Volume 16/Number 1/January 1985

Marine Pollution Bulletin, Vol. 16, No. 1, pp. 29–33, 1985
Printed in Great Britain

0025–326X/85 $3.00+0.00

Embryo Abnormalities in the Periwinkle, *Littorina 'saxatilis'*, as Indicators of Stress in Polluted Marine Environments

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Brood pouches of periwinkles belonging to the *Littorina 'saxatilis'* species complex usually contain, in addition to the five normal stages in development, a proportion of embryos that are morphologically abnormal. The frequency of adults with a high percentage of abnormals in their brood pouches reflects to a great extent the degree of environmental stress, natural and anthropogenic, to which the adult population has been exposed.

Collections of this gastropod from coastal populations along the Gower coast, in South Wales, disclosed higher frequencies of abnormal embryos from sites exposed to pollutant-laden, residual water currents in Swansea Bay, than was recorded from a clean, coastal population at Rhossili, 26 km west of Swansea, where frequencies were similar to those recorded from other unpolluted, coastal populations elsewhere in the British Isles.

While environmental water quality is probably a dominating factor in determining this effect, the presence of a low frequency of high scoring individuals in clean environments points to this phenomenon having a complex aetiology. It is suggested that the underlying causal factor may be one of disease, which is present in all *L. 'saxatilis'* populations, and which is triggered by factors related to reduced environmental water quality.

The reproductive cells and tissues of marine invertebrates are very sensitive to the effects of environmental contaminants (Calabrese *et al.*, 1973; Staveland, 1979) and consequently may be used as sensitive indicators of environmental perturbation (Åkesson, 1983; Dixon, 1983). In the rough periwinkle, *Littorina 'saxatilis'*-species complex (James, 1968; Heller, 1975; Smith, 1981), embryonic development takes place in a specialized brood pouch borne dorsally on the first body whorl of the female. Approximately 60 days are required for development from the egg to the juvenile snail (Berry, 1956; Fretter & Graham, 1962; Dixon, 1983). During the period in the brood pouch, the embryonic *L. 'saxatilis'* passes through five, readily distinguishable stages, corresponding to the gastrula (0–2 days), unshelled veliger (3–8 days), shelled veliger (9–20 days), late veliger (21–36 days), and pre-emergent young (37–60 days) (Berry, 1956). In addition to these normal stages in development, the brood pouch may also contain a proportion of abnormally developed embryos. It is the frequency of these abnormals which can be related directly to the degree of environmental stress, natural as well as anthropogenic, to which the adult population has been exposed (Dixon & Nugegoda, in preparation).

Results are presented for a field study of embryonic abnormalities in different *L. 'saxatilis'* populations from the south coast of the Gower Peninsula, which extends into the Bristol Channel from the southern coast of Wales. This area is exposed to a wide variety of pollutants, most of which emanate from the industrial centre of Swansea—Port Talbot, leading to the presence of a pollution gradient in a westerly direction towards cleaner waters at the mouth of the Bristol Channel (Abdullah & Royle, 1974; Chubb *et al.*, 1980). Recognized pollutant inputs in the region include domestic sewage and industrial wastes containing petroleum compounds, heavy metals and organochlorines (Chubb *et al.*, 1980; Kadhim, 1983), many of which have both mutagenic and teratogenic properties.

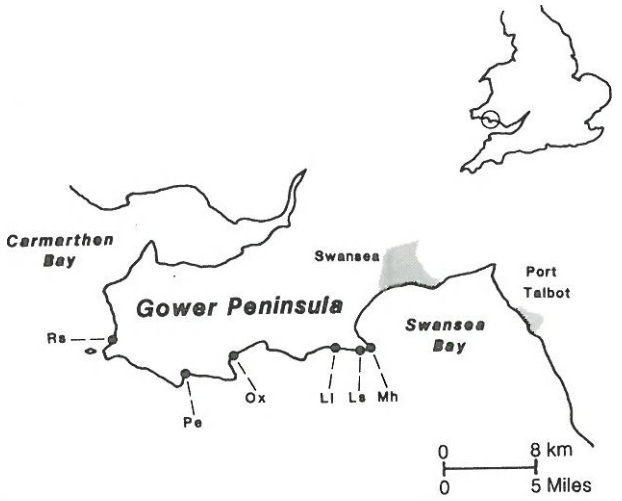

Fig. 1 Map of the Gower Peninsula, on the south coast of Wales, great Britain, showing the localities referred to in the text. Mh = Mumbles Head, Ls = Limeslade Bay, Ll = Langland Bay, Ox = Oxwich Bay, Pe = Port Eynon, Rs = Rhossili. Inset shows the study area in relation to the rest of southern Britain.

