Scand. J. Lab. Anim. Sci. No. 1 . 1994 . Vol. 21

A review of the biology of *Octopus vulgaris* and its use as a laboratory animal

by *J. F. A. Carver*, BVetMed MRCVS.
Laboratory Animal Science & Welfare, Department of Veterinary Pathology and Infectious Diseases, Royal Veterinary College, Royal College Street, London NW1 0TU.

Introduction

The Animals (Scientific Procedures) Act 1986 provides considerable protection for all vertebrate animals that are used for research purposes within the United Kingdom. Following the implementation of this legislation there has been disquiet that some of the higher invertebrates are not covered by the Act.

Given the highly developed nature of their nervous and sensory systems and the complex behavioural that they display, there is a strong body of thought that the cephalopods are capable of suffering, and therefore should be included in the Act. This matter was considered in 1992 by the Animal Procedures Committee, and their annual report (1992) contained discussion of the issue. As a consequence of this report it was decided to include *Octopus vulgaris* in the Act, and an Order to this effect came into force on the 1st October 1993.

This decision is interesting and somewhat controversial in that it raises as many issues as it resolves. The Order is also a landmark in it that represents an amendment to the scope of the Animals (Scientific Procedures) Act 1986, beyond the vertebrates, within the very short period (in legislative terms) of some seven years. It acts, therefore, both as a precedent for, and a possible pointer to, the inclusion of further invertebrate species into the Act.

The aim of this paper is to relate the general biology of *O. vulgaris* to its requirements as a laboratory animal, and to review the usage of this species in biomedical research over the past 19 years. Further consideration will also be given to some of the implications of its inclusion within the Animals (Scientific Procedures) Act 1986.

Taxonomy

PHYLUM: Mollusca
CLASS: Cephalopoda
SUBCLASS: Coleoidea
ORDER: Octopoda
SUBORDER: Incirrata
FAMILY: Octopodidae
GENUS: Octopus
SPECIES: *Octopus vulgaris*

Husbandry

Capture and Transport Methods

Capture. In common with the other cephalopods, *O. vulgaris* has a delicate skin that is very susceptible to physical trauma. It is therefore prone to subcutaneous bruising which subsequently proves to be invariably fatal. This can pose considerable problems with the supply of animals for the laboratory since the most common source of supply of *O. vulgaris* is from commercial trawlers. As one would expect, this method of capture has a very high mortality rate. The method can be improved upon by using small beam trawls for short hauls of 15 to 20 minutes duration (*Boyle* 1991a). However, the best technique for specimen collection is the use of traps or pots. This relies on the fact that the animals' habitat of choice is in crevices. Ideally the pots should be lifted every two to three days and this allows for the capture of undamaged individuals.

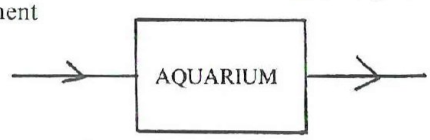
Transport. Given the delicacy of octopus skin it is essential that animals are kept moist at all times during transport to the laboratory. Animals captured at sea are best kept in deck tanks of sea water that are continuously filled with fresh sea water. Individuals can be transported in cooled and sealed polythene bags that are one third-filled with sea water and with air, or preferably oxygen, in the remaining space. Animals can survive under these conditions for 8 to 10 hours.

Housing

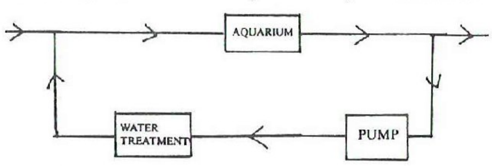
Habitat. O. vulgaris is an exclusively marine, epi-benthic (i.e. living on or close to the sea floor) animal. The classic habitat for the species is coastal stony or rocky areas. These provide both shelter and a source of the preferred range of invertebrate prey species. This feature of octopod biology must be reflected in the way that they are kept in the laboratory.

Aquarium Types. There are three types of aquarium systems:

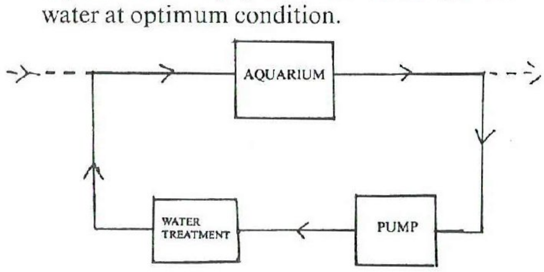
a. Open- Here all water that enters the system leaves it, i.e. there is constant replenishment



b. Semi-Open- Here some of the water that enters the system is recirculated but some is discharged, i.e. there is partial replenishment



c. Closed- Here all water that enters the system is recirculated. Such a system requires sophisticated equipment to maintain the water at optimum condition.



