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# THE SHETLAND ISLES: LONG-TERM OBSERVATIONS ON THE SUBTIDAL MARINE FLORA

IAN TITTLEY & WILLIAM F. FARNHAM

Tittley, I. & W.F. Farnham. 2001. The Shetland Isles: Long-term observations on the subtidal marine flora. *Arquipélago*. Life and Marine Sciences. Supplement 2 (Part B): 1-17. Ponta Delgada. ISSN 0873-4740.

The marine algal flora of Sullom Voe, Shetland, has been surveyed on three occasions in twenty years (1973, 1983, 1993). To date 281 species have been recorded in Shetland, of which 175 species occur in Sullom Voe. The subtidal vegetation of the outer part of the voe is characterised by forest formations of the perennial kelp *Laminaria hyperborea* on bedrock and a species-rich epiflora on stipes and holdfasts. This community was stable with time despite the wave-exposed conditions there. The subtidal vegetation of the inner, sheltered, voe with a sea-bed of sediment, stone and shell, was characterised by the canopy-forming annual *Laminaria saccharina / longicruris*. Comparison of survey results revealed this canopy and associated subflora communities to vary in abundance and extent with time.

*Ian Tittley (e-mail: I.Tittley@nhm.ac.uk), Department of Botany, The Natural History Museum, London SW7 5BD. UK. - William F. Farnham, Institute of Marine Sciences, University of Portsmouth, Portsmouth PO49 9LY, UK.*

## INTRODUCTION

Shetland is an archipelago of about 100 islands in the Atlantic Ocean lying between 60° and 61°N, 170 km north of mainland Scotland and 300 km southeast of the Faroe Islands. Although the archipelago lies within the cold-temperate region, the sea does not freeze due to the warming influence of the Gulf Stream (average surface temperatures are 4°C in winter and 12°C in summer). The Shetlands contain a mainland which is 85 km long and incised by deeply penetrating, fjordic arms (sea-lochs or voes); one of these, Sullom Voe (Fig. 1), is the subject of this paper.

Sullom Voe is a 15 km long, narrow inlet of the sea where there is a gradient of wave-exposure from extremely wave-exposed to sheltered. A few rivulets drain into the voe but cause no significant reduction in salinity. Sullom Voe has a range of subtidal habitat types, including rocky outcrops, extensive sediment areas of shell, stone, sand and silt, and biogenic surfaces such as beds of *Modiolus* (horse-mussel).

Although accounts of the algal flora of the Shetlands go back almost two centuries (e.g. Edmonston 1809), studies have been sporadic and key works are those of Børgesen (1903a, b), Børgesen & Jónsson (1905), Dixon (1963), Irvine (1962, 1974, 1980) and Irvine et al. (1975). The marine algal flora of Shetland is typical of the species poorer cold temperate North Atlantic Ocean with 281 species recorded (50 Chlorophyta, 101 Phaeophyta, 130 Rhodophyta).

Sullom Voe had been scantly studied until the construction of Europe's largest oil terminal in the 1970s (Dunnet & McIntyre 1995). Long-term surveillance of the intertidal biota of Sullom Voe has been undertaken regularly since 1981 (Moore et al. 1995). Studies on the subtidal vegetation were initiated in 1973 (Tittley et al. 1977) as part of a wider study of the algae of Shetland (Irvine 1974), and repeated in 1983 (Tittley et al. 1985) and 1993 (Tittley & Farnham 1997 - unpublished report).

The 1993 algal survey of the Sullom Voe provided an opportunity to revisit previous study sites, assess the current status of the subtidal vegetation, and make comparisons with previous observations; some results are presented in this paper.

## METHODS

The surveys were carried out in July / August 1973, July 1983 and August 1993. In the original 1973 survey, the principal features of the algal vegetation of Sullom Voe were recorded by sampling from quadrats along transects; these transect studies were complemented by sampling from 'spot-dive' study sites at pre-selected depths (TITTLEY et al. 1977). As the original survey was essentially semi-quantitative and descriptive, transect sampling was not replicated; subsequent reassessment was undertaken in the same manner. Transects were relocated as accurately as previous field data allowed. At each site a rope line was laid from an accessible reference point at high water mark to the subtidal region. The transects were 200 m long and the rope forming the transect line was weighted and marked at 10 m intervals. Depths at these points were recorded at noted times and adjusted to Chart Datum. At each marked 10 m interval, vegetation was cleared from a 1 m x 1 m quadrat beside the transect line and brought back to the laboratory for identification. Where possible, whole stones and cobbles were collected for examination of encrusting algae. Laminarians were weighed wet to assess relative abundance; ages of *Laminaria hyperborea* plants were ascertained by sectioning stipes and counting annual growth rings (cf. KAIN 1963). The results for four of the transect surveys (Fig. 1) are presented below (for other sites see TITTLEY & FARNHAM 1997).

Detrended Correspondence Analysis (DECORANA, a numerical, ordination method) was used to facilitate comparison of quadrat data from the three surveys. A database of species presence in quadrats was prepared for the purpose (in TITTLEY & FARNHAM 1997). Ordination arranges the vegetation samples in relation to each other according to their similarity of species composition and associated environmental or temporal controls (KENT & COKER 1992). The results of an ordination are in this case two-dimensional graphs in which each point represents a vegetation sample or quadrat. The distances between the points in the graph are taken as an indication of their similarity or difference.

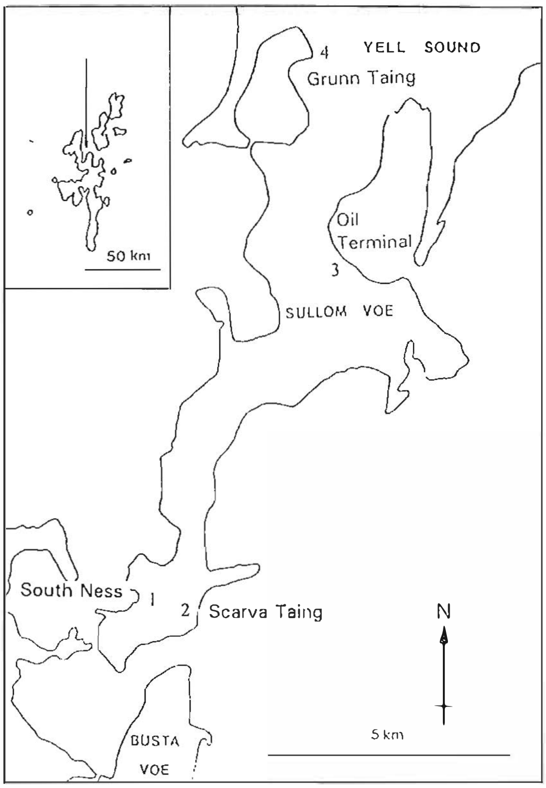

Fig. 1. Sullom Voe showing transects sites.

## RESULTS

### Inner Voe - South Ness

The transect traversed an intertidal area of gently sloping shingle, boulder and outcropping rocks that continued subtidally and, at greater depths (10 m), gave way to a sea-bed of soft mud and shell debris.

*Vegetation description and comparison*

Seventy-nine species were recorded in the three surveys (32 species in 1993, 55 in 1983 and 36 in

1973); only a small component (11 species) of the total flora was found on all occasions.

At shallow sublittoral levels the transect crossed a canopy of mixed of *Fucus serratus*, *Laminaria digitata*, *L. hyperborea* and *L. saccharina/longicruris* (*L. saccharina* and *L. longicruris* may be conspecific cf. SOUTH & TITTLEY 1986). From 30 m offshore (2-3 m depth) the canopy was solely of *L. saccharina / longicruris* (Fig. 2). In 1993 the *Laminaria* canopy extended to only 90 m offshore (9 m depth), contrasting with 1973 when it was present in all quadrats to 140 m offshore (14 m depth), and 1983 when it was patchily present 80-130 m offshore.

The subflora (underflora and epiflora) recorded in shallow waters comprised a diverse species-assemblage but differed in composition in each survey. At 30–80 m offshore (2-8 m depth) only a depauperate underflora was recorded in 1993 compared with species-richer assemblages of 1973 and 1983 (cf. TITTLEY et al. 1977, 1985). The principal algae in 1993 were, epiphytes on *Laminaria* blades (e.g. *Ectocarpus siliculosus*, *Myrionema corunnae*), and crustose forms such as the *Aglaozonia* stage of *Cutleria multifida*, *Lithothamnion glaciale* and *Pseudolithoderma extensum* on stones and shells. At 140-150 m offshore only crustose species were recorded in 1993 in contrast to the foliose and filamentous species found previously; in deepest waters at 25-28 m (160-200 m offshore) vegetation was absent in 1993 contrasting with previous occasions (cf. TITTLEY et al. 1977, 1985). The red alga *Phyllophora crispa* that in 1973 and 1983 was commonly present as extensive, detached, mats 120-200 m offshore, was not recorded in 1993.

### *Quadrat comparison*

Ordination (Fig. 3) produced a single cluster of points. The lack of a clear linear pattern successive points (quadrats along the transect) reflects the patchy mosaic of vegetation crossed by the transect. The first axis of the ordination suggests a depth gradient; quadrats at depths of less than 10 m (characterised by *Laminaria sacharina / longicruris*) are positioned to the left of 200, quadrats from deeper waters (often containing *Phyllophora crispa*) are positioned to the right. The second axis suggests temporal differences with most 1973 and 1983 quadrats in the lower part of the graph (below 200), and 1993 quadrats in the upper part. The ordination does not show the quadrats that in 1993 lacked algae.

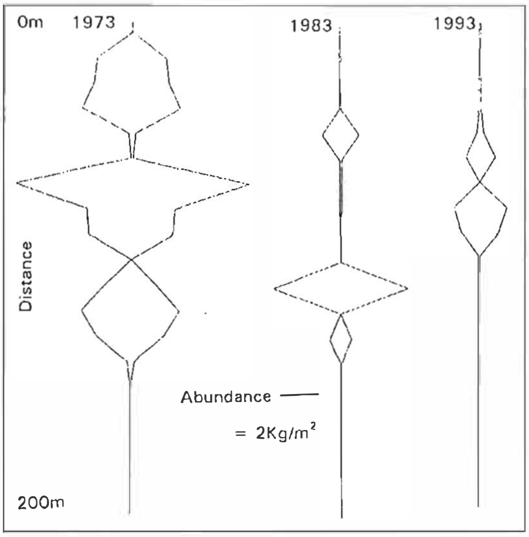

Fig. 2. Transect 1, South Ness. Kite diagram showing *Laminaria saccharina / longicruris* extent and abundance along the transect in the three survey years.

### Inner Voe - Scarva Taing

The transect crossed an intertidal area of shingle with rocky outcrops that gave way at shallow sublittoral levels (3 m depth) to a sea bed of gravel, coarse sand and cobble. At 4-5 m depth the sea-bed was of mud and sand which continued to 100 m offshore. Beyond this point the sea-bed changed to mud-shell debris and *Modiolus* beds, and soft mud and shell debris at the greatest depths investigated (25-30 m).

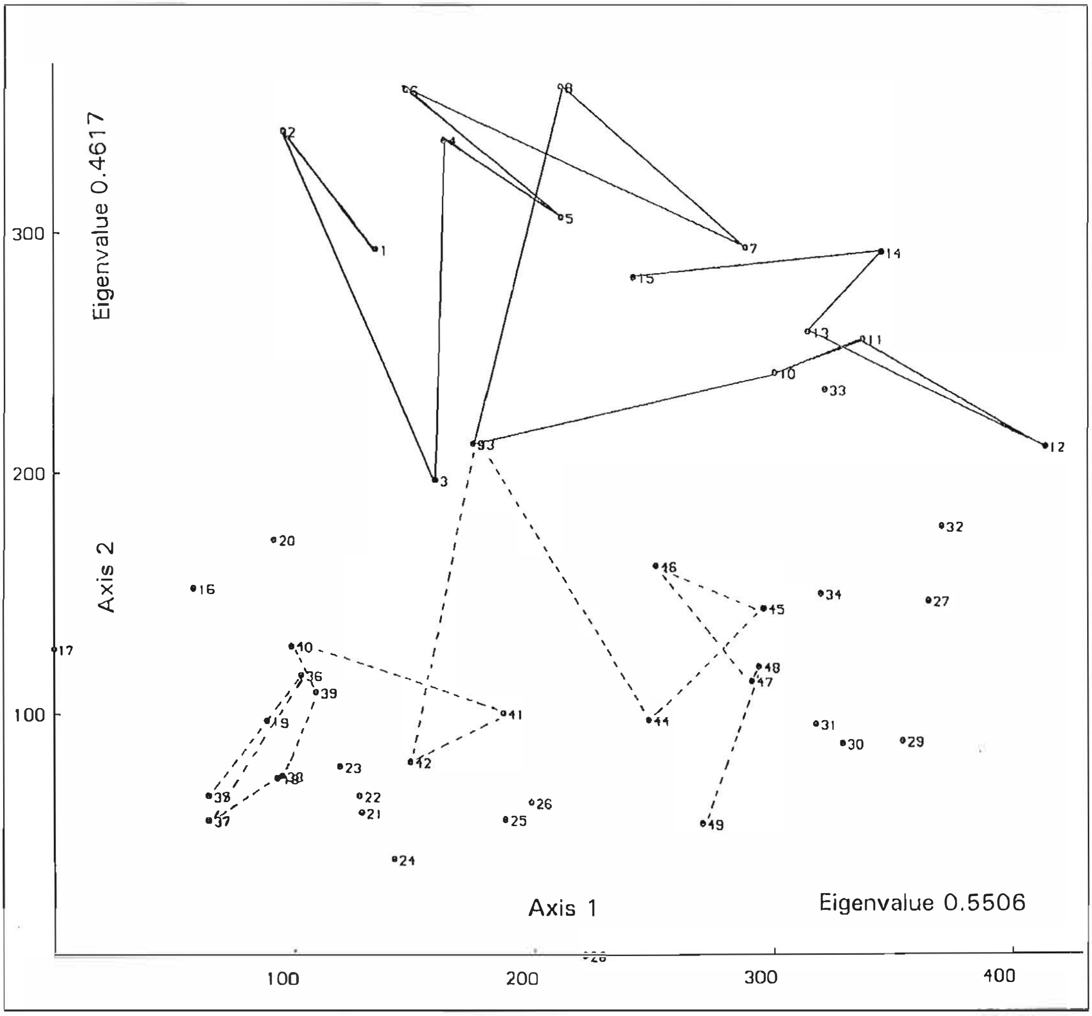

Fig. 3. Transect 1, ordination of quadrat data. Numbered points represent quadrats (successively joined for 1973 and 1993 transects). 1973, hatched line 35 (inshore) - 49 (offshore); 1983, points unjoined 16 (inshore) - 34 (offshore); 1993, continuous line 1 (inshore) - 15 (offshore).

*Vegetation description and comparison*

Eighty species were recorded during the surveys (42 in 1973; 64 in 1983; 18 in 1993) but only eight on all occasions.

In 1993 the principal kelp at sublittoral fringe level was *L. saccharina / longicruris,* contrasting with mixed *Laminaria* spp. recorded in 1973 and 1983. At greater depths a mixture of *L. saccharina / longicruris* and *L. hyperborea* was present on all occasions; *L. saccharina / longicruris* was consistently the dominant species in deepest waters (Fig. 4). Differences in extent of *L. saccharina / longicruris* canopy were observed in each survey occasion (1973 to 110 m offshore; 1983 to 50 m offshore; 1993 to 40 m offshore).

A diverse subflora of mainly red algae (e.g. *Phycodrys rubens*, *Phyllophora crispa*, *Polysiphonia elongata, Pterosiphonia parasitica*) and the crustose brown algae *Aglaozonia* and *Pseudolithoderma extensum*, was recorded in the three surveys. Species composition differed on each occasion and fewer species were recorded in 1993 than previously. The subflora extended to only 80 m offshore in 1993, compared with 150 m in 1973 and 200 m in 1983.

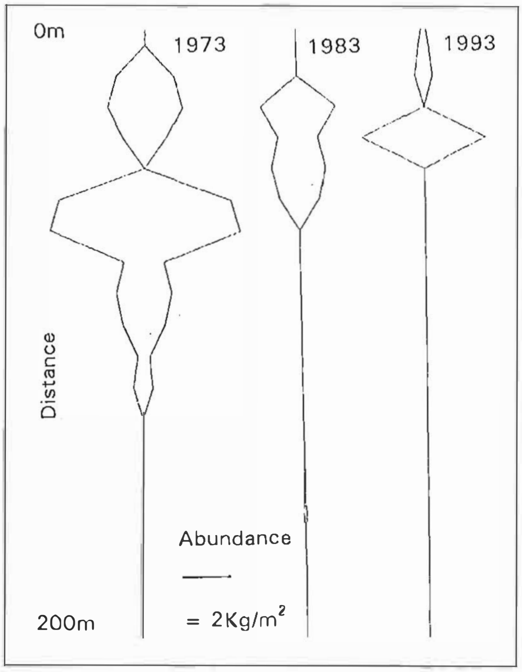

Fig. 4. Transect 2, Scarva Taing. Kite diagram showing *Laminaria saccharina / longicruris* extent and abundance along the transect in the three survey years.

### *Quadrat comparison*

Ordination (Fig. 5) produced a graph with a single cluster of points. Quadrats from below 20 m depth are positioned in the top left of the plot and those from shallow water to the right side of the plot. Quadrats from deep water and lacking in algae in 1993 are not shown.

### Central Voe - Oil Terminal

The transect crossed a man-made boulder foreshore that gave way in shallow waters to a sandy sea-bed. At 4 m depth the sea-bed was of mud and shell debris and changed to soft mud at approximately 10 m depth. In deep water (15-20 m depth) the sea-floor was of soft mud and silt.

### *Vegetation description and comparison*

Seventy-seven species have been recorded in the surveys (47 in 1993, 48 in 1983, 46 in 1973) but only sixteen species were recorded on all three occasions.

In 1993 (and 1973) *Laminaria digitata* and *L. hyperborea* successively formed narrow bands on boulders at shallow sublittoral levels, contrasting with 1983 when both were absent. *L. saccharina / longicruris* was predominant in deeper waters offshore on all occasions (Fig. 6). At the greatest depths investigated in the present survey (20 m), macrophytes were absent. *Laminaria saccharina / longicruris* beds extended to 80 m offshore in 1993 compared with 190 m in 1983.

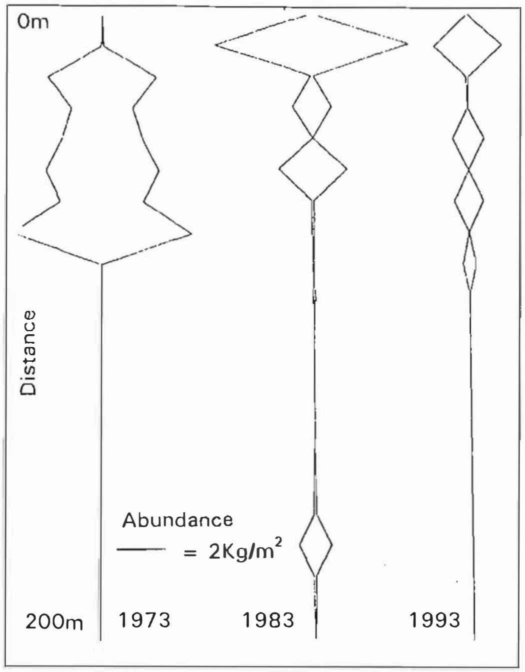

Fig. 6. Transect 3, Oil Terminal. Kite diagram showing *Laminaria saccharina / longicruris* extent and abundance along the transect in the three survey years.

A subflora of *Ulva lactuca*, *Chorda filum*, *Ectocarpus* spp., *Sphacelaria cirrosa* and *Phycodrys rubens* occurred beneath the *Laminaria* canopy on all occasions. The filiform brown algae *Dictyosiphon foeniculaceus*, *Stictyosiphon soriferus*, *Spermatochnus paradoxus* and *Sauvageaugloia griffithsianus* recorded in 1973 and 1983 (cf. TITTLEY et al. 1977, 1985) were not found in 1993. Flocculent mats of *Rhodothamniella floridula* occurred in smaller amounts at 70 m offshore in 1993 compared with 1983. Algae were not found beyond 90 m offshore in 1993 while in 1983 *Coccotylus truncatus, Polysiphonia elongata, Phyllophora crispa*, and *R. floridula* were present, and in 1973 *Polyides rotundus*, *Desmarestia aculeata*, *Sphacelaria plumosa* and *Chaetomorpha melagonium* were also recorded (TITTLEY et al. 1977, 1985).

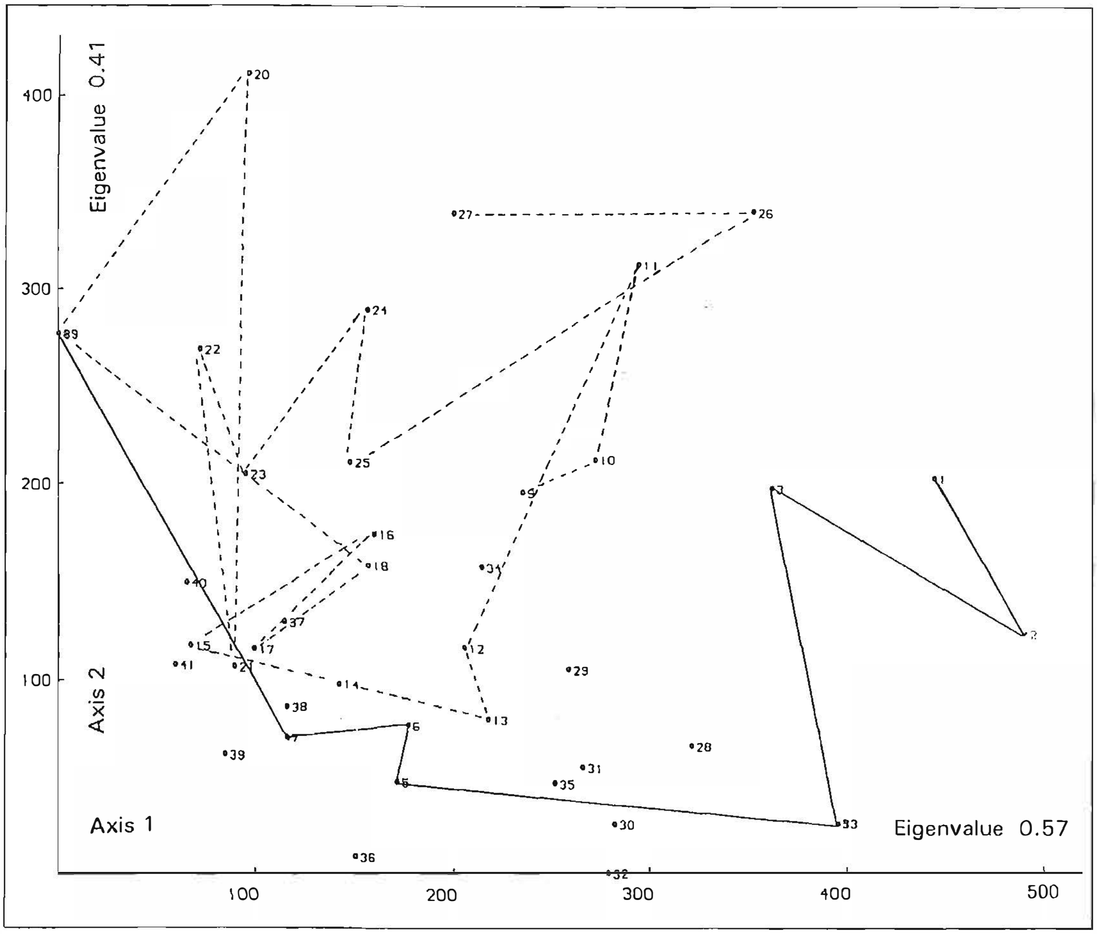

Fig. 5. Transect 2, ordination of quadrat data. Numbered points represent quadrats (successively joined for 1983 and 1993 transects). 1973, points unjoined 29 (inshore) - 41 (offshore); 1983, hatched line 9 (inshore) - 27 (offshore); 1993, continuous line 1 (inshore) - 8 (offshore).

*Quadrat comparison*

Ordination of quadrats (Fig. 7) indicated a depth gradient with those from deeper waters positioned at higher values on axis 1; the arrangement of successive transect quadrats in the graph is not linear but meanders (from shallow to deep water) reflecting the mosaic of communities traversed. The second axis suggests a time series, with 1973 quadrats positioned at low values, 1983 quadrats at middle values, and most 1993 quadrats at high values.

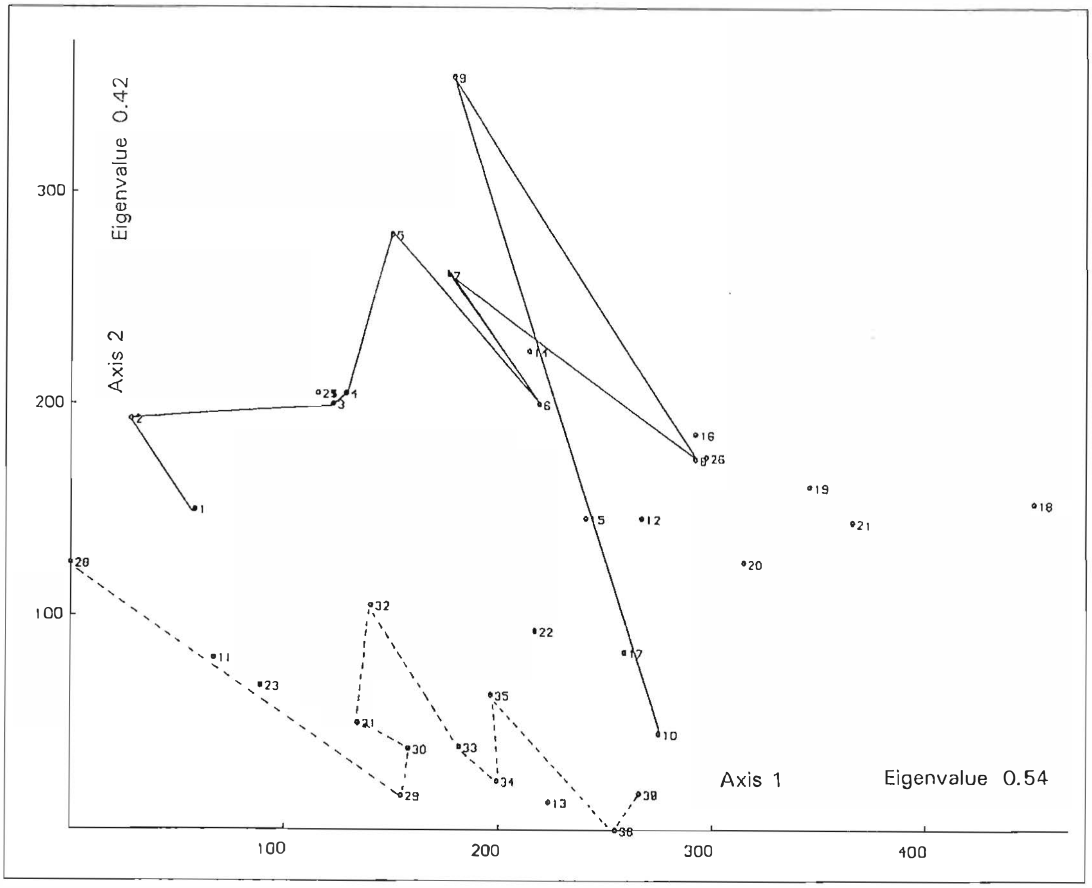

Fig. 7. Transect 3, ordination of quadrat data. Numbered points represent quadrats (successively joined for 1973 and 1993 transects). 1973, hatched line 28 (inshore) - 38 (offshore); 1983, points unjoined 11 (inshore) – 27 (offshore); 1993, continuous line 1 (inshore) - 10 (offshore).

Outer Voe - Grunn Taing

The transect crossed a wave-exposed intertidal sea-shore of shallowly shelving bedrock which extended to 5–6 m below low water level (100 m offshore). Beyond this point were sporadic areas of boulder, stones and gravel over bedrock, and furthest offshore, patches of sand and small boulders over bedrock.

*Vegetation description and comparison*

Fifty-nine species have been recorded in the three surveys (41 in 1973, 32 in 1983, and 39 in 1993). Twenty species have been found on all occasions; seven are unique to this site.

Beyond a narrow inshore band of *L. digitata*, the principal kelp species on all occasions was *Laminaria hyperborea* which formed an extensive stand of large plants to 200 m offshore (Fig. 8). In 1993 plants were aged at (5)-6-(7) years old. Patchy growths of *L. saccharina / longicruris* grew among the *L. hyperborea* canopy along the entire transect in 1993, compared with only beyond 100 m in 1973 and 1983. Another kelp, *Saccorhiza polyschides*, was found occasionally in the three surveys, where sand is temporarily washed away from the bedrock.

The subflora in all surveys comprised (Table 1) a species-rich assemblage on *Laminaria hyperborea* stipes, and a species-poorer assemblage on bedrock and stones beneath the *L. hyperborea* canopy.

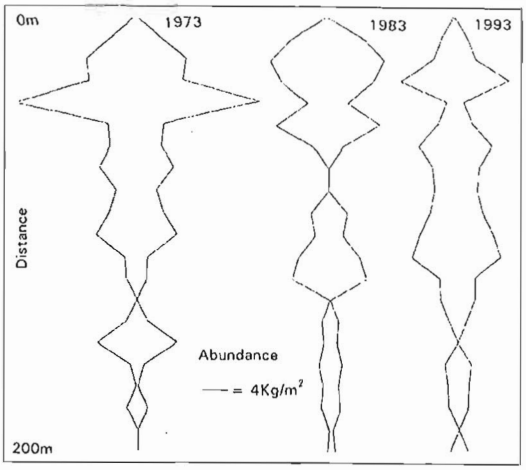

Fig. 8. Transect 4, Oil Terminal. Kite diagram showing *Laminaria hyperborean* extent and abundance along the transect in the three survey years.

Table 1
Subflora at Grunn Taing

**Epiflora on *Laminaria hyperborea***

*Audouinella purpurea*
*Callophyllis cristata*
*Ceramium nodulosum*
*Cladophora rupestris*
*Cryptopleura ramosa*
*Derbesia marina*
*Dermatolithon pustulatum*
*Desmarestia viridis*
*Haraldiophyllum bonnemaisonii*
*Lomentaria clavellosa*
*Membranoptera alata*
*Phycodrys rubens*
*Plocamium cartilagineum*
*Polysiphonia stricta*
*Ptilota gunneri*
*Ptilothamnion pluma*
*Rhodomela confervoides*
*Rhodophyllis divaricata*
*Sphacelaria radicans*
*Ulva lactuca*

**Underflora on bedrock only**

*Hildenbrandia rubra*
*Lithothamnion glaciale*
*Peyssonnelia dubyi*
*Phymatolithon rugulosum*

**Subflora on bedrock and *L. hyperborea***

*Membranoptera alata*
*Phycodrys rubens*
*Polysiphonia stricta*
*Ptilota gunneri*
*Ulva lactuca*

### *Quadrat comparison*

Ordination (see TITTLEY & FARNHAM 1997, p.87) presented a single tight cluster of quadrats in which a clear depth- or time-series could not be discerned.

## DISCUSSION

There exists an extensive body of knowledge on the seasonality, long-term occurrence, change in abundance and depth distribution, of *Laminaria* spp. around the coast of Scotland (e.g. WALKER & RICHARDSON 1955; WALKER 1957). *Laminaria* occurrence around Shetland was less known until recently (cf. CONNOR et al. 1997; TITTLEY et al. 1977). The results of this field survey agree with the conclusion of WALKER (1958) that the greater abundance of *Laminaria* spp. occurs at depths of less than 9 m. The present and previous algal surveys have revealed differences in laminarian communities in the inner and outer parts of Sullom Voe. The extensive areas of subtidal bedrock in the outer voe provide habitat suited to the strongly anchored, long-lived perennial *Laminaria hyperborea*. The three surveys have shown the species to thrive there despite the wave-washed conditions which at an extreme can cause considerable damage by snapping the stiff stipes (BIRKETT et al. 1998). WALKER (1958) noted the dominance of *L. hyperborea* on extensive bedrock in the Orkney Islands. The inner reaches of Sullom Voe have only a restricted area of subtidal bedrock and therefore little *L. hyperborea*. However, the extensive sea-bed of sediment, stones and shells there, create a suitable habitat for the fast-growing, opportunist, annual *L. saccharina / longicruris* and for filiform annual brown algae that do not require firm anchorage. Similar assemblages occurred in sheltered fjords in the Faroes where *L. longicruris* grew on small stones and shells or even unattached (TITTLEY et al. 1982). With its brittle laminae several metres long, *L. saccharina / longicruris* is suited to extremely sheltered conditions with little water movement.

Although the three surveys showed a kelp canopy to be continuously present throughout the voe, results from transect studies (and also the ordination analysis) suggest differences in stability of the vegetation in the inner and outer voe. The *Laminaria hyperborea* forests in the outer areas of Sullom Voe appeared stable in contrast to the changes in extent and abundance of *Laminaria saccharina / longicruris* canopy in the inner voe. However, in the outer voe where mobile sand is temporarily washed away from bedrock, the vegetation on uncovered surfaces is characterised by opportunists such as *Saccorhiza polyschides* and *L. saccharina / longicruris*. Changes recorded in the inner voe are probably natural and possibly cyclical. WALKER (1956a) identified changes in the quantity and composition of Laminariaceae in Scotland and showed an 11 year cycle and coincidence with sunspot activity; WALKER (1956b) suggested that this activity may influence meteorological conditions and thus the marine environment. Elsewhere in the North Atlantic grazing by sea-urchins has significantly reduced the extent of *Laminaria* stands (TITTLEY et al. 1989); this is not the case in Sullom Voe as urchins do not occur in numbers large enough to deplete the kelp assemblage. To speculate, the contraction of vegetation from deeper waters recorded in the present survey may relate to water quality and the flushing of the inner basin of Sullom Voe. Although a de-oxygenation event was recorded in 1978 at the head of Sullom Voe (PEARSON & ELEFTHERIOU 1981; STANLEY et al. 1981), adverse conditions occurred only beneath a summer thermocline at 30-35m.

The age of the *Laminaria hyperborea* population in the outer voe (5-6-7 years) was slightly more than the 4-5 years reported by Walker & Richardson (1957) for open sea populations in Scotland. Although turnover of individual *L. hyperborea* plants has occurred between surveys, recruitment has been sufficient to maintain a broadly similar outward appearance of the kelp bed. WALKER (1958) observed that over a year the standing crop of *L. hyperborea* is generally stable until reduced by rough seas and by the shedding of old laminae in spring. WALKER (1958) also recorded 'perennial changes' in cover and density in *Laminaria* populations in which reductions and gains were noted. BIRKETT et al. (1998) discussed the constant change in the spatial mosaic of kelp communities and suggested time-scales of decades to centuries for such natural dynamic variation. Our observations in Sullom Voe suggest that some kelp communities change more rapidly than others, confirming WALKER & RICHARDSON'S (1955) observation that *L. saccharina* is more noticeably seasonal in its growth than is *L. hyperborea*.

*Laminaria hyperborea* also provides habitat for a dynamically stable subflora of epiphytes. The associated underflora assemblage on bedrock comprised mainly slow-growing species that also formed a dynamically stable facies. The subtidal algal underflora communities, and those at locations in the voe without a kelp canopy (see TITTLEY et al. 1977 for a full description of these communities), in general agree with the biotopes defined by CONNOR et al. (1977) from the north of Scotland.

The 175 species of algae recorded from Sullom Voe (Appendix 1) compares with 199 from the much longer Hardangerfjord in adjacent Norway (JORDE & KLAVESTAD 1963), but considerably exceeds 49 from Skálafjødur (comparable in length to Sullom Voe) in the Faroes (TITTLEY et al. 1982).

These subtidal vegetation surveys, and other studies over the past twenty years (DUNNET & MCINTYRE 1995), have indicated that physical disturbance caused by construction of the oil terminal has been relatively confined. As shown by Walker's many studies *Laminaria* populations are resilient to disturbance; *Laminaria hyperborea* at the oil terminal had recovered in 1993 after an absence in 1983.

The *Laminaria* forests that occur on the temperate sea-shores of the world are biologically diverse and as a consequence are of considerable conservation importance. They are described by BIRKETT et al. (1998) as the marine equivalent of tropical rainforests in terms of their biological productivity, the numbers of individuals living there or dependent on the habitat, and the species diversity present in the habitat. Despite the extensive body of knowledge on kelp forests there are few long-term studies in Britain. Unlike the intertidal monitoring in Sullom Voe that was undertaken at shorter intervals and therefore provides more complete time-series information on algal populations and communities, these subtidal algal surveys only provide snapshots at longer time intervals between which other unrecorded changes may have occurred.

