardson, 2004) and these may have had an impact on the reproduction and survival of animals at higher trophic levels including the pipefish and blue whiting.

The increase in pipefish in both oceanic and shelf waters may have considerable ecological implications. In the waters to between the European continental shelf and the mid-Atlantic ridge the increase in pipefish and in numbers of blue whiting may well be the result of changes in the pelagic ecosystem over a wide area. The forcing factors and mechanisms causing these impacts require further investigation.

## Acknowledgements

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Table 1. Samples from which pipefish were extracted to be made available for genetic analysis. (n.b. these are all samples not included in routine CPR analysis and the locations are not included in Figure 1.

CPR sample	Latitude	Longitude	Date
414BB6	48°57'N,	13°37'W	29/07/05
440W-14	55°52'N	10°57'W	6/07/05
349DA16	56°20'N	26°15'W	24/07/05
349DA20	56°17'N	27°27'W	24/07/05
349DA22	56°16'N	28°02'W	24/07/05
349DA24	56°14'N	28°38'W	24/07/05
350DA16	56°19'N	26°21'W	4/09/05
350DA18	56°18'N	26°57'W	4/09/05

Table 2. Number of Syngnathidae per year (1948-1972) in CPR samples, including “non-analysed” samples. Zero values not shown.

Year	1950	1952	1953	1956	1958	1961	1963	1964	1965	1966	1970	1972
Syngnathidae	2	3	5	2	4	3	2	1	3	4	1	1

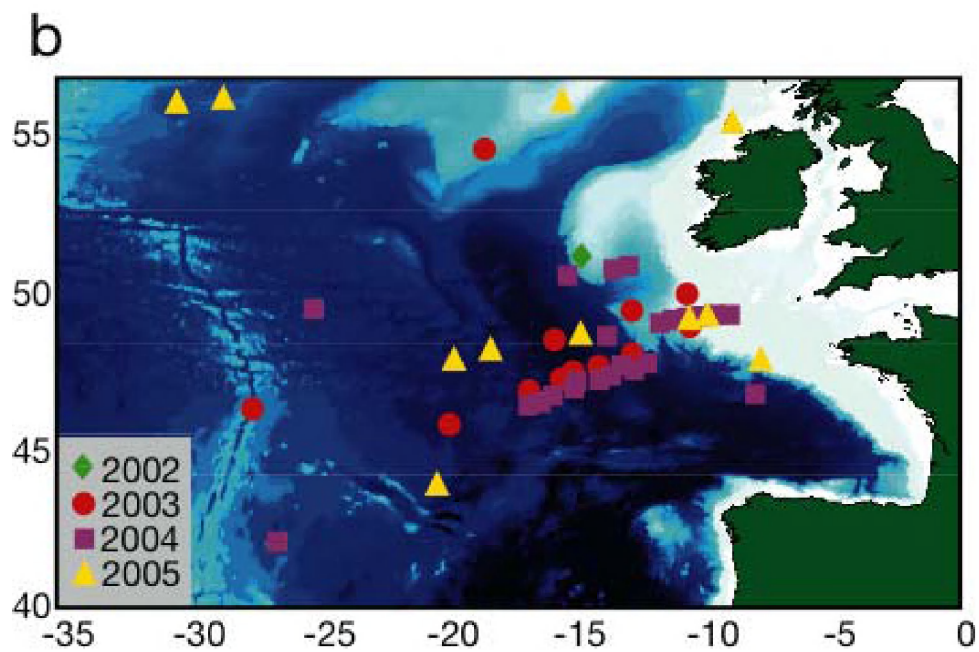
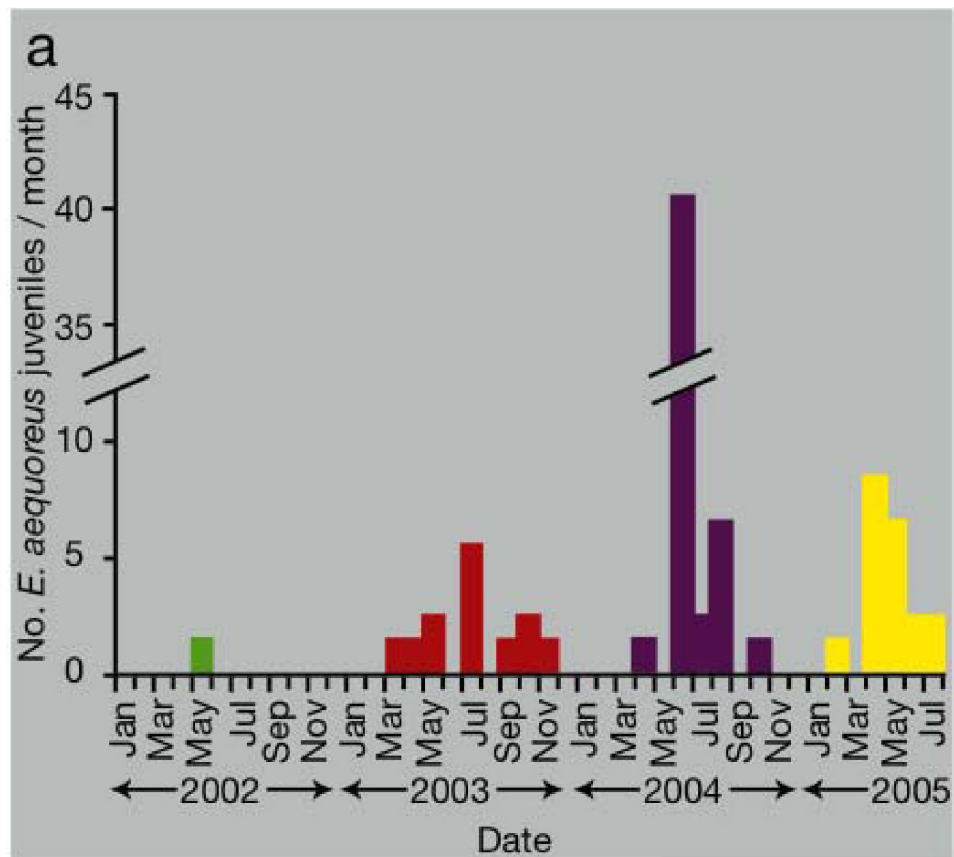