Materials and Methods

The *L. 'saxatilis'*-species complex occupies a zone on the upper part of the sea shore coincident with the one characterized by the brown alga, *Pelvetia canaliculata* (Lewis, 1978). Mature periwinkles were sampled from six coastal sites on the Gower Peninsula in June 1981. In a westerly direction from Swansea Bay these were Mumbles (Middle Head), Limeslade Bay, Langland Bay, Oxwich Bay, Port Eynon and Rhossili (Fig. 1), respectively 3.3, 3.9, 5.5, 16.2, 19.5 and 26.3 km from the docks at Swansea (West Pier).

Between 70 and 100 gravid females were collected from each of the six sites. In the laboratory the snails were housed temporarily in tanks containing artificial seawater (Instant Ocean). Shell length measurements were made with vernier calipers. The shells were cracked open in a small bench vice, and the sex of each individual adult was determined on the basis of the presence of either a penis or a brood pouch containing embryos. In these collections, because very small specimens were excluded from the samples, no neuter individuals were found.

The bodies of the females were detached from the shell by separating the large columella muscle and removed to individual Petri dishes containing seawater. The brood pouches were then teased open with mounted needles. The embryos were examined under a binocular microscope and the frequency of embryo abnormality was determined. Only females containing 100 or more embryos in total were included in the analysis.

In addition to unfertilized, undersized or otherwise non-viable eggs, the brood pouches of *L. 'saxatilis'* can contain several types of embryonic abnormalities. These include twins and other forms of multiple embryos, embryos with abnormal shells (straight-shelled, sinistrally coiled), and early-stage embryos which are characterized by the physical disruption of their soft tissues, often resulting in the presence of a number of separate, floating, cell masses within the egg capsule (Fig. 2). This chaotic pattern of development was the most regular response to changes in environmental water quality (Dixon & Nugegoda, in preparation).

Data analysis

The Kruskal–Wallis one-way analysis of variance by ranks (Siegel, 1956) was used to compare the median levels of embryo abnormality at the different sites. The Mann–Whitney *U*-test at the 0.05 significance level was employed to test for differences between individual median abnormality frequencies. A Chi-square test (Siegel, 1956) was applied to the data to disclose any distributional differences that the samples might display with respect to the frequency of abnormality.

Results

Figure 3 shows percentage abnormality histograms based on the data for the six sites, Mumbles Head, Limeslade Bay, Langland Bay, Oxwich Bay, Port Eynon and Rhossili. While the Port Eynon results are included here