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## APPENDIX 1: SPECIES RECORDED FROM SHETLAND AND SULLOM VOE

Nomenclature and citation mostly follows GUIRY (1997)
* = Voucher specimens at BM
7 = Recorded from Sullom Voe in 1973
8 = Recorded from Sullom Voe in 1983
9 = Recorded from Sullom Voe in 1993

Species without asterisks are not represented by specimens at BM; species without 7,8,9 have been recorded for Shetland but not Sullom Voe.

| | | | | |
|---|---|---|---|---|
| **Bangiophyceae (Rhodophyta)** | | | | |
| *Aglaothamnion bipinnatum* (P Crouan & H Crouan) Feldman-Mazoyer | | | | 9 |
| *Aglaothamnion hookeri* (Dillwyn) Magg & Hommersand) | | 7 | 8 | 9 |
| *Aglaothamnion priceanum* Maggs, Guiry & Rueness | | | | 9 |
| *Aglaothamnion roseum* (Roth) Maggs & L'hardy-Halos | | 7 | | |
| *Aglaothamnion sepositum* (Gunnerus) Maggs & Hommersand | | | | |
| *Ahnfeltia plicata* (Hudson) Fries | * | 7 | 8 | 9 |
| *Antithamnionella floccosa* (O F Müller) Whittick | | | | |
| *Asparagopsis armata* Harvey [Falkenbergia stage] | * | | | |
| *Audouinellla alariae* (Jónsson) Woelkerling | | | | |
| *Audouinella daviesii* (Dillwyn) Woelkerling | * | | | |
| *Audouinella membranacea* (Magnus) Papenfuss | * | | | |
| *Audouinella parvula* (Kylin) Dixon | | | | 9 |
| *Audouinella purpurea* (Lightfoot) Woelkerling | | 7 | | 9 |
| *Audouinella secundata* (Lyngbye) Dixon | | | 8 | 9 |
| *Audouinella virgatula* (Harvey) Dixon | | | 9 | |
| *Bangia atropurpurea* (Roth) C Agardh | | | | |
| *Boergeseniella fruticulosa* (Wulfen) Kylin | | | | |
| *Bonnemaisonia hamifera* Hariot [Trailliella phase] | * | 7 | 8 | 9 |
| *Brongniartella byssoides* (Goodenough & Woodward) Schmitz | * | 7 | 8 | |
| *Callithamnion corymbosum* (J E Smith) Lyngbye | | 7 | 8 | |
| *Callithamnion granulatum* (Ducluzeau) C Agardh | * | | | |
| *Callithamnion tetragonum* (Withering) S F Gray | * | | | |
| *Callocolax neglectus* Batters | * | 7 | | 9 |
| *Callophyllis cristata* (Turner) Kützing | * | 7 | 8 | 9 |
| *Callophyllis laciniata* (Hudson) Kützing | * | 7 | 8 | 9 |
| *Catenella caespitosa* (Withering) L Irvine | | | | |
| *Ceramium cimbricum* H Petersen | | | | |
| *Ceramium deslongchampsii* Duby | | | 8 | 9 |
| *Ceramium diaphanum* (Lightfoot) Roth | * | | | |
| *Ceramium nodulosum* (Lightfoot) Ducluzeau | * | 7 | 8 | 9 |
| *Ceramium shuttleworthianum* (Kuetzing) Rabenhorst | * | | | |
| *Ceramium strictum sensu* Harvey | | | | |
| *Ceratocolax hartzii* Rosenvinge | | 7 | | |
| *Chondrus crispus* Stackhouse | * | 7 | 8 | 9 |
| *Choreocolax polysiphoniae* Reinsch | | | | |
| *Chylocladia verticillata* (Lightfoot) Blidingia | * | 7 | 8 | 9 |
| *Coccotylus truncatus* (Pallas) M Wynne & J Heine | * | 7 | 8 | 9 |
| Colacodictyon reticulatum (Batters) J Feldmann | | | | |
| *Corallina officinalis* Linnaeus | * | | | 9 |
| *Cordylecladia erecta* (Greville) J.Agardh | * | | 8 | |
| *Cruoria pellita* (Lyngbye) Fries | | | 8 | |

| | | | | |
|---|---|---|---|---|
| *Cryptopleura ramosa* (Hudson) Lily Newton | * | 7 | 8 | 9 |
| *Cystoclonium purpureum* (Hudson) Batters | * | 7 | 8 | 9 |
| *Delesseria sanguinea* (Hudson) Lamouroux | * | 7 | 8 | 9 |
| *Dilsea carnosa* (Schmidel) Kuntze | * | 7 | 8 | |
| *Dumontia contorta* (S Gmelin) Ruprecht | * | 7 | | 9 |
| *Erythrocladia irregularis* Rosenvinge | | 7 | | |
| *Erythroglossum laciniatum* (Lightfoot) Maggs & Hommersand | | | | |
| *Erythrotrichia carnea* (Dillwyn) J Agardh | | 7 | 8 | 9 |
| *Fosliella* sp. | | | | |
| *Furcellaria lumbricalis* (Hudson) Lamouroux | * | 7 | 8 | 9 |
| *Gelidium pusillum* (Stackhouse) Le Jolis | * | | | |
| *Gloiosiphonia capillaris* (Hudson) Berkeley | | | | |
| ? *Gracilaria gracilis* (Stackhouse) Steentoft, L Irvine & Farnham | | 7 | 8 | |
| *Griffithsia corallinoides* (Linnaeus) Trevisan | * | 7 | 8 | 9 |
| *Halarachnion ligulatum* (Woodward) Kuetzing | * | 7 | | |
| *Halosacciocolax kjellmanii* S Lund | | | | |
| *Halurus flosculosus* (Lightfoot) Kützing | * | 7 | 8 | 9 |
| *Haraldiophyllum bonnemaisonii* (Kylin) A Zinova | * | | | |
| *Helminthora divaricata* (C Agardh) J Agardh | | | | |
| *Heterosiphonia plumosa* (Ellis) Batters | * | 7 | 8 | 9 |
| *Hildenbrandia crouanii* J Agardh | | | | |
| *Hildenbrandia rubra* (Sommerfeldt) Meneghini | | | 8 | 9 |
| *Hypoglossum hypoglossoides* (Stackhouse) F Collins & Hervey | * | 7 | 8 | 9 |
| *Kallymenia reniformis* (Turner) J Agardh | * | | | |
| *Lithophyllum crouanii* Foslie | | | | |
| Lithophyllum incrustans Phillippi | | | | |
| *Lithophyllum orbiculatum* (Foslie) Foslie | | | 8 | |
| *Lithothamnion glaciale* Kjellman | | | | |
| *Lithothamnion sonderi* Hauck | | | 8 | |
| *Lomentaria articulata* (Hudson) Lyngbye | * | | | 9 |
| *Lomentaria clavellosa* (Turner) Gaillon | * | 7 | 8 | 9 |
| *Lomentaria orcadensis* (Harvey) Taylor | * | | | |
| *Mastocarpus stellatus* (Stackhouse) Guiry | * | | | 9 |
| *Meiodiscus spetsbergensis* (Kjellman) Saunders & McLachlan | | | 8 | 9 |
| *Melobesia membranacea* (Esper) Lamouroux | | | 8 | |
| *Membranoptera alata* (Hudson) Stackhouse | | 7 | 8 | 9 |
| *Nemalion helminthoides* (Velley) Batters | * | | | |
| *Nitophyllum punctatum* (Stackhouse) Greville | * | 7 | 8 | 9 |
| *Odonthalia dentata* (Linnaeus) Lyngbye | * | 7 | | 9 |
| *Osmundea hybrida* (De Candolle) Nam | * | | | |
| *Osmundea pinnatifida* (Hudson) Stackhouse | | | | |
| *Palmaria palmata* (Linnaeus) Kuntze | * | 7 | 8 | 9 |
| *Peyssonelia dubyi* P Crouan et H. Crouan | | 7 | 8 | 9 |
| *Phycodrys rubens* (Linnaeus) Batters | * | 7 | 8 | 9 |
| *Phyllophora crispa* (Hudson) Dixon | * | 7 | 8 | 9 |
| *Phyllophora pseudoceranoides* (S Gmelin) P Newroth & A R A Taylor | | 7 | 8 | 9 |
| *Phymatolithon calcareum* (Pallas) Adey & McKibbin | * | | | |
| *Phymatolithon laevigatum* (Foslie) Foslie | | | | |
| *Phymatolithon lamii* (Lemoine) Chamberlain | | | 8 | |
| *Phymatolithon lenormandii* (Areschoug) Adey | | | 8 | 9 |
| *Phymatolithon purpureum* (P Crouan & H Crouan) Woelkerling & L Irvine | | | 8 | |
| *Phymatolithon tenue* (Rosenvinge) Duwel & Wegeberg | | | | 9 |
| *Plocamium cartilagineum* (Linnaeus) Dixon | * | 7 | 8 | 9 |
| *Plumaria plumosa* (Hudson) Kuntze | | 7 | 8 | 9 |

| | | | | |
|---|---|---|---|---|
| *Pneophyllum caulerpae* (P Crouan & H Crouan) P Jones & Woelkerling | * | | | |
| *Pneophyllum confervicola* (Kuetzing) Chamberlain | | | | |
| *Polyides rotundus* (Hudson) Greville | * | 7 | 8 | 9 |
| *Polysiphonia atlantica* Kapraun & J Norris | * | | | |
| *Polysiphonia brodiaei* (Dillwyn) Sprengel | * | | | |
| *Polysiphonia elongata* (Hudson) Sprengel | * | 7 | 8 | 9 |
| *Polysiphonia elongella* Harvey | | | | 9 |
| *Polysiphonia fibrata* (Dillwyn) Harvey | * | | | |
| *Polysiphonia fibrillosa* (Dillwyn) Sprengel | | | | 9 |
| *Polysiphonia fucoides* (Hudson) Greville | * | 7 | 8 | 9 |
| *Polysiphonia lanosa* (Linneaus) Tandy | * | 7 | 8 | 9 |
| *Polysiphonia nigra* (Hudson) Batters | * | 7 | 8 | 9 |
| *Polysiphonia stricta* (Dillwyn) Greville | | 7 | 8 | 9 |
| *Porphyra amplissima* (Kjellman) Setchell & Hus | * | | | |
| *Porphyra leucosticta* Thuret | * | | | |
| *Porphyra umbilicalis* (Linnaeus) Kützing | * | | | |
| *Porphyropsis coccinea* (Areschoug) Rosenvinge | * | | | |
| *Porphyrostromium ciliare* (Harvey) M Wynne | | | | |
| *Pterosiphonia parasitica* (Hudson) Falkenberg | * | 7 | 8 | 9 |
| *Pterothamnion plumula* (Ellis) Nägeli | * | 7 | 8 | 9 |
| *Ptilota gunneri* P Silva, Maggs & L Irvine | * | 7 | 8 | 9 |
| *Ptilothamnion pluma* (Dillwyn) Thuret | | 7 | 8 | 9 |
| *Rhodomela confervoides* (Hudson) P Silva | | 7 | 8 | 9 |
| *Rhodomela lycopodioides* (Linnaeus) C Agardh | * | 7 | | 9 |
| *Rhodophyllis divaricata* (Stackhouse) Papenfuss | * | 7 | 8 | 9 |
| *Rhodophysema elegans* (J Agardh) Dixon | * | | | |
| *Rhodothamniella floridula* (Dillwyn) J Feldmann | * | 7 | 8 | 9 |
| *Sahlingia subintegra* (Rosenvinge) Kornmann | | | | |
| *Scagelia pusilla* (Ruprecht) Athanasiadis | * | | 8 | |
| *Scinaia furcellata* (Turner) J Agardh | * | 7 | | 9 |
| *Seirospora interrupta* (J E Smith) Schmitz | * | 7 | 8 | 9 |
| *Spermothamnion repens* (Dillwyn) Rosenvinge | | 7 | 8 | 9 |
| *Stylonema alsidii* (Zanardini) K Drew | | 7 | 8 | 9 |
| *Titanoderma corallinae* (P Crouan & H Crouan) Woelkerling, Chamberlain & P Silva | | | | 9 |
| *Titanoderma pustulatum* (Lamouroux) Nägeli | | | 8 | |

### Fucophyceae (Phaeophyta)

| | | | | |
|---|---|---|---|---|
| *Acinetospora crinita* (Harvey) Kornmann | | | | 9 |
| *Acrothrix gracilis* Kylin | * | | | 9 |
| *Alaria esculenta* (Linnaeus) Greville | | | | 9 |
| *Ascophyllum nodosum* (Linnaeus) Le Jolis | * | | | |
| *Asperococcus compressus* W J Hooker | * | 7 | 8 | |
| *Asperococcus fistulosus* (Hudson) W J Hooker | * | 7 | 8 | 9 |
| *Asperococcus bullosus* Lamouroux | * | 7 | 8 | 9 |
| *Chilionema ocellatum* (Kuetzing) Kuckuck | | | | |
| *Chilionema reptans* (P Crouan et H Crouan) Sauvageau | | | 8 | |
| *Chorda filum* (Linnaeus) Stackhouse | * | 7 | 8 | 9 |
| *Chordaria flagelliformis* (O F Müller) C Agardh | * | | | |
| *Cladostephus spongiosus* (Hudson) C Agardh | * | | | |
| *Colpomenia peregrina* (Sauvageau) C Agardh | | | | |
| *Cutleria multifida* (Mertens) De Notaris [includes Aglaozonia stage] | * | 7 | 8 | 9 |
| *Desmarestia aculeata* (Linnaeus) Lamouroux | * | 7 | 8 | 9 |
| *Desmarestia ligulata* (Lightfoot) Lamouroux | * | | | |
| *Desmarestia viridis* (O F Müller) Lamouroux | * | 7 | 8 | 9 |

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|---|---|---|---|---|
| *Dictyosiphon chordaria* Areschoug | * | | | |
| *Dictyosiphon foeniculaceus* (Hudson) Greville | * | 7 | | 9 |
| *Dictyota dichotoma* (Hudson) Lamouroux | * | 7 | 8 | 9 |
| *Ectocarpus fasciculatus* Harvey | | 7 | 8 | 9 |
| *Ectocarpus siliculosus* (Dillwyn) Lyngbye | * | 7 | 8 | 9 |
| *Elachista flaccida* (Dillwyn) Areschoug | | | 8 | |
| *Elachista fucicola* (Velley) Areschoug | * | | | 9 |
| *Elachista scutulata* (J E Smith) Duby | | | | |
| *Endodictyon infestans* Gran | | | | |
| *Eudesme virescens* (Berkeley) J Agardh | * | | | |
| *Fucus ceranoides* Linnaeus | * | | | |
| *Fucus distichus* Linnaeus | * | | | |
| *Fucus evanescens* C Agardh | * | | | |
| *Fucus serratus* Linnaeus | * | | | 9 |
| *Fucus spiralis* Linnaeus | * | | | |
| *Fucus vesiculosus* Linnaeus | * | | | 9 |
| *Giraudia sphacelarioides* Derbès & Solier | * | | | 9 |
| *Gononema aecidioides* (Rosenvinge) P Pedersen | * | | | |
| *Halidrys siliquosa* (Linnaeus) Lyngbye | * | 7 | 8 | 9 |
| *Hecatonema maculans* (F Collins) Sauvageau | | | 8 | |
| *Herponema velutinum* (Greville) J Agardh | | | | |
| *Himanthalia elongata* (Linnaeus) S Gray | * | | | |
| *Hincksia granulosa* (J E Smith) P Silva | * | 7 | 8 | 9 |
| *Hincksia hincksiae* (Harvey) P Silva | | | 8 | |
| *Hincksia ovata* (Kjellman) P Silva | | | | |
| *Hincksia sandriana* (Znanrdini) P Silva | | | | |
| *Hincksia secunda* (Kuetzing) P Silva | | 7 | 8 | |
| *Isthmoplea sphaerophora* (Harvey) Kjellman | * | 7 | 8 | 9 |
| *Laminaria digitata* (Hudson) Lamouroux | * | 7 | 8 | 9 |
| *Laminaria hyperborea* (Gunnerus) Foslie | * | 7 | 8 | 9 |
| *Laminaria longicruris* De la Pylaie | * | 7 | 8 | 9 |
| *Laminaria saccharina* (Linnaeus) Lamouroux | * | | | |
| *Laminariocolax tomentosoides* (Farlow) Kylin | | | | |
| *Leathesia difformis* (Linnaeus) Areschoug | * | | | 9 |
| *Leptonematella fasciculata* (Reinke) P Silva | | | | |
| *Litosiphon laminariae* (Lyngbye) Harvey | * | 7 | 8 | 9 |
| *Mesogloia lanosa* P Crouan & H Crouan | * | | | |
| *Mesogloia vermiculata* (J E Smith) S Gray | * | | | |
| *Mikrosyphar polysiphoniae* Kuckuck | | | | |
| *Mikrosyphar porphyrae* Kuckuck | | | | |
| *Myriactula areschougii* (P Crouan & H Crouan) G Hamel | | | | |
| *Myriactula clandestina* (P Crouan & H Crouan) J Feldmann | | | | |
| *Myrionema corunnae* Sauvageau | * | 7 | 8 | 9 |
| *Myrionema papillosum* Sauvageau | | | | |
| *Myrionema strangulans* Greville | * | 7 | 8 | 9 |
| *Myriotrichia clavaeformis* Harvey | | 7 | 8 | 9 |
| *Pelvetia canaliculata* (Linnaeus) Decaisne & Thuret | * | | | |
| *Petalonia fascia* (O F Müller) Kuntze | * | | | |
| *Petroderma maculiforme* (Wollny) Kuckuck | | | | |
| *Phaeostroma pustulosum* Kuckuck | | | | |
| *Pilayella littoralis* (Linnaeus) Kjellman | * | | | 9 |
| *Pogotrichum filiforme* Reinke | | | 8 | 9 |
| *Protectocarpus speciosus* (Børgesen) Kornmann | | | | |
| *Pseudolithoderma extensum* (P Crouan & H Crouan) S Lund | | 7 | 8 | 9 |

| | | | | |
|---|---|---|---|---|
| *Punctaria latifolia* Greveille | | | | 9 |
| *Punctaria plantaginea* (Roth) Greville | | | | |
| *Punctaria tenuissima* (C Agardh) Greville | | | | |
| *Ralfsia verrucosa* (Areschoug) Areschoug | | | | |
| *Saccorhiza polyschides* (Lightfoot) Batters | | 7 | 8 | 9 |
| *Sauvageaugloia chordariaeformis* (P Crouan & H Crouan) Kylin | * | | | 9 |
| *Sauvageaugloia griffithsiana* (W Hooker) Kylin | * | | 8 | 9 |
| *Scytosiphon lomentaria* (Lyngbye) Link | * | 7 | | |
| *Sorocarpus micromorus* (Bory) P Silva | * | | | 9 |
| *Spermatochnus paradoxus* (Roth) Kützing | * | 7 | 8 | 9 |
| *Sphacelaria arctica* Harvey | | | | |
| *Sphacelaria cirrosa* (Roth) C Agardh | * | 7 | 8 | 9 |
| *Sphacelaria fusca* (Hudson) S Gray | | | | 9 |
| *Sphacelaria mirabilis* (Batters) Prud'homme van Reine | | | | |
| *Sphacelaria nana* Kützing | | 7 | | |
| *Sphacelaria plumigera* Hauck | * | | | |
| *Sphacelaria plumosa* Lyngbye | * | 7 | 8 | 9 |
| *Sphacelaria rigidula* Kuetzing | | | 8 | |
| *Sphaerotrichia divaricata* (C Agardh) Kylin | | | | |
| *Spongonema tomentosum* (Hudson) Kützing | * | | | |
| *Sticytosiphon griffithsianus* (Le Jolis) Holmes & Batters | | 7 | 8 | |
| *Stictyosiphon soriferus* (Reinke) Rosenvinge | * | 7 | 8 | |
| *Stictyosiphon tortilis* (Ruprecht) Reinke | * | 7 | 8 | |
| *Stilophora tenella* (Esper) P Silva | * | | | 9 |
| *Stragularia clavata* (Harvey) G Hamel | | | | |
| *Streblonema breve* (Sauvageau) De Toni | | | | |
| *Streblonema fasciculatum* Thuret | * | | | |
| *Streblonema parasiticum* (Sauvageau) Levring | | | | |
| *Streblonema sphaericum* (Derbès & Solier) Thuret | | | | |
| *Ulonema rhizophora* Foslie | | 7 | | |

**Chlorophyceae**

| | | | | |
|---|---|---|---|---|
| *Acrochaete leptochaete* (Huber) R Nielsen | | | | |
| *Acrochaete viridis* (Reinke) R Nielsen | | | | |
| *Acrochaete wittrockii* (Wille) R Nielsen | | | | |
| *Blastophysa rhizopus* Reinke | | 7 | 8 | 9 |
| *Blidingia minima* (Kützing) Kylin | | | | |
| *Bolbocoleon piliferum* N Pringsheim | | 7 | 8 | 9 |
| *Bryopsis hypnoides* Lamouroux | | | | |
| *Bryopsis plumosa* (Hudson) C Agardh | * | 7 | 8 | |
| *Capsosiphon fulvescens* (C Agardh) Setchell & N Gardner | | | | |
| *Chaetomorpha linum* (O F Müller) Kützing | | | | |
| *Chaetomorpha mediterranea* (Kützing) Kützing | | 7 | | |
| *Chaetomorpha melagonium* (Weber et Mohr) Kützing | | 7 | 8 | |
| *Cladophora albida* (Nees) Kützing | | | 8 | |
| *Cladophora dalmatica* Kützing | | | 8 | |
| *Cladophora hutchinsiae* (Dillwyn) Kützing | | | | |
| *Cladophora pygmaea* Reinke | | | | |
| *Cladophora rupestris* (Linnaeus) Kützing | * | 7 | 8 | 9 |
| *Cladophora sericea* (Hudson) Kützing | | | | |
| *Codium fragile* (Suringar) Hariot ssp. atlanticum (Cotton) P Silva | | | | |
| *Derbesia marina* (Lyngbye) Solier [including Halicystis stage] | * | 7 | | 9 |
| *Enteromorpha clathrata* (Roth) Greville | * | | 8 | |
| *Enteromorpha compressa* (Linnaeus) Nees | | | | |

| | | | | |
|---|---|---|---|---|
| *Enteromorpha flexuosa* (Wulfen) J Agardh | | | | 9 |
| *Enteromorpha intestinalis* (Linneaus) Nees | * | 7 | | 9 |
| *Enteromorpha muscoides* (Clemente y Rubio) Cremades | | 7 | | |
| *Enteromorpha prolifera* (O F Müller) J Agardh | * | | 8 | 9 |
| *Enteromorpha ralfsii* Harvey | | 7 | | |
| *Entocladia perforans* (Huber) Levring | | | | |
| *Epicladia flustrae* Reinke | | | | |
| *Eugomontia sacculata* Kornmann | | | 8 | 9 |
| *Ochlochaete ferox* Thwaites | | 7 | 8 | 9 |
| *Ostroebium queketii* Bornet & Flahault | | | | |
| *Percursaria percursa* (C Agardh) Rosenvinge | * | | 8 | 9 |
| *Prasiola crispa* (Lightfoot) Kützing | | | | |
| *Prasiola stipitata* Jessen | | | | |
| *Phaeophila dendroides* (P Crouan & H Crouan) Batters | | | 8 | |
| *Pringsheimiella scutata* (Reinke) Marchewianka | | 7 | 8 | 9 |
| *Pseudodictyon inflatum* Ercegovic | | | 8 | |
| *Pseudendoclonium fucicola* (Rosenvinge) R Nielsen | | | | |
| *Rhizoclonium tortuosum* (Dillwyn) Kützing | | | 8 | 9 |
| *Spongomorpha arcta* (Dillwyn) Kützing | | | | |
| *Spongonema aeruginosa* (Linnaeus) van den Hoek [Chlorochytrium inclusum stage] | | | 8 | |
| *Ulothrix flacca* (Dillwyn) Thuret | | | | |
| *Ulothrix speciosa* (Harvey) Kützing | | 7 | 8 | 9 |
| *Ulva lactuca* Linnaeus | * | | | |
| *Ulva rigida* C Agardh | | 7 | | |
| *Ulvaria fusca* (Postels & Ruprecht) Ruprecht | | | | |
| *Ulvella lens* P Crouan & H Crouan | | | | |
| *Ulvopsis grevillei* (Thuret) Gayral | | | | |
| *Urospora penicilliforms* (Roth) Areschoug | | | 8 | |

# DEVELOPMENT OF A BIOTIC SCORE FOR THE ASSESSMENT OF THE ECOLOGICAL QUALITY OF THE RIVERS AND STREAMS OF MADEIRA

SAMANTHA J. HUGHES & MIKE T. FURSE

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Macro-invertebrate assemblages are commonly used to assess the impact of anthropogenic activity on rivers. The results are quantified and expressed as biotic index values. Many ecological assessment methods exist and the results are used in catchment management planning. Methods developed for the surface waters of continental Europe are not readily applied to Madeira because of the very low biodiversity of the Madeiran macro-invertebrate fauna. The most diverse macro-invertebrate family in Madeira is the Chironomidae with an estimated 77 species. Statistical analysis of data derived from macro-invertebrate samples collected in 1991-92 resulted in the development of an experimental biotic index, which was tested on a new independent data-set generated from a field collection programme carried out in 1997. Some results of the application of the experimental index and the Chironomid Pupal Exuviae Technique (CPET), a separate ecological assessment method, are analysed and discussed. The need for further testing and development is described.

*Samantha J. Hughes (e-mail: samjhughes@hotmail.com), Laboratório Regional de Engenharia Civil, Departamento de Recursos Naturais e de Hidraúlica, Caminho de Esmeraldo, PT- 9000 Funchal, Portugal. - Mike T. Furse Centre for Ecology and Hydrology CEH Dorset, Winfrith Technology Centre, Winfrith Newburgh, Dorchester, Dorset DT2 8ZD, UK.*

## INTRODUCTION

The island of Madeira (32°30' N – 33°30' N, 16°30' W – 17°30' W) has an estimated 234 streams and rivers within 126 catchments (LNEC 1994). The streams are mostly spring-fed with a short, steep profile running through sheer-sided valleys. Although the species richness of these streams is low, in comparison with mainland Europe (MALMQVIST 1988), approximately 30% of the aquatic macro-invertebrate species found in Madeira's surface waters are endemic (STAUDER 1991; HUGHES et al. 1998).

In common with most regions of the world, the aquatic resources of Madeira are subject to increasing pressure as modern society demands a reliable water supply and a pleasant environment in which to live and work. Thus, increased effluent discharge and habitat destruction occurs as levels of industrialisation and civil construction increase. Effective, integrated management is important in order to monitor and to maintain or improve water and environmental quality.

The European Community has established numerous directives on water policy, including the proposal for an Ecological Quality of Water (Commission of the European Communities 1994). These have recently been brought together under an amended proposal for a Council Directive establishing a framework for Community action in the field of water policy (Commission of the European Communities 1998).

Both chemical and biological monitoring programmes play important roles in water quality management. Chemical monitoring provides valuable information on water quality at the time of sampling. Ecological monitoring systems employ elements of freshwater biological communities (usually macro-invertebrates) in order to appraise the quality of running-water sites. The structure of the community of a site is synthesised into a score representing that site's ecological quality. The score is usually derived via a simple mathematical formula or index. Generally a higher score indicates good ecological quality and a lower score indicates environmental stress.

In most regions of the world, aquatic macro-invertebrates are particularly popular for ecological assessment systems (EAS). This is for several reasons (FURSE et al. 1990): (i) a diverse array of macro-invertebrate taxa can be found at almost all unpolluted sample sites, (ii) the sedentary nature of macro-invertebrates allows the presence or absence of key species to be related to environmental conditions at the sampling site over a period of time, (iii) taxa are comparatively easy to identify to the levels needed for biological indexation, with many taxonomic keys readily available and (iv) sampling is relatively inexpensive.

A wide variety of biotic indices have been developed that make use of macro-invertebrate assemblages to quantify the extent of pollution of watercourses (HELLAWELL 1986; METCALFE-SMITH 1994) and to present this in a form of simple numerical values or colour-coded maps. The use of index values and maps allows information on the condition of rivers to be presented in a way which highlights the problem rivers and sites and permits comparison with a variety of other factors including, for example, catchment geology, land use and the location of known effluent discharges, waste sites or abstraction points. Most European countries have a form of biotic indexation that they use to evaluate the ecological condition of their river network. However none have been developed specifically for Madeira.

A regional EAS for Madeira is now under development, based on the composition of benthic macro-invertebrate assemblages. An essential feature of this system is considered to be that it attributes a high score to sites where rare assemblages or rare endemic species are present.

Ideally, it also needs to take account of the extreme seasonality of the island's streams, the low levels of diversity and individuals occurring at sites, the paucity of pollution intolerant taxa (clean water taxa) and the preponderance of pollution tolerant taxa..

In this paper, the testing of two types of EAS is described and the performance of each is evaluated. The first is an experimental index devised through collaborative work between the Laboratório Regional de Engenharia Civil, Departamento de Recursos Naturais e de Hidraúlica (LREC/DRNH) in Madeira and the Institute of Freshwater Ecology (IFE) in the United Kingdom. This takes account of the full range of macro-invertebrates present.

The second EAS is the Chironomid Pupal Exuviae Technique (CPET), developed in the UK (WILSON 1983). The Chironomidae are ubiquitous members of the macro-invertebrate fauna (CRANSTON 1995) and CPET is used to analyse the diversity and the relative tolerance of selected species of this family. Collections made in 1995 and 1996 revealed the Chironomidae to be the most diverse aquatic macro-invertebrate family on Madeira, with an estimated 77 species. (HUGHES & MURRAY in press). Since aquatic chironomid larvae are exposed to local environmental conditions, but the winged adults ensure effective dispersal and colonisation, characteristic chironomid species assemblages can be related to certain forms of environmental stress. Thus, chironomid communities can be sampled along the whole river system (WILSON 1996), although, in practice, CPET has been more widely used in large, deep rivers (e.g. RUSE & WILSON 1984, 1995; WILSON 1994; WILSON & WILSON 1985).

The Chironomidae are often overlooked in biological monitoring due to perceived difficulties in identification (MURRAY 1996). However, chironomid pupal exuviae (the cast pupal skin left on the water surface as the adult emerges) are both easily collected from the water surface and identified, making them suitable for biological monitoring.

## MATERIAL AND METHODS

### DEVELOPMENT OF THE MADEIRAN BIOTIC SCORE (MBS)

Development of a biotic index termed the Madeiran Biotic Score (MBS) was based on bi-monthly macro-invertebrate collected during 1991 and 1992 (Hughes unpublished). Macro-invertebrate samples were taken over a five-minute period by kick sampling using a hand-net (250 $\mu$m mesh), moving across the river bed and upstream. Coarser substratum was sampled by hand. The biological samples were placed in 70% ethyl alcohol on site. A total of 136 samples from 28 sites in six catchments was considered (Fig. 1, Table 1). All invertebrate taxa were identified to the best achievable level using existing keys and specialist taxonomic advice.

A variety of techniques, including biotic indices, multivariate statistics and diversity indices were explored in order to develop the most appropriate system for Madeira (HUGHES 1995). Also examined were the ratios of the abundance or frequency of occurrence of all individual taxa with the single taxon thought to be most tolerant of pollution.

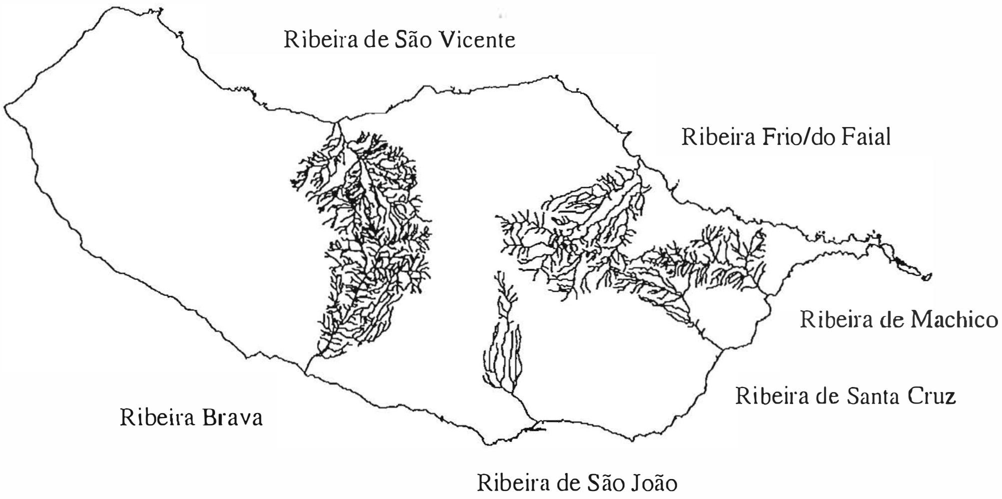

Fig. 1. The 1991 sampling programme.

In the latter approach the chironomid taxon, *Chironomus* cf. *riparius* was the standard pollution-tolerant taxon whose presence was taken as indicative of organic pollution. This cosmopolitan genus is characterised by the presence of haemoglobin in its vascular system which allows it tolerate low levels of dissolved oxygen in the water. Taxa co-occurring with *Chironomus* cf. *riparius*, particularly where both were abundant, were taken to be pollution tolerant and the converse logic was applied to taxa with few, if any, co-occurrences. It was initially intended that the derived pollution tolerance of individual taxa based on their rates of co-occurrence/abundance with *Chironomus* cf. *riparius* could be used to formulate an index system, although this approach was later confined to forming one stage in the development of a more promising alternative.

There were considerable similarities in the range of taxa identified as being indicators of environmental stress by both multivariate analyses and use of *Chironomus cf riparius* to calibrate the pollution tolerance of other taxa. Of the existing European indices, the British Biological Monitoring Working Party (BMWP) system (HAWKES 1997) and its Iberian equivalent (BMWP' - ALBA-TERCEDOR & SANCHEZ-ORTEGA (1988)) seemed to provide the best summary statistic of the ecological quality of the sites in relation to those taxa most associated with the presence of *Chironomus* cf. *riparius* In the

BMWP/BMWP' systems, scores were allocated to macro-invertebrate "families" on the basis of their perceived tolerance to organic pollution. High scores were given to pollution-intolerant taxa and low scores to those that are pollution-tolerant. The BMWP score for a site is the sum of the scores of the individual families present. The ASPT is the Average Score Per Taxa present at the site, (= BMWP site score / number of scoring taxa) and is a measure of the average pollution tolerance of the taxa present. The ASPT is less effort dependant and hence more reliable than the total score and was used as the primary basis for the development of the MBS.

Table 1
Summary of the test sites sampled in 1991.

| River Name | Site no | Perceived sources of environmental impacts |
|---|---|---|
| Machico | 1 | Domestic, organic discharge |
| | 2 | Domestic, organic discharge & detergents |
| | 3 | Domestic, organic discharge |
| | 4 | Domestic, organic discharge |
| | 5 | Domestic, organic discharge |
| Santa Cruz | 1 | None |
| | 2 | Leachates from a open waste tip |
| | 3 | Leachates from a open waste tip |
| | 4 | None |
| | 5 | Organic, piggery discharge |
| | 6 | Organic, piggery discharge |
| | 7 | Diluted domestic, organic discharge |
| | 8 | Domestic, organic discharge |
| São João | 1 | Domestic, organic discharge |
| | 2 | Domestic, organic discharge, sediments in suspension |
| | 3 | Domestic, organic discharge, sediments in suspension |
| Brava | 1 | None |
| | 2 | Organic discharge from abattoir |
| | 3 | Organic discharge from abattoir |
| | 4 | None |
| São Vicente | 1 | None |
| | 2 | None |
| | 3 | None |
| | 4 | Dilute domestic, organic discharge |
| Frio | 1 | None |
| | 2 | Discharge from trout hatchery |
| | 3 | None |
| | 4 | Occasional substratum removal |

Despite the reduction in effort dependency, a shortcoming of the ASPT approach in Madeira was still the difficulty of obtaining reliable index values at sites with naturally low faunal diversity (e.g. upland, spatey, cold sites with low habitat diversity). Index values derived from a wide range of taxa are less prone to sampling variation than those based on a small range of taxa.

In order to overcome the intrinsic problems of low faunal diversity, the new index for Madeira needed to take account of two factors when assessing the quality of sites. Inevitably, diversity had to be considered, since low diversity is a widely used measure of environmental stress (WILSON 1983; WASHINGTON 1984), but account also needed to be taken of the relative number of high and low tolerance taxa present at the site since a species-rich assemblage containing many pollution tolerant taxa is indicative of the early stages of organic pollution.

The following approach to index development was adopted:

- all rare taxa with fewer than ten occurrences out of the 136 samples in the data-set were eliminated as being too infrequent to have reliable indicator values assigned to them. Of the 53 taxa in the data-set, only 31 were retained as potential indicator taxa after this filter was applied.

- the ASPT value of each of the 136 samples was calculated, based on the full complement of taxa present and not just the 31 potential indicator taxa. The Iberian (BMWP') range of taxon scores was used, as being likely to be more appropriate than the British version.

- the occurrences of each of the 31 retained taxa in each sample were correlated with the site ASPTs.

Taxa whose occurrences were indicative of unpolluted sites had positive correlation coefficients and those indicative of polluted sites had negative coefficients. The magnitude of the coefficient was a measure of the indicative strength of the taxon. Correlation coefficients were rounded down to their first decimal point and multiplied by ten to give each of the 31 taxa indicator scores in the theoretical range +10 to -10.

The indicator scores (I.S.) were then used to create an index that took account of both the number of taxa present and their relative quality (i.e. pollution tolerance). The diversity contribution came from the sum of the scores of the positive indicators present. The relative quality came from the quotient of the sum of the positive scores divided by the sum of the negative scores plus one.

The index (MBS), derived from only those indicator taxa present at the site, was therefore expressed by the formula:

$$\mathrm{MBS} = \sum \text{Positive I.S.} + \frac{\sum \text{Positive I.S.}}{(\sum \text{Negative I.S.}) + 1}$$

The addition of one to the negative scores was to avoid division by zero where no negative indicators were present.

Table 2.
The experimental Madeiran Biotic Score (MBS). The index comprises 28 indicator taxa. The highest scoring taxa are pollution intolerant. The negative scoring taxa are increasingly tolerant of (mainly organic) pollution. The scores obtained are introduced into the formula given above. The formula is based upon the diversity of indicator taxa and their pollution tolerance.

| Indicator Taxa | Score |
|---|---|
| *Polycentropus flavostictus* Hagen | 5 |
| Limnephilidae | 3 |
| Tanytarsini | 2 |
| *Oxyethira spinosella* McLachlan | 2 |
| *Hydroptila* sp. | 2 |
| *Ancylus fluviatilis* Müller | 2 |
| *Dugesia gonocephala* group (Duges) | 2 |
| Tanypodinae | 1 |
| *Simulium* sp. | 1 |
| *Hydropsyche maderensis* Hagen | 1 |
| *Dryops luridus* Erichson | 1 |
| *Baetis rhodani* Pictet | 1 |
| Hydracarina | 1 |
| *Physa fontinalis* (Linnaeus) | 1 |
| *Dina lineata* (Müller) | -1 |
| *Eiseniella tetraedra* (Savigny) | -1 |
| *Tubifex* sp. | -1 |
| *Lumbriculus variegatus* (Müller) | -1 |
| *Pisidium casertanum* (Poli) | -1 |
| *Gyraulus parvus* (Müller) | -1 |
| *Lymnaea truncatula* Müller | -1 |
| *Limnophora* sp. | -2 |
| *Asellus aquaticus* Linnaeus | -2 |
| *Physella acuta* Draparnaud | -2 |
| *Pseudamnicola confusa* (Frauenfeld) | -2 |
| *Chironomus* cf *riparius* Meigen | -3 |
| Psychodidae | -3 |
| *Nais* sp. | -3 |

$$\sum \text{positive indicator scores} + \left\{ \frac{\sum \text{positive indicator scores}}{\sum \text{negative indicator scores} + 1} \right\}$$

### THE SCORING SYSTEM

Fourteen positive indicators were identified with a total indicator score of 25 (Table 2). The highest positive indicator was the caseless caddis *Polycentropus flavostictus* Hagen with a score of +5.

