3.4 Diseases, parasites, and contaminants

Shelagh Malham, Paco Bustamante, Santiago Pascual, and Sonia Seixas

3.4.1 Introduction

Recorded incidences of disease and mass mortality events in marine organisms are increasing, a trend thought to be linked to climate-mediated change and anthropogenic inputs. The marine environment is a microbial soup that contains an abundance of potentially pathogenic organisms and parasites, and the spread of disease in the ocean is extremely rapid. Given the various marine habitats occupied by cephalopods during their life cycle, contact with disease-causing organisms is inevitable. However, there are only a few reports of viral, bacterial, or fungal diseases affecting wild stocks of cephalopods.

An understanding of disease dynamics is vital for conserving marine ecosystems. However, compared with terrestrial systems, the role of diseases in most marine ecosystems is relatively poorly known. How diseases affect organisms depends on a range of interacting factors, including whether the disease is specific or non-specific and the effects of other environmental stressors on individuals and populations (e.g. pollution, fishing, and climatic variation). Interactions between hosts and pathogens, such as viruses or bacteria, will alter with changes in climatic features (e.g. temperature), thus affecting the severity of disease outbreaks (Harvell *et al.*, 2002). Results from simulation models of the effects of stress on the population dynamics of infectious diseases suggests that the impact of specific diseases on hosts declines with increasing stress, while that of non-specific diseases increases with stress (Lafferty and Holt, 2003). Effects of diseases on individuals potentially have consequences at population and ecosystem levels. Thus, some diseases have "positive" effects at the ecosystem level by promoting biodiversity.

Importantly, diseases can be pelagic as well as benthic. Suspended sediments can provide food and/or shelter and transport for pathogenic organisms. Water column turbidity can promote waterborne disease outbreaks, with the bottom sediment acting as a reservoir for pathogens. Disturbance, such as dredging or boating activity, can cause resuspension of sediment and release of pathogens. In addition, electrostatic forces that bind viruses/microbes to sediment particles or particulate organic matter can be overcome by hydrological changes that influence the pH, salinity, and temperature of the water column.

The emergence of infectious diseases in coastal and ocean ecosystems is often a result of human-induced environmental change. The underlying causes of emerging infectious diseases range from human encroachment and population increase to climate change, agriculture, increased interaction between humans, domestic animals and wildlife, and immunosuppression in the host owing to environmental stressors or infection by immunosuppressive viruses such as the morbilliviruses (Daszak *et al.*, 2001).

Whether or not the reported increases in diseases and mass mortality events in the ocean are symptomatic of larger problems is unknown. Various factors have been blamed, including climatic change, pollution (see Section 3.5.2), eutrophication, overfishing, and habitat destruction, although obviously not all mass mortality events can be attributed to these factors. Such stressors can increase susceptibility to disease or toxin accumulation in individual cephalopods, with potential consequences at population and community levels. Indeed, where environmental impacts persist over

an extended period (e.g. after an oil spill), more than one generation of cephalopods may be affected. The issue of climate change and its effects on cephalopods is examined in more detail in Section 6.

Cephalopods are known to carry a diverse assemblage of parasites and symbionts, and apparently "healthy" individuals in the wild appear able to survive trauma without suffering major infections (Boyle, 1991). Potentially pathogenic organisms found associated with cephalopods include viruses, bacteria, fungi, protozoans, nematodes, monogeans, digeneans, cestodes, acanthocephalans, polychaetes, hirudineans, crustaceans, copepods, and isopods (Hochberg, 1983; Forsythe *et al.*, 1991).

3.4.2 Viruses

Oceans teem with viruses typically numbering tens of billions litre^{-1} (Fuhrman, 1999). Viruses infect most marine organisms from bacteria to invertebrates and vertebrates. Importantly, viruses appear to play a major role in regulating population density and diversity in their bacterial and phytoplankton hosts, significantly affecting primary production in the world's oceans (Fuhrman, 1999; Wommack and Colwell, 2000).