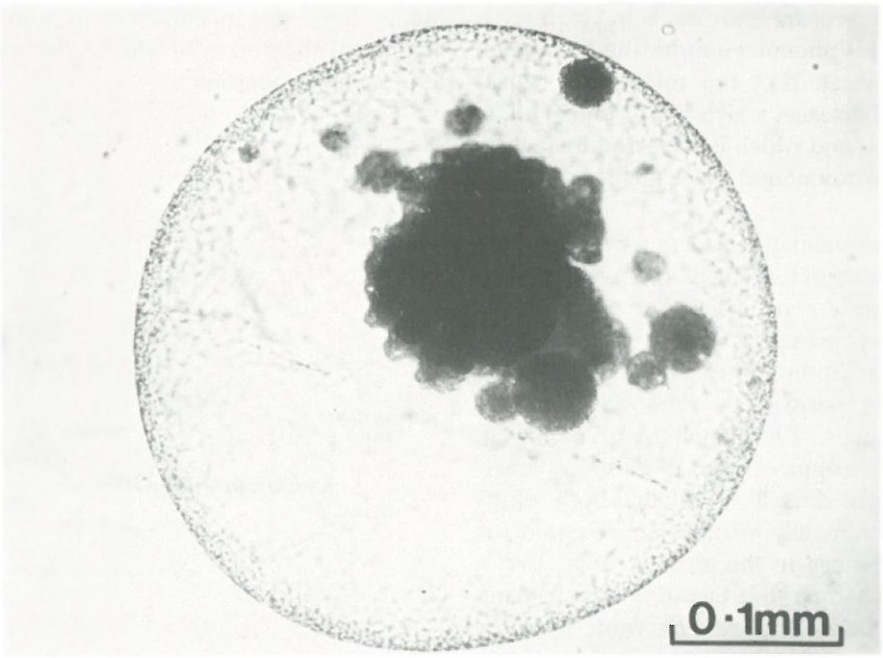

Fig. 2 The type of embryonic abnormality which featured most commonly in environmentally stressed populations of periwinkles along the Gower Peninsula. This stage corresponds to that of an unshelled veliger.

for the sake of completeness, they were omitted from all the statistical treatments as the animals were of small size (average shell length 7 mm, against 9 mm elsewhere) compared to all the other sites, and had correspondingly low fecundity (only 11 individuals out of a total sample size of 72 contained 100 or more embryos). Since abnormal embryos have a tendency to be retained in the brood pouch for a longer period than is usual for normal development [escape from the brood pouch is entirely a result of the activity of the juvenile (Berry, 1956)], and recognizing the cyclical nature of reproductive activity in this organism (Berry, 1961; Raffaelli, 1976; Faller-Fritsch, 1977), the inclusion of low fecundity individuals in these analyses would have led to the over-estimation of the proportion of individuals with a high frequency of abnormals. The smaller form sampled from Port Eynon could be tentatively identified as *L. saxatilis–tenebrosa,* while the larger form elsewhere was *L. saxatilis–saxatilis* (James, 1968). *L. 'saxatilis'* is actually a large species complex comprising several subspecies (Heller, 1975; Smith, 1981).

The Kruskal–Wallis test showed a significant difference at the 0.01 level of probability between the median percentage abnormality at the five sites. Paired comparisons conducted on the same parameter for the different sites, using the Mann–Whitney *U*-test, revealed significant differences at the 0.05 level (0.01 and 0.001 for Oxwich and Mumbles, respectively), between the median percentage abnormality for Rhossili and all the rest. The only other significant difference between sites was for Mumbles and Limeslade (at the 0.05 level); the result for Langland Bay was significant at the 10%, but not the 5% level, from that at Mumbles Head.

The chi-square comparison of all 5 sites showed that a significant difference existed at the 0.001 level of significance, with respect to the distributional characteristics of the data presented as histograms in Fig. 3. Removal of the Rhossili data from this comparison had the effect of removing any significant site difference. These findings combine with those of the other two tests to show that Rhossili was typified by a much lower frequency of abnormalities compared to the other four sites which were closer to the polluting influence of Swansea–Port Talbot.