Conventional aquaria can be used as holding tanks. The tank walls should be opaque and there should be constant diffuse overhead light. Plastic piping, on a sand or gravel substrate, should be provided as dens for the animals. These allow for a greater stocking density, but can cause problems with the rapid and thorough removal of uneaten food that will otherwise decompose. Octopuses are inquisitive and, as a consequence of their arms and suckers, highly mobile. So aquaria need tightly fitting lids or coverings in order to render them escape-proof. This desire to explore beyond their immediate environment is a feature of their high degree of behavioural development and octopuses quickly adapt to capitivity, and can even become tame. This is indicative of both their intelligence and their value as a laboratory species.

Environmental Conditions

(*Boyle* 1991b):

a. **Water Temperature** should be matched to that of the local conditions.

b. **Water Salinity.** Aquarium sea water should be kept at around 34–36 ‰ S as this is the spectrum of sea water and octopuses can only live in a narrow salinity range (stenohalinity).

c. **pH** level should be held above pH 7.5 as cephalopods are sensitive to acidity. Once again the preferred range is that of open sea water i.e. pH 7.8–8.2. Sodium bicarbonate can be used to correct any tendency to acidity.

d. **Oxygen.** Although *O. vulgaris* is quite tolerant of reduced oxygen levels, forced aeration should be used to achieve near saturation oxygen levels.

e. **Recommended Maximum Levels Of Nitrogenous Excretory Products** are as follows: < 0.10 mg/l ammonia, < 0.10 mg/l nitrite, < 20.00 mg/l nitrate.

f. **Space Requirements.** Providing that water quality is adequate, *O. vulgaris* can be held at quite high stocking densities. Circular tanks of 1 m diameter and 0.6 m depth can hold five to ten animals, whilst a tank of the same depth but twice the diameter can house three times this number. The aggressiveness of individual animals has a significant effect on the stocking density.

Feeding

O. vulgaris is a predator of various crustacea, fish and molluscs, which it hunts using visual and chemical cues. The radula and beaks are used to remove flesh from the shell. It is thought that secretions from the posterior salivary glands are used both to immobilise the prey and to externally digest the tissues. The food then passes through the crop, stomach, caecum and intestine prior to entering the large digestive gland, where the majority of digestion and absorption occurs.

Despite the fact that octopuses will attack and eat dead food, such as pieces of skewered sardine that are jiggled about in front of them, it is widely accepted that their maintenance requires regular supplies of live prey. Indeed, there are no artificial diets available for this purpose. This is both expensive and demanding. Although octopuses are capable of killing crustacea of their own size, it is recommended that prey should be about ten per cent of the mass of the octopus that is being fed. ·

Although they can survive fasts of several weeks' duration, octopuses usually have high feeding rates. Daily rates of food intakes are in the range of 1–10 per cent of body weight. Given the fact that flesh retrival from crustacea such as crabs is only in the order of 50 per cent, there is a high daily requirement for fresh prey, especially with large individual specimens. Food should be made available *ad libitum*.

An important side issue of feeding is the fact that *O. vulgaris* will bite their handlers, especially when being fed. Not only is there the traumatic consideration, but octopus saliva contains a number of toxins which make the bite more painful and may even result in partial paralysis. Care must be exercised to keep out of the mouth's way and to prevent the animal from crawling up one's hand or arm.

Breeding

Octopuses are dioecious. Fertilisation is achieved by mating with the male inseminating the female by passing spermatophores to the female via a muscular groove in a specialised arm (the hectocolylus). The sperm enter the female genital tract and lodge in the oviducal glands, prior to entering the ovaries. The female then lays eggs. Both the male and female die after reproduction. The hatchlings are immediately active and survive on the remains of the yolk sac for a few days before they start to take live prey.

The culture and breeding of all of the cephalopods is a very difficult process which requires either open sea water circulation or a very high quality closed system. Consequently it is not widely practised and is, therefore, excluded from the review.

Clinical aspects

Life Span

Estimates of the life span of *O. vulgaris* are in the range of one to two years. However, in aquarium conditions, this is more likely to be one year.

Trauma and Disease

Trauma. As has already been described, wild caught octopuses are often damaged during capture by commercial fishing techniques. This accounts for the high mortality rate that is often seen in the first few days immediately post capture. Muscle bruising appears as marked swellings, which are blue as a consequence of the blood pigment, haemocyanin, which is held in solution as opposed to intra-cellularly. Nerve damage can lead to paralysis of one or more arms, or permanently white skin patches, due to chromatophore failure.