Recent research has demonstrated that the Earth's oceans are a primary reservoir of emerging infectious diseases caused by RNA viruses (the Caliciviridae), which are capable of causing a whole suite of diseases in marine and terrestrial organisms (Smith, 2002). Additionally, viruses are transferring from the land to the sea. Thus, the canine distemper morbillivirus is thought to have been responsible for deaths in pinnipeds, although other reported mass mortalities in marine mammals appear to be caused by different (related) morbilliviruses (De Guardo *et al.*, 2005).

Cephalopods such as *Octopus vulgaris* have been demonstrated to contain virus-like particles associated with tumours (Rungger *et al.*, 1971; Hanlon and Forsythe 1990a, 1990b). In addition, squid axoplasm has been demonstrated to transport neurotropic viruses such as *Herpes simplex* virus type 1 (Bearer *et al.*, 1999), suggesting that these viral particles could survive in cephalopods.

3.4.3 Bacteria

Bacteria are essential to the ocean's biogeochemistry and for their role in the decomposition of organic matter necessary for marine foodwebs. However, oceans are also the conduits for various bacterial diseases affecting humans (usually through contact with contaminated seawater or shellfish), including typhoid fever, meningo-encephalitis, and gastroenteritis. Bacterial organisms that occur naturally in the ocean, and can be pathogenic, include *Aeromonas*, *Clostridium, Klebsiella*, *Rickettsia, Legionella, Listeria*, *Pseudomonas*, and *Vibrio*. Bacterial diseases in the ocean have resulted in large-scale epidemics in marine organisms, ranging from seagrasses to oysters and fish.

A number of bacterial diseases of cultured cephalopods were described by Hanlon and Forsythe (1990a) in their review of cephalopod diseases. These appear to be linked to several bacterial groups including representatives from *Vibrio*, *Aeromonas*, *Pseudomonas*, and *Flavobacterium* sp. Infections often follow injury and result in skin lesions (Hanlon and Forsythe, 1990a; Ford, 1992), with *Vibrio* sp. bacterial infections being the main problem for captive cephalopods (Scimeca, 2006). Additionally, in cuttlefish, fracture of the cuttlebone often causes infections that lead to mortality. A few studies have been undertaken on diseases in wild cephalopods. In wild *Octopus vulgaris*, researchers demonstrated that signs of disease, such as lesions on the arm

and mantle, were associated with the bacterium *Vibrio lentus* (Farto *et al*., 2003). Quantifying the incidence of diseases in cephalopod populations may be difficult because diseased and dead animals (especially as they are soft-bodied) are likely to be rapidly removed by predators or scavengers.

3.4.4 Parasites

Parasites are found in almost all taxa of eukaryotes. Parasite hosts are more susceptible to disease when they contain the added burden of parasites. In turn, reduced fitness and mortality of hosts can affect host population dynamics and community composition. Invasion of parasites into naïve areas is a major cause of biodiversity loss (Lafferty, 1997; Hoberg and Klassen, 2002; Rohde, 2002).

Almost all wild and laboratory-cultured cephalopods contain parasites, which, apart from viruses and bacteria, include polychaetes, copepods, and metazoans. A number of these parasites use cephalopods as a reservoir or second or third intermediate host. Generally, cephalopod parasites are mainly found on the skin and gills and in the digestive gland, digestive tract, and kidneys (Hochberg, 1990; Pascual *et al*., 1996; Gestal *et al*., 1999), although parasites have been associated with most organs. The most frequently encountered cephalopod parasites are the ciliates, which mainly infect pelagic cephalopods, and the dicyemids, present in the renal appendages, digestive glands, and digestive tract. Dicyemids eliminate the ciliate parasites when juvenile benthic cephalopods settle after their initial pelagic stage and are the most common and characteristic parasites of cephalopod renal appendages. Dicyemids have been found in benthic and epibenthic cephalopods, including cuttlefish, octopus, and loliginid squid (Hochberg, 1990; Furuya *et al*., 2003, 2004) and may have a detrimental effect. Recent studies have demonstrated that parasites (both micro and macro) affect cephalopods by diminishing their nutrient absorption capabilities (Pascual *et al*., 2007a). These authors demonstrate that the parasites lyse large areas of functional tissue within the animal, deplete energy stores, and affect the host's immune defence mechanisms. Cephalopods have also been described as harbouring parasitic platyhelminths, including monogeneans, digeneans, and cestodes, probably acquired through eating infected invertebrates and fish (Hochberg, 1990).