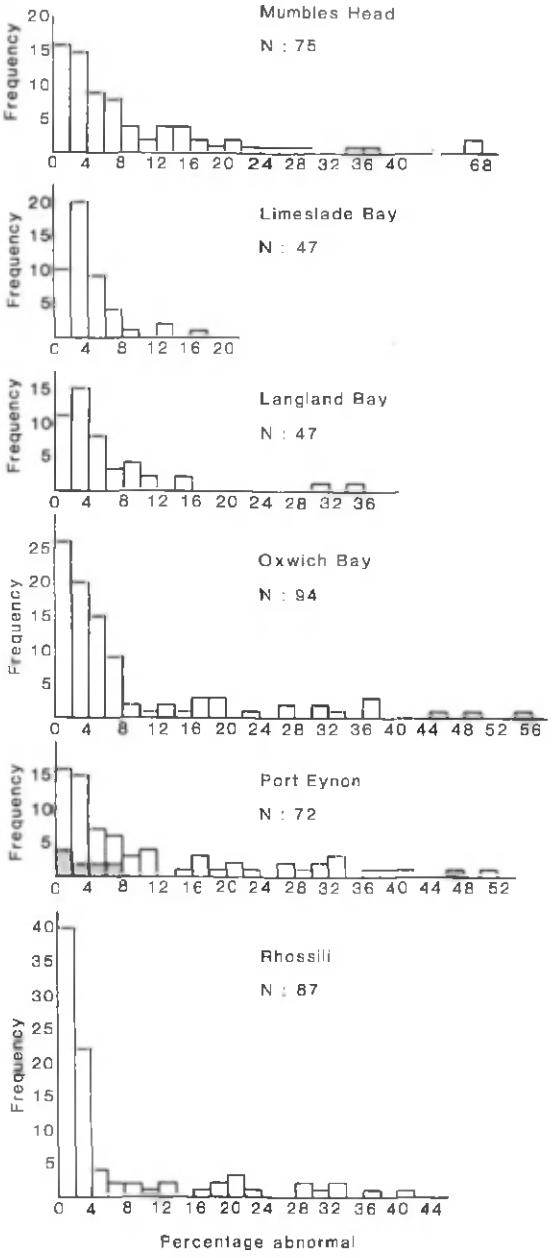

Fig. 3 Percentage abnormality–frequency histograms showing the distribution of embryo abnormalities in the Gower coast collections of *Littorina 'saxatilis'*. The shaded region indicates that portion of the Port Eynon sample that was made up of individuals containing 100 or more embryos.

Discussion

Developmental abnormalities have been described from the brood pouches of *L. 'saxatilis'* from several coastal populations on the Gower Peninsula, and from a number of other coastal and estuarine localities about the British Isles (Dixon, 1983: Dixon & Nugegoda, in preparation). Thorson (1946) described finding embryos with abnormally developed shells in samples taken from Øresund Sound (Denmark), which he attributed to poor water quality (low salinity) in the region, where the animal survives "under conditions so near its extreme limit for life that it is approaching total degeneration". The variety and frequency of embryo abnormalities is actually considerably greater than Thorson described, since he concentrated only on the later, hard-bodied developmental stages and twins. In fact, the earlier, soft-bodies stages are considerably more sensitive to environmental perturbation, and in some populations the individual load of abnormally developed embryos may regularly exceed 60% of all those contained in the brood pouch. Sexually mature individuals contain upwards of 100 embryos, with a maximum of ≃ 900 embryos in large specimens (Pelseneer, 1911; Linke, 1933). Where the frequency of abnormality is high, this must represent a serious reduction in reproductive potential (Monk *et al.*, 1978; Staveland, 1979).

These results indicate that differences exist between populations with respect to the frequency of abnormalities. Although there was no clear evidence of a gradient in effect between Mumbles Head and Rhossili, the latter site was characterized by a much lower overall frequency

of embryonic abnormalities than was typical of the other four sites tested. This is in keeping with the reported dispersion of pollutants in the area, recognizing Swansea–Port Talbot to be a point source for a variety of different pollutant types (Abdullah & Royle, 1974; Chubb *et al.*, 1980). These pollutants include some which are capable of inducing abnormal development in embryos (teratogenic effects), e.g. heavy metals (Ni, Cd, Hg), petroleum hydrocarbons and organochlorine pesticides such as gamma HCH, lindane and dieldrin (IARC, 1976; Wilson, 1976; Staveland, 1979; Léonard *et al.*, 1981).

Barnes & Parry (1983) explained the absence of a gradient in mutagenic activity in marine algae along the same stretch of Gower coastline as being due to the tidal mixing that takes place in the Bristol Channel. The recorded distribution of trace elements in the Bristol Channel (Abdullah & Royle, 1974) tends to suggest that any gradient in pollutant levels may occur over a greater spatial distance than was covered by the present study. The site at Rhossili, apart from being the furthest removed in terms of distance from the polluting influence of Swansea–Port Talbot, has the lowest frequency of abnormalities. This may reflect the fact that it lies on a promontory separating two hydrographically different water masses, and therefore may be more representative of the cleaner conditions in Carmarthen Bay (a non-industrialized embayment), which is contiguous with the Celtic Sea.