Fourteen negative indicators were also identified with a total indicator score of -24 (Table 2). Three taxa had the lowest indicator score of -3. These were *Nais* sp., Psychodidae and *Chironomus* cf *riparius*. Three further taxa had indicator scores of zero and were excluded from calculations. The maximum theoretical index value for a site is 50, where all the positive indicators are present but none of the negative indicators:

$$\mathrm{MBS}_{\mathrm{max}} = \sum \text{Positive I.S.}\,(25) + \frac{\sum \text{Positive I.S.}\,(25)}{(\sum \text{Negative I.S.}(0)) + 1}$$

The minimum theoretical score for a site, when no taxa, or only negative indicators are present, is zero.

$$\mathrm{MBS}_{\mathrm{min}} = \sum \text{Positive I.S.}\,(0) + \frac{\sum \text{Positive I.S.}\,(0)}{(\sum \text{Negative I.S.(any)}) + 1}$$

When applied to the 136 samples used to develop the system, the actual value range was from 24 (a sample from a site at the top of the Ribeira Santa Cruz catchment) to zero (three samples from sites in the Ribeira do São João catchment and one on the Ribeira Brava).

A preliminary recommendation for bands of ecological quality, based on the criteria described in the preceding section, was:

- Band A (good ecological quality); index values $\geq 18$
- Band B (fair ecological quality); index values $= 12 - < 18$
- Band C (poor ecological quality); index values $= 6 - < 12$
- Band D (bad ecological quality); index value $< 6$

On this basis, ten samples (7%) were indexed as good, twenty-eight (21%) as fair, forty-two as poor (31%) and fifty-six (41%) as bad. The further application and testing of this system and its comparison with CPET form the primary objectives of the current study.

### CPET METHODOLOGY

Quantitative collections of floating chironomid pupal exuviae were made over a five minute period at each test. Sampling involved skimming the water surface at the shore with the hand net and picking up the flotsam amongst which pupal exuviae accumulate and often adhere.

The collected material was sorted, enumerated and identified to predetermined taxonomic levels in the laboratory. The assemblage composition of chironomid pupal exuviae samples were quantified using the Shannon Weaver Diversity Index (H) (SHANNON & WEAVER 1949). A minimum number of 200 chironomid pupal exuviae is recommended for index calculation (WILSON 1983).

### FIELD TESTING OF THE MBS

In order to test the MBS on independent macro-invertebrate samples, a new sampling programme was conducted in 1997. A total of 15 sites were sampled in five catchments; Ribeira da Madalena, Ribeira Brava, Ribeira do Porto Novo, Ribeira do Juncal and Ribeira de São Jorge (Fig. 2, Table 3). A preliminary assessment of site quality was made, based on field observations of potential sources of environmental stress (Table 3). Samples were collected bi-monthly throughout the year but, with the exception of four March samples from the Ribeira do Porto Novo, only the May 1997 samples are analysed here.

Two types of standard sample were collected from each site on each visit. The first type was a macro-invertebrate assemblage sample and the second was a collection of chironomid pupal exuviae. MBS index values were derived for each macro-invertebrate assemblage sample (Fig. 3) and Shannon Weaver Diversity Index (H) values for each CPET sample (Fig. 4).

## RESULTS

The results of the macro-invertebrate based MBS showed that the experimental index values conformed well to the perceived sources of environmental impacts operating at the test sites (Table 3, Fig. 3).

The Ribeira do São Jorge sites (an apparently unpolluted river on the north of the island) attained scores that range from good to fair (site 1 MBS = 25.5, site 2 MBS = 14.86 and site 3 MBS = 16.25). Further east, sites on the Ribeira do Juncal, a small stream with some organic loading from domestic discharge, were classified from fair to poor (site 1 MBS = 15.75, site 2 MBS = 15.7 and site 3 MBS = 11.11).

MBS values for Ribeira do Porto Novo in March and May 1997 highlighted the unstressed conditions of site 1 (MBS = 22.5), followed by a sharp decline in ecological quality at site 2 (MBS = 8.4) due to the influence of leachates emanating from the Meia Serra Waste Treatment Station. The poor ecological quality at sites 3 and 4 was due to the physical impact of substratum removal for use as aggregate in civil construction. No values were obtained for sites 3 and 4 in May since they had no flow, due to the seasonal decrease in rainfall and the diversion of surface waters into the levadas for agricultural irrigation.

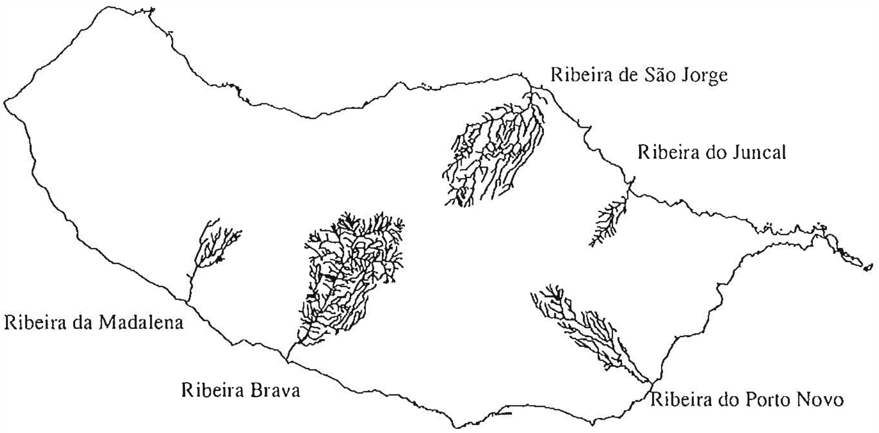

Fig. 2. The 1997 sampling programme.

Table 3.
Summarised data of the test sites sampled in 1997.

| River Name | Site no | Perceived sources of environmental impacts |
|---|---|---|
| São Jorge | 1 | None |
| | 2 | None |
| | 3 | None |
| Juncal | 1 | Domestic, organic discharge |
| | 2 | Domestic, organic discharge |
| | 3 | Domestic, organic discharge |
| Porto Novo | 1 | None |
| | 2 | Leachates from a waste treatment works |
| | 3 | Substratum abstraction, industrial discharge |
| | 4 | Substratum abstraction |
| Brava | 1 | None |
| | 2 | Domestic,organic discharge and rubbish dumping |
| | 3 | None |
| Madalena | 1 | None |
| | 2 | Substratum abstraction |

Site 1 on the Ribeira Brava was subject to virtually no anthropogenic influence and this was successfully reflected by its "good" MBS score of 20. Sites 2 and 3, downstream from the village of Serra da Água were subject to domestic discharge and the dumping of domestic rubbish into the watercourse. A "poor" MBS classification was attributed to these sites (MBS site 2 = 11.67 and MBS site 3 = 11.43) although they were both very close to 12, which is the provisional upper limit of Band C, "poor ecological quality", and the lower limit of Band B, "fair ecological quality".

Ribeira da Madalena site 1, situated in a small laurissilva copse close to source, also exhibited good ecological quality (MBS = 21). Site 2, situated approximately 600m upstream from the river mouth, was heavily impacted by heavy machinery constantly removing substratum for aggregate. The highly unstable nature of the riverbed and sediments in suspension resulted in a very inhospitable environment for aquatic organisms, reflected in the "poor" classification of the site (MBS = 10).

The CPET Shannon Weaver Diversity Index data (denoted as H) also yielded promising preliminary results for ecological evaluation of streams (Fig. 4). However, the average number of pupal exuviae collected per sample was 129 and several samples had less than the minimum number of 200 chironomid pupal exuviae recommended for index calculation (Wilson 1983). Results based on less than the recommended number of specimens must, therefore, be regarded with caution.

Ribeira do São Jorge sites showed relatively high H values, commensurate with the higher diversity found at these unpolluted sites (site 1 H = 1.417, site 2 H = 1.712 and site 3 H = 1.346). Sites 1 and 3 showed slightly lower H values due to large numbers of pupae belonging to a single taxon (site 1) or small sample size (site 3). Ribeira do Juncal had very similar H values to those of Ribeira do São Jorge. The lower two sites suffered from increasing organic input and had slightly lower H values than site 1. It is possible that slight enrichment of these sites may have caused an increase in diversity.

Ribeira do Porto Novo diversity values emphasised the high ecological quality of site 1 (March H = 1.855, May H = 1.997) and the rapid decline in ecological quality at site 2 (March H = 0.95, May H = 0.474). The H value of site 3 in March (H = 1.099) was based upon a sample size of only three pupal exuviae and was not meaningful. No pupal exuviae were collected from site 4.

The diversity index value for site 1 on the Ribeira Brava site 1, with no perceptible sources of pollution, was high (H = 1.90). Sites 2 and 3 had slightly lower diversity values (site 2 H = 1.41, Site 3 H = 1.0276). Low levels of diversity at unpolluted site 1 at Ribeira da Madalena resulted in low H values (H = 0.58), whilst an extremely small sample of four pupal exuviae from site 2 (H = 1.39) meant that no reliable inferences could be made for this site.

Correlation of MBS values and Shannon Weaver Diversity Index (Fig. 5) values showed a positive relationship (r=0.663 ($p<0.01$)). Sites with high MBS values, resulting from their relatively high richness of positive-scoring indicator taxa and low richness of negative-scoring indicators, also tended to have higher chironomid diversity. Sites with low macro-invertebrate taxon richness, relative absence of positive indicators and predominantly negative indicators exhibited low chironomid diversity.

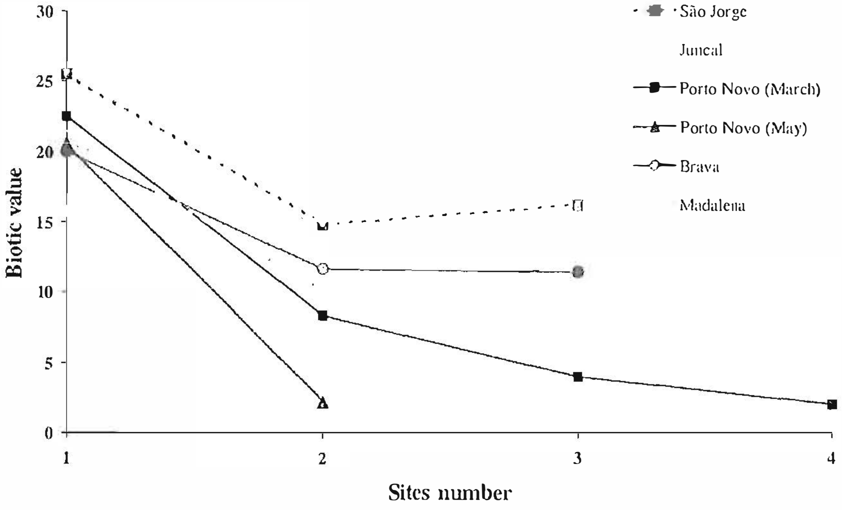

Fig. 3. Madeiran Biotic Score for rivers sampled in March and May 1997.

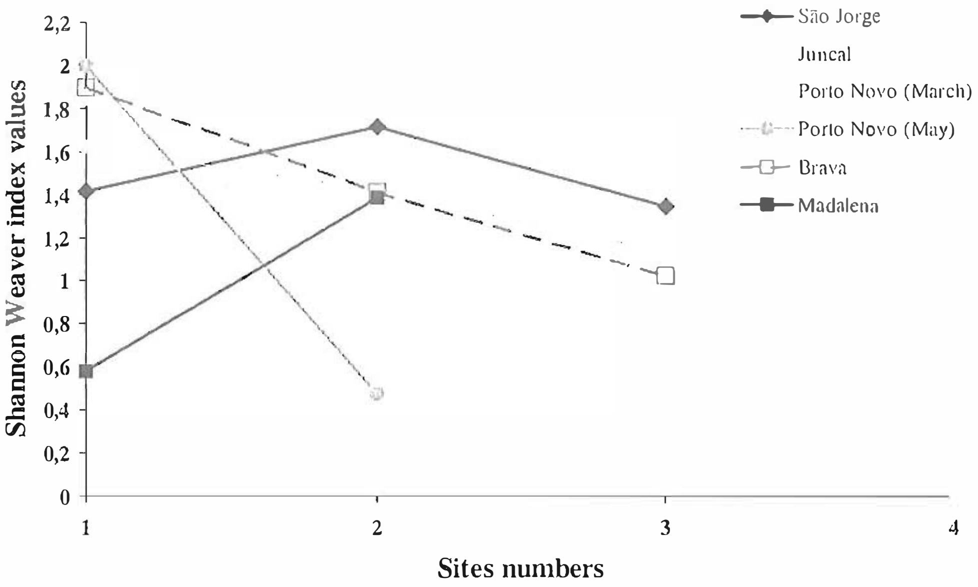

Fig. 4. CPET - Shannon Weaver Diversity Index values for March and May 1997.

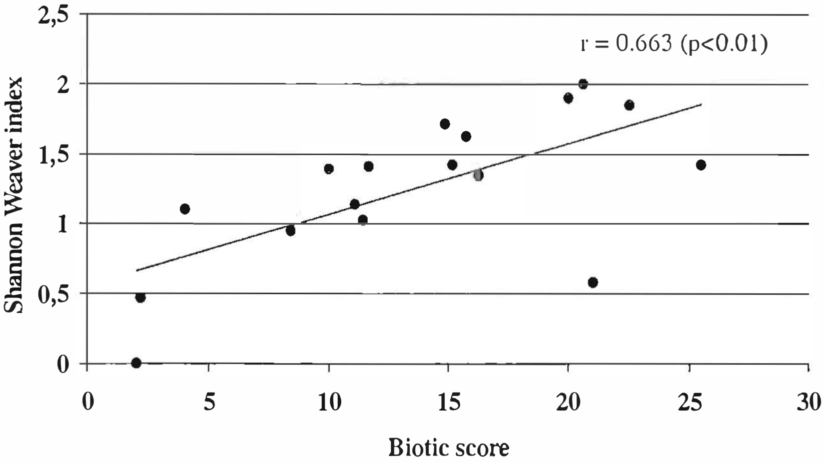

Fig. 5. Correlation of Madeiran Biotic Score v Shannon Weaver Diversity Index values.

## DISCUSSION

Whilst the testing and development of the Madeiran Biotic Score are still in their preliminary phase, initial results of the practical application of the index are promising. A total of five sites were classified as having good ecological quality (class A), four sites were classified as having fair ecological quality (class B), five sites exhibited poor ecological quality (class C) and three sites were of bad ecological quality (class D). These classifications correlated well with the known sources of environmental impacts operating on the test sites (Table 3).

Thus, for example, all five of the MBS Class A sites were the furthest upstream sampling locations in their catchment, some relatively close to source, and were thus most isolated from anthropogenic activity. At each of these sites the top scoring positive indicator species *Polycentropus flavostictus* was present. The second highest scoring positive indicator, Limnephilidae, was present in the samples collected from R. do Porto Novo site 1 in March and May.

The Class B sites were characterised by the presence of lower scoring positive indicator taxa and an increased number of negative scoring taxa. Although low level enrichment may have actually increased diversity at these sites, strongly pollution intolerant taxa were absent.

The Class C sites suffered from leachates, organic pollution or extreme physical disturbance. These sites were characterised by low levels of diversity and the predominance of negative scoring indicator taxa. They thus attained total positive indicator scores of only 1 or 2 whilst the sum of negative indicator scores range between 0 and -9. This reflected the extremely poor species richness of the sites.

Further indication of the usefulness of the MBS was derived from the comparative results obtained using CPET, a well tested and successful index of environmental stress elsewhere in Europe (RUSE & WILSON 1995, WILSON & WILSON 1985). In the present study the correlation between MBS and the Shannon Weaver Diversity Index values obtained from CPET sampling was significant at $p<0.01$ level. Again the tendency was for the upland near source sites to have the highest species richness and highest H values (e.g. Ribeira do Porto Novo site 1, Ribeira Brava site 1 and Ribeira do Juncal site 1), although there were notable exceptions in the most upstream sites on the Ribeira da Madalena site 1 and Ribeira do São Jorge site 1. In the two latter sites, 88% and 60%, respectively, of the specimens examined belonged to a single species. A further exception was site 2 on the Ribeira do São Jorge, which only classified as fair using the MBS but had one of the highest recorded H values of 1.712.

Sites suffering slight organic pollution or physical disturbance had lower H values of between 1.417 – 1.35. These included Ribeira do Juncal site 2, Ribeira Brava site 2, Ribeira da Madalena site 2 and Ribeira do São Jorge site 3. More heavily polluted sites had H values ranging from 1.14 to 0.47. These included the lower Porto Novo sites and Ribeira do Juncal site 3 that suffered from the cumulative effect of organic pollution. Ribeira Brava site 3 also fell into this category.

However, although promising, the tests undertaken on MBS and the application of CPET in Madeira are still preliminary and there are reasons to be cautious about their initial performances.

An example of the need for caution is the role of *Polycentropus flavostictus* and Limnephilidae as positive indicators of good ecological quality. Although both taxa are strongly associated with the unpolluted sites, their restricted distribution in unpolluted, upland headwaters may also be due to other natural influences and these may compromise the range of site type over which they can act as reliable indicators. Both are cold water stenotherms, requiring high levels of dissolved oxygen (HUGHES 1997) and the sites most suited to these ecological requirements tend to be situated at altitude or close to source. Thus, whilst these indicator taxa will contribute to high MBS index values at such sites they will not be present at sites outside of their range of physiological tolerance, i.e. low altitude and gradient sites in the main river channel.

The situation at the two downstream sites on the Ribeira do São Jorge illustrated the problem. The upstream site 1 had an MBS value of 25.5 but the respective values for the next sites downstream were 14.86 and 16.25. At neither downstream site were *Polycentropus flavostictus* or Limnephilidae present. Although, there may have been undetected or diffuse sources of pollution affecting these sites it is possible that the absence of the positive indicators was because the sites, although unpolluted, were outside the normal ranges of the two indicators. Without further research, this remains an issue for speculation but, if true, then two options may exist to modify the MBS.

The first option is to incorporate additional indicators in the form of pollution intolerant taxa characteristic of the lower reaches. This approach is unlikely to succeed because the fauna of Madeira is now extremely well known (HUGHES et al. in press) and no such taxa are evident. The second option is to acknowledge that the MBS values of the downstream reaches of Madeira's rivers are intrinsically lower than those for sites further upstream. In Britain the resolution of this problem is being tackled in two different ways. One is to use site-specific reference states to define the unstressed fauna and index values of individual sites, and then to compare the observed fauna with the expected reference state (WRIGHT et al. 1993). The other is to have a variety of different scores for each of the indicator taxa, according to the environmental character of the survey site. WALLEY & MARTIN (1997) have adopted this approach in modifying the values of the BMWP system according to site type and individual taxon abundances.

Another factor which needs to be resolved before the effectiveness of the MBS can be fully demonstrated is that of sampling variability. As stated earlier, the low species richness of Madeiran rivers may result in large differences in MBS values through the failure to capture the majority of the taxa present at a site at the time of sampling. CLARKE (2000) has demonstrated the importance of a knowledge of all sources of variation in bioassessment, using macro-invertebrates, before confident judgements can be made about the existing condition of sites and "true" temporal and spatial differences in their ecological quality. A system of replicate sampling, as adopted by Clarke, will be necessary to evaluate sampling variation in Madeiran rivers.

Analyses of chironomid pupal exuviae collections, although superficially successful, also demonstrated the difficulties associated with the species-poor Madeiran fauna. Thus, the range of H values (maximum H = 1.99, minimum H = 0.47) was much narrower than would be the case for an equivalent set of freshwater sites in mainland Europe. Sample size, therefore, strongly influenced the reliability of the results obtained. Some samples comprised only four pupal exuviae and these were clearly inadequate for calculating reliable diversity index values. It is possible that the sampling method employed was not suitable for collecting a representative sample and setting drift nets over longer periods of time may prove more reliable

Other issues which need to be considered in applying CPET techniques in Madeira are (i) the applicability of the technique to the small, near-source streams which form the majority of the apparently unstressed sites in Madeira, (ii) the applicability of the number of pollution tolerant v pollution intolerant taxa ratio which characterise the interpretation of results in mainland Europe (WILSON 1966) and (iii) the seasonality of index values.

To date the use of CPET in mainland Europe has concentrated largely, but not exclusively, on large lowland rivers where conventional macro-invertebrate pond-netting techniques are impractical (WILSON 1996). Until more extensive use of CPET in small stream sites has conclusively demonstrated its worth in small streams in mainland Europe its application and interpretation in Madeira must be undertaken with caution.

The index of proportion of pollution intolerant taxa was calculated for each test sample but failed to give meaningful results. This may be partly because of low numbers of taxa and specimens and partly because of the unsuitability, in Madeira, of the allocation of taxa to pollution tolerance categories. The availability of freshwater niches on Madeira, generally pre-occupied by other taxa in equivalent continental freshwater habitats, may mean that measurement of chironomid pollution tolerance will have to be modified for Madeira. Furthermore endemic species of chironomids from Madeira, some only recently discovered (HUGHES & MURRAY in press), may have different ecological tolerances than closely allied continental species with known tolerances.

Finally, the initial trials have been conducted entirely on spring samples collected in a single year, whereas it is known that both diurnal and seasonal emergence patterns will affect the results of diversity indices (HUGHES 1978). The same shortcomings exist in the testing of the MBS and the performance characteristics and potential promise of the index at other times of the year and flow conditions also remain to be examined.

The next stage in the development of the MBS is a more extensive, multi-seasonal testing of the index, including replicate sampling. This may lead to modifications in the list of indicator taxa and the scores assigned to each, including the possibility of two different but compatible score systems for upper and lower stream reaches. Parallel testing and development of the CPET, including new, endemic taxa and the potential re-assignment of pollution tolerance categories of other, more widespread taxa, will also be conducted.

## CONCLUSIONS

Recent studies of the freshwater fauna of Tenerife (MALMQVIST et al. 1993, 1995) and Gran Canaria (NILSSON et al. 1998) have clearly demonstrated the threat posed to the specialist species and assemblages of Macaronesia by the reduction and degradation of flow in the islands rivers. If this threat is to be monitored and reversed then it is essential to have suitable evaluation procedures and survey programmes in place to document and manage the changes. Both the MBS and CPET represent promising techniques. However, both are in need of further testing, and possible refinement, in order that they are appropriate to meet the specific character of Madeira's streams and their distinctive and often endemic fauna.

Once perfected, these methods will produce an accurate and urgently required interpretation of the state of the aquatic environment on Madeira. The information obtained will have a wide diversity of applications ranging from integrated resource management, catchment planning targets and post-project appraisal to environmental impact surveys, conservation programmes, regional reports and environmental education.

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# COMMENTS ON THE PUPA OF *Zavrelimyia* sp. (INSECTA: DIPTERA, CHIRONOMIDAE) FROM MADEIRA

SAMANTHA J. HUGHES & DECLAN A. MURRAY

HUGHES, S.J. & D.A. MURRAY. 2001. Comments on the pupa of *Zavrelimyia* sp. (Insecta: Diptera, Chironomidae) from Madeira. *Arquipélago*. Life and Marine Sciences. Supplement 2 (Part B): 33-37. Ponta Delgada. ISSN 0873-4704.

*Zavrelimyia nubila* (Meigen, 1830) is the only species of *Zavrelimyia* FITTKAU (1962) recorded previously from Madeira based on collections of adult specimens. However, pupal exuviae recently obtained in Madeira differ from those of *Z. nubila* in their overall smaller size, presence of a corona on the thoracic horn and in the abdominal pigmentation and shagreen pattern. An illustrated description of these features is given here to distinguish this morphotype from related Palaearctic species.

*Samantha Jane Hughes (e-mail: samjhughes@hotmail.com), Laboratório Regional de Engenharia Civil, Departamento de Recursos Naturais e de Hidraúlica, Caminho de Esmeraldo, São Martino, PT – 9000 Funchal, Portugal. - Declan A. Murray, Department of Zoology, University College Dublin, Belfield, Dublin 4, Ireland.*

## INTRODUCTION

Qualitative collections made in 1995 and 1996 to investigate the distribution and occurrence of Chironomidae in selected freshwater habitats of Madeira yielded 53 taxa at species level, of which 16 were new to the island's fauna (HUGHES & MURRAY 2000). Additional pupal exuviae material has since been obtained during the 1997 biological monitoring survey of Laboratório Regional de Engenharia Civil, Departamento de Recursos Naturias e de Hidraúlica (LREC/DRNI) to assess the ecological quality of Madeira's surface waters. The majority of specimens collected were readily identifiable using existing taxonomic determination keys but 13 exhibited morphological differences in comparison with descriptions of currently known species and possibly represent variant forms or species endemic to Madeira.

This paper presents some of the characteristics of the pupal exuviae of a species of *Zavrelimyia*, collected from several sites on Madeira, that differ from the exuviae of *Z. nubila* (Meigen, 1830) the only species of *Zavrelimyia* previously recorded (as an adult) from the island. The pupal exuviae, the cast "skin" of the pupa remaining on the water surface following eclosion of the adult, possesses a wealth of species-specific diagnostic features. Examination of collections of pupal exuviae rapidly provides reliable information of the resident fauna, species composition and phenology at a given site (WILSON 1996).

## METHODS

During field sampling carried in September 1995, April 1996 and throughout 1997, collections of Chironomidae were obtained from 57 sites covering several types of freshwater habitat. Exuviae were collected by drift net in selected flowing-water sites, augmented by skimming the water surface with a hand net (250 µm mesh) and picking up flotsam to which pupal exuviae may adhere.

Although adult Chironomidae were collected at many sites, adult male or female specimens of *Zavrelimyia* were not obtained. However pupal exuviae of *Zavrelimyia* were obtained at the sites indicated (Fig. 1, Table 1). Exuviae of *Z. nubila* from Ireland were used for comparison with the Madeiran specimens. All exuviae for examination by light microscopy were processed and slide mounted according to methods outlined in WIEDERHOLM (1986). Scanning electron microscopy was carried out on selected specimens. Identifications are based on key works of FITTKAU (1962), FITTKAU & MURRAY (1986) and LANGTON (1991).

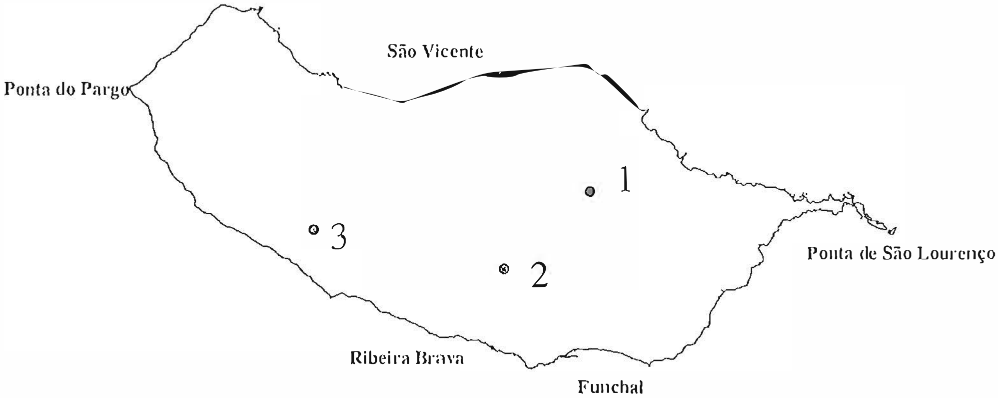

Fig. 1. Sites where specimens of *Zavrelimyia* sp have been collected. Details of each site are given in Table 1.

Table 1.
Sites where samples during 1995 and 1996 on Madeira and Porto Santo (S= Sweep netting, A= Aquatic collections). Site co-ordinates were obtained using a *GARMIN* GPS 45 receiver.

| Date | # | Location | Method | Co-ordinates | Alt (m) |
|---|---|---|---|---|---|
| 15.09.95 | 1 | Fajã de Nogueira hydro electric reservoir | S/A | N32°44’909 / W16°55’411 | 530 |
| 13.04.96 | 2 | Curral das Freiras (Terra Chã) - reservoir | A | N32°42’714 / W16°58’335 | 450 |
| 21.05.97 | 3 | Perennisal stream in laurissilva copse Arco da Calheta | A | N32°43’597 / W17°07’511 | 780 |

Material examined:

*Zavrelimyia* sp. Madeira
Pe, 15/09/1995, hydroelectric station reservoir, Fajã de Nogueira.
Pe, 13/04/1996, artificial irrigation reservoir, Terra Chã, Curral das Freiras.
Pe, 21/05/1997, 1$^{st}$ order stream in small copse of laurel forest, Arco da Calheta.

*Zavrelimyia nubila*.
Pe, 20/05/1986, water tank, Ardsallagh, Co. Meath, Ireland.
Pe, 29/09/1997, artificial small garden pool, Meadesbrook, near Ashbourne, Co. Meath, Ireland.

## RESULTS

The pupal exuviae of *Zavrelimyia* sp. from Madeira differ from Z *nubila* in the overall size, pigmentation pattern of the abdominal tergites, structure of the abdominal armature (shagreen) and in features of the respiratory thoracic horn.

The overall length of the Madeiran specimens, 5.0 mm, is shorter than that of Z. *nubila* at 6.5 mm. The tergites of the Madeiran specimens have a distinctly homogenous pigmentation pattern with a rounded anterior median mark on tergites II to VI (Fig. 2a and b) whereas in *Zavrelimyia nubila* dark anterior and posterior bands on each tergite are joined by a single median band (Fig. 3a and b). The shagreen on tergite 2 of the Madeiran specimens is composed of individual spinules (Fig. 2c) while in Z. *nubila* the spinules are grouped and occasionally branched (Fig. 3c). The thoracic horns of all specimens obtained in Madeira have a distinct rim or corona that appears, under light microscopy, as a translucent area surrounding the plastron plate. The corona, which is absent in Z. *nubila*, is clearly visible in scanning electron micrographs of the Madeiran specimens (Fig. 4). Reaching 0.075x the length of the thoracic horn, the plastron plate is broader than the neck of the respiratory atrium, which fills ¾ of the horn respiratory chamber.

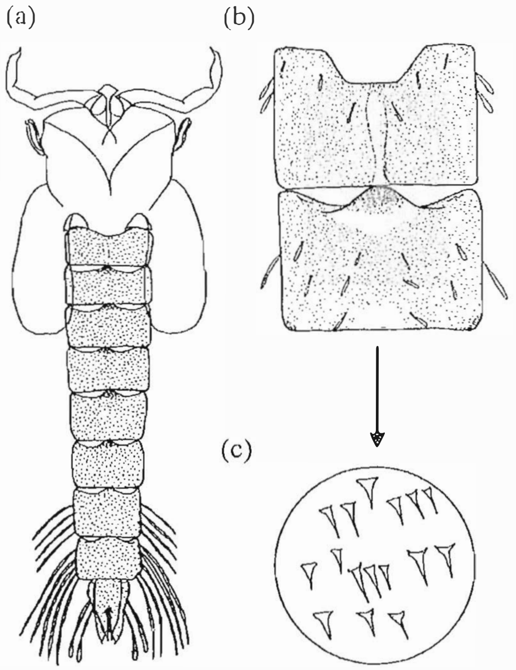

Fig. 2. Pupal Exuvia of *Zavrelimyia* sp. from Madeira. (a) pigmentation of the abdominal tergites (b) detail of tergites I and II (c) diagram of the spines on tergite II.

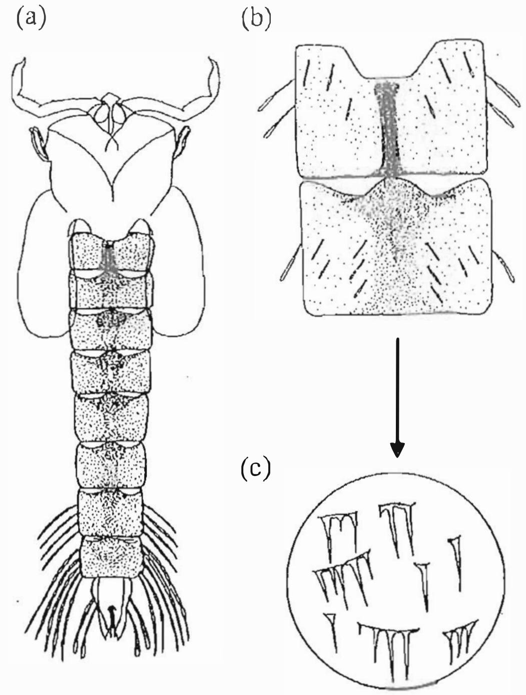

Fig. 3. Pupal Exuvia of *Zavrelimyia nubila* (a) pigmentation of the abdominal tergites (b) detail of tergites I and II (c) diagram of the spines on tergite II.

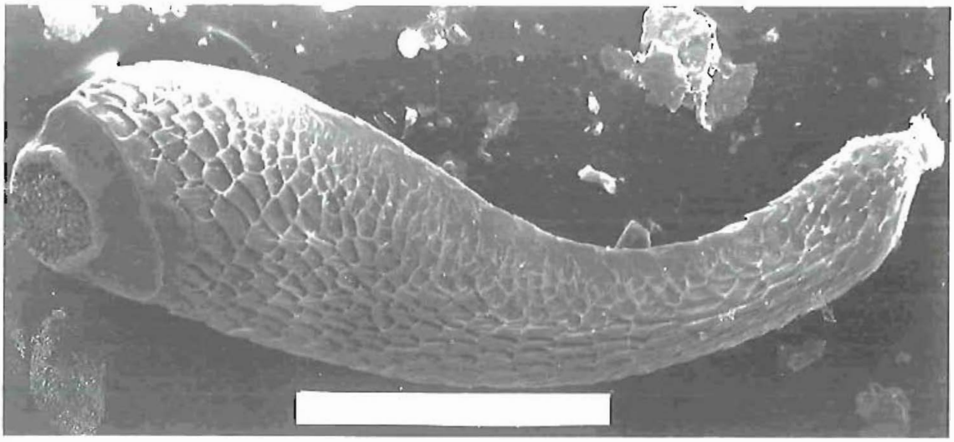

Fig. 4. Electron micrograph of the thoracic horn of *Zavrelimyia* sp. Madeira. Note the rim around the plastron plate. This characteristic is absent in *Zavrelimyia nubile* (Meigen, 1830). Scale bar 100 µm.

## DISCUSSION

Observations on the *Zavrelimyia* pupal exuviae examined indicate that the specimens in question differ from *Zavrelimyia nubila,* the only species of *Zavrelimyia* recorded from Madeira until now (as adult only) by STORÅ (in FREY 1949), FREEMAN (1959) and BAEZ & ARMITAGE (1990). *Z. nubila* has also been recorded (likewise as adult only) from the Azores (STORÅ in FREY 1945) and the Canary Islands (CRANSTON & ARMITAGE 1988).

Pupal exuviae of all known *Zavrelimyia* species have a background golden/yellow colour. However, pigmentation patterns are evident in different species (FITTKAU 1962). Whereas the gross structure of the thoracic horn of *Z. sp.* Madeira most closely resembles *Z. melanura,* the abdominal tergites of *Z. melanura* possess dark transverse anterior and posterior bands connected by median and lateral longitudinal bands. In *Z. nubila*, a median longitudinal band connects dark anterior and posterior bands. The pattern in *Z.* sp. Madeira resembles neither of these species and is restricted to a distinct rounded, median anterior mark adjacent to the apophyses. Such a pattern is known in *Z. berberi* Fittkau, 1962, from Morocco, but in that species the horn atrium is about half the width of the horn in contrast with the Madeiran specimen where the atrium is at least ¾ as wide as the thoracic horn.

The shagreen on abdominal tergite 2 of the pupa of *Z. sp.* Madeira also differs from that of *Z. nubila.* In *Z. nubila* the shagreen is composed of grouped and occasionally branched spinules, somewhat similar to *Z. barbatipes* (Kieffer), in contrast to the individual or single spinules of the Madeiran specimens.

It is clear from these observations that the exuviae of *Z. sp.* Madeira do not belong to *Z. nubila.* Without re-examination of previously collected specimens and until definitive, reared and associated, pupal and adult material is obtained is not possible to confirm the status of previous records or to state whether one or two species of *Zavrelimyia* occur on the island.

## ACKNOWLEDGEMENTS

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# ENZYMATIC IDENTIFICATION OF *Glyptapanteles* sp. (INSECTA: HYMENOPTERA) FROM MADEIRA ISLAND

L. OLIVEIRA, V. VIEIRA, J. TAVARES & P. GARCIA

OLIVEIRA, L., V. VIEIRA, J. TAVARES & P. GARCIA. 2001. Enzymatic identification of *Glyptapanteles* sp. (Insecta: Hymenoptera) from Madeira Island. *Arquipélago*. Life and Marine Science. Supplement 2 (Part B): 39-42. Ponta Delgada. ISSN 0873-4704.

During a scientific expedition carried out in Madeira Island in September 1997, *Pseudaletia (=Mythimna) unipuncta* (Lepidoptera: Noctuidae) larvae were collected in maize fields and pastures. These larvae were parasitized by a braconid belonging to *Glyptapanteles* genus. This population was characterised biochemically to identify the species. Seven enzyme systems studied by electrophoresis were analysed: aldehyde oxidase (AO), a-glycerophosphate dehydrogenase (a-GPD), tetrazolium oxidase (TO), malate dehydrogenase (MDH), glucose-6-dehydrogenase (G6PD), malic enzyme (ME), isocitrate dehydrogenase (IDH). All systems showed only one band, with two exceptions: α-GPD and ME which had two bands which corresponded to different loci. No polymorphic enzymes were detected. Comparing this results with those obtained from *G. militaris* collected in Azores Islands and in Quebec-Canada we can suggest that the population of *Glyptapanteles* collected in Madeira Island belongs to *G. militaris* species.

*L. Oliveira (e-mail: ziza@notes.uac.pt), V. Vieira, J. Tavares & P. Garcia, Universidade dos Açores, Departamento de Biologia, Rua da Mãe de Deus 58, PT – 9501-801 Ponta Delgada, Portugal.*

## INTRODUCTION

*Pseudaletia (=Mythimna) unipuncta* (Lepidoptera: Noctuidae) is a cosmopolitan species, considered as the most important pest in Azorean gramineous fields (TAVARES 1989). Several studies concerning the establishment of biological control programs against this pest have been carried out in our laboratory (TAVARES 1989; VIEIRA 1992; OLIVEIRA 1996). In this sense, it is important to survey the pest natural enemies and preserve their characteristics during the several steps of biological control programs, such as, the mass rearing processes and field releases. However, some problems may arise when such programs are under development such as species mis-identification (especially for sibling species) leading to a loss of effort, time and money (POWELL & WALTON 1989). Electrophoretic study, as a tool for identification, may help to overcome some of such errors (PINTUREAU & VOEGELÉ 1980; PINTUREAU 1987; POWELL & WALTON 1989).

In the Azores, *P. unipuncta* has few species of parasitoids from Hymenoptera (TAVARES 1989; OLIVEIRA 1996), with the braconid *G. militaris* being the main native biological control agent. Therefore, a larger survey for *P. unipuncta* biological control agents was expanded to other Macaronesian Islands, such as Madeira.

During a scientific expedition carried out in Madeira Island in September 1997, *P. unipuncta* larvae were collected in maize fields and pastures. These larvae were parasitized by a braconid belonging to *Glyptapanteles* genus, as in Azores Islands.