Figure 1. *Entelurus aequoreus* in CPR samples, 2002 to July 2005 (a) Numbers of specimens taken in routinely analysed samples per month. (b) The geographical distribution of the records.

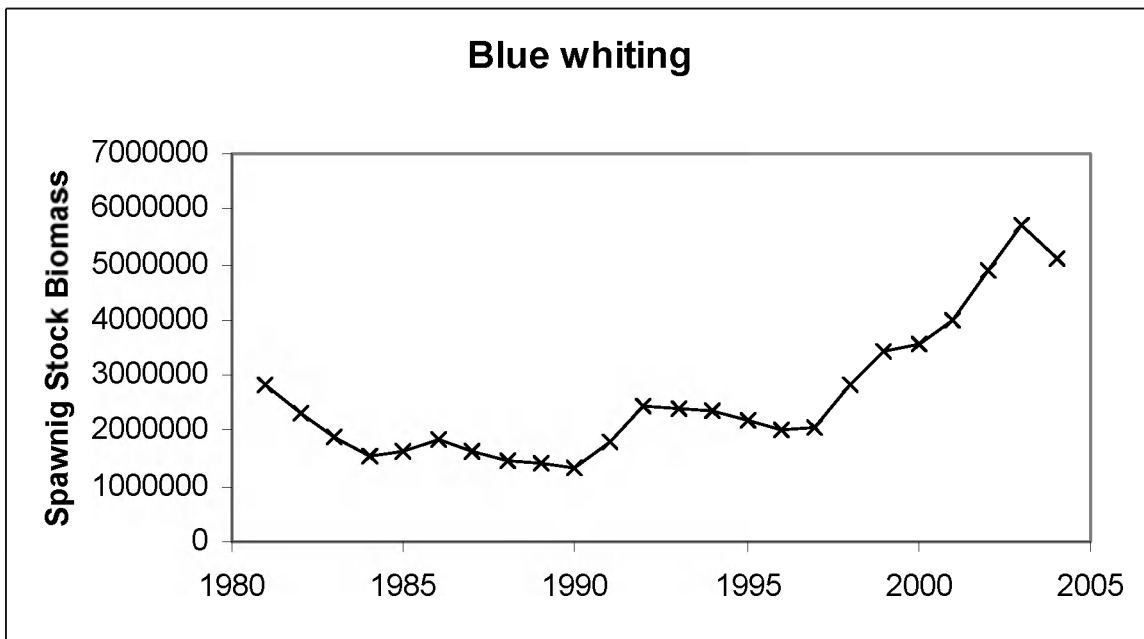


Figure 2. Blue whiting. Spawning Stock Biomass from virtual population analysis. ICES data from Anon 2005.