Throughout this discussion it has been assumed that the observed increases in the frequency of embryonic abnormality are the result of contact between the organism and pollutants. While this assumption is borne out by the findings of a number of separate investigations at polluted localities in the British Isles (Dixon, 1983; Dixon & Nugegoda, in preparation), a variety of physical and infectious agents can also modify or prevent the development of genetically determined structures, leading to reduced embryonic viability and congenital malformations (Tuchmann-Duplessis, 1980). Consequently, the observed effect, which is assumed to be related to pollutant stress, could arise through the interplay of several independent factors resulting in a complex aetiology.

Salinity, which has been shown to influence the frequency of abnormality in *L. 'saxatilis'* embryos (Thorson, 1948; Dixon & Nugegoda, in preparation), shows little variation about a mean of full strength sea water along the Gower Peninsula (Abdullah & Royle, 1974). This can therefore be discounted as a contributory factor in the present investigation.

An alternative explanation is that the abnormalities may be caused by a latent pathogenic agent which is activated to a disease condition by environmental stress. A number of pathogenic organisms, notably viruses, are known to cause congenital malformations in the embryos of higher vertebrates, in addition to their recognised role in disease conditions including certain types of neoplasm (Cooper, 1966; Nilsson, 1978). Farley (1980) has considered the case for the role of viruses in neoplastic disorders in shellfish, while Jensen and co-workers (review by Jensen, 1983) have isolated an iridovirus from lesions in diseased cod which they believe may be responsible for the ulcers recorded from fish taken in polluted waters.

Physical features of the dominant type of embryo abnormality recorded in environmentally stressed populations of periwinkles are reminiscent of the kind of uncontrolled breakdown in the processes associated with normal growth and development which one might expect from a neoplastic disorder, albeit in this case confined within the egg capsule (Fig. 2). Histological examinations have so far failed to reveal any evidence of any pathogenic condition associated with the somatic tissues of adult periwinkles containing a high incidence of abnormals (Dixon & Lowe, unpublished). Similarly, electron micrographs have shown no evidence of virus particles in the cells of abnormal embryos (courtesy of Dr D. C. Kelly, N.E.R.C. Institute of Virology, Oxford).

Abdullah, M. I. & Royle, L. G. (1974). A study of the dissolved and particulate trace elements in the Bristol Channel. *J. Mar. Biol. Ass. U.K.*, **54**, 581–97.

Åkesson, B. (1983). Methods for assessing the effects of chemicals on reproduction in marine worms. In *Methods for Assessing the Effects of Chemicals on Reproductive Functions*, Scope 20 (V. B. Vouk & P. J. Sheehan, eds.) pp. 459–482. John Wiley, Chichester.

Barnes, W. S. & Parry, J. M. (1983). The mutagenicity of acid digests from several common marine macroalgae (*Fucus serratus, Porphyra umbilicalis, Enteromorpha* spp.). *Marine Environ. Res.*, **10**, 127–145.

Berry, A. J. (1956). *Some factors affecting the distribution of* Littorina saxatilis *(Olivi).* Ph.D. thesis, University of London.

Berry, A. J. (1961). Some factors affecting the distribution of *Littorina saxatilis* (Olivi). *J. Anim. Ecol.*, **30**, 27–45.

Calabrese, A., Collier, R. S., Nelson, D.A. & MacInnes, J. R. (1973). The toxicity of heavy metals to embryos of the American oyster *Crassostrea virginica*. *Mar. Biol.*, **18**, 162–166.

Cooper, L. Z. (1966). German measles. *Scient. Am.*, **215**, 30–37.

Chubb, C. J., Dale, R. P. & Stoner, J. H. (1980). Inputs to Swansea Bay. In *Industrialised Embayments and their Environmental Problems* (M. B. Collins, F. T. Banner, P. A. Tyler, S. J. Wakefield and A. E. James, eds.), pp. 307–327. Pergamon Press, Oxford.

Dixon, D. R. (1983). Methods for assessing the effects of chemicals on reproductive function in marine molluscs. In *Methods for Assessing the Effects of Chemicals on Reproductive Functions*. Scope 20 (V. B. Vouk & P. J. Sheehan, eds.), pp. 439–457. John Wiley, Chichester.