Since electrophoretic studies have been used to identify and distinguish between sibling species (PINTUREAU & VOEGELÉ 1980; PINTUREAU 1987; PINTUREAU et al. 1990) we analyzed seven enzyme systems by gel electrophoresis to compare the parasitoids from Madeira with the Azorean population of *G.*

*militaris*. This study allowed an increase on the records of Madeira native species and the genetic characterization of the wasp's populations, contributing to the proper development of biological control programs.

## MATERIAL AND METHODS

Adult parasitoids were obtained from larvae of *P. unipuncta*, randomly collected at Madeira Island Miguel Island only in pastures. The encountered *P. unipuncta* larvae, more than 100, were individually isolated in plastic cages (4.5x3 cm), and kept in the laboratory at 22±1 °C, 75±5% RH and L:D 16:8, for daily observation of parasitoid emergence. Twenty-eight of these larvae were parasitized by *G. militaris*, producing an average of 32 cocoons per host. After parasitoid adult emergence, the insects were stored frozen at -20 °C until subsequent electrophoresis (frozen period < 1 month).

Each adult was isolated and homogenized in 15 microliter of "Trudgill" solution and centrifuged for 5 minutes at 9 000 rpm, according to the methodology used by PINTUREAU (1987).

A vertical electrophoresis on polyacrilamide gel was performed on seven enzyme systems: aldehyde oxidase (AO), α-glycerophosphate dehydrogenase (α-GPD), tetrazolium oxidase (TO), malate dehydrogenase (MDH), glucose-6-dehydrogenase (G6PD), malic inzyme (ME) and isocitrate dehydrogenase (IDH). The techniques used generally followed the methodology described by PINTUREAU (1987), PINTUREAU et al. (1991) and OLIVEIRA (1996), with specific details in table 1. Eighteen individuals (each one from an individual parasitized host) collected in Madeira Island were run for each enzymatic system. As control, two positively identified specimens of *G. militaris* from São Miguel Island (OLIVEIRA 1996) were run for each enzymatic system.

## RESULTS AND DISCUSSION

The electrophoretic patterns observed in *G. militaris* were similar for the populations of Madeira and Azores (Fig. 1). Aldehyde Oxidase, Tetrazolium oxidase, Malate dehydrogenase, Glucose-6-dehydrogenase and Isocitrate dehydrogenase exhibited a single band, for each of these five systems, therefore only one locus was found. However, α-Glycerophosphate dehydrogenase and Malic enzyme exhibited two bands, corresponding two different loci.

Table 1
Electrophoretic conditions used to study seven enzymes systems in *G. militaris*.

| Protein | Buffers |  | Migration |  | Revelation |  |
|---|---|---|---|---|---|---|
|  | Bridge | Gel | time (Minutes) | voltage (Volts) | time (Minutes) | solution |
| Aldehyde oxidase (AO) | Tris/HCl<br>4 %<br>pH 6.7 | Tris/HCl<br>7 %<br>pH 8.9 | 30<br>90 | 150<br>300 | 30<br>roomtemp. | Tris/HCl<br>Benzaldeide<br>NBT<br>PMS |
| α-Glycerophosphate D,L,Glycerophosphate dehydrogenase (α-GPD)<br>Tetrazolium oxidase (TO) | " | " | 30<br>120 | 150<br>300 | 40<br>37°C | Tris/HCl<br>α-<br>NAD<br>NBT<br>PMS<br>MTT |
| Malate dehydrogenase(MDH) | " | " | 30<br>90 | 150<br>300 | 40<br>37°C | Tris/HCl<br>L-Malic acid<br>NAD<br>NBT<br>PMS |
| Glucose-6-dehydrogenase (G-6-PD) | " | " | 30<br>90 | 150<br>250 | 40<br>37°C | Tris/HCl<br>Glycerol-6-phosphate<br>NADP<br>$MgCl_2$<br>NBT<br>PMS<br>NBT |
| Malic inzyme (ME) | " | " | 30<br>90 | 150<br>300 | 15<br>37°C | Tris/HCl<br>L-Malic acid<br>NADP<br>$MgCl_2$<br>PMS<br>MTT<br>Agar 2 % |
| Isocitrate dehydrogenase (IDH) | " | " | 30<br>90 | 150<br>300 | 60<br>37°C | Tris/HCl<br>D.L-Isocitric acid<br>NADP<br>$MgCl_2$<br>PMS<br>MTT |

The two populations of *G. militaris* were examined for nine allozyme loci, but all of them appeared entirely monomorphic. The enzymes used in this study are useful to characterize genetically *G. militaris*, but they constitute only a part of the species genome. Most species of Hymenoptera are reported to have low electrophoretic variation (PINTUREAU 1987; OMWEGA & OVERHOLT 1996). Our data indicated that *G. militaris* falls in the former group with less variability, as *Cotesia glomerata* (unpublished data).

The comparison of both populations suggests that the *Glyptapanteles* population collected in Madeira Island belong to *G. militaris* species. The same bands were observed for a population of *G. militaris* from Quebec-Canada (OLIVEIRA, 1996). The results of the morphological study carried on both populations confirm the enzymatic identification of the species (OLIVEIRA et al.1999).

According to BAEZ (1993) and GRAHAM (1986a, 1986b), the genus *Apanteles* (= *Glyptapanteles*, *Cotesia*) is cited from Madeira, but the species were not identified. Therefore, the species *G. militaris* is the first time recorded for this island.

The presence of this parasitoid in Madeira Island can be a good indicator of the natural control exerted by this wasp on the populations of the agricultural pest *P. unipuncta*. Furthermore, the population of *G. militaris* should be protected from the indiscriminate use of pesticides.

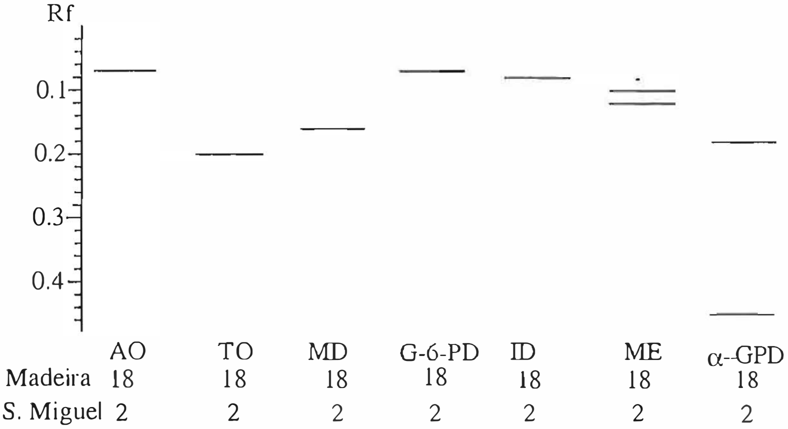

Fig. 1. Electrophoretic partterns of seven enzyme sistems observed in two populations of *G. militaris* from Madeira and Azores Islands; AO - Aldehyde oxidase; TO - Tetrazolium oxidase; MDH - Malate dehydrogenase; G-6-PD - Glucose-6-dehydrogenase; IDH - Isocitrate dehydrogenase; ME - Malic enzyme and α-GPD - Glycerophosphate dehydrogenase.

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# EFFECT OF HOST AVAILABILITY ON *Trichogramma cordubensis* (INSECTA: HYMENOPTERA) REPRODUCTIVE STRATEGIES

PATRÍCIA GARCIA & JOÃO TAVARES

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The effect of host availability on *Trichogramma cordubensis* Vargas & Cabello (Hymenoptera, Trichogrammatidae) fecundity and survival rates was analysed in this study. When hosts were daily provided (either with an unlimited or limited number), wasps had the highest rate of reproduction in the first day of parasitism, decreasing with oscillating values thereafter. When a limited number of hosts was provided with 3 days intervals, the parasitoids reproduction rate was significantly reduced, tending to be equally distributed throughout lifetime. A positive linear relation was found between reproduction and survival: wasps that had daily oviposited had greater longevity than those that had oviposited only every 3 days or did not oviposit. These results suggest that *T. cordubensis* is able to adjust fecundity schedule as an adaptation to changing hosts resources, which is an important survival feature for this species as host shortage is likely to occur in nature.

*Patrícia Garcia (e-mail: patriciag@notes.uac.pt) & João Tavares, Universidade dos Açores, Departamento de Biologia, Rua da Mãe de Deus 58, PT – 9501-801 Ponta Delgada, Portugal.*

## INTRODUCTION

Numerous parasitoids are efficient natural enemies of important agricultural and forestry pests, and among these, the genus *Trichogramma* Westwood is worldwide used in several pest management programs (LI-YING 1994). *Trichogramma* are minute wasps that oviposit into the eggs of other insects, and depend upon these hosts as food resources for their larvae. According to several authors the lifetime reproductive success of the wasp increases as progeny number allocated to the host decreases (KLOMP & TEERINK 1978; PAK & OATMAN 1982; WAAGE & LANE 1984; WAJNBERG et al. 1989; NENON 1993). Therefore, to allocate the progeny suitably, these wasps must respond to the number of host eggs locally available, by ovipositing few eggs per host if the probability of finding enough hosts is high. Optimal strategies for exploiting host resources are essential for survival of the species and their usefulness as biological control agents. However, the role of host availability on the reproductive success of *Trichogramma* species has been poorly investigated despite its importance in natural biotopes and biological control programs (SCHMIDT & SMITH 1985; BAI & SMITH 1993; FLEURY & BOULÉTREAU 1993; MANICKAVASAGAM et al. 1994).

*Trichogramma cordubensis* Vargas & Cabello (Hymenoptera, Trichogrammatidae) is a native species for the island of São Miguel (Azores) (PINTUREAU et al. 1991). The evaluation of the effectiveness of this species as a biological control agent for agricultural pests existing in the Azores islands has been investigated in our laboratory, by studying the parasitoid biology (PINTO & TAVARES 1991; GARCIA & TAVARES 1995, 1997), population dynamics (GARCIA et al. 1995) and rearing techniques (TAVARES & VIEIRA 1992). As part of this research, the evaluation of the effect of host availability on *T. cordubensis* fecundity and survival rates was analysed in this study.

## MATERIAL AND METHODS

*T. cordubensis* was obtained from parasitized eggs of *Autographa gamma* Linnaeus (Lepidoptera, Noctuidae), collected at Ribeira do Guilherme (São Miguel island). Parasitoid laboratory rearing was made on eggs of the host *Ephestia kuehniella* Zeller (Lepidoptera, Pyralidae) according to the methods of TAVARES & VIEIRA (1992).

In the experiments, eggs of *E. kuehniella* were presented to parasitoids as egg cards that were prepared by mixing one surface of a rectangular piece of index card with a water solution of non-toxic glue, and then spreading the required number of host eggs on this surface. A drop of honey was poured on the surface of the egg card to provide the parasitoids a carbohydrate source. The host eggs were less than 24 hours old and had been previously ultra-violet irradiated for 20 minutes.

To test the effect of host availability on the fecundity and longevity of *T. cordubensis*, the following four treatment groups were assembled: group A (n = 33 females), each female was provided, per day, with a host unlimited egg card (i.e., with 140 ± 8 eggs per egg card); group B (n = 32 females), each female was provided, per day, a host limited egg card (i.e., 10 eggs per egg card); group C (n = 31 females), each female was provided, every 3-day intervals, a host limited egg card (i.e., 10 eggs per egg card); group D (n = 31 females), no host eggs were available for each female. For all treatment groups, each egg card was exposed to the female wasp only during 24 hours. Females from group C were deprived of hosts during the first three days of their lives, therefore the first supply of hosts was only given on day 4. The experiments ran in temperature cabinets at 20 ± 0.5 °C, 75 ± 5 % R.H. and L:D 16:8 h. Cards with parasitized eggs were maintained under the same conditions for wasp progeny development. Wasp fecundity was determined by counting the number of parasitized host eggs (weather parasitoids emerged or not), since the eggs of *E. kuehniella* are relatively small and *Trichogramma* females always oviposit only one wasp per host egg. However, adult offspring was counted to check the any occurrence of superparasitized eggs. The number of parasitized hosts was determined by counting the host eggs that turned black, using a dissecting microscope at 25X. Egg chorions of insects infested by *Trichogramma* turn black when parasitoids are in the prepupal stage. This is caused by black deposits that forms a cocoon-shaped cuticle around the body of the larva and inner surface of the chorion of the host egg (SAAKIAN-BARANOVA 1991). Longevity was determined by checking the number of dead parental females once a day, starting from the day of their adult emergence. Wasps who drowned in the honey were eliminated from the statistical analysis.

Fecundity and longevity comparisons between different treatment groups were performed using an analysis of variance (ANOVA). When statistical differences existed between data sets, a Sheffé test was made to separate the differing means. Fecundity data was transformed by $\sqrt{(x+0.5)}$ to reduce variance differences (ZAR 1996). A regression between lifetime fecundity and longevity data was calculated.

## RESULTS

For all treatment groups only one parasitoid emerged from each host egg, indicating the absence of superparasitism.

When hosts were daily supplied, the greatest progeny production (mean ± s.e.) was observed on the first day for group A (12.9 ± 2.2 parasitized eggs per female), and the first and second days for group B (7.1 ± 1.2 and 7.5 ± 1.3 parasitized eggs per female, respectively), sharply decreasing with oscillating values thereafter on both treatment groups (Fig. 1). However, fecundity for the first day was significantly higher (ANOVA, F=14.527, df=2 & 102, $P<0.0001$) in host unlimited (group A) than in both host limited treatments (groups B and C). The mean (± s.e.) fecundity of parasitoids from group C (host limited with 3-day intervals) was inferior on the first day of parasitism (4 ± 0.7 parasitized eggs per female), but afterward was almost equally distributed throughout female's lifetime. In this group, wasps parasitized almost the totality of available hosts during their existence, except for parasitoids with expanded longevity. For these last, fecundity decreased after the 21st day probably due to depletion of female's egg supply (Fig. 1).

Mean (± s.e.) lifetime fecundity of each female was significantly higher (ANOVA, F=34.570, df=2 & 102, *P* <0.0001) when hosts were daily supplied in unlimited (82.8 ± 6.3 parasitized eggs per female of group A) or limited (85.3 ± 6.5 parasitized eggs per female of group B) numbers, than when a limited number of hosts was provided with 3 day intervals (26.3 ± 2.9 parasitized eggs per female of group C) (Fig. 2).

Longevity did not differ significantly (Sheffé Test, *P* =0.9086) between wasps that were daily given host eggs (group A, mean ± s.e. = 22 ± 1.6 days; group B, mean ± s.e. = 23 ± 1.5 days). However, longevity was significantly higher (ANOVA, F=19.780, DF=3 & 123, *P* <0.0001) for parasitoids that had daily available hosts than those that did not (group C, mean ± s.e = 16 ± 1.1 days; group D, mean ± s.e. = 11 ± 0.7 days) (Fig. 2). A positive (rather than a negative) linear relation was found between the averages of longevity and lifetime fecundity of the wasps (F = 117.919, df = 1 & 94, *P* <0.0001, Fig. 3).

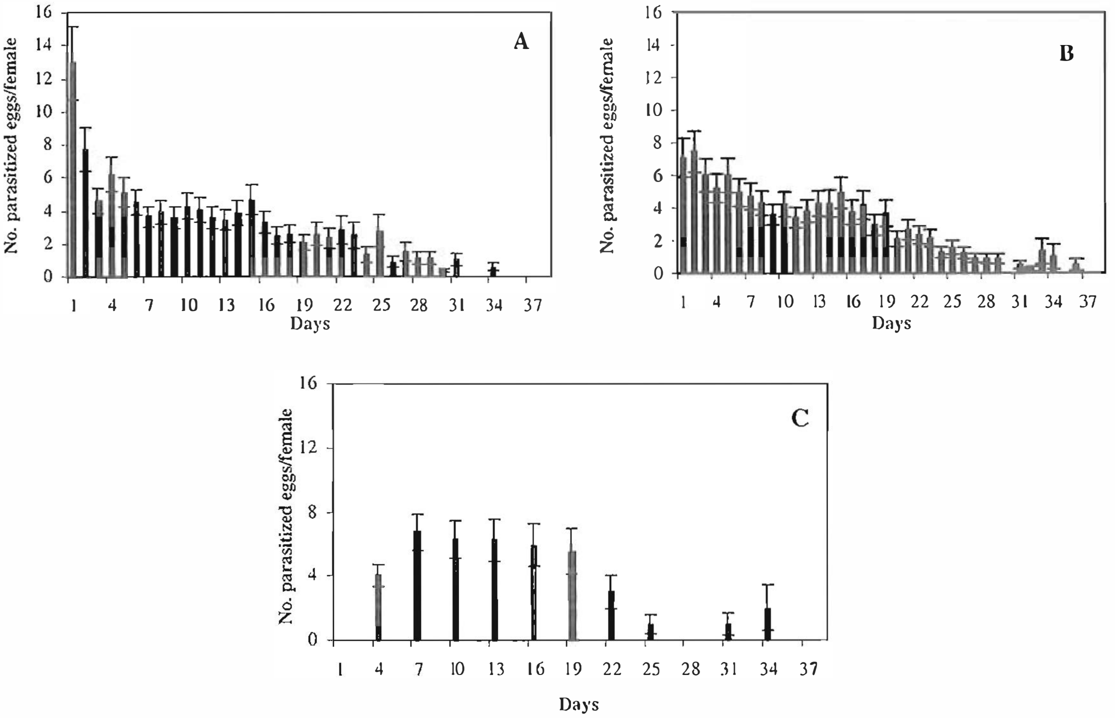

Fig. 1. Number (mean ± s.e.) of parasitized eggs per female of *T. cordubensis*, when daily provided with an unlimited (A) or limited (B) number of hosts, and supplied with a limited number of hosts every 3-day (C).

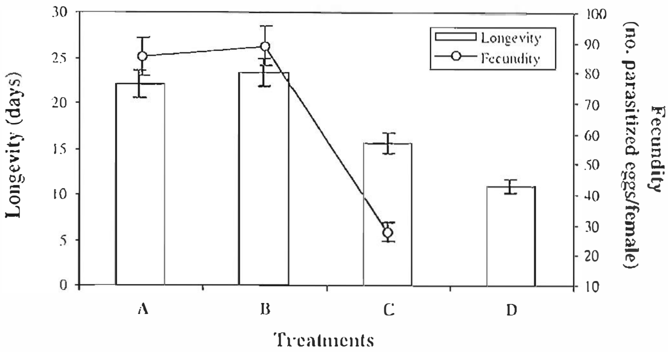

Fig. 2. Longevity (mean ± s.e.) and lifetime fecundity (mean ± s.e.) of *T. cordubensis* females, when daily provided with an unlimited (A) or limited (B) number of hosts, and supplied with a limited number of hosts every 3-day (C).

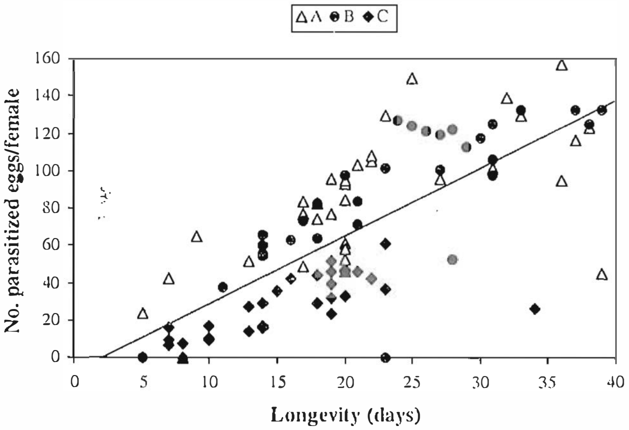

Fig. 3. Relationship of the total number of parasitized eggs per wasp with longevity; $y = -6.224+3.622x$, $r^2 = 0.556$, $P < 0.0001$.

## DISCUSSION

Our results show that the longevity of the wasps was influenced by host availability: wasps that had daily-oviposited (provided daily with hosts) had greater longevity than those that had oviposited only every 3 day or did not oviposit. Adult feeding by the parasitoids on a carbohydrate source increases longevity and fecundity (CABELLO GARCIA & VARGAS PIQUERAS 1985; HOMANN et al. 1989; LAETEMIA et al. 1995). In our experiments all the parasitoids had access to honey as a carbohydrate source. *Trichogramma* has been observed to feed on hosts occasionally after oviposition (TAVARES 1985; MANWEILER 1986; HOMANN et al. 1988). This feeding behaviour allows the intake by the ovipositing wasp of food resources that exist in the host and are not available in honey. The daily consumption of such resources probably increased the wasp's longevity, as our results point out: wasps that were given hosts only every 3-day or lacking hosts available, had a shorter life span in spite of being fed with honey.

A positive linear relation was found between average longevity and lifetime fecundity: females that produced the greatest number of progeny also lived the longest, whereas those that did not produce any progeny had the shortest life span. BAI & SMITH (1993) also found a positive correlation between reproduction and survival of *Trichogramma minutum* Riley but they did not found evidence for a trade-off between wasps' fecundity and longevity. On the other hand, MANICKAVASAGAM et al. (1994) observed opposite relations between fecundity and longevity depending on the species: a negative correlation was observed for *Trichogramma pretiosum* Riley and a positive for *Trichogramma chilonis* Ishii.

Host availability influenced *T. cordubensis* lifetime fecundity: lifetime fecundity was higher when hosts were daily supplied. The reduction in the overall fecundity of the wasps without daily available hosts could result either from their shorter life spans (e.g., parasitoids with lower longevity have a shorter period to oviposit) or due to egg oosorption by the wasp. ANUNCIADA & VOEGELÉ (1982), by dissecting females of *Trichogramma brassicae* Bezdenko, observed that resorption of chorionated eggs occurred after 3 days of host deprivation. Later, FLEURY & BOULÉTREAU (1993) verified that *T. brassicae* parasitization capacity was drastically reduced due to egg resorption when parasitoids were submitted to host deprivation for 4 days. Oosorption is viewed as an adaptive strategy allowing females to conserve their metabolic resources instead of laying eggs under unfavourable conditions. Therefore, if oosorption occurs in *T. cordubensis* when wasps are deprived of hosts and is accompanied by reduction in overall fecundity, then it would be adaptive for this species only if other components of fitness are improved. Such components could be the dispersion of parasitization that would either enhance the avoidance of predation or hyperparasitization. FLEURY & BOULÉTREAU (1993) found that the dispersion of parasitization of *T. brassicae* increased when host deprivation exceeded one day.

*Trichogramma* is considered to be pro-ovigenic (oogenesis is completed prior to adult emergence), therefore females lay most of their eggs shortly after emergence (PAK & OATMAN 1982). However, WANG & SMITH (1996) and VOLKOFF & DAUMAL (1994) showed that thelytokous parasitoids are more similar to synivogenic wasps because they carry few mature eggs in their ovaries at emergence and display a steady pattern of ovarian development. We verified that *T. cordubensis* (a thelytokous wasp) had the highest fecundity on the first day when hosts were daily offered in unlimited or limited numbers, dropping with oscillating values in the subsequent days. If the ovipositing female could count the total number of hosts locally available, then its optimal strategy would be to partition progeny over all available hosts, therefore increasing the fitness of each offspring by reducing the stress of predation and hyperparasitization. However, "host counting" is not possible, so when hosts are available the wasp exploits them as much as possible during the first few days of adult life. Moreover, according to our results when hosts are unavailable for some days, females are able to compensate the reduction in fecundity by dispersing the ovipositions more equally throughout their life, parasitizing most of the hosts they are able to find. These results suggest that *T. cordubensis* is able to adjust fecundity schedule as an adaptation to changing host's resources, which is an important survival feature for this species as host shortage is likely to occur in nature.

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# THE EXPLOITATION OF THE CLAM, *Tapes decussatus* (MOLLUSCA: BIVALVIA), IN SANTO CRISTO LAGOON, SÃO JORGE, AZORES.

ROGÉRIO R. FERRAZ & JOÃO M. GONÇALVES

FERRAZ, R.R. & J.M. GONÇALVES. 2001. The exploitation of the clam, *Tapes decussatus* (Mollusca: Bivalvia), in Santo Cristo Lagoon, São Jorge. *Arquipélago*. Life and Marine Sciences. Supplement 2 (Part B): 51-58. Ponta Delgada. ISSN 0873-4704.

An analysis of the exploitation of the clam. *Tapes decussatus*, in the coastal lagoon of Santo Cristo on São Jorge Island (Azores) is presented from 1991, when a new regime of controlled exploitation was implemented, to 1998. During these years about 6.8 t of clams were caught, with a maximum of 1.75 t in 1993. Annual landings, mean shell length, and catch per unit effort (CPUE – kg/hour) have been decreasing since 1994 (when a maximum of 1.6 kg/hour was reached). In 1998 the annual mean shell length was the lowest since 1991 (47.0 mm). These facts indicate a decrease of the population, at least in the more exploited areas of the lagoon, and/or the inefficiency of the present management model. Alternative management models are discussed.

*Rogério R. Ferraz (e-mail: ferraz@horta.uac.pt) & João M. Gonçalves, Universidade dos Açores, Departamento de Oceanografia e Pescas, Cais de Santa Cruz, PT - 9901-862 Horta, Portugal.*

## INTRODUCTION

The clam *Tapes decussatus* (Linnaeus, 1758) (syn. *Venerupis decussatus*, *Ruditapes decussatus*) occurs in the north-eastern Atlantic, distributed from SE-W England to NW Africa (Morocco to Senegal), including the Iberian Peninsula and the Mediterranean (TEBBLE 1976). The species lives buried in muddy-gravel bottoms from the intertidal to a few metres depth in the subtidal (POUTIERS 1987; POPPE & GOTO 1993).

In the Azores, the clam *T. decussatus* is confined to a unique habitat: the Santo Cristo lagoon on the N. coast of S. Jorge island, which has an area of 0.9 km$^2$. Moreover, this occurrence constitutes the westernmost locality in the geographical range of this species. Several authors have published general descriptions of the Santo Cristo coastal lagoon and its biological communities (e.g. MORTON 1967, SANTOS & MARTINS 1988, FONSECA et al. 1995, MORTON et al. 1998). The work of SANTOS et al. (1990) was focused mainly on the clam population. Santo Cristo lagoon is the only true marine coastal lagoon in the Azores archipelago and was declared as a protected natural reserve in 1989 (Special Ecological Area - DLR 6/89/A, July 18).

*T. decussatus* is believed to have been introduced in Santo Cristo lagoon by man (MORTON 1967). The genetic studies of WILLEMS et al. (1995) suggested that the Santo Cristo population has been isolated from those of the continent only recently.

The clams constitute a small resource that has been traditionally exploited by the local residents. This exploitation was uncontrolled until 1984, when the inhabitants objected to the unregulated harvesting. This led to the prohibition of catches from 1984 until 1989. A new regulated exploitation period begun in 1990 following recommendations by SANTOS & MARTINS (1988). Ten exploitation licences, allowing a monthly catch of 50 kg, are issued annually by the Regional Directory of Fisheries (DRP). A seasonal closure (from 15 May to 15 August) and a minimum catch size (shell length) of 30 mm was fixed. The resident warden of the reserve administered this new regime and filled in the catch control forms.

The exploitation of *T. decussatus* in Santo Cristo lagoon is normally carried out in the intertidal margins of the lagoon and in the upper subtidal using rakes. The rakes have 3 to 4 m long handles and a minimum of 3 cm gap between the teeth.

The number of licences was raised to 13 in 1994, and to 14 from 1995 to 1997. Annual reports of exploitation, based on these forms, were produced by the Department of Oceanography and Fisheries (DOP) of the University of the Azores, as well as recommendations for the next year's activity (GONÇALVES & MARTINS 1991; GONÇALVES 1992, 1993, 1994; GONÇALVES et al. 1995, 1997; FERRAZ & GONÇALVES 1997, 1998).

The clam biomass was estimated in 1988 to ca. 9.4 t. in the intertidal zone and to 36.6 t. in the subtidal (SANTOS et al. 1990). In 1992, clam biomass was estimated to 4.1 t. in the intertidal and to 15.1 t. in the subtidal (GONÇALVES 1994).

In this paper we review the clam exploitation during an eight year regime that ended in 1998.

## METHODS

The reserve warden recorded each catch by the licensed harvesters on appropriate forms. The forms contain the identification of the harvester, total weight per catch, the area of the lagoon where the clams were caught, the number of hours of activity and the characteristics of the tool used. Measurements of a sub-sample of 50 clams (shell length to the nearest mm) from the total catch were also included. The logbooks were sent every month to DOP where the data were introduced in a computer database (Microsoft Access©).

The following parameters were analysed: annual and monthly catches, catch per unit of effort (CPUE – kg/hour), mean shell length, and length frequency histograms (5 mm size classes).

## RESULTS

### 1. LANDINGS

The total declared clam weight increased substantially from 1991 to 1993, which could be due to the increase of the number of licences attributed, and also to a more efficient use of them. Since 1993, the annual catches have decreased slowly (with small oscillations) in spite of an increase in the number of licences. In 1995 and 1997 the landings were the lowest since 1991. The 1998 value consists only of catches from the first four months (Fig. 1) since the resident warden of the reserve resigned from May 1, 1998.

The monthly variation of landings tends to increase from January to April, followed by a subsequent decrease to November, and rising again in December (Fig. 2).

The exploitation was concentrated in certain zones of the lagoon: C, D, F and I, mainly in C and D. (Fig. 3).

### 2. CATCH PER UNIT OF EFFORT (CPUE - KG/HOUR)

From 1991 until 1994 the mean annual CPUE tended to increase. After 1994, when the highest value was reached (1.6 kg/hour), the CPUE decreased slowly. The high variability of the means is indicated by the high standard deviations (Fig. 4).

### 3. SHELL LENGTH

The mean shell length has always been close to 50 mm, with a slight tendency to decrease since 1994. The smallest mean (47.0 mm) was recorded in 1998 (Fig. 5).

The shell length shows a decrease in the predominant size classes since 1991 (modal value of 60 mm) to 1998 (modal value of 35 mm) (Fig. 6).

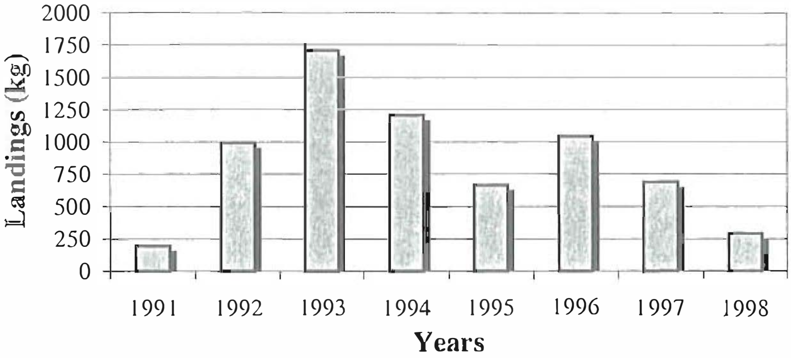

Fig. 1. Annual landings of clams from 1991 to 1998.

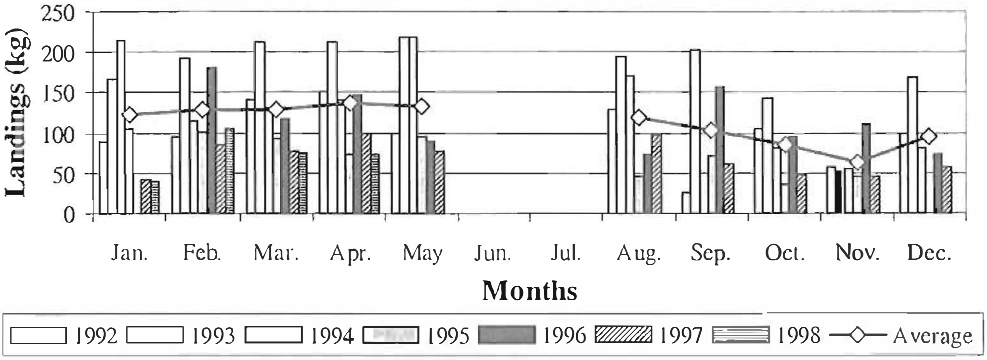

Fig. 2. Monthly variation of landings. Bars indicate values for various years with the average value also given.

## DISCUSSION

The landings of clams (*T. decussatus*) from Santo Cristo lagoon (< 2 t. annually from 1991 to 1998) are very small when compared with other regions (e.g. POUTIERS 1987), even when compared with other artisanal Azorean fisheries (see SANTOS et al., 1995). However, these figures should be considered in the context of the small area of the lagoon. The importance of these clams should not be disregarded as they, and the lagoon, in which they occur, are highly relevant in the Azorean context, especially for the island of S. Jorge. The main reason for declaring the Santo Cristo lagoon as a natural reserve (Special Ecological Area) was the presence of clams.

The general pattern of decrease in landings during the period of regulated exploitation (from 1991 to 1998), indicate a reduction of the stock. The number of catch permits increased slightly during the same period, while the CPUE decreased. Also, the slight decrease in the annual mean shell length as well as the decrease in size of the modal class, support this view. However, it is to be expected that average size should decrease with time in an under-exploited or a new fishery (KING 1997). MORTON & CUNHA (1993) mentioned that the lack of large empty shells in the lagoon is a typical sign of a managed fishery. The average size of *T. decussatus* recorded from Santo Cristo, however, is relatively high when compared with the common catch size on the Portuguese mainland (SANCHES 1992).

The increase of landings and CPUE during the first years (before 1994) of regulated exploitation

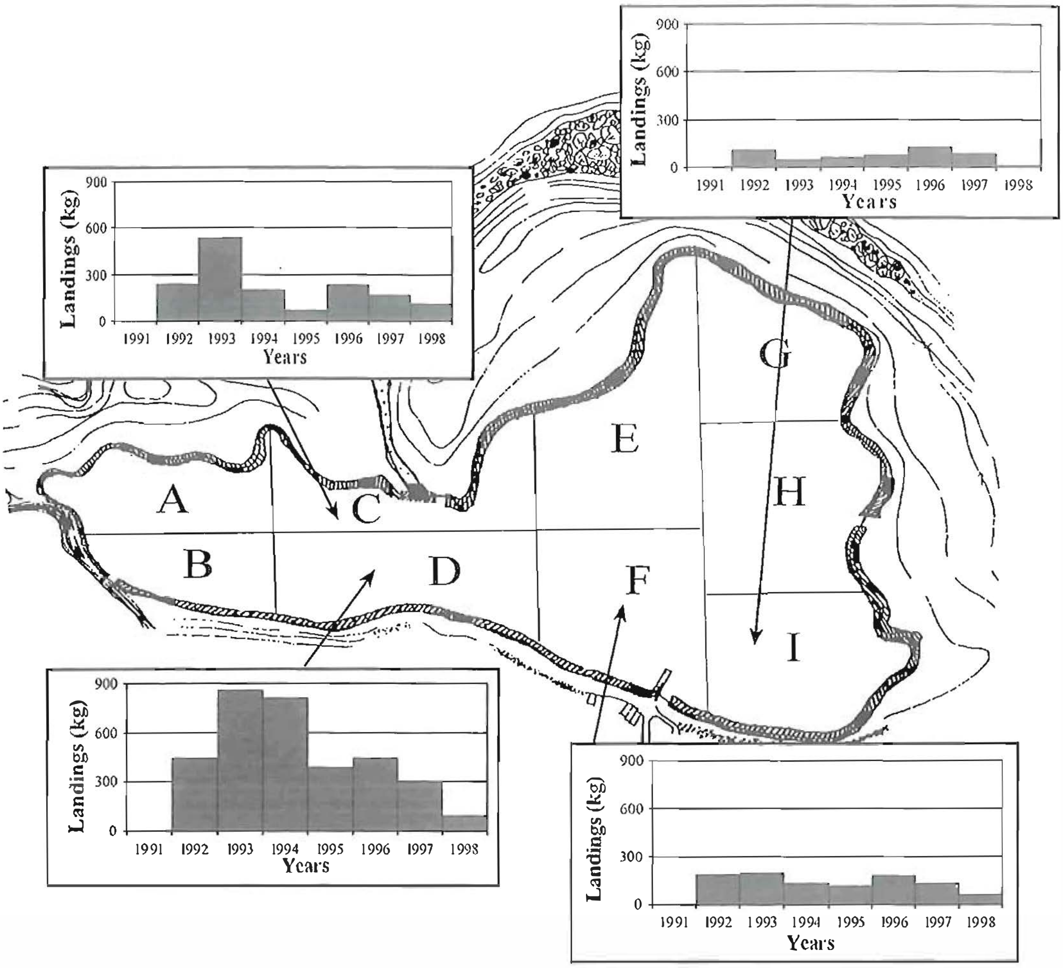

Fig. 3. Annual landings from the main exploited areas in the lagoon.

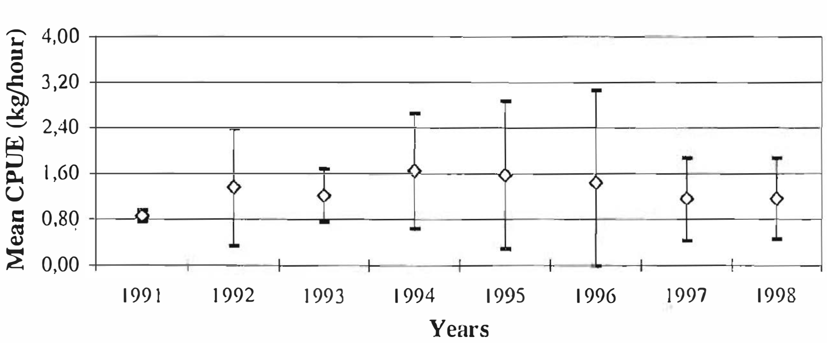

Fig. 4. Annual means of catch per unit of effort (CPUE – kg/hour). Vertical bars represents standard deviation.

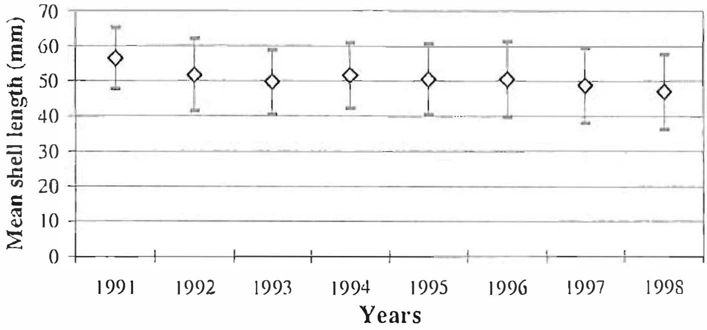

Fig. 5. Variation of annual mean shell length. Vertical bars represent one standard deviation.

