

the world's oceans. Clearly a strong lead is required from an international environmental organization such as UNEP if this problem is to be seriously tackled. Only in this way can satisfactory pollution control measures for plastic waste become the norm and be effective.

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# Abnormal Development of *Dentalium* due to the *Amoco Cadiz* Oil Spill

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**A comparison was made between the development of *Dentalium* eggs, spawned by animals, collected before and after the *Amoco Cadiz* oil spill. Development of eggs from animals collected before the oil spill was significantly better than development of eggs from animals collected after the oil spill. It is suggested that development is affected by oil hydrocarbons, accumulated during oogenesis in the lipid-rich phases of the oocytes.**

During our stay at the Station Biologique at Roscoff in August 1978, the planned embryological investigations with *Dentalium vulgare* have been continuously frustrated by abnormal development of the embryos. There are, however, some results suggesting that this might be due to the *Amoco Cadiz* oil spill in March 1978.

By coincidence, a number of females of *Dentalium* were kept from July 1977 in the running sea-water system of the laboratory, thus escaping the oil-contamination in their natural habitat. This fortunate circumstance enabled us to make a comparison between the development of embryos and larvae from oocytes of females, collected before and after the oil spill. The results reported here indicate that the disturbances of normal development may be explained by accumulation of oil hydrocarbons in the oocytes.

## Materials and Methods

*Dentalium vulgare* (da Costa) (Scaphopoda, Mollusca) was collected in the Bay of Morlaix, 300–400 m North of 'La Vieille' (depth 25–30 m) and kept in the laboratory in a layer of bottom-grit, either in running sea-water at 17°C or in daily changed sea-water at 12°C. The animals, collected in 1977 passed the winter in running seawater in the aquarium of the laboratory. Spawning was induced by transferring the animals to glass containers (with about 100 ml of sea-water), left to equilibrate to room temperature. Oocytes were fertilized by adding a sperm suspension of adequate dilution. Cleaving embryos and larvae were kept in millipore-filtered sea-water, which was changed daily.

## Results

As a rule, fertilization of oocytes spawned by females freshly collected in 1978 was extremely poor. In the batches of oocytes, in which fertilization succeeded, no more than 30% of the eggs started cleavage; adding more sperm in these cases did not improve fertilization rate. First cleavage in about 20% of the cases was abnormal. The following deviations of normal cleavage were seen: retarded cleavage, unequal cleavage and/or premature regression of the polar lobe constriction and regression of cleavage furrows.

Trochophores, formed 25 h after first cleavage, were frequently malformed. Development was retarded and the trochophores were swimming slowly and were incoordinated. Mortality of trochophores was marked (up to 100% in some batches). The larvae, which metamorphosed normally, seemed to be affected no more.

One experiment needs special attention. Oocytes were obtained from females, collected before and after the oil spill (i.e. in 1977 and 1978, respectively). These batches of oocytes were fertilized each with an equal amount of the same sperm-suspension, obtained from males collected in 1978. While the percentage of fertilization was low in both cases, development of eggs spawned by females collected in 1977 was significantly better than development of eggs, spawned by females collected in 1978 (Table 1). For reasons of brevity the first groups of larvae will be designated 1977-larvae, while the latter will be called 1978-larvae.

The 1977-larvae are regularly formed (Figs 1 and 2(a)). The prototroch forms a continuous belt and a foot can be clearly distinguished. The pretrochal region is small in these larvae (compare Kowalewsky, 1883; Lacaze-Duthiers, 1856, 1857). The trochophores are swimming rapidly and coordinated near the water-surface. The 1978-larvae, in contrast, are irregularly formed (Figs 1 and 2 (b) and (c)). The prototroch is less pronounced than in the 1977-larvae, while the foot and posttrochal region cannot always be clearly delineated. The pretrochal region is larger than in the 1977-larvae. The trochophores are swimming badly coordinated; they are turning around and rotating near the bottom of the culture vessel without any recognizable direction.

The eggs spawned by animals collected in 1979 developed normally. Therefore, the development of *Dentalium* seems to be affected only the first reproductive season after the oil spill.