Various studies indicate that the ecological niche of a cephalopod species is more important than its phylogeny in determining its risk of parasitic infection (González *et al*., 2003). However, various biotic and abiotic environmental drivers will determine the extent of the infection. Stressed laboratory animals demonstrate increased parasitic burdens (Forsythe *et al*., 1990; S. Malham, pers. comm.). Pollution may also make marine animals more susceptible to infections and lead to changes in parasite loads. Parasites will themselves be affected by pollution and may be useful in monitoring environmental conditions (Rohde, 2002). Parasites, including those found in cephalopods, have been used as biological tags (Rohde, 2002).

3.4.5 Cephalopod immune system

The immune system of an animal is its defence against invading pathogenic organisms and reflects its state of health. However, the use of the status of the immune system as an indicator of ecosystem health or pollution stress is complicated by the range of different factors involved in any immune response. Sublethal exposure to environmental contaminants can have measurable effects on many physiological processes, but different degrees of exposure to a stressor can elicit different types of immune system response. The ability of animals and plants to protect themselves from infection relies mainly on the detection of non-self and the

capacity of the immune system to mount a response. The ability of animals or plants to defend themselves against disease can be linked directly to the "quality" of the surrounding environment (Oliver and Fisher, 1999; Nürnberger and Brunner, 2002). Animals are likely to be at risk of disease and/or mortality if the normal functioning of the immune system is disrupted.

Immune systems in invertebrates involve complex combinations of cellular and humoral components, including external barriers, which act to prevent invasion by pathogens. Invertebrate immunity tends to be thought of as innate, non-adaptive, and non-specific. However, recent research has revealed similarities and conserved mechanisms, at the cellular and molecular levels, between vertebrates and invertebrates, such as the complement pathway (Cooper, 2002).

The cephalopod immune system is capable of recognition of non-self and appears, at present, to be innate. Cephalopods, unlike other molluscs, have a closed circulatory system with a central systemic heart and two branchial hearts (Wells, 1978, 1983; Wells and Smith, 1987). In addition, the arterial, venous, and capillary bed circulatory system of cephalopods is similar to that of vertebrates (Browning, 1979; Wells, 1983; Shadwick and Nilsson, 1990). The blood consists of haemolymph (plasma), haemocyanin (respiratory pigment), and haemocytes (blood cells). Little detailed information is available on cephalopod immunology (Ford, 1992; Malham and Runham, 1998). Changes linked to climate-mediated or anthropogenic impacts (e.g. in relation to water quality, temperature, pollution, or fishing) will affect the immune system of cephalopods (Lacoue-Labarthe *et al.*, 2009a) and their parasites, and potentially lead to mortality. Immune-compromised animals tend to have higher infection rates, although, as noted above, this may not be noticeable in soft-bodied cephalopods because diseased animals would quickly be picked off and eaten, leaving very little trace.

3.4.6 Contaminants

Anthropogenic inputs to the marine environment, such as excess nutrients and pollutants, including heavy metals and persistent organic pollutants (POPs), all have numerous, usually negative, effects on marine organisms and the animals that eat them. Anthropogenic inputs can directly or indirectly affect a whole marine community or ecosystem.