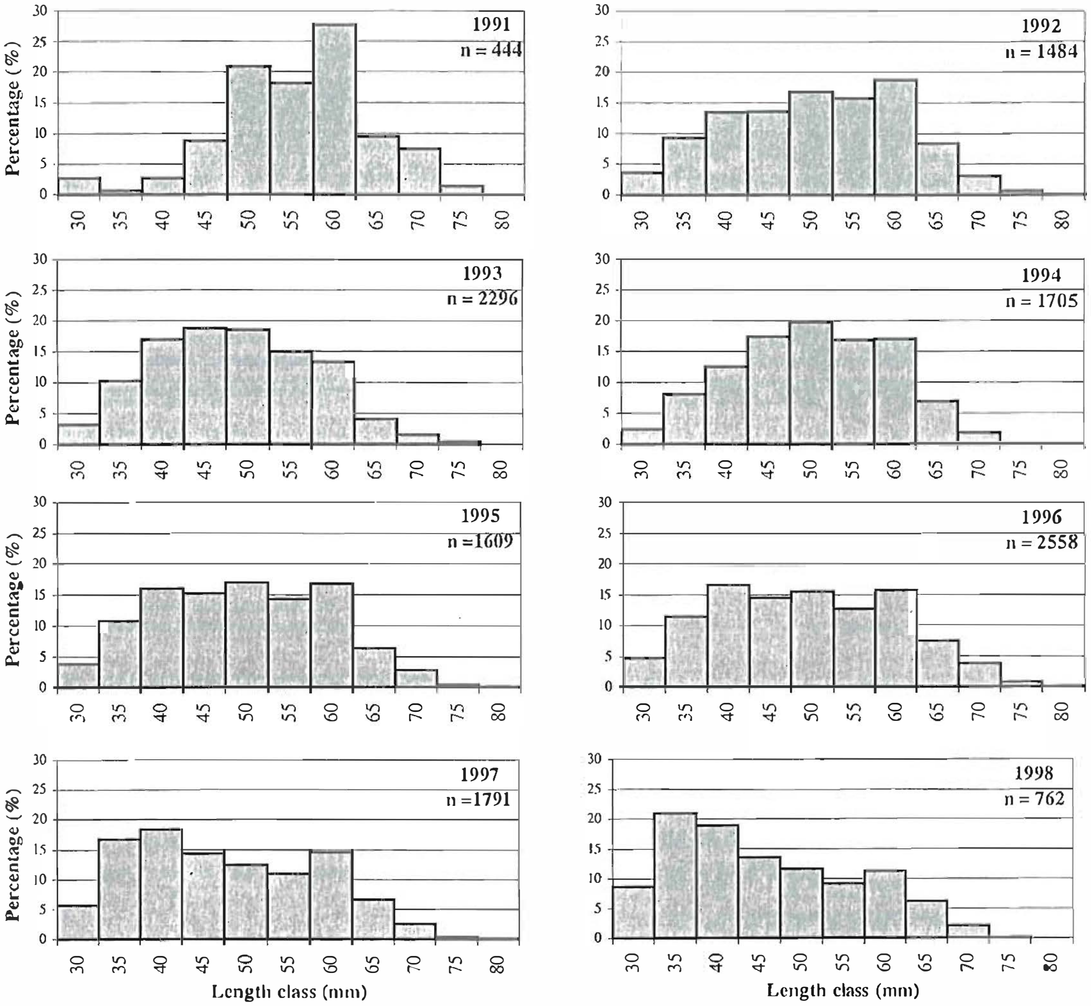

Fig. 6. Histograms of shell length of *Tapes decussatus* in 5 mm size classes, from the Santo Cristo lagoon for the years 1991-1998.

could be due to a higher availability of the resource and/or to an improved efficiency while the effort remained the same. In some cases CPUE's can increase while the resource is already declining (KING 1997).

However, it should also be noted that the decline in landings could be an artefact as undeclared catches could have occurred, making an interpretation of the fishery difficult. As the catch limit is restricted to 50 kg per month, under-declaring or not declaring catches are ways to avoid control. The exploitation of clams was concentrated in a few areas of the lagoon (C, D, F, I - see Fig. 3). There are two main reasons for this. These areas are more accessible for exploitation and they also benefit from the seawater inflow. The type of sediment seems to be less important as various sediment types occur in these areas (FONSECA et al. 1995). Although the density of *T. decussatus* in Santo Cristo lagoon might be higher on the intertidal shore, the biomass is higher in the subtidal, due to the presence of bigger and heavier clams (SANTOS et al. 1990; GONÇALVES 1994).

The accumulated landings of the last 8 years (ca. 6.8 t.) represents 40% of the biomass estimation for clams larger than 30 mm (intertidal + subtidal = 17 t.) estimated by GONÇALVES (1994), which is less than the estimation by SANTOS et al. (1990). However, both biomass estimations should be considered with caution, as the sampling of subtidal clams was insufficient, due to the difficult access.

CAMACHO (1980) refers that 6-7 years is needed by *T. decussatus* in Ria Arosa (NW of Spain) to achieve an average size of 5 cm. The growth rates reported by SANTOS et al. (1990) for the Santo Cristo clams are higher, at least for smaller size classes. This is not surprising since the water temperature in this Azorean lagoon is higher, all year round, than in the Spanish localities. (cf. SANTOS & MARTINS 1988; SANTOS et al. 1990; MORTON & CUNHA 1993; CAMACHO 1980). Unfortunately there are no data on the lagoon's productivity, neither primary or from the clams, to be compared with other regions.

The current regime of controlled exploitation of the clams in Santo Cristo lagoon has now ended, since there is no resident warden to control the catches after April 1998, and it has been very difficult to find a substitute. For 1999 a modification in the management regime was introduced and 9 catch permits were issued. These licensed harvesters will self-register the data from their own catches. If this new exploitation regime does not work, it has to be changed.

As an alternative regime we suggest the implementation of a total allowed catch (TAC) per year based on assessment and productivity studies. The annual TAC might be exploited by licensed fishermen or completely free to be exploited by anyone. The only fixed rules might be the minimum catch size (30 mm) and an exploitation ban during the reproductive season (15 May to 15 August). Monthly analysis of the landings will follow closely the evolution of the exploitation. When the TAC is achieved the exploitation will be stopped. This means that the end of the season might be different from year to year. To implement this model it will be necessary to have a good knowledge of the stock (biomass estimation and productivity) and trustworthy landing data. In this case it will be irrelevant if the fishermen have permits or not. However, it is easier to regulate licensed fishermen, as they will tend to control each other. Another possibility is to use the same system as for limpet exploitation where two kinds of permits are used (FERRAZ et al. 2001). The first, for the general public only for self-consumption, without the issuing of licences, and restricted to a small amount. This will satisfy an old demand from the local inhabitants. The second category, for commercial catches, would need the issue of temporary (annual) permits, in which case the catches should be declared for statistical purposes. The problem with these management models is that it will be necessary to perform annual biomass estimations, which represent an additional cost.

Leaving this resource uncontrolled and overexploited would certainly lead to its destruction and possibly extinction. The area of the lagoon is very small and the high fecundity of clams could be insufficient to counteract the high exploitation. The clams could also become extinct in the Azores if the natural conditions of the lagoon change. A decrease of seawater inflow caused by enclosure of the main channel to the open sea could modify the salinity and other important parameters. The opposite might happen if the seawater entrance is enlarged, which will destroy the sheltered environment. In both cases the environment might be inappropriate for the clams as well as other species.

Another aspect that should be considered is the harvesting method. At present only rakes are allowed. However, this instrument has the disadvantage of digging in the bottom and leaving it disturbed. This action might in fact have adverse impacts on other biological bottom communities, which are also unique and need to be protected (MORTON et al. 1998). Other lesser damaging methods (e.g. snorkelling) should be considered in the future, if really advantageous.

Whatever the model of exploitation, the enforcement and control will always be necessary to ensure the implementation of the regulations. This enforcement should not only be focused on the catch in the lagoon but also elsewhere where the clams are traditionally sold to the public (restaurants, snackbars, etc.).

From a pure conservation perspective for the overall lagoon and its biological communities, the best solution would be to ban the exploitation of clams altogether. However, this will mean the end of an old gastronomic tradition in São Jorge Island, and should be considered.

For a better management of this resource in the future it will be necessary to improve the research as several aspects of the population dynamics of the clams are still unknown. Growth parameters, maturation, mortality, in relation to age and size, as well as biomass estimation are some of the biological parameters that need to be studied.

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# LIMPET (*Patella* spp.) (MOLLUSCA: GASTROPODA) EXPLOITATION IN THE AZORES, DURING THE PERIOD 1993-1998

ROGÉRIO R. FERRAZ, GUI M. MENEZES & RICARDO S. SANTOS

FERRAZ, R.R., G.M. MENEZES & R.S. SANTOS. 2001. Limpet (*Patella* spp.) (Mollusca: Gastropoda) exploitation in the Azores, during the period 1993-1998. *Arquipélago*. Life and Marine Sciences. Supplement 2 (Part B): 59-65. Ponta Delgada. ISSN 0873-4704.

In 1993 the Regional Government of the Azores published a new law in order to regulate limpet harvest in the Azores. Six years after the implementation of this law, the effect on the population was analysed, using the limpet capture diaries filed by the harvesters. In this study we analyse the catch per unit of effort (CPUE) in g/min and give the variability and evolution of the captures in each island. The main conclusions of this study are that the populations appear to have recovered and are stable with regard to their biomass. These results suggest that their exploitation in Central (Faial, São Jorge, Pico, Terceira, and Graciosa) and Western groups (Flores and Corvo), should be allowed as long as the implemented measures are followed. On the other hand, the limpet populations in the Eastern group (Santa Maria and São Miguel Islands) seem to be dangerously low. The implemented rules are believed to have helped the recovery of the limpet population in the Central group. For this reason, we believe that it is necessary to improve the efficiency of the legislation by an active reinforcement by the local authorities as well as by giving relevant information to the general public.

*Rogério R. Ferraz (e-mail: ferraz@horta.uac.pt), Gui M. Menezes & Ricardo S. Santos, Universidade dos Açores, Departamento de Oceanografia e Pescas, Cais de Santa Cruz, PT – 9901-862 Horta, Portugal.*

## INTRODUCTION

The exploitation of limpets in the Azores has probably been taken place since human colonisation of the islands in the 15th century. Until the 1980's the limpet harvest in the Azores was moderate. The limpets were mainly gathered for self-consumption or for door to door bartering. The two co-occurring species (*Patella ulyssiponensis aspera* Röding, 1792 – "Lapa brava"; *Patella candei* d'Orbigny, 1840 – "Lapa mansa") were both harvested, and much appreciated by the local population as an important source protein in some villages (see CORTE-REAL et al. 1992 & 1996 for details on taxonomy). Since no records exist for the few transactions that were made, it is impossible to obtain statistical data prior to 1980 (MARTINS et al. 1987).

With access to snorkelling and scuba diving activities in the 1980's the exploitation of limpets increased drastically. This fact, together with an improvement in refrigeration methods, increased the commercial value and lead to a decline of the stocks, mainly in the Central group (Faial, Pico, Terceira, São Jorge, and Graciosa) during 1983-84. In these islands, the decline was at the time associated with a hypothetical "limpet disease" with unknown causes. In 1985, harvesting of limpets was banned in the central group (SANTOS et al. 1990; MENEZES 1994). This measure, however, increased the exploitation in the other islands, mainly in São Miguel and Santa Maria (Eastern group), which lead to a disruption of the stock. In an effort to save the stocks, a law was approved in 1989, which prohibited all limpet harvest in the Azores. However, in September 1990, non-commercial catch quotas were established for the Western group (Flores and Corvo) (MENEZES et al. 1991).

In 1993, it became evident that the adopted measures were not sufficient to control illegal captures. The limpet price on the "black-market" rose and the number of harvesters increased. For this reason, the Regional Directorate of Fisheries (DRP) in collaboration with the Department of Oceanography and Fisheries (DOP) made contacts with harvesters in order to draft a law that would allow a sustainable harvest and management of the limpet resource. The resulting legislation was published in July 1993.

The following measures were taken:

1. Harvesters have to obtain a license, and declare all the catches by maintaining a capture logbook - limpet capture diaries;
2. A closed season was implemented from October 1 to May 31;
3. A minimum landing size was imposed for both species: 55 mm for *Patella ulyssiponensis aspera* and 30 mm for the *P. candei*;
4. Several reserves and zones with regulated harvesting were created on each island;
5. Since 1996 the DRP has only issued licences to harvesters who declared catches in the previous year.

The aim of this paper is to analyse the results obtained by the implementation of the 1993 law, to suggest modifications that should lead to a sustainable management of this resource in the Azores.

## METHODS

The analysis is based on data from the individual "limpet capture diaries", which are filled in by each harvester every time they sell catches at the official auction (*LOTAÇOR* – Azorean market services). In each diary entrance, besides the total weight of each species, the identification of the harvester, date, locality and method of capture, effort (time), and the environmental conditions are registered. These diaries are sent to DOP every month where the data are introduced into a Microsoft Excel computer database.

In this study we analyse the catches per unit effort (CPUE) variations and the evolution of the captures in each island. The percentage of captures of each species is calculated based on the total landings of this period. The CPUE (in g/min) were calculated for each individual catch (dividing the capture weight - g, by the effort - minutes).

## RESULTS

The number of limpet capture diaries received each month at DOP is presented in Table 1. After 1996 the number of diaries submitted increased substantially.

### LANDINGS

Ninety three percent of the total weight of catches belong to the species *P. u. aspera* the remaining 7% belong to the species *P. candei*.

The total landings declared during 1993 was very low (37 kg) with only 3 landings recorded for that year (Fig. 1). In the following year the total landings increased to 1.734 kg. In 1995 there was a decline, but from 1996 to 1998 the landings increased every year. The highest volume of landings since the implementation of the 1993 law, was recorded in 1998 (6.187 kg).

Table 1

Number of limpets capture diaries received each month at DOP, during the period 1993-1998.

| | **Year** | | | | | |
|---|---|---|---|---|---|---|
| **Month** | **1993** | **1994** | **1995** | **1996** | **1997** | **1998** |
| **June** | | 42 | 37 | 25 | 98 | 100 |
| **July** | | 29 | 27 | 59 | 108 | 119 |
| **August** | 1 | 48 | 23 | 30 | 75 | 74 |
| **September** | 2 | 28 | 3 | 12 | 71 | 47 |
| **Total** | 3 | 147 | 90 | 126 | 352 | 340 |

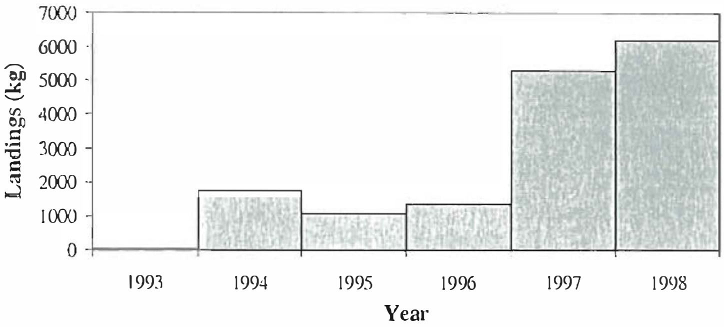

Fig. 1. Total landings (in kg) recorded for all islands of the Azores from 1993 to 1998.

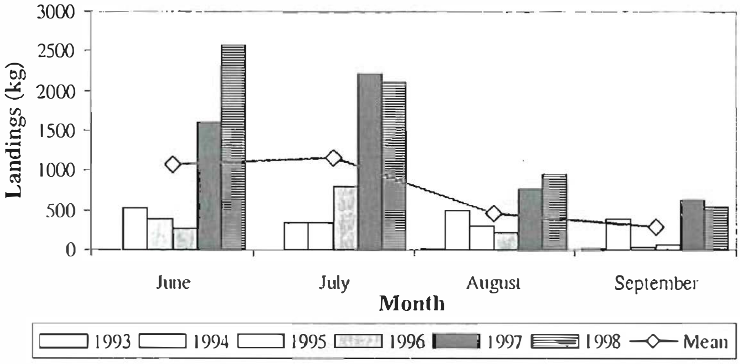

Fig. 2. Total annual landings (in kg) per month and the monthly mean during the open season from 1993 to 1998.

For all years the landings are highest at the beginning of the open season, in June and July, after which they start to decrease (Fig. 2).

Terceira is the only island with landings declared for the whole 6 year period (Fig. 3). In Pico the landings increased every year from 1994 to 1998 and this island presents the highest total landing weights in the archipelago. In Faial and São Jorge the landings have increased almost every year and in 1998 they reached 394 and 914 kg, respectively. The others 3 islands showed very low landings. In 1998, Pico landings (4,229 kg) represent about 70% of the total landings in the archipelago in this year.

Only 32 out of 54 possible zones of exploitation have been effectively exploited (Fig. 4). The results show that only in Pico and São Jorge all possible zones of exploitation were effectively exploited. Zones 24 and 25 in Terceira, and 10, 14, 15 and 16 in Pico correspond to the regions were the harvesters declared the highest limpet catch. In the remaining zones the declared catches were less than 200 kg/year. In the archipelago the most exploited zone during this period, is zone number 16 (in Pico) with a total catch of 2,225 kg.

## Catches per unit effort (CPUE in g/min)

The mean capture per unit effort (CPUE in g/min) increased from 1993 to 1994, and decreased in 1995 (Fig. 5). After 1995, the mean CPUE increased annually and in 1998 the highest effort was recorded (144.94 g/min).

The CPUE differs from island to island (Fig. 6). Santa Maria and Faial islands have the lowest CPUE, 42.4 and 81.5 g/min., respectively. Graciosa (153.6 g/min.), Pico (155.9 g/min.) and São Jorge (150.1 g/min.) presents the highest CPUE values.

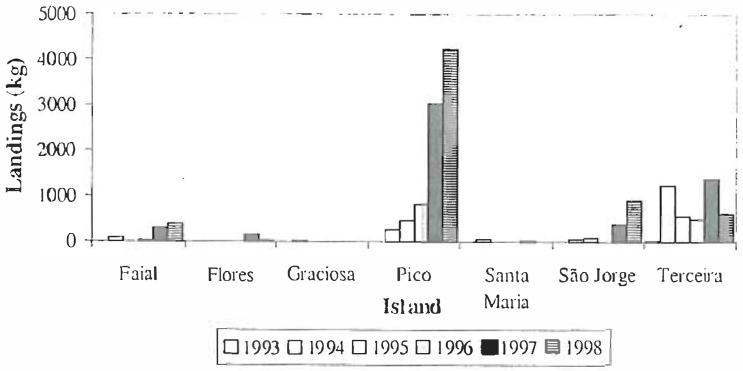

Fig. 3. Limpet landings (kg) recorded for the Azores from 1993 to 1998.

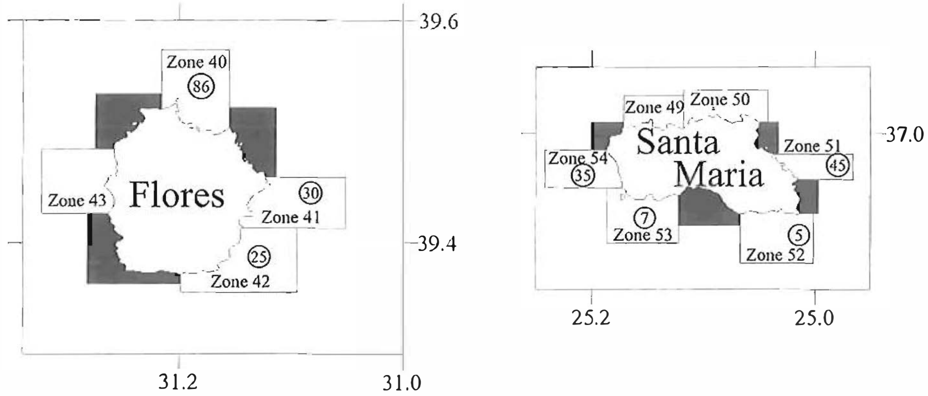

Fig. 4. The total limpet catches declared for each exploited zone in Flores and Santa Maria islands, from 1993 to 1998. Inside each circle are the total landings (in kg) declared for that zone.

## DISCUSSION

The majority of limpets were harvested in the Central group mainly on Pico and Terceira islands. On São Jorge and Faial the harvests were lower but showed an increasing trend. The catches declared from Santa Maria, Graciosa and Flores islands were sporadic. This is probably due to the fact that these three islands are less populated and the few registered harvesters probably sell their catches to their neighbours. From Corvo and São Miguel islands no catches were recorded in this period. These variations seem to be related to different factors (e.g. low stock levels in Santa Maria and São Miguel).

The preliminary results from a biomass survey in progress at DOP show that on islands most affected by the stock decline in the mid 1980s (Pico and Faial) the limpet populations have recovered (FERRAZ et al. 1999). On the other hand, on Santa Maria where the stock was over-exploited, the population has been depleted. In general, the Central group populations have recovered from the mortality that affected them in the 1980's and commercial exploitation is now possible. The Eastern group has not recovered from over-exploitation and as a preventive measure the exploitation should be stopped or at least reduced.

The substantial increase of landings in 1997 and 1998 appear to be connected with the licence issuing policy implemented by the DRP after 1996. Several harvesters who had licences since the beginning of the present legislation (1993)

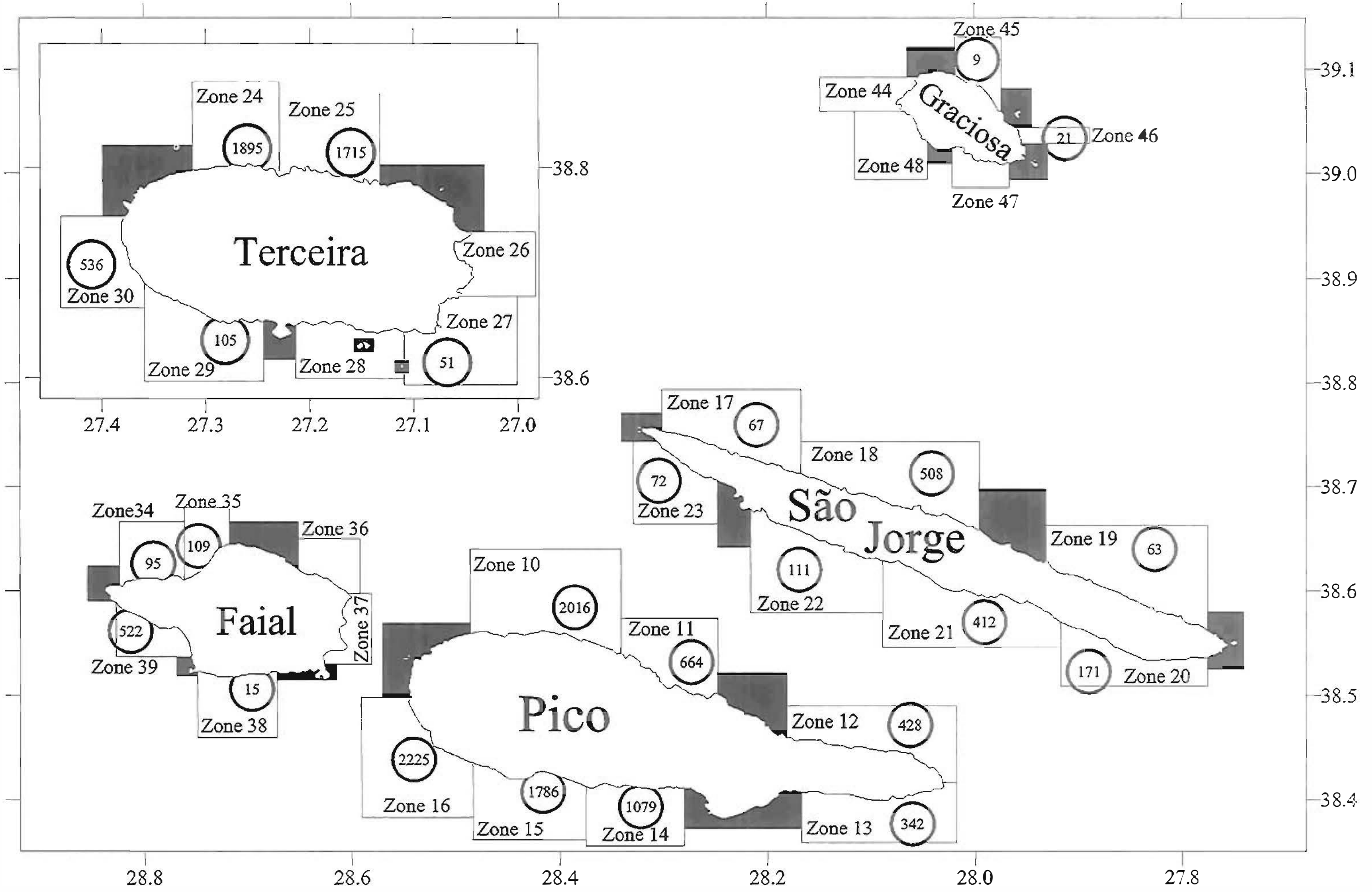

Fig. 4 (continued). The total limpets catches declared for each exploited zone in central group of islands, from 1993 to 1998. Inside each circle are the total landings (in kg) declared for that zone.

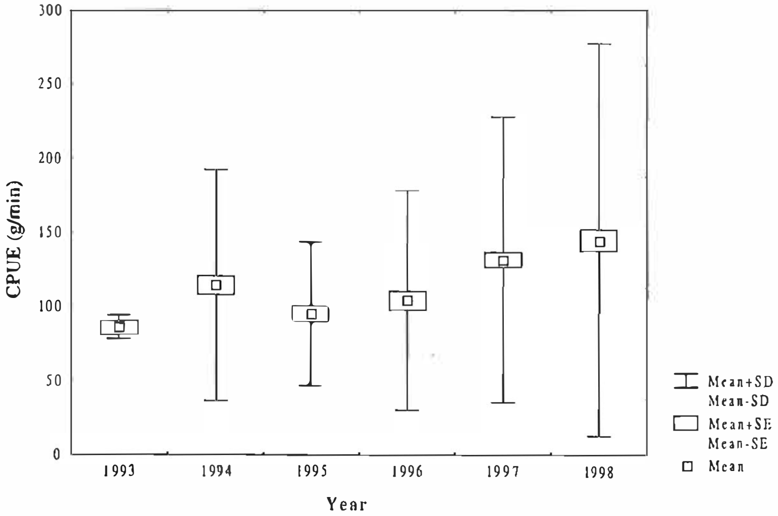

Fig. 5. Mean captures per unit of effort (CPUE in g/min) during the period 1993-1998. SD - Standard deviation; SE - Standard error.

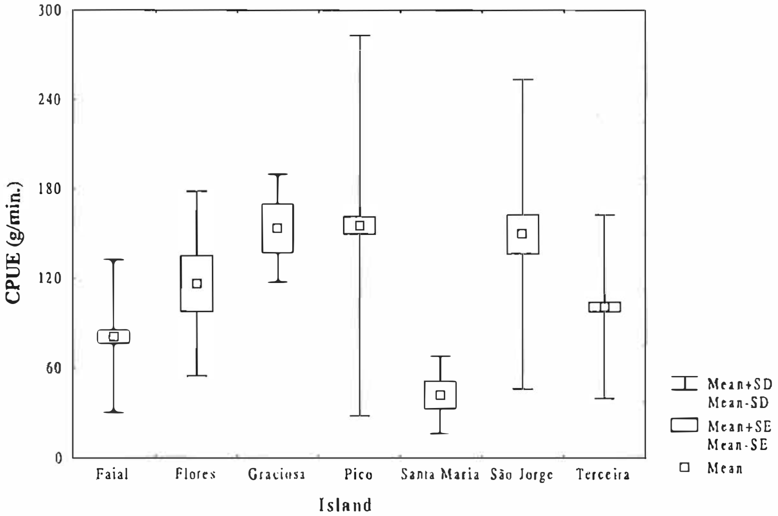

Fig. 6. Mean captures per unit of effort (CPUE in g/min) for each island from 1993 to 1998. SD - Standard deviation; SE - Standard error.

only started to declare their catches after 1996. We believe that this measure has helped to prevent some of the illegal catches recorded by FERRAZ (1998).

The implemented rules (1993 law) are believed to have helped the growth of the limpet population in the Central group. However, they have not been so successful in the Eastern group. For this reason it is necessary to improve current legislation and promote active inforcement by the local authorities as well as increase public awareness and information on this issue.

## ACKNOWLEDGEMENTS

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# INFLUENCE OF TEMPERATURE AND PHOTOPERIOD ON THE MATURATION OF THE SEMINAL VESICLE AND ALBUMEN GLAND IN *Oxychilus* (*Drouetia*) *atlanticus* (GASTROPODA: PULMONATA)

TRISTÃO DA CUNHA, R., A. S. RODRIGUES & H. SOUSA

Tristão da Cunha, R., A.S. Rodrigues & H. Sousa. 2001. The influence of temperature and photoperiod on the maturation of the seminal vesicle and albumen gland in *Oxychilus* (*Drouetia*) *atlanticus* (Gastropoda: Pulmonata). *Arquipélago*. Life and Marine Sciences. Supplement 2 (Part B): 67-70. Ponta Delgada. ISSN 0873-4704.

The land snail *Oxychilus* (*Drouetia*) *atlanticus* is endemic to São Miguel (Azores). In this species, the reproductive system, especially the glandular organs, changes in size and shape during the reproductive cycle. As maturation-diagnostic organs, the seminal vesicle and the albumen gland have complementary interpretations. While the former is appropriate to establish the phase of the pre-copulatory and copulatory periods the latter appears a more accurate way to define pre-egg-laying and egg-laying periods. Concerning the effects of photoperiod and temperature on both organs, it seems that while the former triggers the physiological phenomena related to reproduction, inducing the seminal vesicle and the albumen gland to mature, the latter acts mainly as a regulatory factor for these organs, delimiting the distinct phases of reproduction.

*R. Tristão da Cunha (e-mail: rcunha@notes.uac.pt), A. Rodrigues & H. Sousa, Universidade dos Açores, Departamento de Biologia, Rua da Mãe de Deus 58, PT – 9501-801 Ponta Delgada, Portugal.*

## INTRODUCTION

*Oxychilus* (*Drouetia*) *atlanticus* is a land snail endemic to São Miguel (Azores). In this species, as in Stylommatophora in general, the reproductive system changes in size and shape during the reproductive cycle. These changes, which are better seen in glandular organs such as the seminal vesicle and the spermoviduct, may be used to diagnose maturation (Rodrigues 1995).

Although stylommatophorans are simultaneous hermaphrodites, some species reveal a form of protandry (Baur 1994) in which the male function reaches maturity before that of the female (Sokolove & McCrone 1978). In *Oxychilus atlanticus*, gonadal maturation is characterized by an earlier development of spermatogenesis followed later by vitellogenesis of the oocytes, subsequently showing spermatozoa and oocytes side by side in the same acinus (Rodrigues 1995).

The albumen gland, which is a female organ, is a compound alveolar gland (Tompa 1984), very similar in Stylommatophora in general (Noyce 1973) and it is the last part of the reproductive system to develop, changing in size and colour in adult snails (Rigby 1963; Runham & Laryea 1968; Els 1978; Tompa 1984).

Reproductive development is controlled by endocrine mechanisms, induced by environmental parameters, mainly photoperiod and temperature (Tompa 1984; Baker 1988).

According to Beck (1968) photoperiod promotes the synchrony of internal functions that regulate biological systems, as well as affecting every organism in its geographical distribution, seasonal biology, growing, shape, metabolic rates and behaviour.

In Order Stylommatophora, Parivar (1978) found that temperature affects the gonadal development and the relative proportion of male and female gametes, although it affects spermatogenesis more than oogenesis (Smith

1966), and it is the key factor to initiate spermatogenesis and egg laying (TOMPA 1984). Life cycles and life spans are also affected by changes in both temperature and photoperiod (UMINSKI 1975; TOMPA 1984; SACCHI 1990).

The aim of this study is i) to test the validity of the albumen gland as a new diagnostic organ of sexual maturity, using the seminal vesicle as the reference organ (RODRIGUES 1995), and ii) to relate the general development of both organs with photoperiod and temperature along the reproductive cycle of the species.

## SAMPLING SITE

The climate of the island is of the humid Atlantic type, characterised by prolonged rainy periods in winter and spring, mild temperature (mean 17.5°C), very high air relative humidity (>80%) and soil moisture, even during the summer months (BETTENCOURT 1977). Photophase in the Azores, located between 36°55'N - 39°45'N and 24°45'W - 31°17'W, ranges from around 10 hours in January, to 14h30' in June (Fig. 1A), decreasing at the same rate after July (BECK 1968).

The sampling site, Abelheira, lies about 4 km north-east of Ponta Delgada, 130 m above sea level. Data on temperature from the meteorological station Afonso de Chaves, was used to characterize Abelheira during the sampling period.

## MATERIALS AND METHODS

Sampling of *Oxychilus atlanticus* at Abelheira started in November 1993 and lasted until December 1995. For the study of the seminal vesicle and the albumen gland, 10 individuals with the highest shell diameter were selected from each monthly sample, since this parameter is considered to be the most reliable estimator of age (CHARRIER & DAGUZAN 1978).

In order to examine the correlation between abiotic parameters and the seminal vesicle and albumen gland, data were organized in four sampling periods, winter 1993-spring 1994, summer-autumn 1994, winter 1994-spring 1995, and summer-autumn 1995.

The seminal vesicle was classified according to one of the three degrees of development established by RODRIGUES (1995), based on external morphology: 1) narrow and straight; 2) of intermediate thickness and slightly folded (coiled); 3) thick and heavily coiled.

The albumen gland was measured with EDUSKAN-3.1, a program developed by BELLIDO (1993). Monthly values of the albumen gland were divided into four size classes: 1) small [$<5.0\times10^5$ $\mu^2$]; 2) of intermediate size [$5.0\times10^5$-$10.0\times10^5$ $\mu^2$]; 3) large [$10.01\times10^5$-$20.0\times10^5$ $\mu^2$], and 4) very large [$>20.01\times10^5$ $\mu^2$]. These size classes were considered to reflect the sequence of physiological changes exhibited by the albumen gland during growing.

## RESULTS

In the individuals of *Oxychilus atlanticus* studied, the maximum shell diameter (SD) ranged from 5-6 mm in January-February to 8-8.5 mm in late autumn and early winter. The pattern of growing was very similar from year to year, indicating an annual life cycle with a maximum longevity of 18 months (TRISTÃO DA CUNHA 1995).

According to the degree of maturity shown by the seminal vesicle, individuals were immature or undergoing maturation between January and May and mature between June and November-December (Fig. 1B).

Measurements of the area (size) of the albumen gland (Fig. 1B) show that the organ was not developed between January and June, slowly began to increase between June and October, and reached the highest area in November-December.

### PHOTOPHASE VS. SEMINAL VESICLE AND ALBUMEN GLAND

There is a strong correlation ($r^2=0.942$, $P=0.01$, $N=260$) between photophase and the degree of maturity exhibited by the seminal vesicle. The organ is fully developed in June, when photophase is at its maximum (14h30'), and remains mature until November-December, despite the decrease of photophase (Fig. 1A and B).

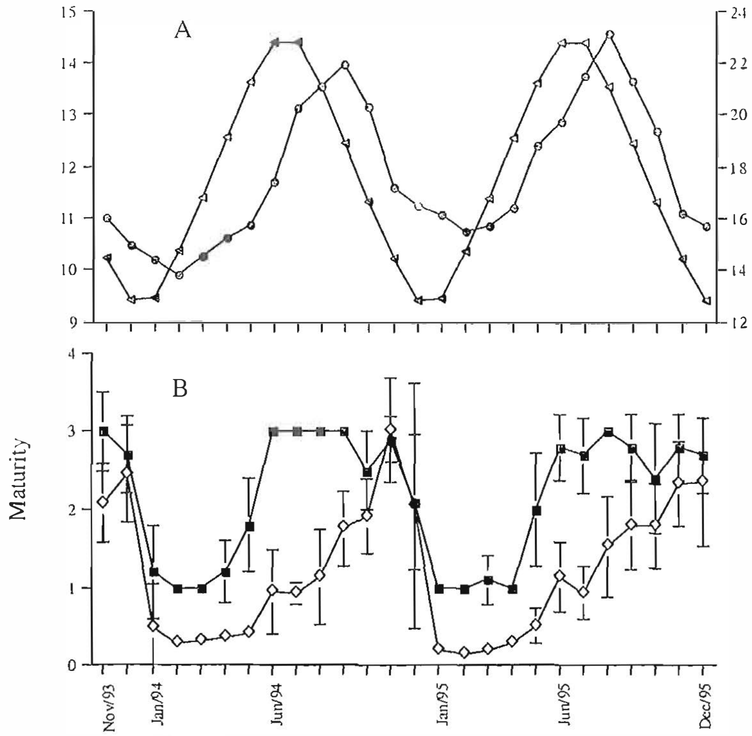

Fig. 1. A) Photophase (hr) (—◀—) for the Azores latitude and Temperature (°C) (—●—) at Abelheira, between November 1993 and December 1995; B) Degrees of maturity of the Seminal Vesicle (average ± sd) (—■—) and size ($\mu^2$) of the Albumen Gland (average ± sd) (—◇—) of *O. atlanticus*, between November 1993 and December 1995, at Abelheira.

Photophase and albumen gland exhibit a similar general pattern but the highest values of the former anticipate those of the latter by 6 months. The correlation between them is low ($r^2$=0.523, P=0.10, N=260). In June when photophase is at its maximum, the albumen gland starts to develop, reaching the highest size in December, despite the decrease in photophase (Fig. 1A and B).

### TEMPERATURE VS. SEMINAL VESICLE AND ALBUMEN GLAND

There is a strong correlation between temperature and seminal vesicle ($r^2$=0.95, P=0.01, N=260). The organ is immature when temperature is lower than 16° C and fully developed when temperature is higher than 18° C (Fig. 1A and B).

The correlation between temperature and albumen gland development is high ($r^2$=0.847 P=0.01, N=260). Maximum values of temperature exhibit a delay of two months relative to the highest measured area of the albumen gland (Fig. 1A and B).

## DISCUSSION

### i) IS THE ALBUMEN GLAND A NEW DIAGNOSTIC ORGAN OF SEXUAL MATURITY?

From data on shell diameter, seminal vesicle maturity and albumen gland size, it is possible to establish that the reproductive system of *Oxychilus atlanticus* develops gradually as the animal grows and it appears to be fully developed when it reaches around 7.5 mm diameter, between June and November.

However, the pattern of maturation in both organs is distinct. The seminal vesicle is mature between June and November, which is in accordance with RODRIGUES (1995), with specimens immature the rest of the year. The albumen gland starts to develop by June but the rapid enlargement only occurs in October - November, signaling final maturation and vitellogenesis of the oocytes in the gonad. A similar general pattern was also observed by RIGBY (1963), in *O. helveticus*, and by SOKOLOVE & MCCRONE (1978), in *Limax maximus*.