## Discussion

After adding water-soluble fractions of different oils to the sea-water, low percentage of fertilization, poor development of embryos and mortality of larvae of the sand dollar and some species of marine bivalves has been observed (Renzoni, 1975; Byrne & Calder, 1977; Nicol *et al.*, 1977; Nunes & Benville, 1978). Vacuolization of embryos and inability to swim has been described for *Mulinia* larvae

under experimentally oiled conditions (Renzoni, 1975). The concentrations of hydrocarbons, causing these effects are in the range of 0.1–10 mg l<sup>-1</sup>. As the concentration of oil hydrocarbons, measured in the sea-water at Roscoff in July 1978 was 0.02 mg l<sup>-1</sup> maximally (Cabioch, personal communication), it is unlikely that the poor development of *Dentalium* embryos and larvae reported here, is due to the presence of oil hydrocarbons in the sea-water. Furthermore, normal development of the 1977-larvae confirms that oil hydrocarbons, if present in the sea-water, are not directly responsible for causing a disturbance of development. Possibly, the low percentage of fertilization can be explained in that way (sperm being sensitive to much lower concentrations of oil hydrocarbons than oocytes (Renzoni, 1975; Nicol *et al.*, 1977).

A more feasible hypothesis to explain the observed disturbances in the development of *Dentalium* eggs is that abnormal morphogenesis is caused by oil hydrocarbons, accumulated in the oocytes. Accumulated hydrocarbons are likely to cause malformations similar to hydrocarbons, added to the sea-water. In both cases the hydrocarbons are affecting development indirectly by their incorporation in lipid-rich phases of the embryos (lipid-yolk and membranes). Abortion of lobster-eggs, due to oil hydrocarbon accumulation following the *Amoco Cadiz* oil spill is reported (Laubier, 1978).

Unfortunately, no data about the composition of *Dentalium* oocytes are available. In general, however, the ovaries of marine molluscan species have high lipid contents, which increase during oogenesis (Williams, 1970; Webber, 1970; Lawrence, 1976). In *Dentalium* oogenesis occurs in spring, the oocytes being mature from June till September. Similar to the oocytes of marine molluscan species, the eggs of the polychaete *Nereis* are very rich in neutral lipids (Pocock *et al.*, 1971). Under experimental conditions oil hydrocarbons are specifically accumulated in the oocytes of the polychaete *Neanthes* (Rossi & Anderson, 1977).

The sediment from which *Dentalium* was collected is known to be contaminated by oil hydrocarbons. In August 1978 (i.e. 5 months after the oil spill) concentrations of about 30 mg kg<sup>-1</sup> dry weight were measured (Cabioch, personal communication). It is feasible that this sediment-contamination causes low levels of oil hydrocarbons in the interstitial water for a long period (Cabioch *et al.*, 1978).

TABLE 1

Development of embryos and larvae of *Dentalium vulgare* from oocytes, spawned by females collected in 1977 and 1978. The batches of oocytes were fertilized with an equal amount of the same sperm-suspension, spawned by males collected in 1978.

	Development up to fourth cleavage			Development of trochophores (95 h)		
	No cleavage	Abnormal cleavage*	Normal cleavage	Dead	Abnormal †	Normal ‡
A. Oocytes from females collected in July 1977, kept at 17°C	100/140 (72%)	5/140 (4%)	33/140 (24%)	7/33 (21%)	2/33 (6%)	24/33 (73%)
B. Oocytes from females collected in August 1978, kept at 17°C	138/183 (75%)	5/183 (4%)	38/183 (21%)	26/38 (68%)	12/38 (32%)	0/38 (0%)
C. Oocytes from females collected in August 1978, kept at 12°C	310/345 (90%)	11/345 (3%)	24/345 (7%)	8/24 (33%)	16/24 (67%)	0/24 (0%)

\*Polyspermic eggs not included.

† Trochophores are swimming uncoordinated near the bottom of the culture vessel. The larvae are irregularly formed (see Figs 1 and 2 (b) and (c)).

‡ Trochophores are swimming rapidly near the water surface. The larvae are regularly formed (see Figs 1 and 2 (a)).