The release of a large quantity of pollutants can cause immediate mortality, whereas lower levels of pollutant discharge may lead to accumulation of the pollutant within animals, with consequences such as immunosuppression and reproductive failure. Once in the ocean, pollutants can be distributed over wide areas through physical processes (e.g. currents and gyres). Generally, movement of contaminants from organism to organism is through trophic links (i.e. by direct assimilation following ingestion of the prey). Bioaccumulation is basically an equilibrium phenomenon. A contaminated environment leads to contaminated animals, and certain compounds (e.g. lipophilic POPs) have a greater affinity for animal tissues than for seawater, so that concentrations in animals can greatly exceed those in their surroundings. Heavy metals can accumulate in specific tissues, depending on the detoxification mechanisms that an animal possesses. In addition, concentrations of persistent organic pollutants (POPs), and also of some heavy metals, typically increase through food-chain transfers (biomagnification). Thus, cephalopods are responsible for transfer of cadmium to top marine predators (Bustamante *et al.*, 1998a).

There is considerable evidence that marine animals may be more susceptible to disease if they are contaminated with pollutants. Harvell *et al*. (1999) state that mass mortality of marine mammals appears to be associated with heavily polluted coastal areas of the North Atlantic. Other areas "identified" by these authors as being "hot spots" for disease include the Caribbean basin and the Indo–Pacific, although, in practice, the problem appears to be worldwide. Sources of anthropogenic pollutants entering coastal and ocean ecosystems range from atmospheric deposition to industrial and agricultural run-off involving both point sources (such as rivers and dumping) and diffuse sources (such as atmospheric deposition and groundwater). All of these sources have the potential to disturb ecosystems and increase the risk of disease, harmful algal blooms, and viral epidemics.

Generally, chemical pollution (e.g. from metals or organic compounds), has been linked to various disease problems worldwide and is suggested to be a potential cause of mass mortalities and changes in biodiversity. Chemicals such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) have been linked with deformities, cancer, compromised immune systems, and increased susceptibility to parasitic infection and disease. Environmental pollution can alter the host–pathogen relationship, thus increasing the probability of disease and significantly affecting the host population (Arkoosh *et al*., 1998). In addition, some chemicals, such as the PAHs, can serve as growth-stimulating nutrients for pathogenic bacteria, such as some species of *Vibrio*.

Pollutants may have impacts on areas far removed from their point of discharge. Oceans transport substances and organisms over very long distances; for example, the Arctic is polluted with PCBs and pesticides that originated mainly at lower latitudes. Migratory and planktonic marine organisms, including cephalopods and their paralarvae, which swim or drift over long distances, may be exposed to a variety of environmental hazards. Impacts of pollution at some intermediate location may seriously affect the population at the end of the migration or drift route. There is also often a significant time-lag between the release of the pollutant and the manifestation of its impact on the marine environment. Thus, dichlorodiphenyltrichloroethane (DDT) accumulated in the marine environment from the mid-1940s to the mid-1970s and, even 20 years later, was still found in high levels in species such as dolphins and birds (Addison, 1989; Fry, 1995). The timing of a pollution event is also important, because even a small release at the wrong time or season in a sensitive environment can cause considerable harm and leave organisms susceptible to disease.

Cephalopods are arguably very suitable biological indicators of ecosystem health. They are short-lived, fast-growing animals in which individual and population parameters, such as growth and recruitment, are known to depend strongly on environmental conditions. Importantly, many cephalopod species (e.g. octopus) inhabit coastal waters or come to mate and spawn close to the coast (e.g. cuttlefish). Therefore, they are more subject to direct contamination with high concentrations of pollutants released by industrial, domestic, and agricultural run-off than species, which live their entire lives away from the coast (e.g. some squid). The effects of these pollutants are many and varied, ranging from the molecular level, through individual organisms, to the level of communities and ecosystems. Chemicals may affect the physiology and immune responses of individuals, as well as reproductive success and sex ratios within populations. Biological effects may include direct toxicity, stress, disease, and reproductive failure. Invertebrate embryos and the first stages of

development are particularly sensitive to pollutants (e.g. Calabrese *et al.*, 1984), which may be particularly critical for the success of population recruitment of cephalopods.