As maturation-diagnostic organs of sexual maturity, the albumen gland and seminal vesicle have complementary interpretations. The seminal vesicle may be used to establish the phase of the pre-copulatory and copulatory periods, the albumen gland seems more accurate to define pre-egg-laying and and egg-laying periods.

### ii) EFFECTS OF PHOTOPHASE AND TEMPERATURE ON THE SEMINAL VESICLE AND ALBUMEN GLAND

According to the data presented, photophase correlates with and probably triggers the physiological phenomena related to reproduction, inducing the seminal vesicle and the albumen gland to increase in size, while temperature acts mainly as a regulatory factor for these organs, making a clear demarcation between the distinct phases of reproduction. Further work will be needed to test these hypotheses among.

## ACKNOWLEDGEMENTS

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# MORPHOLOGY AND HISTOLOGY OF THE DIGESTIVE GLAND OF *Oxychilus* (*Drouetia*) *atlanticus* (MORELET & DROUËT) (GASTROPODA: PULMONATA)

M. LOPES, A. RODRIGUES & I. MARIGOMEZ

LOPES, M, A. RODRIGUES & I. MARIGOMEZ. 2001. Morphology and histology of the digestive gland of *Oxychilus* (*Drouetia*) *atlanticus* (Morelet & Drouët) (Gastropoda: Pulmonata). *Arquipélago*, Life and Marine Sciences. Supplement 2(Part B): 71-76. Ponta Delgada. ISSN 0873-4704.

Little information exists on the histology of Zonitidae digestive system. This study deals with a detailed characterisation of the different types of cells comprising epithelium lining the digestive gland of *Oxychilus atlanticus*. From light and scanning electron microscope (SEM) studies, three types of differentiated cells can be identified in the digestive gland: digestive cells, excretory cells and calcium cells. Digestive cells are the most numerous, and are present in two forms, one believed to be absorbing food material and the other secreting material. Excretory cells are distinguished by having a large central vacuole, containing excretory granules. Calcium cells contain spherules of calcium salts, which have a characteristic birefringence.

*M. Lopes, A. Rodrigues,(e-mail:rodrigues@notes.uac.pt), Universidade dos Açores, Departamento de Biologia, Rua da Mãe de Deus 58, PT – 9501-801 Ponta Delgada, Portugal; I. Marigomez, Biologia Zelularra Arloa, Zoologia eta Animali Zelulen Dinamika Saila, Euskal Herriko Unibertsitatea. 644 P. K. ES – 48080 Bilbao, Spain.*

## INTRODUCTION

*Oxychilus* (*Drouetia*) *atlanticus*, a terrestrial zonitid gastropod endemic to São Miguel island (Azores), has been successfully subjected to predatory experiments on *Lymnaea truncatula*, thus showing the carnivorous behaviour of the species under laboratory conditions (CUNHA 1991). Further works, concerning the analyses of stomach contents from individuals collected in the field, have shown the omnivorous tendency of this group in natural conditions (LOPES et al. 1997).

Some studies have been undertaken concerning the alimentary preferences within the family Zonitidae (BOYCOTT 1934; FRÖMMING 1954; RONDELAUD 1975; MORDAN 1977; RONDELAUD 1977; DIDIER & RONDELAUD 1989) but only a few on the anatomy and histology of the alimentary tract (RIGBY 1963; LOPES et al. 1998).

The digestive system of gastropods includes, besides the alimentary tract, the digestive gland, a voluminous organ, which develops from the endoderm and constitutes a large part of the visceral mass (KRESS et al. 1994). This gland, also called mid-gut gland, is a much-branched organ ramifying through most of the visceral mass and ending in numerous blind, small tubules. The tubules open into ductules which join to form ducts, and latter combine to form the hepatic ducts which empty into the stomach (GRIEBEL 1993).

According to OWEN (1966) the digestive gland is thought to perform the following functions: absorption of ingested food material, extracellular and intracellular digestion, secretion, excretion and osmoregulation.

Histological studies on the gastropod digestive gland have shown that cells structure and function vary even within the same group (KRESS et al. 1994). Accordingly, several different types of cells have been identified and described in the digestive gland. The most common types of cells are: digestive cell, microtubule-containing cell, mineral-containing granule cell, excretory cell and calcium cell (RIGBY 1963; LUFTY et al. 1967;

WALKER 1970; KRESS et al. 1994; GRIEBEL 1993).

The present study provides a detailed characterisation of the different types of cells lining the digestive gland epithelium of *O. atlanticus* and, in the process, attempts to describe the morphological organisation of the gland.

## MATERIAL AND METHODS

Specimens of *Oxychilus atlanticus* were collected at Abelheira near Ponta Delgada (São Miguel Island).

For light microscopy, specimens were dissected and the digestive gland removed and fixed in Bouin. Following fixation, the material was dehydrated and then, embedded in paraplast and stained with Haematoxylin/Eosin (MARTOJA & MARTOJA-PEARSON 1970). For the detection of calcium, material was fixed in Carnoy and subject to the Stoeltzener process (MARTOJA & MARTOJA-PEARSON 1970). The blocks of paraplast were cut to the thickness of 7 µm.

For scanning electron microscope (SEM) examination, a particular procedure was followed. The material was fixed in Bouin and embedded in paraplast, a method usually followed for routine optical microscopy. Following this procedure, 60 µm sections were cut, producing blocks with regular surfaces. The blocks were then submitted to a dehydration procedure and finally immersion in hexamethyldisilazane. The liquid phase of the hexamethyldisilazane evaporates in a few minutes, leaving cells whose ultrastructure is comparable to that of the cells dried by critical-point drying (GOLDSTEIN et al. 1992). For stereology studies, in order to obtain the relative frequency of the different cells types, was used the reticule Weibel Multipurpose Test System M168 (WEIBEL 1979). The reticule was superimposed on the image of the digestive gland tubules using a drawing-tube adapted to a microscope Leitz Laborlux S (Leica). The sections of each portion of the digestive gland were randomly selected and viewed at 100x.

## RESULTS

The hepatic ducts of *O. atlanticus* are lined by a ciliated columnar epithelium laying on a thin basement membrane.

The fresh digestive gland is generally yellowish-brown and consists of a single layered epithelium, supported by a thin basement membrane bound by connective tissue and muscle fibres.

According to structural features, three types of cells are identified within the digestive gland epithelium: Cell type I, Cell type II and Cell type III (Figure 1).

### Cell type I (Figure 2, A)

The Cell type I is the most abundant in the digestive gland, corresponding to 82% of the lining epithelium. They are columnar in shape with apical walls either level or round in contour. The cells with level apical surfaces possess a well developed brush border, in contrast with the ones that have rounded apical surfaces, where the same structure is less conspicuous. These cells average 50 µm in height and 17 µm in basal width. Nuclei are basal and oval, and nuclear chromatin can be observed as small granules. Much of the cell volume is filled with granules that give a range of colours, according to their stain reaction.

### Cell type II (Figure 2, B)

These cells are globular, averaging 30 µm in height and 13 µm in basal width. Much of the cell volume is taken up by a spherical central vacuole. Within this, there are excretory granules, which either occur singly or are joined in small groups. The free surface of the cell presents a well defined brush border. The nucleus is basal when the cell reaches the mature stage, corresponding to the vacuole maximum volume. The cytoplasm presents granules that give a similar stain reaction as the ones found in the cell type I. Cell type II corresponds to 10% of the lining epithelium.

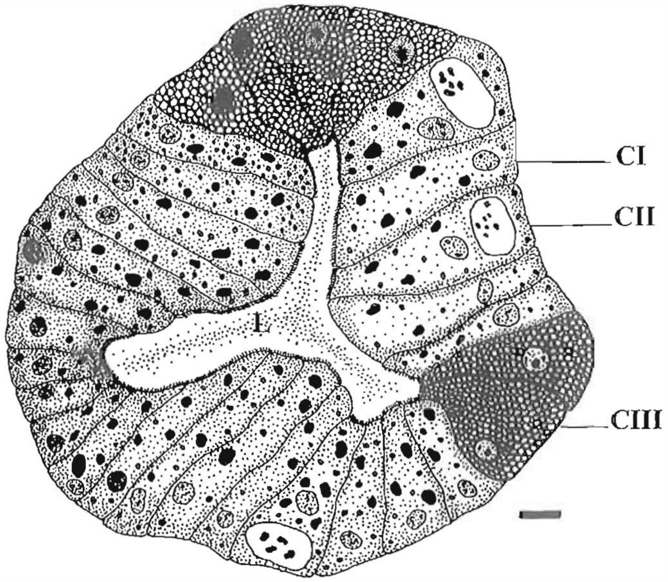

Figure 1. Digestive gland epithelium. C I, Cell type I; C II, Cell type II; C III, Cell type III. Scale bar 10 µm.

Cell type II (Figure 2, B)

These cells are globular, averaging 30 µm in height and 13 µm in basal width. Much of the cell volume is taken up by a spherical central vacuole. Within this, there are excretory granules, which either occur singly or are joined in small groups. The free surface of the cell presents a well defined brush border. The nucleus is basal when the cell reaches the mature stage, corresponding to the vacuole maximum volume. The cytoplasm presents granules that give a similar stain reaction as the ones found in the cell type I. Cell type II corresponds to 10% of the lining epithelium.

## DISCUSSION

The hepatic ducts are responsible for the transport of materials to and from the digestive gland. This function is assisted by features of its lining epithelium, where the movement of the cilia creates a current that aids the transport of particles into the digestive gland, whereas a continuously produced secretion flows out of it (VAN WEEL 1950).

The digestive gland epithelium of *O. atlanticus* is composed of three distinct types of cells: Cell type I, Cell type II and Cell type III.

Following the structural description of the digestive gland of *Agriolimax reticulatus* (WALKER 1970), the cell types found in *O. atlanticus* correspond, respectively, to digestive, excretory and calcium cells. Therefore, our results are in contrast with the two types of cells described by RIGBY (1963) for *O. cellarius*, digestive and excretory cells. Other authors have also described the same three types of cells for various pulmonates (VAN WEEL 1950; GUPTA 1977; DIMITRIADIS & HONDROS 1992).

As suggested by DIMITRIADIS & HONDROS (1992) for *Helix lucorum*, in *O. atlanticus* the digestive cells constitute the major part of the lining epithelium.

The range of colours observed in the digestive cells cytoplasm of *O. atlanticus* could be explained, according to KRESS et al. (1994), by food vacuoles at different stages of digestion.

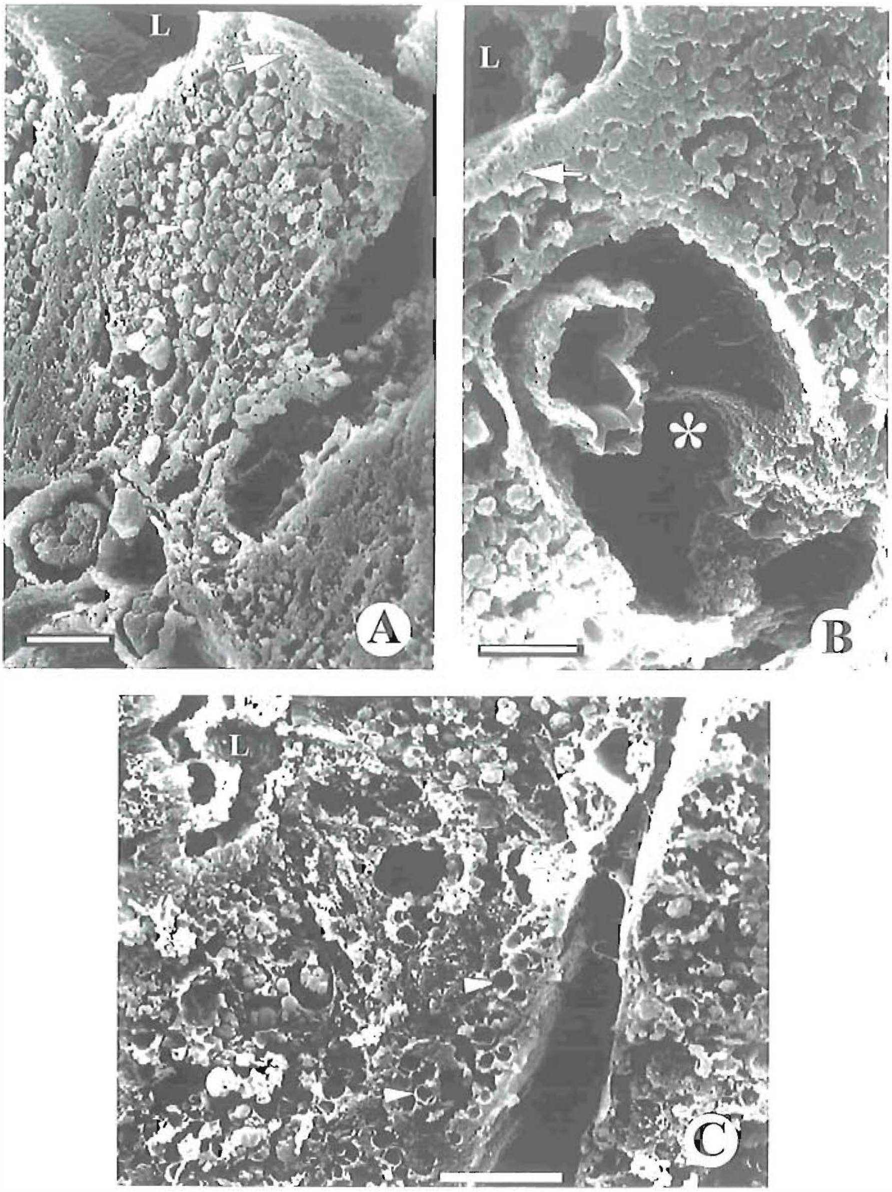

Fig. 2. Digestive gland epithelium (S.E.M.). A (scale bar 10 µm), Digestive cell; B (scale bar 5 µm), Excretory cell; C (scale bar 16 µm), Calcium cell. L, Lumen; * Vacuole; ➡ Brush border; ▸ calcium spherules.

On account of their structural features, the digestive cells can be divided in two different forms: one, believed to be absorbing food material, has a level apical surface that possess a well developed brush border, while the other, mainly involved in secretion, presents a rounded apical surface with a less conspicuous brush border. The presence of two digestive cell forms has been referred to in other pulmonates (DAGUZAN 1985; WALKER 1970).

The similar reaction of the granules found in digestive and excretory cells of *O. atlanticus* suggests a close affinity between both types of cells. This reaction was also noted for other species by WALKER (1970) and DAGUZAN (1985), who considered that excretory cells are derived from digestive cells.

Calcium cells are characterised by the presence of calcium salts, giving a strong positive reaction to the Stoeltzener test. The function of the calcium reserves is not yet certain but, according to the literature, it is believed to participate in many important calcium dependent metabolic reactions, such of shell construction and repair, pH regulation at the digestive tract, and in the reproductive process (WALKER 1970; GUPTA 1977; SIMKISS & MASON 1983; IRELAND & MARIGOMEZ 1992).

The use of special procedures to prepare SEM material provided clear cuts (see figure 2, A, B and C) for 3-D observation and interpretation. Besides, they could be compared with histological sections for light microscopy, which were used as templates.

## ACKNOWLEDGEMENTS

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# COMPARATIVE STUDY OF THE GONADAL INDEX OF *Diadema antillarum* (ECHINODERMATA: ECHINOIDEA) BY TWO METHODS AT GRAN CANARIA ISLAND (SPAIN)

M.J. GARRIDO & R.J. HAROUN

GARRIDO, M.J. & R.J. HAROUN. 2001. Comparative study of the gonadal index of *Diadema antillarum* (Echinodermata: Echinoidea) by two methods at Gran Canaria Island (Spain). *Arquipélago*. Life and Marine Sciences. Supplement 2 (Part B): 77-81. Ponta Delgada. ISSN 0873-4704.

During the last decades the populations of the sea urchin *Diadema antillarum* Philippi, 1845 have experienced a great increase in density and distribution along the Canarian coasts. Diverse aspects of its biology have been reported elsewhere; however, no data are available on the reproductive effort of this sea urchin species at Canary Islands. The objective of this study is to evaluate the monthly evolution of the gonadal index of *D. antillarum* during an annual cycle comparing two different methods in order to determine which is more suitable. Samples were collected monthly in Gran Canaria Island from January 1997 to March 1998 at two locations (Puerto Sardina and Puerto Rico), with different oceanographic conditions. The gonadal index was calculated monthly by two methods previously tested in sea urchins by other authors: (a) the ratio of gonadal volume to dry body weight and (b) the ratio of gonadal volume to body volume. Both gonadal indexes showed the same trend with a maximum in April-May and minimum in September-October.

*Mateo J. Garrido (e-mail: mgarrido@biologia.ulpgc.es), Ricardo J. Haroun Dpto. de Biología. Fac. de Ciencias del Mar. Universidad de Las Palmas de Gran Canaria. ES - 35017 Las Palmas de Gran Canaria, Spain.*

## INTRODUCTION

Gran Canaria island is located between Tenerife and Fuerteventura islands in the Canarian Archipelago at 28° North latitude (Fig. 1). The prevailing North-East trades drive the surface waters to South, in the so called Canary Current. The mean temperature of coastal surface waters from 1981 to 1985 in Gran Canaria Island attained the minimum between February to April (18°C) and the maximum from August to October (25°C) (LLINAS 1988). Due to the shadow effect of the island, the southern sea surface temperature remains warmer during the cooling of the winter months, reaching a difference of about 1°C in February. This situation may be a common feature in the south of the Canary Islands (ARÍSTEGUI et al. 1989).

The main objective of this contribution is to select the easiest method to quantify the reproductive effort of *D. antillarum* at the Canary Islands. The annual evolution of the gonadal index was therefore monthly measured in the two areas, north and south of Gran Canaria island by the two different methods.

Breeding periods of tropical echinoids have been described for different populations by a number of authors. In the Caribbean sea CAMERON (1986) showed at Puerto Rico that, *Lytechinus variegatus* Lamarck, 1816; *Echinometra viridis* A. Agassiz, 1863; and *Echinometra lucunter* Linnaeus, 1758 exhibit reproductive peaks in the warmest months of the year. In contrast, reproduction of *D. antillarum* in the tropical western Atlantic showed little variation among different populations. Animals were reported to spawn throughout the year in Curaçao and the Virgin Islands (RANDALL et al. 1964), but mainly in spring in Barbados (LEWIS 1966), and in fall in the Florida Keys (BAUER 1976), without an evident relationship between reproduction and sea temperature fluctuations.

## MATERIALS AND METHODS

### Field sampling

Two collecting stations, Puerto Sardina and Puerto Rico (Fig. 1), which are subjected to different oceanographic conditions, were sampled at monthly intervals from January 1997 to March 1998. During this study, temperatures were measured with a field thermometer every sampling day (Fig. 1). Monthly collections of sea urchins were made at approximately the same phase of the moon (four days after full moon) to minimise bias introduced by a possible lunar subcycle in spawning (PEARSE 1975). The specimens were collected randomly from both stations within 24 hours. In these easily accessible stations, large natural populations of *D. antillarum* were found, large enough to sustain our regime of sampling without observing any change in population density. Every month twenty sea urchins were caught in depth less than 10 m by snorkelling using long forceps.

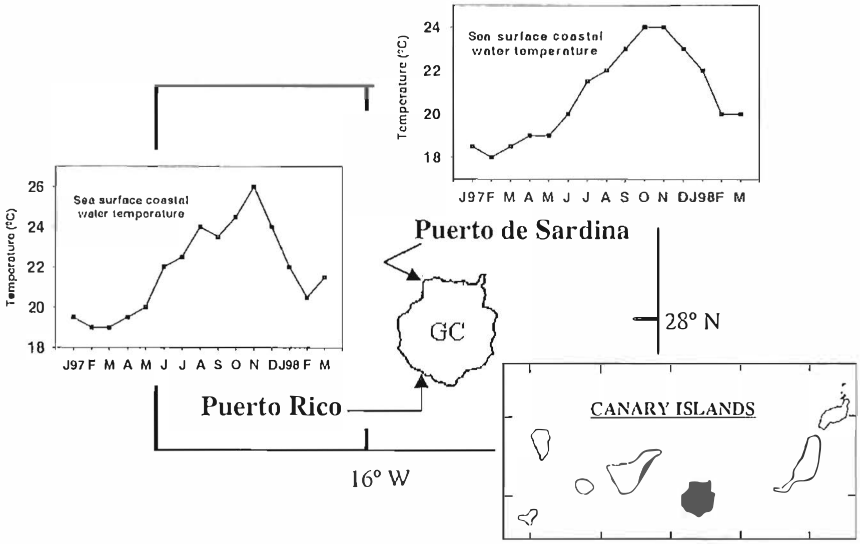

Fig. 1. Sampling stations of *D. antillarum* at Gran Canaria island, Puerto Sardina and Puerto Rico with the sea surface coastal temperature water variations.

### Laboratory procedures

The sea urchins collected were dissected within a day after collection. Each sea urchin was opened around the peristome using scissors, without taking out the spines. The lantern and the gut were removed to obtain the gonads. The volume of the gonads was measured to the nearest 0.1 ml by displacement in seawater, in a 50 ml graduated cylinder. Before dissection, the test of each specimen was measured to the nearest 0.1 mm with a calliper.

Later, each sea urchin with test, lantern, spines, gut, and gonads was labelled and allowed to dry for 24 hours at 110°C in a drying oven, after which they were weighed to the nearest 0.01 g.

Table 1
Results of the t-test analysis between the means of the gonadal index by both methods, dry weight and volume, in the studied locations. A and B, corresponding with Puerto Sardina and Puerto Rico respectively.

| | | J-97 | F | M | A | M | J | J | A | S | O | N | D | J-98 | F | M |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | t | .003 | -.450 | -1.17 | 1.63 | .731 | 1.12 | -.209 | 1.59 | -.658 | -.007 | .039 | -.158 | .366 | .069 | .324 |
| | p | .996 | .655 | .248 | .110 | .469 | .216 | .835 | 2.11 | .514 | .993 | .968 | .875 | .716 | .949 | .747 |
| B | t | .659 | -1.58 | -1.02 | .579 | -.855 | 1.50 | -.586 | .464 | -.639 | -.855 | -.795 | .484 | .455 | .083 | -.193 |
| | p | .513 | .874 | .253 | .565 | .397 | .139 | .560 | .644 | .526 | .397 | .431 | .630 | .651 | .934 | .847 |

The gonadal indices (GI) were calculated by two methods, (a) dry weight, the ratio of gonadal volume to dry body weight, multiplied by 100 (LESSIOS 1981) and (b) volume, the ratio of gonadal volume to body volume calculated by the formulate $V= (4/3)\ \pi\ a^2\ b$, where "a" is the radius of the ambitus and "b" the radius through the oral-aboral axis (ILLIFE & PEARSE 1982).

## Statistical analysis

The t-test was used to compare the near monthly gonadal indices obtained each month by the two methods, dry weight and volume, in Puerto Rico and Puerto Sardina.

To compare the variations of the gonadal indices between locations and through time, two-way analysis f variance was conducted one for each method, dry weight and volume.

To carry out the statistical analysis we used a Box & Cox transformation (GEORGE et al. 1989) and tested for homogeneity of variances using Cochran's test (GALINDO VILLARDÓN 1984).

## RESULTS

The *p* values of the t-test (Table 1) are all above 0.05, indicating no significant differences between means for each method, dry weight and volume, at the two studied locations through the studied period, which means that both methods have equivalent results.

The maximum gonadal index by the dry weight and volume method (Figs. 2 and 3) were in April-May whereas the minimum was on September-October. The same results were obtained by the volume method.

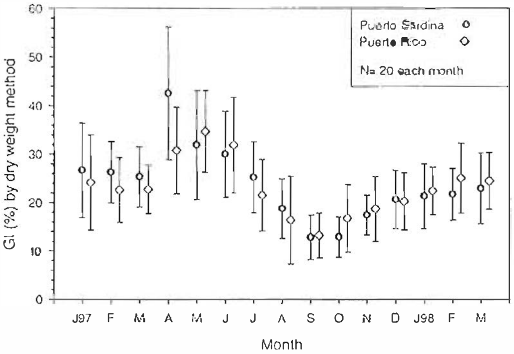

Fig. 2. Monthly Gonadal indices of *D. antillarum* by dry weight method at Gran Canaria island. Mean and standard deviation (vertical bars).

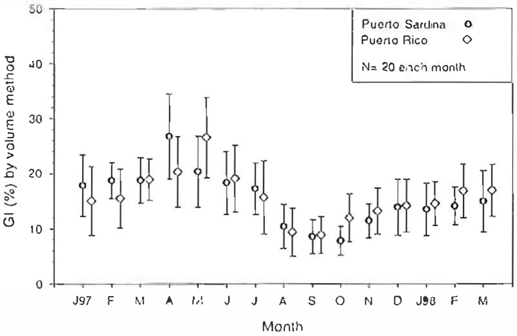

Fig. 3. Monthly Gonadal indices of *D. antillarum* by volume method at Gran Canaria island. Mean and standard deviation (vertical bars).

The result of the two way analysis of variance for each method, volume and dry weight (Table 2 and 3), showed that there were no significant differences in the GI between locations, independent of the method used. However, the Month and the interaction between Location-Month were statistically significant at the 95.0% confidence level.

Table 2
Dry weight method analysis of variance with two fixed factors, Location and Month. (N. S. = non significant).

| Source | Sum of Squares | Df | Mean Square | F-Ratio | Probability |
|---|---|---|---|---|---|
| Main Effects | | | | | |
| Location | 0.660919 | 1 | 0.660919 | 0.90 | N.S. |
| Month | 321.608 | 14 | 22.972 | 31.24 | <0.001 |
| Interactions | | | | | |
| LxM | 22.5809 | 14 | 1.61292 | 2.19 | <0.05 |
| Residual | 419.097 | 570 | 0.735257 | | |
| Total | 763.947 | 599 | | | |

Table 3
Volume method analysis of variance with two fixed factors, Location and Month. (N. S. = non significant).

| Source | Sum of Squares | Df | Mean Square | F-Ratio | Probability |
|---|---|---|---|---|---|
| Main Effects | | | | | |
| Location | 0.384422 | 1 | 0.384422 | 0.65 | N. S. |
| Month | 273.296 | 14 | 19.5211 | 33.06 | <0.001 |
| Interaction | | | | | |
| LxM | 27.8496 | 14 | 1.98926 | 3.37 | <0.001 |
| Residual | 419.097 | 570 | 0.590414 | | |
| Total | 638.065 | 599 | | | |

## DISCUSSION

The same trend in gonadal reproductive effort was observed irrespective the location and the method used (Fig. 2 and 3, Table 1). It is necessary to point out that the volume method is faster and easier to perform than the dry weight method because it can be done completely in the field. The size of the monthly sample used was large enough (n=20) for our research purposes as was indicated by the similar standard deviations for the different months.

In Gran Canaria Island, the gonadal index of *D. antillarum* showed a continuous reproduction along the year with a general maximum in both locations when the sea surface coastal water temperature was still cold, about 20°C, April-May, and the minimum was reached when the coastal water temperature was warm, about 26°C, September-October. Our results show that the monthly variations in GI are not random and some external factors such as temperature and/or primary production could have played an important role. The field temperature data obtained monthly from the coastal waters were highly consistent with the mean temperature data for 1985 to 1989 (LLINAS 1988).

Authors at different locations have related seasonal variations of water temperature with sea urchins spawning. This relation was reported for *D. antillarum* by RANDALL (1961), RANDALL et al. (1964) who observed spawning in winter and early spring in Virgin Islands and Curaçao, while LEWIS (1966) in Barbados observed spawning in May 1963 and April 1964 when sea temperatures were about 27°C. LESSIOS (1981) found a major peak in October at Fort Randolph but no seasonality at Maria Chiquita, Panama (Atlantic coast). BAUER (1976) reported the spawn in Florida in November (1968) in the period of low temperatures and suggested a strong relation between seawater temperatures and reproduction in *D. antillarum* at the northern limit of the distribution of this species. ILLIFE & PEARSE (1982) also observed this relation in Bermuda: the gonads grow mainly in spring when sea temperatures are increasing, and spawning occurs from early summer to early winter. These observations together with our data suggest that the relationships between temperature and reproduction are stronger for *D. antillarum* in higher latitudes of the Atlantic Ocean. However more seasonal variables as primary production, should be related with sea urchin reproduction to obtain a final conclusion concerning the factors that controls their reproduction effort.

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# ASPECTS OF THE REPRODUCTION OF SIX EXPLOITED DEMERSAL FISH SPECIES IN THE AZORES ARCHIPELAGO

S. ESTÁCIO, A. MENDONÇA, H. KRUG, G.M. MENEZES, & M.R. PINHO

ESTÁCIO, S., A. MENDONÇA, H. KRUG, G.M. MENEZES, & M.R. PINHO. 2001. Aspects of the reproduction of six exploited demersal fish species in the Azores archipelago. *Arquipélago*. Life and Marine Sciences. Supplement 2 (Part B): 83-94. Ponta Delgada. ISSN 0873-4704.

Important reproduction aspects of six demersal species from Azores were studied: red (blackspot) seabream (*Pagellus bogaraveo*), bluemouth (*Helicolenus dactylopterus*), two alfonsinos (*Beryx splendens and Beryx decadactylus*), yellow-orange scorpionfish (*Pontinus kuhlii*) and silver scabbardfish (*Lepidopus caudatus)*. Sex ratios were calculated and tested by chi-square analysis. In order to determine the size at first maturity, maturation ogives were estimated using Logistic or Gompertz models. The gonadosomatic index was determined for those species for which the reproductive period was not well known. The ratio of males to females was significantly different ($p<0.05$) from 1:1 for all species: *P. bogaraveo* (1:0.20*)*, *H. dactylopterus* (1:0.86), *B. splendens* (1:1.25), *B. decadactylus* (1:1.38), *P. kuhlii* (1:1.75) and *L. caudatus* (1:1.74). Length and corresponding age (years) at first maturity were: *P. bogaraveo* (females - 29.2 cm FL, age 4; males - 26.2 cm FL, age 3), *H. dactylopterus* (females - 21.8 cm TL, age 3; males - 28.1 cm TL, age 5), *B. splendens* (females - 23.0 FL, age 2; males - 22.9 cm FL, age 2), *B. decadactylus* (females - 32.5 FL, age 4; males - 30.3 cm FL, age 4), *P. kuhlii* (females - 22.6 cm TL, age 5; males - 29.9 cm TL, age 9) and *L. caudatus* (females - 114.9 cm TL, age 3; males - 85.0 cm TL, age 1). The results were compared with other studies and contributed to a better knowledge of the biology of these species in the Northeast Atlantic.

*Susana Estácio (susana@drp.raa.pt), Secretaria Regional de Agricultura e Pescas, Rua Cônsul Dabney, Colónia Alemã, PT – 9900-014 Horta, Portugal. - Ana Mendonça, Helena Krug, Gui M. Menezes & Mário R. Pinho, Universidade dos Açores, Departamento de Oceanografia e Pescas, Cais de Santa Cruz, PT– 9901-862 Horta, Portugal.*

## INTRODUCTION

Since 1982, a significant part of the research efforts developed in the Department of Oceanography and Fisheries (University of Azores) has been the study of the biology and ecology of demersal fishes of Azores in order to monitor the status of the stocks.

The demersal fishery is a multi-species fishery involving more than 20 species and representing economically the second most important fishery in the Azores region. Distribution of these species is limited to the edge of the islands, banks and seamounts. Distribution patterns and biological characteristics such as hermaphroditism and high longevity contribute to a particular vulnerability of these demersal species to overexploitation (SILVA et al. 1994; KRUG 1995).

The published information on red (blackspot) seabream, bluemouth, the two alfonsinos and yellow-orange scorpionfish includes studies of reproduction from the Azores (KRUG 1990, 1995; ISIDRO 1989, 1996). Because there is still a lack of information, this study will contribute additional baseline life-history knowledge of these species in Azorean waters. To date, no studies of reproduction of silver scabbardfish are known for this region.

The aim of this work was to study reproductive aspects of six important commercial species from the Azores: red (blackspot) seabream (*Pagellus bogaraveo*), bluemouth

(*Helicolenus dactylopterus*), two alfonsinos (*Beryx splendens* and *Beryx decadactylus*), yellow-orange scorpionfish (*Pontinus kuhlii*) and silver scabbardfish (*Lepidopus caudatus).*

## MATERIALS AND METHODS

### Fish Sampling

Samples of six demersal species from Azores archipelago were collected yearly in the local market from 1985 until December 1997. Thereafter, monthly sampling was carried out until July of 1998. During the spring/summer survey cruises of 1995, 1996 and 1997 onboard the R/V ARQUIPÉLAGO using bottom longline, a great number of specimens was also collected. The sampling efforts produced a total of 2895 specimens of *P. bogaraveo*, 2293 of *H. dactylopterus*, 806 of *B. splendens*, 355 of *B. decadactylus*, 456 of *P. kuhlii* and 909 of *L. caudatus.*

Fork length (cm) was measured in *P. bogaraveo, B. splendens* and *B. decadactylus* and total length (cm) in *H. dactylopterus, P. kuhlii* and *L. caudatus*. The total weight (g) and the gonadal weight (g) were also obtained. Whenever possible, the sex and maturity stages were identified macroscopically.

Three types of ovotestis were identified in hemaphrodites, as observed by LAMRINI (1986) in *Pagellus acarne* and by KRUG (1990) in *Pagellus bogaraveo*: functional testes with ovarian tissue (the ovarian part is halted at the early stage of gametogenesis), gonads in which male and female parts were approximately the same size but the functional part was not well defined (transitional stage) and functional ovaries with a degenerated testicular part.

The maturity stages were determined based on the following scale, adapted from HOLDEN & RAITT (1974): 0 - Immature, 1 - Resting, 2 - Developing - Beginning and development of maturation, 3 - Pre-spawning, 4 - Mature - Running, 5 - Spent.

### Data Analyses

Sex-ratios were calculated and tested for deviation from 1:1 by chi-square analysis. The length classes were grouped by 10 cm intervals in *Lepidopus caudatus* and by 2 cm intervals in the other species.

Size at first maturity was considered as the length at which 50% of the individuals are mature ($L_{50}$). Maturity stages 1, 3, 4 and 5 were considered sexually mature. As in LOZANO et al. (1990), the size at first maturity ($L_{50}$) was obtained applying the Gompertz model or the Logistic model and the choice between them was determined by considering the best coefficient of determination ($r^2$) obtained. The models are expressed in the following way (KAUFMANN 1981; ISIDRO 1996):

$$P = 100 \times e^{\left[-e^{-a\left(L+L_i\right)}\right]} \qquad \textit{Gompertz Model}$$

$$P = \frac{100}{1+e^{-r(L-L_{50})}} \qquad \textit{Logistic Model}$$

P: Percentage of mature individuals,
a: Constant; the slope of the asymmetrical curve,
r: Constant; the slope of the symmetrical curve,
L: Length class,
$L_i$: Length at the inflection point,
$L_{50}$: Length at which 50% of the individuals are mature or at the inflection point.

The age at first maturity was obtained for both sexes by using the age-length key previously determined by KRUG et al. (1998) to determine the most frequent age in the length class represented by $L_{50}$.

For *H. dactylopterus* and *L. caudatus*, the season of reproductive activity was identified from a gonadosomatic index (GSI) for females and males separately. This index was calculated from the relationship between gonadal (GW) and total (TW) weights (GSI = GW / TW x 100). For the others species, in spite of the great number of samples, data were not collected every month of the year and could not be used to analyse monthly changes of GSI.

## RESULTS

### Pagellus bogaraveo

Of a total of 2895 specimens examined, 357 (12.3%) were females, 1795 (62.0%) males and 743 (25.7%) hermaphrodites. The overall ratio of males to females was 1:0.20 and $\chi^2$-test revealed a significant difference from 1:1 ratio ($P<0.05$). Females predominated in large length classes, while males were abundant at smaller lengths (Fig. 1).

Length at 50% maturity was 29.2 cm ($r^2=0.87$) for females and 26.2 cm ($r^2= 0.99$) for males respectively (Fig. 2), based on the Gompertz model. These sizes at first maturity corresponded to the ages of 4 (females) and 3 (males) years (KRUG et al. 1998).

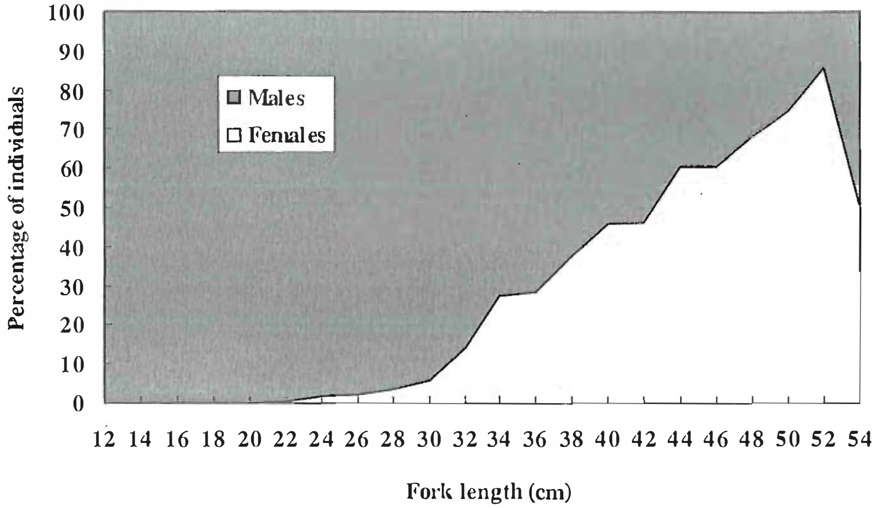

Fig. 1. Sex composition of *Pagellus bogaraveo* by 2 cm length classes.

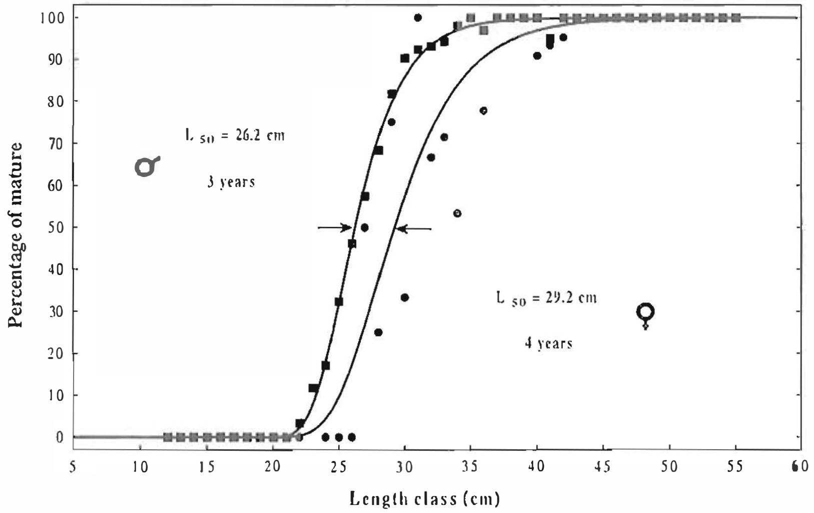

Fig.2. Maturation ogives for male (N=1795) and female (N=357) *Pagellus bogaraveo*.