In the wild lost arms will regenerate but, in the aquarium such lesions rarely heal and the animal usually suffers from a fatal systemic infection.

Disease. Ulcerations are frequently seen in laboratory octopuses. These spread rapidly and deepen to include the dermis and underlying musculature. Unless treated they usually prove fatal in two to four days. Fungi and bacteria have been cultured from similar lesions in other octopod species. Infected skin lesions are much more common in groups of animals held at high stocking density. Periodic dipping in solutions of nifurpirinol is effective in reducing mortality and promoting healing (*Boyle* 1991c).

Parasitism

Cephalopods in general carry the whole gamut of parasites and symbionts from viruses to nematodes. None of these are of any particular significance in a laboratory environment. There is a potential zoonotic risk from the larval stages of *Ascaris* and *Anisakis* but, to date, there has not been any evidence of cross-infection.

Cannibalism

Small specimens may be killed and eaten by larger ones. However, this is influenced by the size range of tank mates, the stocking density, and provision of adequate food and shelter places. As such, therefore, the problem can be avoided by good husbandry practices.
Sick or dying animals are often eaten by tank mates, and animals with injuries (particularly to the arms) may eat their own necrotic tissue (autophagy). Such animals should be euthanased.

Anaesthesia. The brain is located between the eyes and is enclosed in a cartilaginous cranium. The suboesophageal areas of the brain are concerned with the control of muscle groups. It is possible to identify clear divisions of function between the various lobes. Higher motor centres are located in the lateral and supra-oesophageal areas. There are large optic lobes laterally on each side of the brain. In addition to the brain there are a number of peripheral ganglionic masses whose functions are restricted to the organs where they are located. For instance, this allows for some degree of localised control of movement. This latter point is important when anaesthetising octopuses as spontaneous arm movements can occur at a surgical plane of anaesthesia.

No work has been done with injectable anaesthetics. Consequently anaesthesia by immersion is the only current technique. The animal is transferred into a container of sea water which contains the anaesthetic. Depth of anaesthesia is controlled by the agent's concentration and the period of immersion. As anaesthesia deepens there is progressive loss of activity and paling of the skin. Anaesthesia is judged complete when ventilatory movements cease. Despite this local arm movements do still occur, as does reflex contraction of the mantle.

Given the fact that anoxia is an immediate consequence of the correct plane of anaesthesia there is a limit on the period of anaesthesia of 10 to 20 minutes. Recovery is achieved by returning the animal to clean aerated sea water. It can be further assisted by flushing water through the mantle cavity and massaging the mantle. Full recovery takes two to five minutes.

It would seem that the agent of choice is an isotonic solution of 7.5 per cent $MgCl_2$ $6H_2$. This is a very atraumatic technique. In contrast, the other agents (3 per cent urethane and 2–2.5 per cent ethanol) seem to cause distress to a proportion of animals. It should be noted that cold water is probably analgesic in effect, as opposed to anaesthetic.

Euthanasia. As with other laboratory animals perhaps the simplest, and most humane, method of euthanasia is terminal anaesthesia. Alternatively, an animal involved in a terminal procedure can be killed by having its brain destroyed whilst anaesthetised. The identical technique can be used in animals which have to be killed without anaesthesia.

Laboratory usage

General. O. vulgaris is probably the most robust of all the cephalopods and this goes a long way to explain its usage as a laboratory animal. It certainly tolerates a much higher degree of handling than other octopuses (such as *Eledore cirrhosa*). Such handling can be either directly or by hand nets, but should take place underwater as much as possible.

Table 1. Incidence of use of *O. vulgaris*, in biomedical research, as recorded by Medline™ over the period 1974 – Jan 1994, in comparison with other octpods.

Genus/Species	*Eledone*	*Octopus*	*Octopus vulgaris*
Number of Papers	15	606	75

Literature Search. In order to make an assessment of how frequently *O. vulgaris* has been used in biomedical investigations, a literature search was conducted using Medline™ for the period 1974–January 1994. The keywords used were *Eledone, Octopus* and *Octopus vulgaris*. These were chosen as the main two genera of laboratory octopods are either *Eledone* or *Octopus*. Medline™ was chosen as the intention was to concentrate on purely biomedical research.

Table 1 shows that over the period in question, *O. vulgaris* was used in only 12.1 % of work that involved octoped species. Abstracts were then printed out for all the papers that dealt with *O. vulgaris* and the papers were allocated to a number of different research topics. The findings are summarised in Table 2.