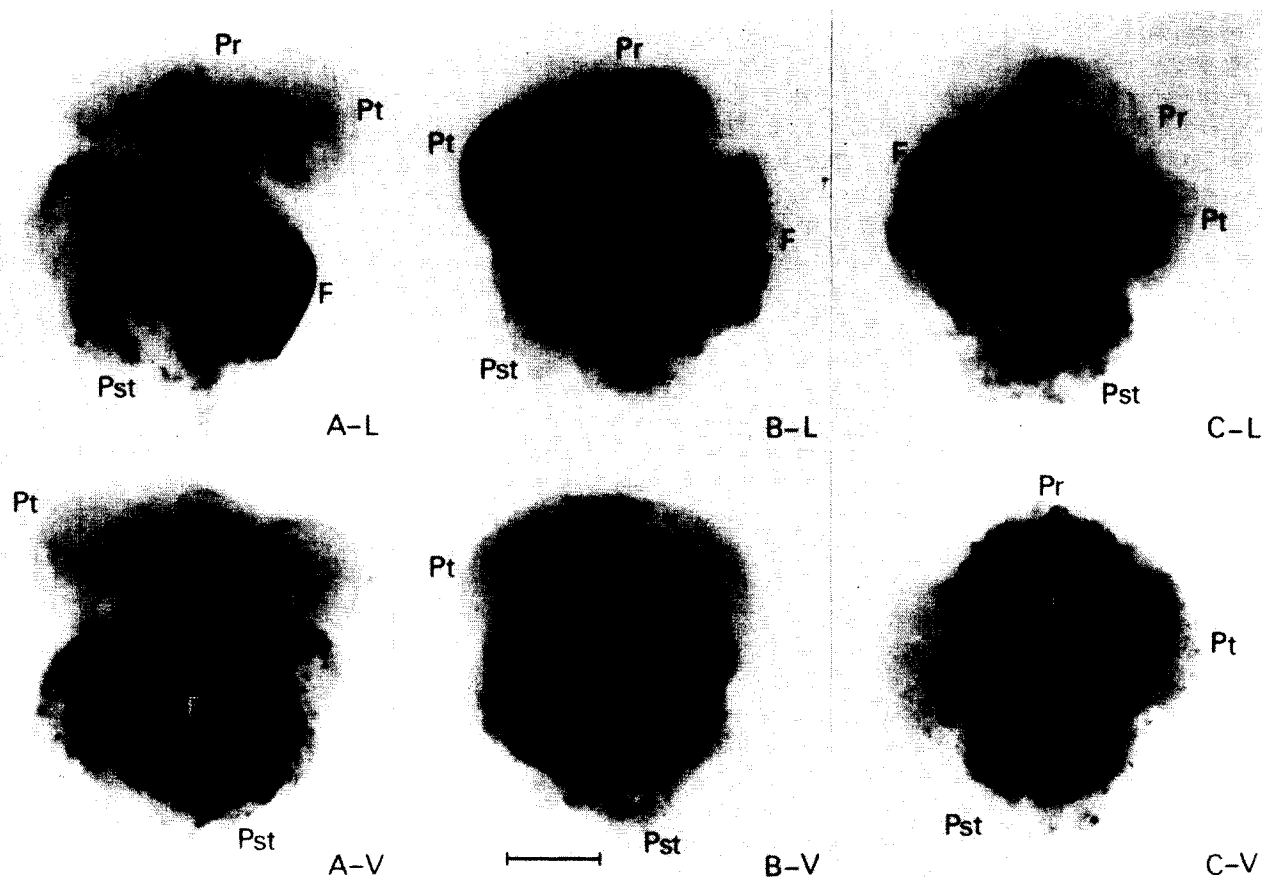


Fig. 1 Trochophores (50 h after first cleavage) of *Dentalium vulgare* developed from oocytes, spawned by females collected in 1977 (a) and 1978 (b) and (c). The females were kept at 12°C (c) or 17°C (a) and (b). Each larva is seen from the lateral (L) and ventral (V) side. F = foot, Pr = pretrochal region, Pst = posttrochal region, Pt = prototroch. The larvae were fixed in zenker and stained with gallocyenin (0.15% in 15% chrome-alum, pH 0.84). Scale: 0.2 mm.