Faller-Fritsch, R. J. (1977). Reproductive strategies of the winkle *Littorina rudis* in relation to population dynamics. In *Biology of Benthic Organisms* (B. F. Keegan, P. O'Ceidigh & J. S. Bodden, eds.), pp. 225–231. Pergamon Press, New York.

Farley, C. A. (1980). Phylogenetic relationships between viruses, marine invertebrates and neoplasia. In *Phyletic Approaches to Cancer*. Proceedings of the Eleventh International Symposium of the Princess Takamatsu Cancer Research Fund, Tokyo, pp. 75–87.

Fretter, V. & Graham, A. (1962). *British Prosobranch Molluscs*. Ray Society, London.

Heller, J. (1975). The taxonomy of some British *Littorina* species with notes on their reproduction. *Zool. J. Linn. Soc. Lond.*, **56**, 131–151.

International Agency for Research on Cancer (1976). Cadmium and cadmium compounds. In *IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man*, Vol. 11, *Cadmium, Nickel, some Epoxides, Miscellaneous Industrial Chemicals and General Considerations on Volatile Anaesthetics*. Lyon: WHO/IARC, pp. 39–74.

James, B. L. (1968). The distribution and keys of species in the family Littorinidae and their digenean parasites, in the region of Dale, Pembrokeshire. *Field Studies*, **2**, 615–650.

Jensen, N. J. (1983). The ulcus syndrome in cod (*Gadus morhua*); a review. *Rapp. P.-v. Reun. Cons. int. Explor. Mer*, **182**, 58–64.

Kadhim, M. (1983). The detection of mutagens in the marine environment. Ph.D. thesis, University of Wales.

Leonard, A., Gerber, G. B. & Jacquet, P. (1981). Carcinogenicity, mutagenicity and teratogenicity of nickel. *Mutat. Res.*, **87**, 1–15.

Lewis, J. R. (1978). *The Ecology of Rocky Shores*. Hodder & Stoughton, London.

Linke, O. (1933). Morphologie und physiologie der genitalapparates der Nordseelittorinen. *Wiss. Meresuntersuch, Abt. Helgoland*, **19**, 3–52.

Monk, D. C., Cowell, E. B. & Syratt, W. J. (1978). The littoral ecology of the area around Mongstad refinery, Fensfjordan, during the three years

after refinery commissioning, 1975–1977. Report to Rafnor a/s and Co., Mongstad, London: British Petroleum Company.
Nilsson, K. (1978). Transformation of lymphoid cells by herpesviruses: a review with special reference to the phenotypic properties of transformed cells. In *Oncogenesis and Herpesviruses III. Part 1: DNA of Herpesviruses, Viral Antigens, Cell–Virus Interaction* (G. de-The, W. Henle, F. Rapp, eds.), pp. 451–472. WHO/IARC, Lyon.
Pelsenneer, P. (1911). Recherches sur l'embryologie des Gastropodes. *Mem. Acad. Roy.*, ser. II, **3**, 1–167.
Raffaelli, D. G. (1976). The determinants of zonation patterns of *Littorina neritoides* and the *Littorina saxatilis* species–complex. Ph.D. thesis, University of Wales.
Siegel, S. (1956). *Non Parametric Statistics for the Behavioural Sciences.* McGraw-Hill, New York.
Smith, J. E. (1981). The natural history and taxonomy of shell variation in the periwinkles *Littorina saxatilis* and *Littorina rudis*. *J. mar. Biol. Ass. U.K.*, **61**, 215–241.
Staveland, J. T. (1979). Effects on hatching in *Littorina littorea* after an oil spill. *Mar. Pollut. Bull.*, **10**, 255–258.
Thorson, G. (1946). Reproduction and larval development of Danish marine bottom invertebrates, with special reference to the planktonic larvae in the Sound (Øresund). *Medd. Komm. Havundersøg.* (ser. Plankton), **4**, 170–173.
Tuchmann-Duplessis, H. (1980). The experimental approach to teratogenecity. *Ecotox. Environ. Safety*, **4**, 422–433.
Wilson, J. G. (1976). Environmental chemicals. In *Handbook of Teratology* (J. G. Wilson & F. Clarke Fraser, eds.), pp. 357–385. Plenum Press, New York.