*Helicolenus dactylopterus*

Of the 2293 fish examined, 1058 (46.1%) were females and 1235 (53.9%) males. The overall sex ratio of males to females was 1:0.86 and $\chi^2$-test revealed a significant difference from 1:1 ratio ($P<0.05$). Males were more abundant in the largest length classes (Fig.3).

Length at 50% maturity was 21.8 cm ($r^2= 0.98$) for females and 28.1 cm ($r^2= 0.95$) for males based on the Gompertz model (Fig. 4). The corresponding ages at first maturity were 3 and 5 years respectively (KRUG et al. 1998).

The most significant reproductive period seemed to be between January and May for females (Fig. 5). For males, the highest GSI values occurred during June and July, although data were lacking for August through November, (Fig. 6). An asynchrony was observed between reproductive cycles of females and males.

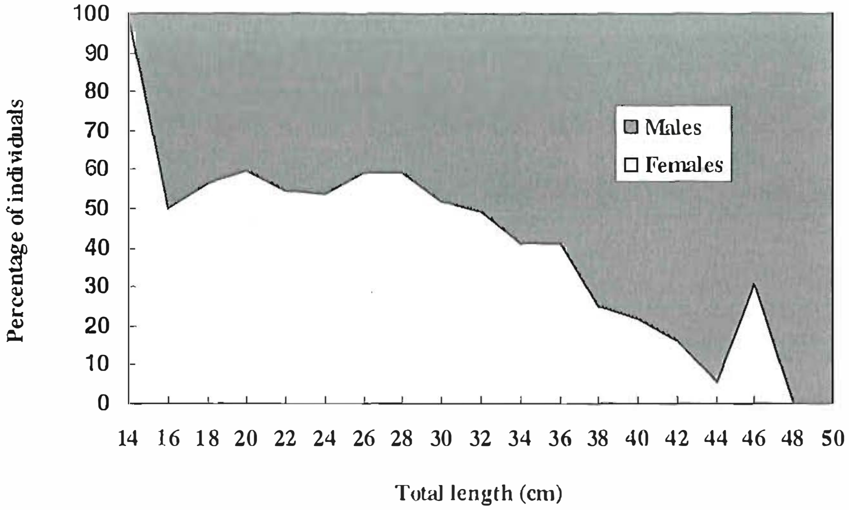

Fig. 3. Sex composition of *Helicolenus dactylopterus* by 2 cm length classes.

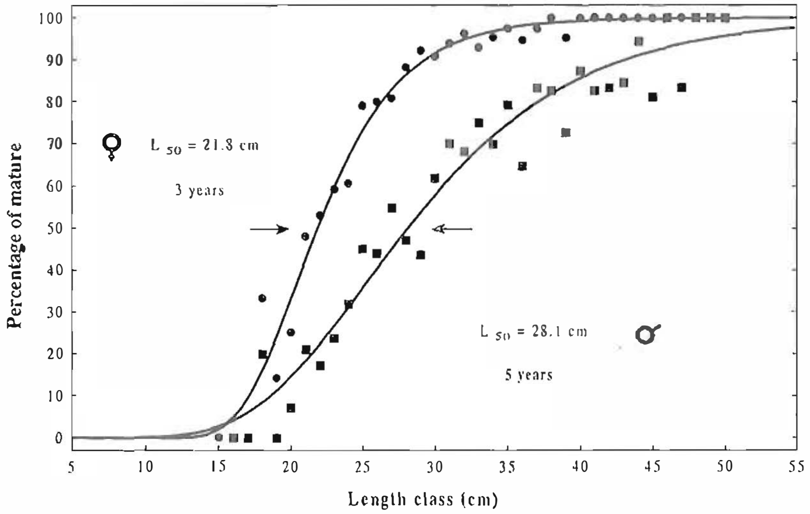

Fig. 4 . Maturation ogives for male (N=1235) and female (N=1058) *Helicolenus dactylopterus*.

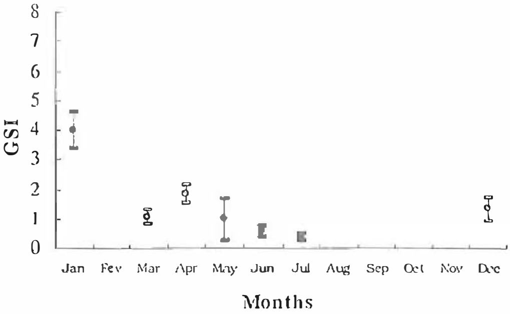

Fig. 5. Mean monthly gonadosomatic index (GSI) of females *Helicolenus dactylopterus* (± SD).

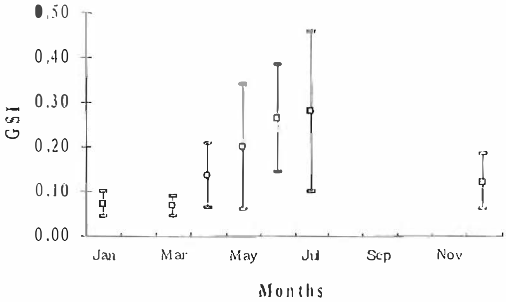

Fig. 6. Mean monthly gonadosomatic index (GSI) of males *Helicolenus dactylopterus* (± SD).

*Beryx splendens*

Of the 806 individuals examined, 447 (55%) were females and 359 (45%) males. The overall ratio of males to females was 1:1.25. The $\chi^2$-test revealed a significant departure from 1:1 ratio (P<0.05) (Fig. 7). Females were more abundant, especially in the length classes ≥ 32 cm.

Length at 50% maturity was 23.0 cm ($r^2$= 0.90) for females and 22.9 cm ($r^2$= 0.94) for males respectively, based on the application of the Logistic curve (Fig. 8). These values correspond to an age at first maturity of 2 years for both sexes (KRUG et al. 1998).

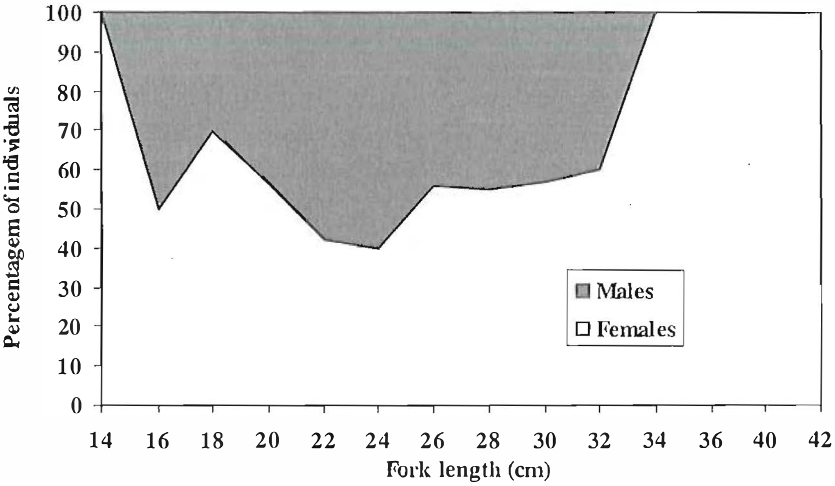

Fig. 7. Sex composition of *Beryx splendens* by 2 cm length classes.

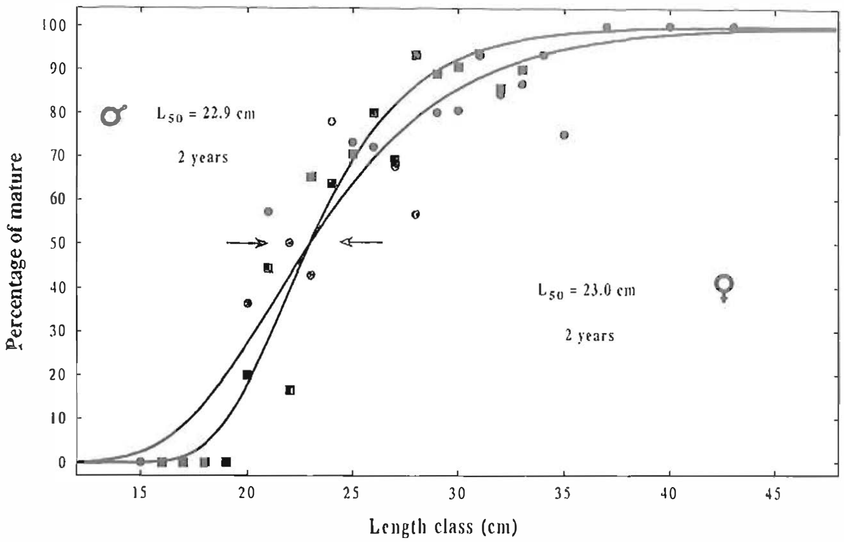

Fig. 8. Maturation ogives for male (N=359) and female (N=447) *Beryx splendens*.

### *Beryx decadactylus*

Of a total of 355 individuals, 206 (58%) were females and 149 (42%) males. The overall ratio of males to females was 1:1.38 and $\chi^2$-test revealed a significant departure from 1:1 ratio (P<0.05) (Fig. 9). No defined trends in sex ratio seemed to exist.

For females the best fit was achieved through the Logistic curve that indicated a length at 50% maturity of 32.5 cm ($r^2$= 0.93) and for males it was the Gompertz curve that showed the best fit with a length at 50% maturity of 30.3 cm ($r^2$= 0.91). The age at first maturity was 4 years for both sexes (Fig. 10) (KRUG et al. 1998).

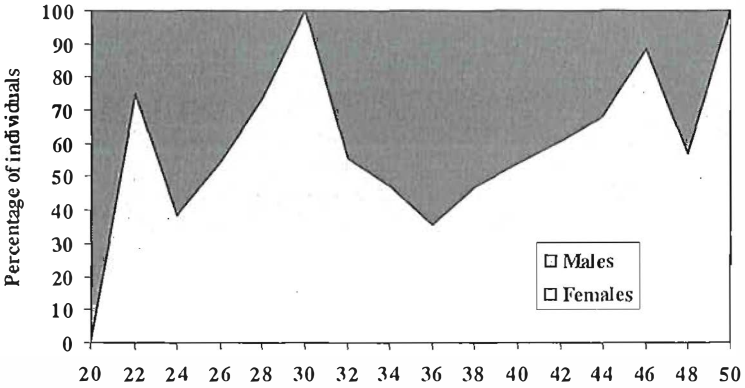

Fig. 9. Sex composition of *Beryx decadactylus* by 2 cm length classes.

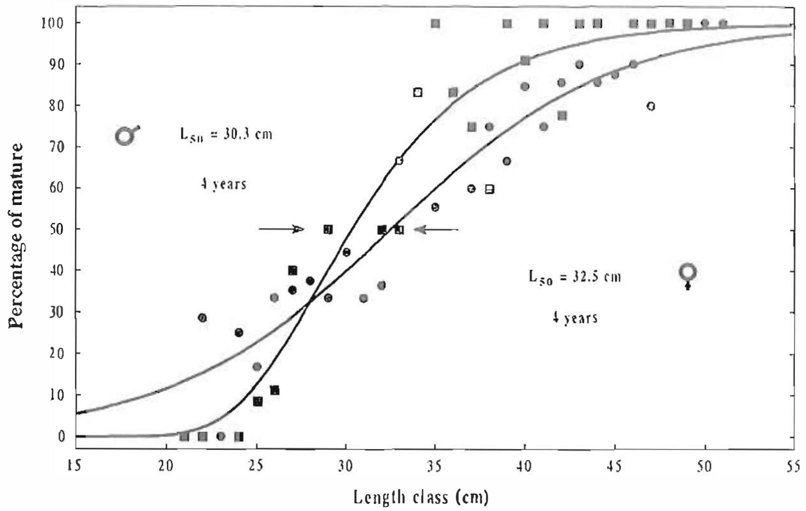

Fig. 10. Maturation ogives for male (N=149) and female (N=206) *Beryx decadactylus*.

*Pontinus kuhlii*

Of a total of 456 specimens collected, 290 (64%) were females and 166 (36%) males. The overall ratio of males to females was 1:1.75 and $\chi^2$-test revealed a significant departure from 1:1 ratio (P<0.05) (Fig. 11). Males predominated in large length classes, while females were abundant in smaller lengths (Fig. 12).

The results obtained by the plot and the fit of the Logistic model for females and males were a length at 50% maturity of 22.6 cm ($r^2$= 0.97) and 29.9 cm ($r^2$ =0.91) respectively and the corresponding ages were 5 and 9 years (Fig. 12) (KRUG et al.1998).

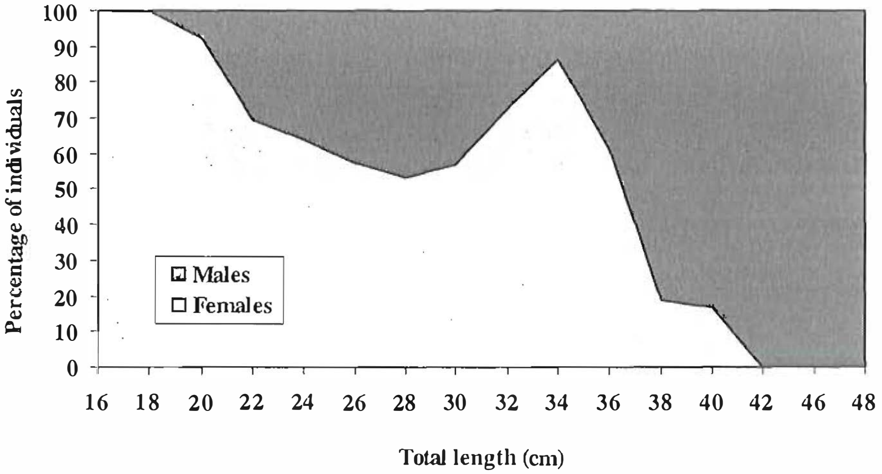

Fig. 11. Sex composition of *Pontinus kuhlii* by 2 cm length classes.

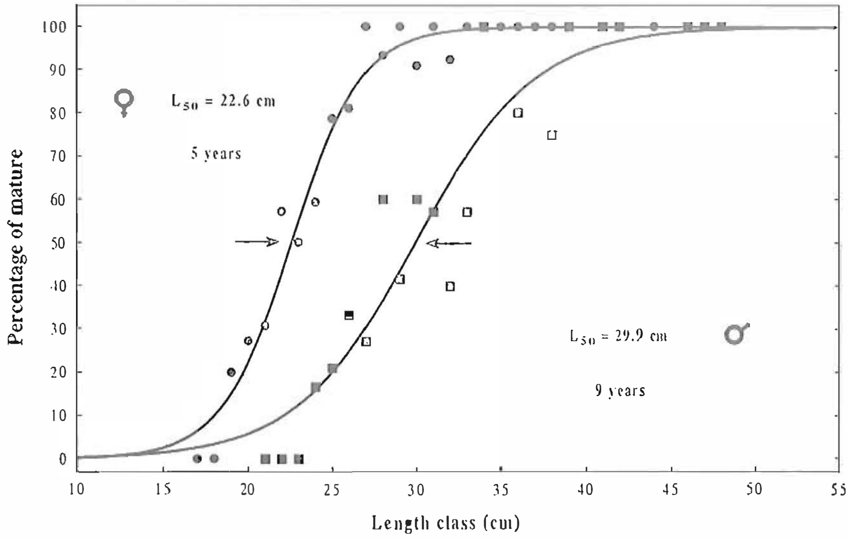

Fig. 12. Maturation ogives for male (N=166) and female (N=290) *Pontinus kuhlii*.

*Lepidopus caudatus*

Of the 909 fish examined, 577 (63.5%) were females and 332 (36.5%) males. The overall ratio of males to females was 1:1.74 and $\chi^2$-test showed significant differences from 1:1 ratio (P<0.05). Females predominated in the largest size classes (Fig. 13).

A Gompertz curve fitted well the females and males data, indicating an length at 50% maturity of 114.9 cm ($r^2$=0.96) and 85.0 cm ($r^2$=0.91), respectively and the corresponding ages were 3 years and 1 year, (Fig. 14) (KRUG et al. 1998).

The GSI showed a similar reproductive period for both sexes, located between September-December for females (Fig. 15) and August-October perhaps until January for males (Fig. 16).

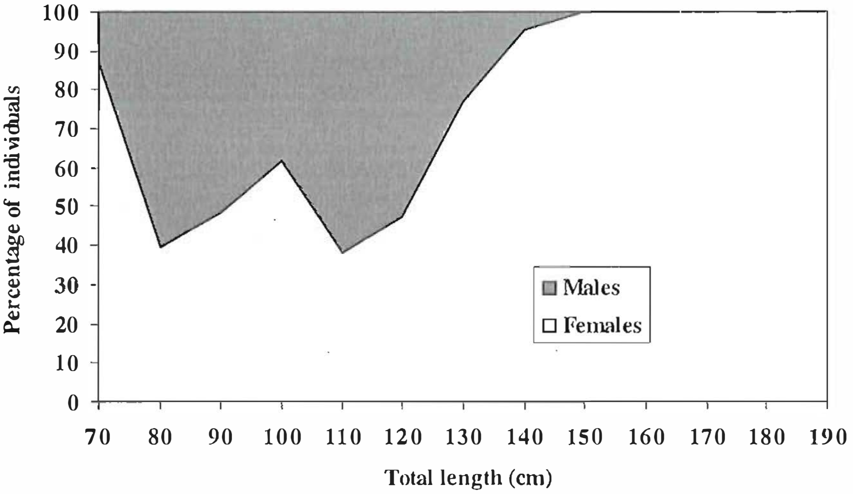

Fig. 13. Sex composition of *Lepidopus caudatus* by 10 cm length classes.

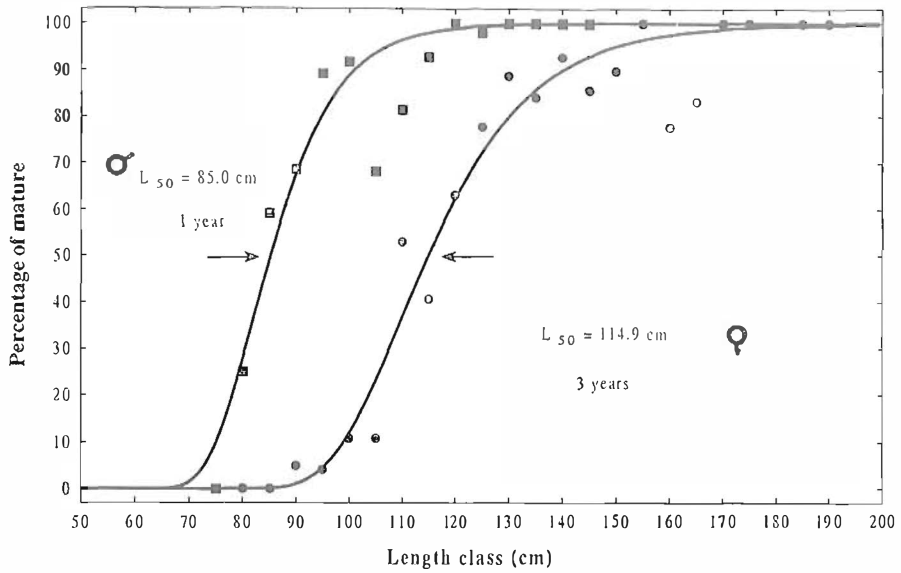

Fig. 14. Maturation ogives for male (N=332) and female (N=577) *Lepidopus caudatus*.

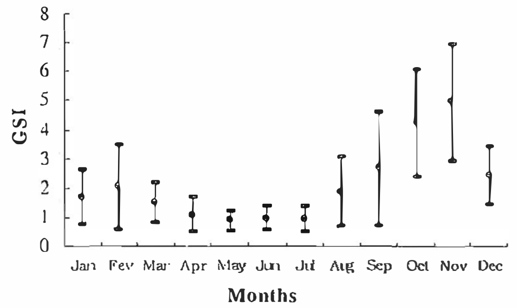

Fig. 15. Mean monthly gonadosomatic index (GSI) of females *Lepidopus caudatus* (± SD).

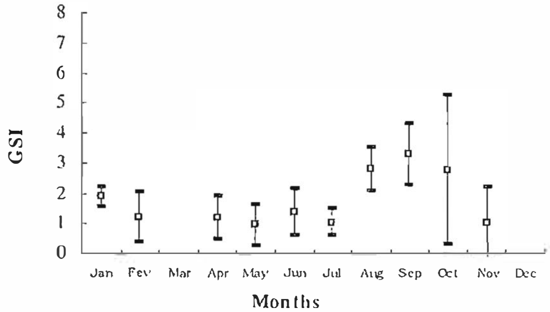

Fig. 16 - Mean monthly gonadosomatic index (GSI) of males *Lepidopus caudatus* (± SD).

## DISCUSSION

### *Pagellus bogaraveo*

Since 1982 the sizes and ages at first maturity of this species have been monitored. Through the present study it was verified that the size at first maturity of males was similar to the value observed for the period 1982-83, but smaller than the results achieved for the periods of 1984-86 and 1991 (KRUG 1995). The current study showed consistently lower size at first maturity for females than those obtained for the periods 1982-83, 1984-86 and 1991. The ages at first maturity decreased one year for both sexes compared to the data of 1991 (KRUG 1995), indicating an earlier maturation. Variation in size/age of first maturity should be expected since fish populations may change the size/age at first maturity as an adaptive response to population changes. During recent years high levels of exploitation of this stock seemed to reflect this variation of values. KRUG (1995) warned about the serious consequences of the overexploitation of this stock and suggested the reduction of fishing effort. However, at present, red (blackspot) seabream continues to be exposed to a high level of exploitation.

As in KRUG (1990, 1995) this study presents evidence of protandric hermaphroditism in males of the smallest length classes and females of the largest length classes. Variations in size at first maturity between males and females were expected.

In the Azores the reproductive period occurs during winter, January-April, with a maximum of activity in February and March (KRUG 1990, 1995). This species has the same reproductive period in the Cantabric Sea (SANCHEZ 1983).

### *Helicolenus dactylopterus*

The size at first maturity of females obtained was similar to the value presented by ISIDRO (1989), for the period 1985-88. For the same period males revealed a lower value, than in the present work.

In spite of lack of information between late summer/autumn, the most significant reproductive period is similar to that verified by Isidro and to other regions, for instance the SE coast of United States (WHITE et al. 1998). This species is a partial spawner, with internal fertilization occurring in June-October and spawning in January-May (ISIDRO 1989). Isidro observed that a notable feature of the females is the retention of sperm for a long period, reaching 6-7 months, until the start of spawning. WHITE et al. (1998) also described a delay, 1-3 months, between insemination and fertilization. This reproductive behaviour is well reflected in the results obtained.

### *Beryx splendens*

The size at first maturity obtained for females and males were similar. These results are very similar to those previously published for Azores (ISIDRO 1996). However, Isidro obtained an age at first maturity higher for both sexes - 3.4 years for males and 4.3 years for females.

In Japanese waters the age at first maturity was 4 years for this species (MASUZAWA in HORN & MASSEY 1989). However in the Atlantic, Pacific and Indian Oceans, most alfonsino attain sexual maturity at 5-6 years although a few are mature at 2 years (KOTLYAR 1987).

In the Azores, the female GSI tends to be higher from the late summer through autumn and winter (January/February). For males, this index suggested a protracted spawning season from September to March/April (ISIDRO 1996). In this study only a few females were found in spawning stage (stage 4). This result is in agreement with ISIDRO (1996). A more detailed study on the reproductive behaviour of this species is necessary to confirm these results.

### *Beryx decadactylus*

The size at first maturity obtained for females was higher than that indicated for the period 1991-93 (ISIDRO 1996), although the size at maturity males was similar. Comparing the two studies for both sexes, the ages at first maturity were similar.

The GSI for males showed some tendency for a maximum between May and July and a minimum between August and December (ISIDRO 1996). Females GSI is of low amplitude and does not suggest any particular restricted period of spawning activity (ISIDRO 1996). Also for this species, only a few females were found in spawning stage (stage 4). This result is in agreement with ISIDRO (1996). The reproductive behaviour of this species needs to be studied in more detail.

### *Pontinus kuhlii*

In this study a great percentage of females was smaller than males. This was also verified by ISIDRO (1996) for the period 1990-92. Given these size differences between sexes, the size at first maturity value for males was greater than that for females. In this study the sizes at first maturity observed were higher than those obtained for the period 1990-92 (ISIDRO 1996).

In the Azores this species spawns between June and November with a peak between July and October (ISIDRO 1996).

### *Lepidopus caudatus*

This species is described for the Mediterranean Sea as a partial spawner (ORSI-RELINI et al. 1989; DEMESTRE et al. 1993). In the present study, the difference between the sizes at first maturity of females and males showed a sexual size dimorphism in Azorean waters, as also noted in Morocco region by DOMANEVSKLY in MIKHAYLIN (1977). These values and the respective ages found, were very similar to those achieved for monthly samples examined during 1994 (GONZÁLEZ et al. 1998) for Azores archipelago. Similar results were observed in the Catalan Sea (DEMESTRE et al. 1993).

The most important period for reproduction is from the late summer through autumn. A similar reproductive period occurs in the Mediterranean and Adriatic Sea (ORSI-RELINI et al. 1989, DEMESTRE et al. 1993, KARLOVAC & KARLOVAC 1976 & FISCHER et al. 1987).

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# A VISUAL SURVEY OF THE INSHORE FISH COMMUNITIES OF GRAN CANARIA (CANARY ISLANDS)

JANOS G. HAJAGOS & JAMES L. VAN TASSELL

HAJAGOS, J.G. & J.L. VAN TASSEL. 2001. A visual survey of the inshore fish communities of Gran Canaria (Canary Islands). *Arquipélago*. Life and Marine Sciences. Supplement 2 (Part B): 95-99. Ponta Delgada. ISSN 0873-4704.

An *in situ* visual survey technique (5 minutes and 100 $m^2$ area) was used to assess the inshore fishes off Gran Canaria. In 1996, 211 visual surveys were conducted at 7 localities. Locations differed significantly among each other with regards to the number of species per survey (ANOVA: $p < 0.01$). The five most abundant species were *Chromis limbatus, Boops boops, Pomadasys incisus, Abudefduf luridus, and Thalassoma pavo* with respective mean abundances of 65.6, 37.4, 16.7, 8.7, and 4.5 per 100 $m^2$. Detrended Correspondence Analysis, a multivariate ordination technique showed that the major determinant of community structure is substrate type. The majority of the surveyed species had low axis 1 ordination scores indicating a strong association with a hard substrate. The step-wise linear regression models explained 45.3 % and 11.4% of the variation in the first and second axis survey ordination scores, respectively.

*Janos G. Hajagos (e-mail: jhajagos@life.bio.sunysb.edu), Department of Ecology and Evolution, State University of New York, Stony Brook, New York, U.S.A. - James L. Van Tassell, New Jersey State Aquarium, Camden, New Jersey, U.S.A.*

## INTRODUCTION

Approximately 30 years ago commercial development began along the coastlines of the Canary Islands, primarily on the islands of Tenerife and Gran Canaria. In order to meet the needs of the tourist industry, artificial harbors, beaches and hotels were constructed. Few environmental precautions were taken, for example, silt was dumped and massive alterations to the shoreline were made. The effect of these changes has been a serious decline in the economically important fisheries associated with the archipelago (BACALLADO et al. 1989). Basic knowledge of the ecology and well planned BACI (Before and After Control Impact) studies will prove important in assessing the effects of future development on the inshore fish.

Most of the literature on the ecology of inshore fish deals with coral reefs and the debate over stochastic versus deterministic factors in the structuring of these communities (SALE et al. 1994) (AULT & JOHNSON 1998). Literature on non-coral reef fish communities has primarily been concentrated on the kelp forests of California (HOLBROOK et al. 1994) and New Zealand (CHOAT & AYLING 1987). The community ecology of the inshore fishes of the Canary Island Archipelago has received less attention.

The inshore fish community associated with the Canary Islands is unique in its composition (VAN TASSELL 1988; BRITO 1989, BRITO et al. 1995). Throughout the 1990's there has been an effort to quantify the inshore fish of the archipelago by BORTONE et al. (1991) and FALCON et al (1996); however, efforts to quantify the communities on Gran Canaria have been limited to smaller scale investigations (BORTONE et al. 1994; HAROUN et al. 1994). This study is part of a larger, long term temporal assessment (1991-1998) of the inshore fish of Gran Canaria. The goals here are to define the basic community structure, and examine spatial variations in this structure as well as the relation of environmental factors to community organization.

## MATERIALS AND METHODS

### Description of Study Area

The Canary Island Archipelago is located 70-450 km off the northwest shore of Africa (fig. 1). Gran Canaria (1,531 km²) is the third largest island and is inhabited by approximately 600,000 permanent residents (BACALLADO et al 1989). The shoreline consists of a basalt terrace dropping 10 - 15 meters before entering into sand. Each of the seven surveyed localities is referred to numerically. The surveyed localities are 1- Punta de la Cuesta de la Burra, 2- Punta Cruz de Piedra, 3- Punta de los Frailes, 4- Puerto Rico West, 5- Punta del Canario, 6- Punta del Canario Artificial Reef, and 7- Pasito Blanco Offshore Reef.

### Survey Technique

We used an underwater visual survey, the point-count (BORTONE et al. 1989) that is among the least disruptive of all survey techniques currently used to assess shallow aquatic communities (BARDACH 1959). An individual using SCUBA establishes an imaginary circle with a radius of 5.6 m (100 $m^2$), and horizontally counts all fish species up to 10 m above the substrate for a period of five minutes. If one fish of a school enters the survey area, the whole school is recorded as having entered (BROCK 1954). Fish that leave and reenter the survey area are counted only once. For some genera, species were not easily distinguishable and were recorded as genera only. For schooling species abundance was estimated in units of 50.

We recorded the following environmental data for each survey: time, date, depth, slope, substrate, and percentage of sand cover. Slope was estimated on a scale from 1(totally flat) to 4 (vertical). Substrate refers to vertical relief: 1 = no relief (e.g. a sandy surface), 2 = less than 0.5 m, 3 = 0.5 m to 3 m, and 4 = greater than 3 m. Biotic data for each survey included: the percentage of macroscopic algae above 1 cm covering the survey area, and the number of long spined sea urchins *(Diadema antillarium)*. All data were recorded with a pencil on roughened plastic slates and transferred to a computerized database for later analysis. For each sample, species abundance, number of species, number of individuals, and species diversity (H') according to the Shannon-Weaver Index (PIELOU 1966) were determined. All results from the analysis are expressed in units per 100 $m^2$.

### Data Analysis

Detrended Correspondence Analysis (DCA; HILL & GAUCH 1980) is an enhanced eigenvector ordination technique based on reciprocal averaging (RA; HILL 1973). DCA was performed using PC-ORD (MCCUNE & MEFFORD 1997). PC-ORD uses a modified version of DECORANA (HILL 1979) with improvements in the rescaling algorithm suggested by OKSANEN & MINCHIN (1997). Species abundances were $\log_{10}$ transformed in order to reduce the high variation present in the data set.

We constructed step-wise linear regression models to explain variation in the dependent variables (number of species, number of individuals per survey, species diversity (H'), sample ordination scores along the first axis, and sample ordination scores along the second axis). Five independent variables (slope, substrate, percentage of sand, percentage of algae, and number of sea urchins) were entered into each model. Variables were removed from the models if their $p > 0.05$.

Single classification ANOVA was performed on all independent and dependent variables to determine if statistical differences exist among surveyed localities. The dependent variables were also tested by ANOVA against slope and substrate type. Post-hoc tests were conducted using the T-method. Unless otherwise noted the significance level for a statistical test is $p < 0.05$. Data were analysed using programs written in Visual DBASE 7 (BORLAND 1997) and SYSTAT 7 (SPSS 1997).

## RESULTS

### Descriptive

The 48 species recorded in 211 visual surveys comprise 29 different families. Descriptive species statistics are listed in Table 1. The five most abundant species were *Chromis limbatus, Boops boops, Pomadasys incisus, Abudefduf luridus*, and *Thalassoma pavo* with respective mean abundances of 65.6, 37.4, 16.7, 8.7, and 4.5. Mean abundances and standard deviations by locality for each species are listed in table 2. Sixteen species were represented by at most 1 or 2 individuals in a survey. The greatest number of individuals recorded for a single survey was 2500 *(Pomadasys incisus).* The five most frequently observed species were *Chromis limbatus, Abudefduf luridus, Canthigaster rostrata, Thalassoma pavo, and Diplodus vulgaris* with percent occurrences of 82.0, 76.8, 74.4, 72.0, and 51.7 respectively.

### Community Structure

Surveys and species were ordinated along three axes using detrended correspondence analysis. The eigenvalues for each axis are 0.7224 for axis one, 0.3674 for axis two, and 0.2230 for axis three. The two-dimensional configurations for species and surveys are shown in Figures 2 and 3, respectively. Species that scored low along axis 1 are species which inhabit rocky areas of high substrate relief (e.g. *Tripterygion delaisi, Mycteroperca rubra, Ophioblennius atlanticus, Scorpaena maderensis*). At the midpoint of axis one are *Stephanolepis hispidus, Diplodus sargus, Canthigaster rostrata*, and *Boops boops*, which inhabit the transition zone from hard substrate to sand. The highest scores along axis 1 are species solely associated with a sandy substrate (e.g. *Uranoscopus scaber, Heteroconger longissimus, Trachinus* spp., *Bothus podas*, and *Xyrichthys novacula*). The patterns of distribution along the second axis are less clear however. Species with high scores on the second axis tend to have less affinity for the substrate (e.g. *Belonidae* spp. and *Seriola* spp.), whereas lower scores indicate strong association with the substrate such as *Canthigaster rostrata* and *Apogon imberbis.* The low score of *Boop boops* does not fit the trend since it is a schooling species, nor does the high score of *Synodus saurus*, a benthic species.

The ordination of the surveys along the first axis follows a pattern similar to the species. The surveys with the lowest scores are composed of species such as *Apogon imberbis, Thalassoma pavo* and *Sparisoma cretense* which inhabit rocky areas. The highest score surveys are primarily composed of sand associated species (e.g. *Bothus podas* and *Xyrichthys novacula).* Along the second axis, the surveys with the top-ranking scores are composed of free swimming species such as *Trachinotus ovatus* and *Belonidae* spp., whereas lower scores are associated with high abundances of *Xyrichthys novacula* and *Boops boops.* The first axis in the ordination separates both species and survey by their association with substrate type (e.g. rocky versus sand). The gradient the second axis represents is less clear; however, it appears to be loosely linked to the degree of species vagility.

### Step-Wise Regression Models

The percentage of variation explained in the dependent variables by the linear models ranged from 9.4% for number of individuals in a survey to 53.3% for number of species per survey. In the model of number of species, depth, substrate, sand, algae and urchins were significant. The linear model explained 27.6% of the variation in species diversity (H'). For the survey ordination scores along the first axis the linear model explained 45.3% of the variation with depth, substrate, and percent of sand significant. In the model of the second axis scores 11.4% of the variation was explained while only percentage of sand was significant in the model. When the seven localities coded as dummy variables were added to the step-wise models the percent of variation explained increased to 63. 1 % for the number of species, 16.9% for the number of individuals, 33.9% for species diversity, 55.4% for DCA axis 1, and 17.8% for DCA axis 2.

### Analysis of Variance

See Table 3 for means of environmental and biotic variables and their significance in the ANOVA. The number of species recorded at Pasito Blanco differed statistically from the other 6 sites means, this result is due Pasito Blanco being an offshore reef. The means for the number of species, number of individuals, and species diversity were significant with regard to substrate type. Substrate 1 was significantly lower than substrate 2, 3, and 4 for number of species and H'. For slope, number of species and species diversity were significant. Slope one means were significantly lower than slope two means for number of species and H'.

## DISCUSSION

BORTONE et al. (1994) in a more limited survey off Gran Canaria recorded 37 species using the point-count method, seven of which were not recorded in the present study. Three of them are from the family Gobiidae, are cryptic in color and were probably overlooked in the present study because the underwater visual survey technique does not provide an accurate assessment of cryptic species (BROCK 1982). FALCON et al. (1996) in a comparable and more thorough study of the inshore fauna of the Canary archipelago recorded 76 species. However DOOLEY & VAN TASSELL (1985) consider 217 species to be part of the regular inshore fauna of the archipelago. Since there are inter-faunal differences between islands, and we surveyed only a limited section of the Gran Canaria coastline, the 49 species in 29 families in our study represent a considerable portion of the island's fish community.

Spatial scale is an important consideration in any ecological study (LEVIN 1992), and we found high habitat heterogeneity across the seven localities. For the majority of environmental variables and for the number of species per survey the ANOVA models showed significant differences among localities. On a finer scale, looking within habitat types the ANOVA models revealed significant differences among number of species, species diversity, and number of individuals. A substrate of 1 tested significantly lower than the other substrates. An explanation for this is that the community associated with this substrate is sand specific. This community is less diverse and usually less abundant than its rocky counterpart. Part of this is likely due to the high territoriality exhibited by the dominant species (*Bothus podas* and *Xyrichthys novacula*) of the community.

The patterns of community structure revealed in the ANOVA models are supported by the ordination. The first axis in the DCA ordination is strongly associated with substrate type, a finding supported by the species scores and the significant variables (depth, substrate. and percent of sand) in the regression model of the axis scores. A large number of species are clustered along the initial part of the axis. These species, representing a considerable portion of those surveyed are primarily associated with a hard substrate. BORTONE et al. (1991) on El Hierro found a similar pattern in the DCA ordination along the first axis. Since the sandy substrate is relatively absent from El Hierro the effect of clustering was even more pronounced. On El Hierro, BORTONE et al. (1991) found the second axis scores to represent affinity for the substrate. Overall we found a similar pattern for the second axis scores but on finer examination there are a considerable number of exceptions. Most of the anomalies are of rare species, less than 1% overall occurrences in the study. Occurrence of a rare species is more an event of chance than an ecological phenomenon and therefore may obscure ordination results (GAUCH 1982).

It appears that substrate type is important in the spatial structuring of the community of inshore fish into two different assemblages. This conclusion has important consequences for development along the shore. The basalt terrace extending from most of the coastline is rather narrow between 50 and 100 meters. Since a considerable portion of the species are associated with the hard substrate, disruption of the shelf by filling with sand and sediment from harbour and artificial beach construction will have a negative impact on this community.