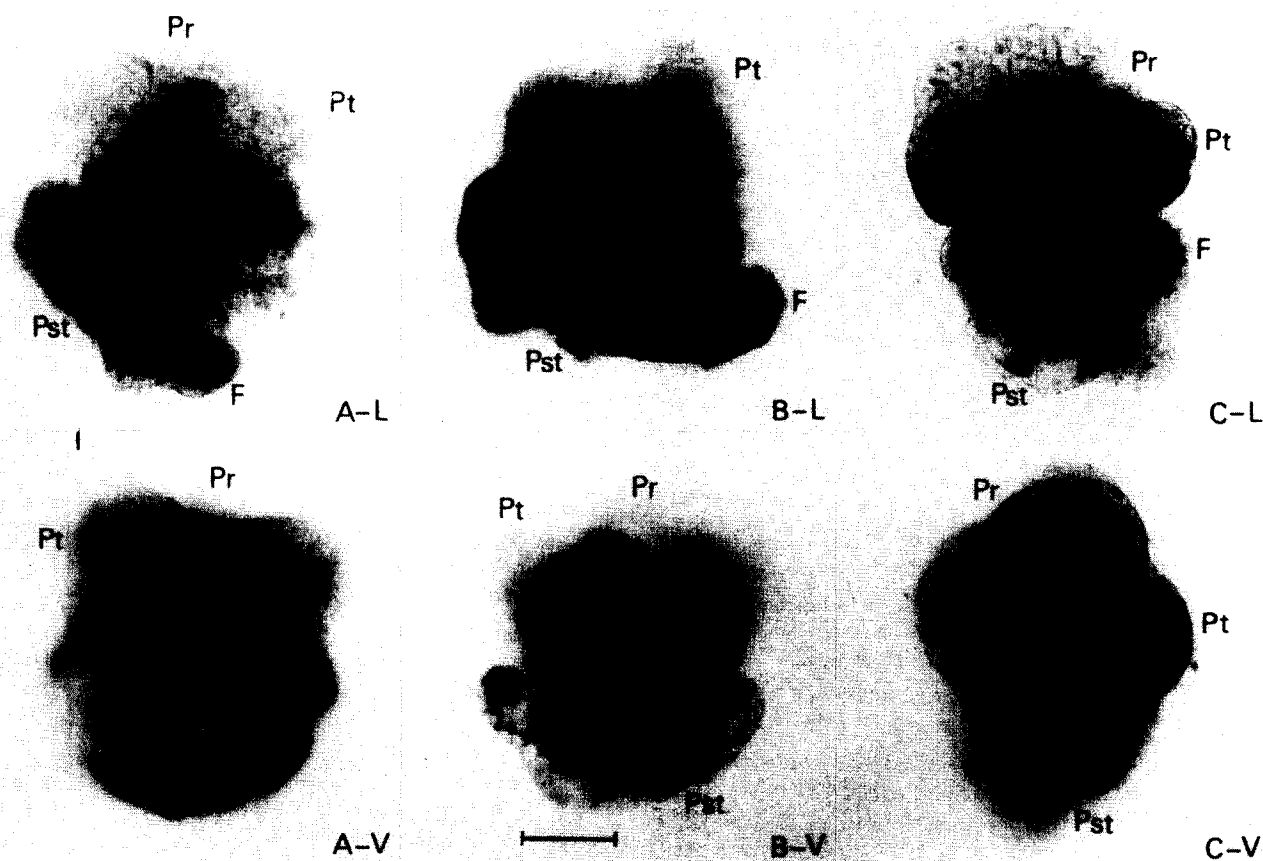


Fig. 2 Trochophores (70 h after first cleavage) of *Dentalium vulgare* developed from oocytes, spawned by females collected in 1977 (a) and 1978 (b) and (c). The females were kept at 12°C (c) or 17°C (a) and (b). Each larva is seen from the lateral (L) and ventral (V) side. F = foot, Pr = pretrochal region, Pst = posttrochal region, Pt = prototroch. The larvae were fixed in Zenker and stained with gallocyenin (0.15% in 15% chrome-alum, pH 0.84). Scale: 0.2 mm.

During long-term exposure to sea-water, containing relative small amounts of oil hydrocarbons, molluscan species accumulate these hydrocarbons in considerable amounts. Concentration-factors as high as 1000 have been reported (Stegeman, 1974, Grahl-Nielsen *et al.*, 1978; Roesijadi *et al.*, 1978). Another way by which oil hydrocarbons could be accumulated is via the marine food web (Blumer *et al.*, 1970). With regard to *Dentalium* it is not clear to what extent both processes could attribute to possible accumulation of oil hydrocarbons (see Morton, 1959).

Summarizing, it is feasible that oil hydrocarbons, deposited in the sediment of the Bay of Morlaix, are accumulated in the oocytes of *Dentalium* during oogenesis, either via ingested material or via direct absorption from the interstitial water. These accumulated hydrocarbons can disturb morphogenesis by their incorporation in the lipid-yolk and the membranes. These disturbances of embryogenesis probably will have no long-lasting effects on the population-dynamics of this species, because development appeared to be normal in animals collected in the second reproductive season following the oil spill.

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# Effect of Non-Petroleum Oil Spills on Wintering Birds near Vancouver

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Within the period of our records, spills of vegetable oils at Vancouver harbour have caused greater losses of birds than spills of petroleum oils. Vegetable oils affect birds by feather wetting but do not exhibit odour and slick characteristics of petroleum oils. Because most vegetable oils are edible their potential danger to aquatic birds may go unnoticed; sites of storage and transshipment of vegetable oils may be overlooked in oil spill contingency planning.

The danger of oil pollution to aquatic birds is widely acknowledged; petroleum oils are well known for their ability to reduce or eliminate feather water-proofing, resulting in exposure and ultimately death. However petroleum products are not the only potential source of oil pollution to aquatic birds. Recently, the greatest effects of oil pollution on birds in the harbour at Vancouver, British Columbia, have come from two small spills of vegetable oils.