Several studies have revealed that cephalopods have the capacity to accumulate heavy metals at high levels in their tissues (e.g. Miramand and Bentley, 1992; Rossi *et al.*, 1993; Bustamante *et al.*, 1998a, 1998b, 2000, 2002a, 2002b; Storelli and Marcotrigiano, 1999; Seixas and Pierce, 2005a, 2005b; Seixas *et al.*, 2005a, 2005b; Pierce *et al.*, 2008a). Heavy metals include both toxic elements (e.g. mercury, cadmium, lead, silver) and numerous trace elements that are essential to biota (e.g. copper, cobalt, or zinc), but may be toxic in high concentrations. These elements accumulate in the sediment, and can be re-mobilized after natural events (e.g. storms) or anthropogenic modification (e.g. dredging). Of the cephalopod tissues, the digestive gland and branchial hearts appear to play a major role in the bioaccumulation of toxic elements, such as cadmium and silver, which are detoxified and sequestrated, sometimes over a very long time-scale (Beuerlein *et al.*, 2002; Bustamante *et al.*, 2002a, 2004).

Metals such as cadmium can have direct effects on embryogenesis in squid (e.g. Şen and Sunlu, 2007). Recent experimental studies have demonstrated that some heavy metals reduce the survival and growth of both eggs and juveniles of the cuttlefish *Sepia officinalis* (E. Le Bihan, pers. comm.). Nonetheless, the eggshell limits the incorporation of cadmium, lead, and mercury during embryogenesis in cuttlefish, but silver is strongly accumulated and can affect survival (Lacoue-Labarthe *et al.*, 2008a). Furthermore, there is evidence that maternal transfer of metals occurs in cuttlefish (Lacoue-Labarthe *et al.*, 2008b). Indeed, invertebrate embryos and the first stages of development are particularly sensitive to pollutants, which may be critical for the success of population recruitment of cephalopods.

Heavy metals can also be a concern for human health. For example, some concentrations of cadmium and lead, determined in edible tissues of octopus in Portugal, exceeded the legal limits set by the European Community for those metals (Seixas, 2004). Moreover, over 75% of the mercury accumulated in cephalopod muscle is in the organic form, which is the most toxic chemical species of this metal (Bustamante *et al.*, 2006).

Other chemicals, mainly anthropogenic in origin, can also have profound effects on marine systems. Examples include POPs, such as PCBs, pesticides, PAHs, and radioactive compounds. Compared with heavy metals, fewer studies have focused on the metabolism of POPs in cephalopods, most of them investigating baseline levels in the flesh (e.g. Goerke *et al.*, 2004; Perugini *et al.*, 2004; Storelli *et al.*, 2006). In general, relatively low POP levels have been reported for cephalopod flesh. Nonetheless, both field and experimental analysis of POPs in cephalopod tissues revealed that they are accumulated at high concentrations in the digestive gland (e.g. Ueno *et al.*, 2003; Danis *et al.*, 2005). Recent work has identified POPs in nine species of deep-sea cephalopods, demonstrating persistence of these chemicals at oceanic depths to 2000 m; the physiological effects on these animals are unknown (Unger *et al.*, 2008). In general, very little is known about toxic effects of POPs on cephalopods.

In countries where humans consume the digestive gland and other viscera (e.g. Japan), exposure to bioaccumulated pollutants could be much higher than if only the muscular mantle and arms were eaten. For example, the toxic equivalent (TEQ) concentrations of dioxins in gut samples of the Japanese common squid were 50-fold larger than those in muscle tissues; indeed the gut contained ca. 95% of the total dioxin load of the squid (Tsutsumi *et al.*, 2007).

Overall, there is a need to collect further information on the effects of pollutants on cephalopods, especially those living in the coastal zone, which form part of the human food chain. This issue is of special concern in the context of ocean acidification and ocean warming, which increase the metal uptake of the early life stage (Lacoue-Labarthe *et al.*, 2009b).