Marine Pollution Bulletin, Vol. 16, No. 1, pp. 33–34, 1985
Printed in Great Britain

0025–326X/85 $3.00+0.00

Characteristics of Selenium in Marine Animals

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The biochemical association and chemical form of selenium in the muscle tissues of four marine animals has been examined. Selenium was predominantly associated with soluble proteins (72–80%), and not present as characterizable inorganic selenium species (SeO_3^{2-}, SeO_4^{2-}).

Selenium enters the atmosphere during the combustion of fossil fuels (Bertine & Goldberg, 1971) and is considered to be a potential marine pollutant (Fowler & Benayoun, 1976a). Selenium is of interest as it is an essential element for animals (Schwartz & Faltz, 1957), toxic at elevated levels (Schroeder & Mitchener, 1971) and influences the accumulation of other toxic elements such as mercury (Koeman *et al.*, 1973). Relatively little is known about the behaviour of selenium in marine organisms (Lunde, 1970, 1972, 1973; Fowler & Benayoun, 1976a, b, c; Wrench, 1979). The present investigation was undertaken to identify some of the properties of the selenium compound(s) present in marine animals.

Materials and Methods

Sample preparation

Animals were collected from St Vincent Gulf, South Australia, during August–December 1981. Muscle tissues were separated, freeze-dried and ground (to <200 μm).

Selenium analysis

Total. After digestion of organic matter with nitric and perchloric acids selenium was isolated by coprecipitation with lanthanum hydroxide, then a piazselenol was formed between selenium and 2,3-diaminonapthalene at pH 1.0 and extracted into cyclohexane. Selenium was determined fluorometrically (λ_{ex} 377 nm; λ_{em} 519 nm) in the extract. The accuracy of the determination procedure has been previously assessed by recovery experiments and the analysis of a standard reference material (Maher, 1982). Recoveries of selenium added to selected marine tissues (fish and scallops) were in the range 97–100%. The selenium concentration obtained by replicate analysis of Orchard Leaves NBS SRM 15 71 was 0.076 ± 0.002 μg g^{-1} and in agreement with the certified value (0.08 ± 0.01 μg g^{-1}).

Inorganic species. Samples were extracted with 5 M hydrochloric acid to release inorganic selenium species. Inorganic selenium was isolated by reduction to hydrogen selenide using sodium tetrahydroborate (III) (Maher, 1983a) and concentrated in a liquid nitrogen trap (Cutter, 1978). Selenium was determined by decomposition of the trapped hydride in a silica T tube furnace and by measurement of the atomic absorption of selenium at 196.0 nm. The detection limit was 0.001 μg and the relative standard deviation at the 0.010 μg level was 1.8%.

Biochemical fractionation

The sequential extraction scheme used for biochemical fractionation is summarized in Table 1. Extractions were performed in centrifuge tubes with mechanical shaking and sonification to mix solutions. Following each

TABLE 1

Sequential extraction procedure for tissue biochemical fractionation

Nominal fraction extracted	Procedure
Lipids/lipoproteins	2:1 v/v $CHCl_3/CH_3OH$, 2×50 ml, 25°C, continuous agitation, 36 h. Addition of 0.2 v/v of 0.5 M KCl. Lower phase lipids, upper phase non lipids.
Amino acids, organic acids, sugars	9:1 v/v C_2H_5OH/H_2O 2×50 ml, 25°C, continuous agitation 12 h.
Proteins	0.1 M Tris/HCl containing 0.1% w/v NaCl, 1% w/v sodium dodecylsulphate and 0.05 M dithiothreitol, 3×30 ml, pH 7.5, 25°C; continuous agitation, 12 h.

TABLE 2

Selenium associated with biochemical fractions

Tissue	Fraction		
	Chloroform/ methanol	Ethanol/ water Selenium (%)*	Tris/hydrochloric acid
Penaeus latisulcatus	5±2	19±5	72±4
Pecten alba	3±1	20±6	80±5
Sepioteuthis australis	2±2	14±3	78±7
Hemir hamphus australis	4±1	17±4	76±3

*Four extractions.

extraction, the mixtures were centrifuged, the supernatants decanted and the solvent removed under reduced pressure. The residues were dissolved in nitric acid and selenium determined as previously described for total selenium.