Neurophysiology and Sensory Receptor Physiology. Although the squid is classically associated with neurophysiology as a consequence of its giant axons, the highly developed nervous system of the octopus makes it a very valuable research animal. This is illustrated by the fact that this area of work accounts for nearly 30 % of all research involving this species. *O. vulgaris* is particul-

Table 2. Incidence of use of *O. vulgaris*, as recorded by Medline™ over the period 1974 – Jan 1994, on the basis of research topics.

Research Topic	Number of Papers	Percentage of Total
Neurophysiology and Sensory Receptor Physiology	22	29.3
Enzymology	15	20
Haemocyanin	11	14.7
Circulatory System (including innervation)	7	9.3
Endocrinology	5	6.7
DNA/RNA	3	4
Behavioural Studies	2	2.7
Pharmacokinetics	2	2.7
Metabolic Studies	2	2.7
Miscellaneous	6	8
TOTAL	75	

arly useful as it is able to withstand a wide range of drastic surgical procedures. Considerable research has been carried out on their diverse (and highly developed) sense organs.

Enzymology. The enzymology of the hepato-pancreas is a particular area of interest.

Haemocyanin. A large amount of research has been carried out on *O. vulgaris* haemocyanin.

Behavioural Studies. O. vulgaris is a highly intelligent invertebrate which displays complex behavioural patterns, including problem solving (*Fiorito et al.* 1990). Indeed, the same institute demonstrated that the species is capable of observational learning (*Fiorito & Scotto* 1992).

Pharmacokinetics. Andrews & Tansey (1981) developed a technique for central drug administration by means of indwelling catheters.

Miscellaneous. This category includes such diverse areas as embryology, ontogeny of lens crystallins and the purification of *O. vulgaris* melanin.

Legislative and ethical consequences

The decision to bring *O. vulgaris* within the scope of the Animals (Scientefic Procedures) Act 1986 is an interesting one. Not only does it extend this legislation beyond the vertebrates for the first time, but it also infers acceptance of the underlying arguments that these animals do have the capacity to suffer. Logic and scientific commonsense would suggest that if *O. vulgaris* has the requisite neuroanatomy and neurophysiology to experience pain, suffering, distress and lasting harm then, at the very least, so must the other members of the family and perhaps even the Order and/or Class.

Another key issue is why a species that only features in approximately 12 % of biomedical research involving octopods (as recorded by Medline™) was singled out for inclusion in the Act.

As has been already described *O. vulgaris* is a very popular laboratory cephalopod, primarily as a consequence of its general robustness. However, since the Animals (Scientific Procedures) Act 1986 places a large administrative burden on research institutes, it is quite likely that research workers will switch to other species (such as *Eledone cirrhosa*) which are equally capable of suffering, but which do not incur a bureaucratic penalty. Ironically this may cause greater suffering, since these other species may well be less resilient and/or adaptable than *O. vulgaris*.

Another consequence of the 1993 Order is that non-biomedical research workers, such as those looking at issues of basic cephalopod biology, will have to comply with the Act.

Now that *O. vulgaris* has been included in the legislation further consideration should be given as to whether it is logical to exclude other cephalopods. It is probably inappropriate to include the whole Class (Cephalopoda) as this incorporates *Nautilus*, which is very much less advanced. It would seem appropriate to set the limit either at the Subclass Coleoidea (which includes the Order that contains the squids) or at the Order Octopoda.

Within the field of mainstream laboratory animal science there is a concerted move towards using purpose-bred animals, wherever possible. As has alredy been described the laboratory production of *O. vulgaris* is so difficult as to be impractical. However, the method of collection from the wild ought to be carried out as ethically as possible. Therefore it will be interesting to see if there is a move towards the use of pot-traps as opposed to the commercial fishing techniques, with their associated high mortality rates.

Summary
The high degree of neurophysiology and behavioural complexity of *Octopus vulgaris* had led to its inclusion in the Animals (Scientific Procedures) Act 1986. This paper reviews the biology of *O. vulgaris* and consider its usage as a laboratory animal.

References
Andrews PL & EM Tansey: Journal of Neuroscience Methods 1981, *4*, 243–247.
Boyle PR: The UFAW Handbook on the Care and Management of Cephalopods in the Laboratory, pp 29, UFAW Potters Bar, 1991a.
Boyle PR: The UFAW Handbook on the Care and Management of Cephalopods in the Laboratory, pp 31, UFAW Potters Bar, 1991b.
Boyle PR: The UFAW handbook on the Care and Management of Cephalopods in the Laboratory, pp 38 UFAW Potters Bar, 1991c.
Fiorito G, C von Planta & P Scotto: Behav. Neural. Biol. 1990, *53*, 217–230.
Fiorito G & P Scotto: Science 1992, *256*, 545–547.