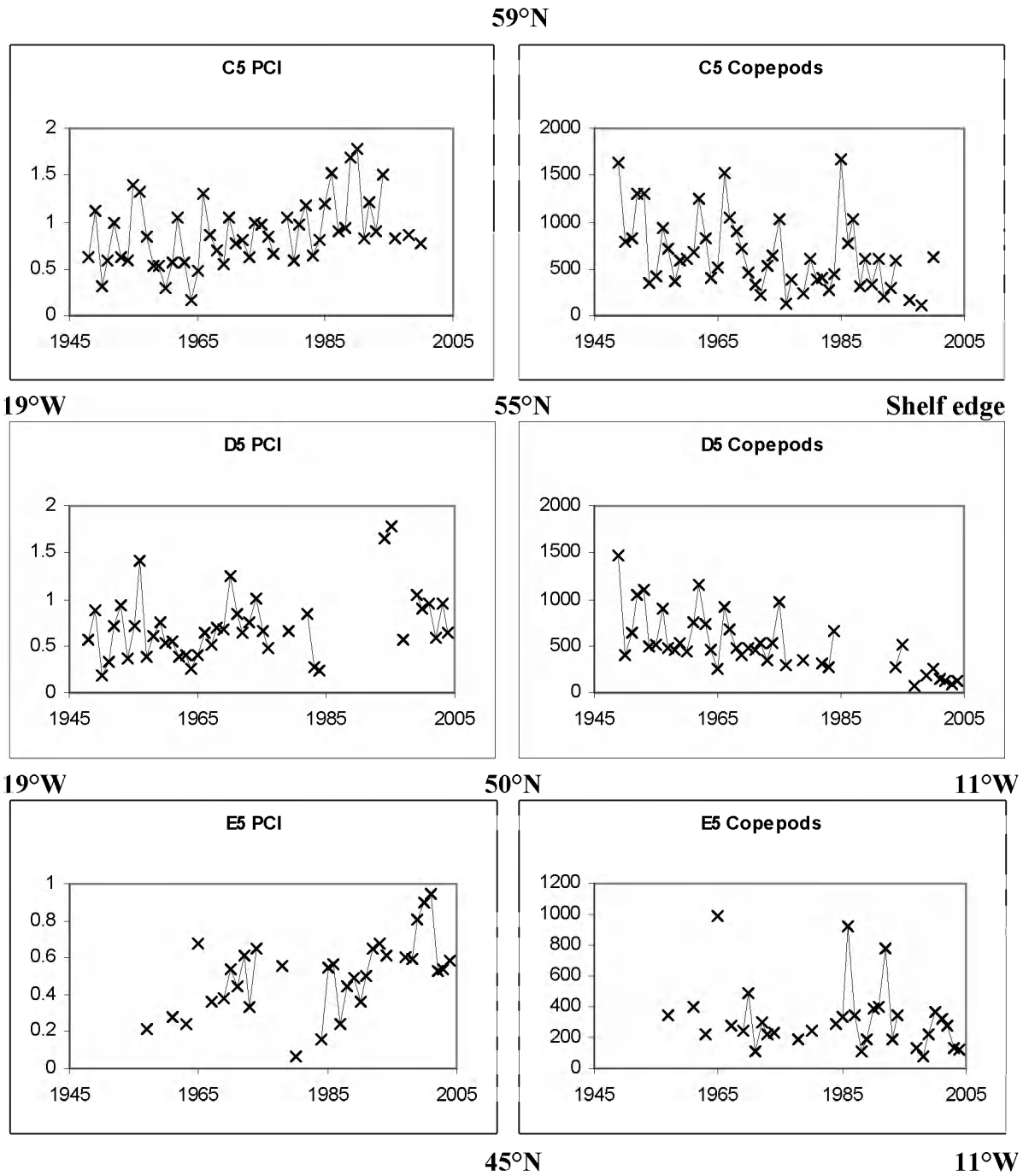


Figure 3. Phytoplankton Colour Index (PCI) and total copepods in the CPR survey. Interannual variations in abundance in 3 standard areas between 19°W and 11°W or the continental shelf edge.