Results and Discussion

The muscle tissues of four marine animals, a prawn *Penaeus latisulcatus,* a scallop *Pecten alba*, a squid *Sepioteuthis australis* and a fish *Hemir hamphus australis* were examined. The prawn muscle tissue contained 4.01 μg g^{-1} Se dry wt, the scallop tissue 1.24 μg g^{-1} Se dry wt, the squid tissue 1.74 μg g^{-1} Se dry wt and the fish tissue 1.49 μg g^{-1} Se dry wt. The selenium concentrations found were similar to those measured in other organisms collected from the same area (Maher, 1983b).

Biochemical fractionation of the muscle tissues (Table 2) revealed that 72–80% of the selenium present is extracted by tris/hydrochloric acid buffer solution. The addition of ethanol to the buffer extracts to precipitate protein also quantitatively precipitated selenium (94–98%). Thus a large fraction of the selenium in the muscle tissues is associated with proteins.

Selenium in the muscle tissues was not present as characterizable inorganic selenium species (SeO_3^{2-}, SeO_4^{2-}). Inorganic selenium species ionically complexed by tissues will be released by acid extraction. Selenium incorporated into seleno amino acids would not have been released by the extraction procedure employed.

The results reported here suggest that selenium in the muscle tissues of marine animals is not present as ionic inorganic species but incorporated into proteins.

Bertine, K. K. & Goldberg, E. D. (1971). Fossil fuel consumption and the major sedimentary cycle. *Science*, **173**, 223–235.

Cutter, G. (1978). Species determination of selenium in natural waters. *Anal. chim. Acta*, **98**, 59–66.

Fowler, S. W. & Benayoun, G. (1976a). Accumulation and distribution of selenium in mussel and shrimp tissues. *Bull. Envir. Contam. Toxic.*, **16**, 339–346.

Fowler, S. W. & Benayoun, G. (1976b). Influence of environmental factors on selenium flux in two marine invertebrates. *Mar. Biol.*, **37**, 59–68.

Fowler, S. W. & Benayoun, G. (1976c). Selenium kinetics in marine zooplankton. *Mar. Sci. Commun.*, **2**, 43–64.

Koeman, J. H., Peeters, W. H. M., Koudstaal-Hol, C. H. M., Tjioe, P. S. & De Goeij, J. J. M. (1973). Mercury–selenium correlations in marine mammals. *Nature, Lond.*, **245**, 385–386.

Lunde, G. (1970). Analysis of arsenic and selenium in marine raw materials. *J. Sci. Fd Agric.*, **21**, 242–247.

Lunde, G. (1972). Location of lipid soluble selenium in marine fish to lipoproteins. *J. Sci. Fd Agric.*, **23**, 987–994.

Lunde, G. (1973). The presence of lipid-soluble selenium compounds in marine oils. *Biochem. biophys. Acta*, **304**, 76–80.

Maher, W. A. (1982). Fluorometric determination of selenium in some marine materials after digestion with nitric and perchloric acids and coprecipitation of selenium with lanthanum hydroxide. *Talanta*, **29**, 1117–1118.

Maher, W. A. (1983a). Determination of selenium in marine organisms using hydride generation and electrothermal atomic absorption spectroscopy. *Anal. Lett. A*, **16**, 801–809.

Maher, W. A. (1983b). Selenium in marine organisms from St Vincent Gulf, South Australia. *Mar. Pollut. Bull.*, **14**, 35–36.

Schroeder, H. A. & Mitchener, M. (1971). Toxic effects of trace elements on the reproduction of mice and rats. *Archs. envir. Hlth*, **23**, 102–106.

Schwarz, K. & Foltz, C. M. (1957). Selenium as an integral part of factor 3 against dietary necratic liver dengeration. *J. Am. Chem. Soc.*, **79**, 3292–3296.

Wrench, J. J. (1979). Uptake and metabolism of selenium by oysters. *Mar. Sci. Commun.*, **5**, 47–59.