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SHORT COMMUNICATION

# DRAGON-TREES (*Dracaena draco* L.) WITH ABERRANT GROWTH FORMS (MONOCOTYLEDONES: LILIIFLORAE: AGAVACEAE)

FRIEDRICH E. BEYHL

BEYHL, F.E. 2001. Dragon-trees (*Dracaena draco* L.) with aberrant growth forms (Monocotyledones: Liliiflorae: Agavaceae). *Arquipélago*. Life and Marine Sciences. Supplement 2(Part B): 101-103. Ponta Delgada. ISSN 0873-4704.

This paper reports on some individuals of Dragon-trees (*Dracaena draco* L.), which do not show the normal, umbellular and regularly branched canopy but exhibit aberrant growth forms. The possible reasons for the formation of these abnormal shapes are discussed.

*Friedrich E. Beyhl, Nonnbornstr. 23, DE - 65 779, Kelkheim, Germany.*

## REGULAR GROWTH FORM

Dragon-trees have a unique, very regular growth form which varies only in some minor features (BEYHL 1995a) and which is the result of strong morphogenetic control. This control system seems to be dependent both on internal and external factors (RAUH 1939) and is so strict that the ramifications of the canopy of a Dragon Tree (fig. 1) are of self-similar character, in the sense of MANDELBROT (1991). Normally, only malnutrition and traumatic events (insect or rust infections, fire, storms) interfere with this regular growth pattern and result in aberrations as seen in the old Dragon-trees of Tacoronte (Tenerife) (Fig. 2) and Garajau (Madeira) and the historical Dragon-tree of La Orotava (Tenerife).

Overnutrition may occur in specimens that are planted in gardens or parks, causing excessive growth of branches so that they are in danger of breaking under their own weight. The trunks of very old trees will become hollow, and this may increase the danger of rotting. Damage may also be caused by cutting of the trunk or branches, by over-exploitation for sap ("Dragon's blood") or by interference with the trees' water and nutrient supplies.

This same situation holds true for the Socotran Dragon-tree, *D. cinnabari* Balf. fil.. (BEYHL 1995b, 1998), except that there are virtually no trees of this species planted in gardens or parks (only one young tree is known from a house garden in the small town of Hadîbû on Socotra). An extraordinary way of doing damage to Dragon-trees is reported from this island where they served as military targets for the training of soldiers in firing exercises.

## ABERRANT GROWTH FORMS

Several examples of Dragon-trees have been observed which do not develop the normal, regular, umbrella-like canopy. A tree in a house garden in La Orotava, Tenerife and another in a house garden of Santa Cruz, Tenerife (Fig. 3) each has an abnormally tall trunk and lack an elaborate canopy. Instead there are only a few branches, which are themselves branched and bear large rosettes of leaves at the tip of the twigs. Both trees are growing in the shade of buildings.

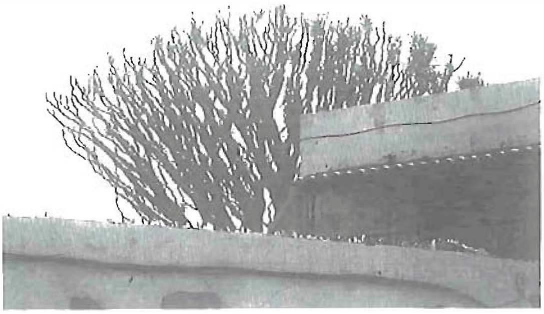

Fig. 1. View of the canopy of a Dragon-tree, *Dracaena draco*.

Fig. 2. The old dragon tree in Tacoronte (Tenerife). Its branches, previously damaged by a storm, are supported by scaffolding.

Fig. 3. Dragon-tree growing in a house garden in the city of Santa Cruz (Tenerife).

Fig. 4. Dragon-trees in the municipal park of the city of Arucas (Gran Canaria).

Although their age is unknown, they are presumably young trees, which have grown very tall in a short time in order to reach the light. It is likely that they are simply not yet old enough to build up a "normal", dense canopy.

By contrast, some Dragon-trees with normal, umbrella-like canopies, ramify very early and develop only a very short trunk. This is the case with one in the garden of the ayuntamiento of Gáldar, Gran Canaria and with one in the botanical garden of Floriana at La Valetta, Malta.

Several Dragon-trees growing in the park of Arucas on Gran Canaria also bear more than one storey of branches (fig. 4). They are similar to the two examples mentioned above from Tenerife in their branching pattern and scant canopies. The reason for the aberrant growth of these trees cannot be easily identified. However, in all the cases reported here it seems clear that the strict morphogenetic control governing canopy formation in Dragon-trees and other plants of dracoid growth (SCHENCK 1907) has broken down.

Overall, these trees are reminiscent of specimens of the Nubian Dragon-tree. *D. ombet* Kotschy & Peyer, and of the South Arabian Dragon-tree *D. serrulata* Baker, (BEYHL 1999) which also often do not produce a closed canopy but have only branches which may be branched themselves and which bear the leaves in extensive terminal rosettes. The African species *D. steudneri* and *D ellenbergiana* as well as the Madagascan/Mascarenian *D. marginata* and the Comoran *D. xiphophylla* are also of this type, with irregular branching, terminal leaf rosettes and lack of a canopy.

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SHORT COMMUNICATION

# DNA EXTRACTION FROM LEAVES OF *Vaccinium cylindraceum* SMITH (ERICACEAE). THE USE OF RAPD MARKERS TO DETECT GENETIC VARIATION. PRELIMINARY RESULTS.

JUAN MARTÍN-CLEMENTE, M. JOÃO PEREIRA, & CÉSAR PEREZ-RUIZ

MARTÍN-CLEMENTE, J., M.J. PEREIRA, & C. PEREZ-RUIZ. 2001. DNA extraction from leaves of *Vaccinium cylindraceum* Smith (Ericaceae). The use of RAPD markers to detect genetic variation. Preliminary results. *Arquipélago*. Life and Marine Sciences. Supplement 2 (Part B): 105-109. Ponta Delgada. ISSN 0873-4704.

*Vaccinium cylindraceum* Smith is an endemic *Ericaceae* from Azores archipelago. This species is being produced by seed germination, micropropagation and stem cuttings. The produced plants are then reintroduced in their natural but disturbed environment and used to repopulate protected areas meanwhile infested with more or less invasive exotic flora. In order to protect the genetic variability within the species, a study of genetic variation between and within populations from different islands was started. DNA extraction was achieved on fresh, dried and criopreserved leaves and the first results using the PCR technique are also presented.

*Juan Martín-Clemente & César Perez-Ruiz, Departamento de Biología Vegetal, Universidad Politécnica de Madrid, Ciudad Universitaria, ES-28040 Madrid, Spain. - M. João Pereira (e-mail: mjpereira@notes.uac.pt), , Universidade dos Açores, Departamento de Biologia, Rua da Mãe de Deus 58, PT – 9501-801 Ponta Delgada, Portugal.*

## INTRODUCTION

One of the most difficult issues in conservation is how best to assess the minimal area needed for a communities survival. That implies at least the knowledge of the density of each species within their several types of natural communities and also their reproduction strategies. One way to assess the minimum area is to determine the area that includes those individuals that together possess most of the allelic variation within the species. This kind of knowledge is also necessary when reproduction *ex situ* is used as a tool to raise the number of individuals in a population and therefore raise the number of certain genotypes.

Information concerning these aspects is required to develop safe strategies for sampling, for example sampling the highest number of mother-plants, and introducing their descendants in the same proportions into the original populations. The assessment of genetic diversity in endangered plants can therefore help to enhance conservation efforts and strategies (HOLLINGSWORTH et al. 1998). Various molecular methods have been employed to investigate levels of variation within and between populations (BACHMANN 1994).

*Vaccinium cylindraceum* Smith is an endemic shrub present only in seven of the nine Azorean islands, generally above 400 m and is a key species in different natural communities (DIAS 1996). Sampling all the individuals in a wild population for reproduction is often impossible due to their inaccessibility. Also, the obligation to introduce the produced plants in the same population of origin leads us to a considerable effort of tagging the plants. Another aspect to consider is the possibility of raising the inbreeding depression, if the sampled population have already attained a reduced level of genetic variation (GUSTAFSSON & GUSTAFSSON 1994). These aspects lead us to the study of some natural reproduction aspects (breeding system: self fertilisation or outcrossing; flowers, fruits and seeds productions, seeds viability and seedlings vigour), and also to the study of genetic variation between and within populations. Spontaneous autogamy is not significant in this species, where fruit and seed development depends mainly on the pollinators activity (PEREIRA et al. 1998).

In 1996 a preliminary investigation was made at 'Universidad Politécnica de Madrid' focused on the capacity to extract DNA from fresh and preserved material and also to survey the potential use of RAPD markers to assess genetic variation.

This preliminary investigation was presented at the 3rd Symposium of Fauna and Flora of the Atlantic Islands.

## MATERIAL AND METHODS

The Random Amplified Polymorphic DNA (RAPD) is a Polymerase Chain Reaction (PCR) based technique, which has been used to assess genetic variation and kinship relationships in plants (CHALMERS et al. 1992; GUSTAFSSON & GUSTAFSSON 1994; HADRYS et al. 1992; HUFF et al. 1993,1994; NEWBURY & FORD-LLOYD 1993). Since almost all RAPD markers are dominant or rarely co-dominant (WILLIAMS et al. 1990), these markers are capable of distinguishing homozygous individuals but to distinguish heterozygous individuals other, more sensitive, molecular techniques are required (NEWBURY & FORD-LLOYD 1993). NYBOM (1993) states that the RAPD technique can be used in the study of population's genetic variability when the individuals are genetically distant due to xenogamy. Conceptually less complex and expensive than other PCR based techniques, this technique works on anonymous genomes and the profile of amplification products detects individual polymorphisms (HADRYS et al. 1992).

In this study, fresh leaves from nine seedlings of *Vaccinium cylindraceum*, all from the same population but with different parents, were used to determine the effectiveness of the DNA isolation technique and the leaf weight required to obtain profitable DNA extractions. Four other seedlings also from the same population, were used to assess the possible use of dried and crio-preserved leaves for DNA extractions.

DNA was isolated according the method described by TORRES et al. (1993) (except the final total of RNAse) and measured by electrophoresis in agarose gel, using samples with standard DNA weights. Nanograms of total genomic DNA were subjected to PCR using 3 different 10-mer nucleotide primers of random sequence from Operon Technologies Inc. (P3, P7 and P9, from kit O) in the Perkin Elmer 480 DNA thermal cycler. The PCR was initiated at 94°C for 1 minute and then cycled 35 times: 92°C, 30 seconds + 37°C, 1 minute + 72°C, 2 minutes; with a terminator of 72°C for 3 minutes. After completion the reactions were kept at 5°C until electrophoresis. Fragments generated by amplification were separated on 1,2 % agarose gel run in 1XTBE (pH 8,0) and stained with ethidium bromide and made visible by illumination with ultraviolet light.

## RESULTS AND DISCUSSION

We have been able to extract DNA successfully from *Vaccinium cylindraceum* following the method described by TORRES et al. (1993). From starting weights of 50 to 200 mg of fresh seedling leaves, we have been able to extract 1250 ng to 6500 ng of DNA, with a mean value of 34.2 ng / mg of fresh tissue (approximate values) (table I and figure 1). These extractions yielded sufficient amounts of DNA for RAPD reactions.

When fresh leaves are not available, criopreserved leaves can be used for DNA isolation and RAPD reactions while extractions from dried leaves do not yield sufficient amounts of DNA for RAPD reactions (figure 2).

The primers used revealed some polymorphism (figure 3) indicating the potential use of RAPD markers to assess genetic variation within this species. Further studies will be continued at the University of the Azores.

The used primers revealed some polymorphism (figure 3) indicating the potential use of RAPD markers to assess genetic variation within this species. Further studies will be continued at the University of the Azores.

Table 1
DNA quantification (estimated values).

| Sample number (gel) | Plant | Leaves weight (mg) | Number of leaves used | Extraction Solution (µl) | DNA Concentration (ng/µl) | Total DNA (ng) | DNA (ng) / leaves (mg) |
|---|---|---|---|---|---|---|---|
| 4 | e | 50 | 4 | 50 | 45 | 2250 | 45 |
| 1 | a | 52 | 3 | 100 | 35 | 3500 | 67 |
| 5 | f | 55 | 4 | 50 | 25 | 1250 | 23 |
| 3 | c | 95 | 3 | 50 | 65 | 3250 | 34 |
| 2 | b | 114 | 2 | 100 | 35 | 3500 | 31 |
| 6 | d | 140 | 3 | 100 | 55 | 5500 | 39 |
| 7 | g | 150 | 3 | 100 | 65 | 6500 | 43 |
| 8 | h | 200 | 3 | 50 | 45 | 2250 | 11 |
| 9 | i | 200 | 2 | 100 | 30 | 3000 | 15 |
| Mean value | | | | | | | 34.2 |

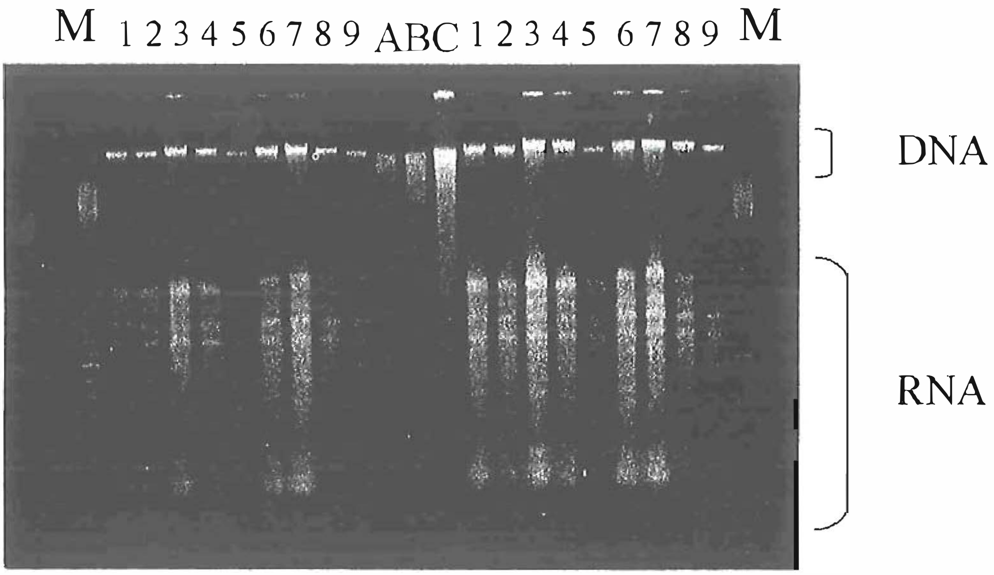

Fig.1 DNA isolated from fresh juvenile leaves. From the left to the right: M=Control size marker; samples number 1,2,3,4,5,6,7,8,9; A=100ng DNA; B=200ng DNA; C=400ng DNA; samples number 1,2,3,4,5,6,7,8,9; M=Control size marker. The samples placed at right are 2 times more concentrated then the samples placed at left.

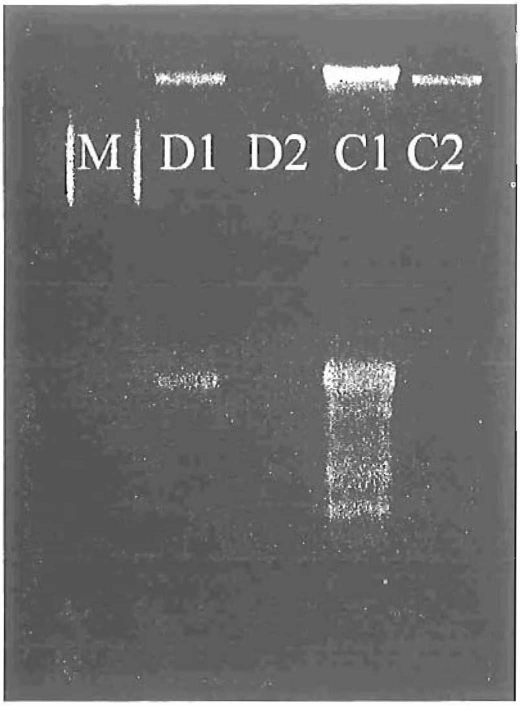

Fig. 2. Quantity of DNA isolated from dried and crio preserved leaves. M = Control size marker. D1 and D2 = DNA from dried leaves. C1 and C2 = DNA from frozen leaves. C1 and C2 samples are 3 times more diluted then D1 and D2 samples.

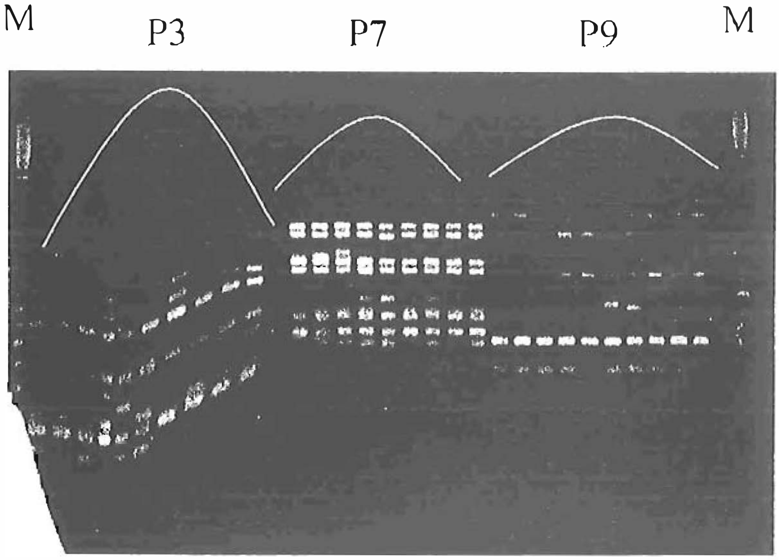

Fig. 3. Random Amplified Polymorphic DNA (RAPD). From the left to the right: M = Control size marker; P3 = Primer 3: samples 1,1,2,3,4,5,6,7,8,9; Negative control; P7 = Primer 7: samples 1,2,3,4,5,6,7,8,9; P9 = Primer 9: samples 1,2,3,4,5,6,7,8,9; Negative control, M = Control size marker. Kit O_from Operon 10-mer primers. 9 samples from 9 seedlings with origin in 9 different shrubs from de same population on S. Miguel Island.

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*Accepted 31 July 2000*

SHORT COMMUNICATION

# RECUPERATION OF A MEDITERRANEAN MONK SEAL PUP, *Monachus monachus*, IN DESERTAS ISLANDS, MADEIRA ARCHIPELAGO: CONDITIONS FOR ITS SUCCESS

H.C. NEVES & R. PIRES

NEVES, H.C. & R. PIRES. 2001. Recuperation of a Mediterranean monk seal pup, *Monachus monachus*, in Desertas Islands, Madeira archipelago: conditions for its success. *Arquipélago*. Life and Marine Sciences. Supplement 2 (Part B): 111-116. Ponta Delgada. ISSN 0873-4704.

During the nursing period, young monk seals depend on their mothers to survive. Sometimes, however, some monk seal pups loose contact with their mothers due to heavy seas. In situations like these, human intervention is crucial for pups' survival. In December 1995, a pup was found on Deserta Grande, separated from its mother and was picked up and taken to the Desertas station at Doca. There it was given shelter and exposed to frequent human contact and the corresponding possibility of infections. Although it appeared to be healthy, the animal died 12 days later. Autopsy results confirmed that the death of the animal has been caused by septicemia. This case led to the construction of a rehabilitation centre for the monk seals in the Desertas Islands. In December 1997, another pup was found washed up on the beach at Doca, Deserta Grande, in conditions identical to the previous one. Based on previous experience, and benefiting from the facilities of the Monk Seal Rehabilitation centre, plus the fact that the pup's mother was located, it was possible to rehabilitate the animal and later return it to the wild. This time, the existing conditions permitted the use of a strict protocol for the rehabilitation of the seal, since the first encounter until its release in the natural habitat. Aseptic conditions were maintained to the extreme, and human contact avoided to a minimum. This care was an important contribution for the rehabilitation of this seal.

*H.C. Neves & R. Pires, Parque Natural da Madeira (e-mail: pnm@mail.telepac.pt), Quinta do Bom Sucesso, Caminho do Meio, PT - 9050 Funchal, Portugal.*

## INTRODUCTION

The Mediterranean monk seal (*Monachus monachus*) is Europe's most endangered marine mammal (JOHNSON & LAVINGNE 1998) with no more than about 300-400 individuals left (AGUILAR 1998). In Portugal, the only herd of monk seals is found in the Desertas Islands (Fig. 1), which were classified as a Natural Reserve in 1990, and by that time the population size was estimated in 10 individuals (NEVES 1991). Since that time, the size of the population has been increasing, and it is presently estimated to number 19 individuals. This is the result of conservation work, which has been carried on there, in effectively protecting the monk seal and its habitat. Monitoring the monk seal and studying its biology and ecology are fundamental to determining the most appropriate strategies for its protection. In the course of this work, it has been found that one of the threats to the population is linked to the fact that the season for births and the pups' first outings into the sea, from November to February, coincides with the season in which ocean storms are the most frequent in the Desertas Islands (PIRES 1997). The pups are still very vulnerable in this phase, not being able to withstand the action of the waves. Thus they run the risk of being driven into areas of strong breakers next to the rocky coast, or pushed by the ocean currents far from the cave where they were born, losing contact with their mother, upon whom they are still dependent for feeding.

This kind of situation appended twice in the Desertas Islands. One in 1995, when the rehabilitation process was unsuccessful, and the second in 1997 when the pup was rehabilitated with success. The objective of this article was to describe procedures followed during the recuperation of the two pups, and to analyse the factors that contributed to failure in the first case and success in the second.

Fig. 1. Location of the Desertas Islands and the locations where the pups were found and stayed.

## MATERIAL AND METHODS

### Pup 1

### *Location and identification of the pup*

The pup was found by one of the wardens of the Madeira Natural Park on December 3, 1995, while manning one of the observation posts used in monitoring and studying the monk seal. The post is located on the beach of an inlet called Calhau das Areias. There he noticed that the monk seal tried several times to get to the sea, but always ended up being thrown back up on land by the action of the waves. Because the sea conditions were tending to worsen, the warden decided to pick up the pup and bring it to the Desertas Islands station at Doca.

Meanwhile, an unsuccessful search for the mother of the pup was undertaken, using a boat to run along the SW coast of Deserta Grande, which is used by the monk seals for raising their young (PIRES 1997).

The pup was a female weigh 19 kg, with 100 cm of standard length and 106 cm of total length. The age was calculated to be 3-4 weeks, based on the absence of the umbilical cord, which probably falls off by the 5th day (VISSER 1991); on the fact that the new growth of fur which appears between the fourth and sixth weeks of life (HART & VEDDER 1990; VISSER 1991; DENDRINOS et al. 1996) had not yet begun; and the fact that its teeth were ready to come through, which happens between 3 and 7 weeks of life (HART & VEDDER 1990; VISSER 1991). The name given to this pup was "Maria".

"Maria" was dehydrated (no humidity around the eyes) and injured at the base of the flippers.

### *Treatment and feeding*

"Maria" remained at the station at Doca, cohabiting the same space with the Nature Wardens who work there, being constantly in contact with them. Although the space and materials used by the seal was desinfected daily with sodium hypochloride, "Maria" was exposed, every day, to a potentially contaminated environment for a baby monk seal.

The care given the pup was essentially in keeping it fed and hydrated. The evaluation of its condition was made by keeping track of its rectal temperature and weight, and by observing its behaviour.

The pup was fed and hydrated 7 times a day, on average, with a mixture consisting of: 100 - 200 g of scabbard fish, 100 - 250 g of oatmeal, and 250 - 650 ml of water with hydrating salts. The first two days, 850 g of fish were used, and this was gradually increased to 1300 g. Contrary to what happens with the majority of pinnipeds in this situation, "Maria" ate voluntarily (Fig. 2) and "force feeding" was not necessary seeking to keep the pup hydrated and in permanent contact with its natural habitat, it was taken to the sea every day and bathed. Although the pup was carried by one of the wardens the first few times, it later became able to accompany them on its own, following them.

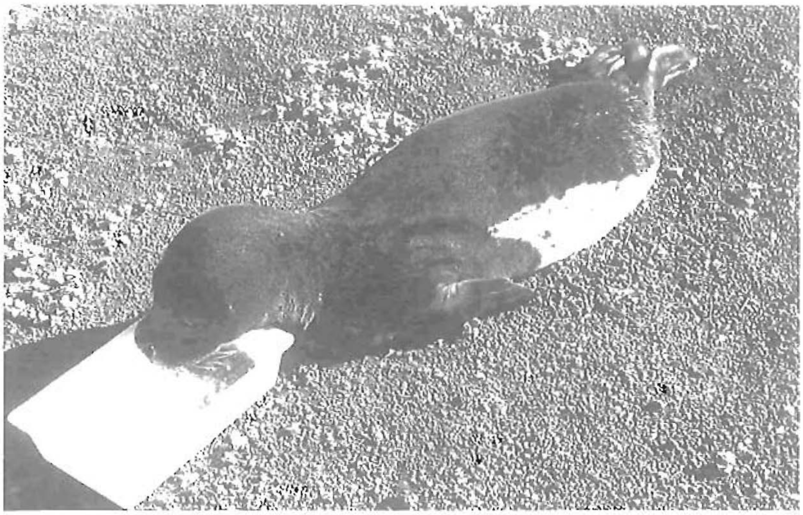

Fig. 2. "Maria" feeding by herself.

## Pup 2

### *Location and identification of the pup*

At 14:30 h. on December 3, 1997, exactly two years from the day "Maria" was found, a pup was found washed ashore at Doca. The observation work at that time was being carried out in the Tabaqueiro inlet, where 3 days earlier it had been noted that the beach was being used by a pup and two females. The fact that both females nursed the pup indicated that one of them had lost her pup.

The pup was dehydrated, with injuries at the base of the flippers. A thick and white mucus in its nostrils evidenced an obstruction of the respiratory tract, and the temperature was high, 38.8°C.

### *Treatment and feeding*

The animal was immediately taken to the Monk Seal Rehabilitation Unit, built at Doca, on the Desertas Islands in 1997. This special care unit consists of a kitchen where food is prepared, and hygienic measures (change of clothes, use of gloves and masks and disinfection of utensils) are followed, when entering the isolated room for seals. The isolating room has a pool with 3 x 2.5 m wide, 0.5 deep and a heated floor of 1.5 x 4 m , where hygienic conditions are maintained.

The pup's condition was checked and "first aid" help was implemented in order to improve the condition of the seal. The pup was hydrated with a saline solution prepared from 500 ml of sterilised water to which various vitamin complexes were added (A, B-1, 2, 6, 12, C, D3, and E). This mixture was given by means of a funnel and a tube inserted in the pup's oesophagus. The wounds were disinfected and the respiratory passages were unblocked with the aid of a drug, hexamidine, sprayed into the nasal passages in liquid form.

The pup was female weighing 17.40 kg and measuring 102 cm standard length, and 108 cm total length. The age was estimated at 1-3 weeks. This was based on the fact that the navel was not healed, and the gums of the upper jaw, which are soft when the monk seals are born (HART & VEDDER 1990), were hard, and by comparison with "Maria". The pup was named "Autonomia".

Since the pup's probable mother (Fig. 3) had been located, the pup's stay in the Rehabilitation Unit was limited to bring the temperature to normal and effect rehydration, so the animal could be returned as soon as possible to its natural surroundings. By 13:00h of the following day, the pup was hydrated 4 times and its temperature recorded.

Fig. 3. "Autonomia" with her potential mother after two days of placement on the beach of Tabaqueiro.

## RESULTS

### Pup 1

#### *Evolution of the pup's condition*

Over a period of 11 days, the pup was apparently healthy. Although its weight remained stable and its temperature varied between 34.5°C and 37.1°C, it was active and the fact that it was eating regularly indicated that it was healthy. However, on the 12th day, the pup demonstrated lethargic behaviour and died at 11:30h.

The Regional Veterinary Laboratory in Funchal, did the autopsy and the analyses. It was carried out an anatomic-pathologic analysis and also a histophatologic analysis using samples of the lung, kidney, spleen and liver. A hemoculture and macerate of viscera where used for microbiological analyses.

The results of the autopsy confirmed that the pup's death was caused by septicemia. Congestive haemorhagic lesions were found in the lung, kidney, spleen and parenchyma. The liver presented a subcapsular haemorhage, and the intestine presented a catarral enteritis. The following pathogenic agents were isolated: *Salmonella arizonae, Staphylococcus aureus,* grup B *Strepotococcus*, *Streptococcus dysgalactiae,* *Streptococcus* *bovis* I, *Streptococcus equisimilis*, hemolytic *E. coli* β, and type I *E. coli.*

#### *Measures taken as a result of this experience*

The Monk Seal Rehabilitation Unit was built, and a biologist from the Madeira Natural Park was trained at the Seal Rehabilitation Centre at Pieterburen (SRRC) to accompany the rehabilitation of monk seals.

### Pup 2

#### *Evolution of the pup's condition*

Soon after a 4-hour period of isolation in the Unit, the rectal temperature dropped to 37.8°C and remained stable until the pup was placed in its natural environment.

#### *Integration of the pup in its environment*

At 13:45h on December 4, the pup was placed on the beach of Tabaqueiro, about 30 m from the other three animals, which were sleeping. The pup called out and the female, which was alone, answered immediately and came up to the pup to establish a contact, typical of mother and young, and soon afterwards nursed the pup. On the following days, it was the other female who adopted the pup and began nursing it and accompanying it on excursions into the sea outside the Tabaqueiro inlet.

## DISCUSSION

The death of "Maria" was the result of a rehabilitation process, which was undertaken without the necessary conditions and experience to ensure its success. Conditions of asepsis were practically non-existent, which according to the autopsy conclusions, were the cause of death of the pup. This, due to the fact that the pup was deprived of its maternal food, essential to mammals in the first weeks of life as a way of obtaining antibodies and organic defences against infections from the surrounding environment. Besides this, the frequent contact with the personnel on duty at the Desertas station would lead to the dependence of the seal on humans, which would be prejudicial to the pup. A repeated close contact with humans could result in a dependence on them, which could cause problems to the animal after its release into the wild. In addition, contact between humans and the pup should be avoided, because infections and diseases can be transmitted.

Although this was a negative experience, two years later it contributed to the success in rehabilitating "Autonomia", because it had alerted us to the need for creating conditions for the rehabilitation of monk seals in the Desertas Islands. Thus, "Autonomia" was treated in the Rehabilitation Unit according to the protocol for the recuperation of seals followed by the Centre at Pieterburen. However, in the case of this pup, the fact that its probable mother was located was, no doubt, the factor that led to the success of this operation, principally because she was nursing another pup. As a general rule among seals, mothers end up abandoning their pups after these are absent for 3 days, because they stop producing milk due to the lack of the sucking stimulation (L.VEDDER pers. comm).

The recuperation of this pup is an excellent example of the importance of the work of monitoring and studying the monk seal for its conservation, which permitted the survival of this pup. This, together with the existence of conditions for the recuperation of abandoned pups reduces their mortality to a minimum.

## ACKNOWLEDGEMENTS

We wish to express our gratitude to the wardens of the Madeira Natural Park for their dedication in the work of saving the monk seal; to SRRC, for their important long-distance collaboration during the recuperation process of "Autonomia"; and finally to International Fund for Animal Welfare (IFAW), in the person of Dr. Petra Deimer, for the financial support to the Monk Seal Rehabilitation Unit of the Desertas Islands.

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SHORT COMMUNICATION

# REMOVAL OF RABBITS FROM DESERTA GRANDE ISLAND, MADEIRA ARCHIPELAGO

BRIAN D. BELL

BELL, B. D. 2001. Removal of rabbits from Deserta Grande Island, Madeira Archipelago. *Arquipélago*. Life and Marine Sciences. Supplement 2(Part B): 117-119. Ponta Delgada. ISSN 0873-4704.

In 1995, the Parque Natural da Madeira negotiated a Life Project through the European Community to remove the introduced mammals from Deserta Grande Island, Madeira Archipelago, an important Nature Reserve. In 1996, a specialist island restoration company from New Zealand, Wildlife Management International Limited, was contracted to carry out the eradication of rabbits (*Oryctolagus cuniculus*) on Deserta Grande. The rabbits were removed during the period of September 1st to December 2nd, using a grain based pellet with brodifacoum as the active ingredient. The methodology and results are outlined. A visit in October 1997 confirmed that all rabbits had been removed from Deserta Grande.

*Brian D. Bell (e-mail: wmil@clear.net.nz), Wildlife Management International Limited, PO Box 14-492, Wellington, New Zealand.*

## INTRODUCTION

Madeira archipelago provides the nesting grounds of the two most endangered seabird species in Europe, *Pterodroma madeira*, nesting on the main island of Madeira, and *P. feae*, breeding on Bugio. The former is currently the subject of strenuous conservation efforts, but *P. feae* is seriously threatened by soil erosion caused by goats and rabbits (ZINO & BISCOITO 1994). Measures to remove these problem animals where recommended in the action plan for *Pterodroma feae* (HEREDIA et al. 1996). A schedule was prepared for removing alien mammals from the small islands near Madeira, and the Parque Natural da Madeira put forward a request for funding to the European Community's Life Programme. Although Bugio was the breeding ground for the endangered *P. feae*, Deserta Grande (approximately 1000 ha) was chosen for the eradication project. Mice were excluded from the eradication project because the terrain was such that every mouse could not be put at risk through a hand laid bait operation.

There were several reasons for the choice, the main one being the very difficult terrain on Bugio and the lack of any facilities. It was considered that a hand laid programme would be impractical. It is also claimed that the goats *Capra hircus* present are from a unique strain of feral goat which may be genetically significant (this needs to be proven). As Deserta Grande already had a ranger station and some old buildings which could be renovated, it appeared the best option, even though it too has some difficult country. Also if Deserta Grande could be cleared then the smaller islands would be seen as more practical.

## MATERIAL AND METHODS

The main target species was the rabbit so the bait selected was "Wanganui No. 7", a grain based pellet manufactured by Animal Control Products in New Zealand. It has been the main bait used for rabbit control in New Zealand but usually with sodium monofluoroacetate (1080) as the active ingredient. On the Deserta Grande project the toxin chosen was brodifacoum at 0.02 g/kg.This was chosen as it requires only one feed to kill the animal and is suitable for any rodent which may be present. It is toxic to mammals and birds but not to reptiles and was used successfully and without problems on Round Island (MERTON 1987). However it is known to be highly toxic to raptors through secondary poisoning. The bait was dyed green to reduce its attractiveness to birds (MOORS et al. 1992).

The 20 tonnes of bait required was shipped from New Zealand. It was transferred to Deserta Grande by Parque Natural boat and taken to depots on the top of the island by helicopter.

On the plateau of the island the bait was laid out in bait stations (small heaps) on a 25 metre grid by both specialist staff (Wildlife Management International Ltd. from New Zealand) and Parque Natural wardens. On the cliff faces the bait was broadcast by personnel walking the cliffs where possible and throwing the bait. On some extreme cliff faces the bait was spread from the helicopter. Some bait was laid on the shore line by personnel landed from inflatable zodiac. The objective was to have every rabbit exposed to the bait.

The feeding out was done twice on the plateau but only once on the very difficult country. There was evidence to show that the rabbits were travelling quite long distances to feed and were not favouring the extreme slopes. This gave us confidence that all rabbits were getting access to the bait.

There were seven WMIL personnel, supported by an average of six Parque Natural staff, working on the project. The programme began at the northern end based at an old renovated building at the head of the Castanheira Valley. The southern end was covered from Doca, the warden station, but the specialist party established a temporary camp for much of the time in cave rooms at the top of the access track while operating at the south. It was hoped to cover Fajã Grande (a major central slump to the west) by landing from an inflatable boat but the adverse sea conditions meant this had to be covered by climbing down into it. Basically the island was covered by a rolling front working from the north to the south. As far as possible, over a week was allowed between the bait laying to give the animals time to die. The entire programme (laying and monitoring) took three months.

## RESULTS

In only a few days there was evidence that the bait was being taken by both mice and rabbits. Dead mice were being seen and rabbit droppings began to turn green. Signs of rabbits became less frequent as time went on and dead rabbits began to be found after a week. Near the end of our stay no rabbits or fresh signs were being found despite searches being made. There was also a spectacular recovery of the grass once the first rains occurred.

As far as could be assessed there were no long-term effects on non-target species. Some deaths had been accepted as one of the temporary costs of the operation. The buzzard *Buteo buteo* and kestrel *Falco tinnunculus*, populations were reduced significantly as a result of feeding on poisoned rabbits and mice. Both species had already re-colonised the island before the party had left. There was a minor kill of pipits, *Anthus berthelotii madeirensis*, and canaries, *Serinus canaria*, but there was no noticeable decline in the populations.

Following the departure of WMIL personnel, Parque Natural Wardens continued to watch for signs of rabbits without seeing any. A small party from WMIL returned to the island twelve months after the poisoning to check for evidence of rabbits. None was found and it can be claimed that all the rabbits have been removed.

Although mice took the bait some survived as expected. On neighbouring Ilhéu Chão surplus bait from the Deserta Grande project was laid out in a similar way and the mice appear to have been eradicated.

With the rabbits gone, the vegetation is rapidly regenerating. However, the other problem species, goat, was not eliminated. They were hunted and shot during the rabbit programme by the park wardens. It will be necessary to remove all the goats if the eradication of rabbits is to show lasting benefits.

## FUTURE MANAGEMENT

The removal of remaining goats must be a priority. The removal of browsing animals often opens the way for weeds to become established before the indigenous vegetation gets firmly re-established and this will need to be monitored closely. Vegetation recovery and the reduction in soil erosion can be accelerated by the use of debris dams to trap soil run off and by strategic planting of desirable species.

The stabilisation of the soil and increased plant growth will be beneficial to the petrel species using the island and may also provide a breeding niche for *P. feae* which periodically prospects the island.

Yellow-legged gulls (*Larus cachinnans*) have developed a technique for hunting the smaller petrels and this may restrict colonisation by *P. feae*. Like many gull species they have increased to an unnaturally high population as a result of poor waste disposal on Madeira itself. Improved rubbish disposal methods should be introduced and in the meantime some direct gull control may be warranted.

With Deserta Grande cleared of rabbits the way is now open to extend similar projects to other islands where problem exotic mammals occur e.g. Bugio, Selvagens etc.

## ACKNOWLEDGEMENTS

I am grateful for the help and assistance provided by Henrique Costa Neves, Director of Parque Natural da Madeira and his staff. The project was funded as part of a European Community Life Grant Life proposal number 95/P/A22/P/125/MAD under Contract number B4 – 3200/95/521

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CONTENTS: PAG.

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