A Preliminary Report on Bioaccumulation in Octopuses (*Enteroctopus dofleini*)

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Abstract
The Seattle Aquarium opened a new exhibit in 1999 for large giant Pacific octopuses (*Enteroctopus dofleini*). The exhibit was designed to allow the natural hunting and feeding behaviors of these octopuses on live crabs (*Cancer productus*). These crabs, collected from Seattle's harbor, made up 50% of the octopuses' diet. The first five male octopuses held in the tank neither grew as large nor lived as long as expected (mean 27 kg). Since water quality parameters were excellent, the diet of crabs was suspected as contributing to the early onset of the octopuses' senescence, and these crabs were eliminated from the diet. The next five octopuses grew statistically larger. Local crabs and the octopuses fed on them had high accumulations of metals in the hepatopancreas and the digestive glands respectively. Octopuses from the Seattle area and from the outer coast of Washington State were also examined for PCBs, which were high in both samples. Octopuses from the coast were low in heavy metals. Heavy metals were likely picked up by the octopuses eating crabs living on and in the historically industrial sediments of Seattle's harbor.

Introduction
In 1999 the Seattle Aquarium opened a new 12,000-liter exhibit for giant Pacific octopuses (*Enteroctopus dofleini*), the largest octopus species in the world (High 1976) and the species most exhibited in public aquariums (Carlson and Delbeek 1999). This was the largest tank dedicated to octopus at the Aquarium to date, and one of the largest in the country. It was hoped that a very large octopus would grow in this exhibit. As we did not want females maturing and laying eggs in the latter stage of their life (Mangold 1987), only males were exhibited. Males typically grow to a large size before reaching senescence when they stop eating and eventually die (Anderson et al. 2003).

The first five males grown in this exhibit reached a mean weight of 27 kg before going into senescence, losing weight, and dying. This was smaller than octopuses grown in other Aquarium tanks (unpubl. data). This was considered to be relatively small as this species can grow much larger (Hochberg 1998), although typical weights in the wild are in this range (Hartwick 1983). Factors that might affect octopus weights and longevity were then investigated.

The water quality in the tanks was excellent, with low ammonia, normal dissolved oxygen, and low contaminants (except for the occasional spike of *E. coli* due to nearby sewage overflows during high rainfall). Organics were low in the incoming water and the fiberglass and acrylic tank was not contributing to longevity problems. Coinhabitants of the tank were compatible. The food given to the octopuses became suspect.

These octopuses were fed a diet of 50% raw sea food (human quality) and 50% live red rock crabs (*Cancer productus*) caught at the Aquarium on Elliott Bay. Previous to this exhibit, octopuses held at the Aquarium had not been fed crabs, due to the difficulty of removing the crab shells off the bottom. The new large tank was cleaned periodically by divers and live crabs were fed for food and enrichment (Anderson and Wood 2001). This investigation then concentrated on these crabs and their possible effect on the octopuses.

Prior to this work, only minimal studies of pollutants found in cephalopods and body areas in which they are concentrated have been reported (Miramand and Guary 1980; Miramand and Bentley 1992; Gerpe et al. 2000; Sanguansin et al. 2003; Seixas 2003). There is nothing in the literature that describes the effect of pollutants on animal health. There are reports of the effects of pollutants on other mollusks, such as the affliction of imposex on snails caused by the antifouling substance TBT (de Wolf **et al.** 2001). Caldwell (pers. comm.) recently stopped feeding shore crabs from San Francisco Bay to his octopuses (*Hapalochlaena* spp) because he believed the crabs contributed to early onset of senescence and death of the octopuses. But there are no recorded effects on cephalopods of increased heavy metals or PCBs in their tissues. It was hypothesized that early senescence and death were caused by high pollutant level in animals that were fed locally collected crabs. This is a preliminary report of that investigation.

Procedures
Five male giant Pacific octopuses (*Enteroctopus dofleini*) originally captured in Puget Sound or the Strait of Juan de Fuca were held at the Seattle Aquarium (Seattle, WA). They were fed *ad libitum* a diet of 50% raw sea food (herring, capelin,

pollock fillets, and squid) and 50% live red rock crabs (*Cancer productus*) caught with crabs traps at Elliott Bay. Another five male octopuses were just fed raw sea food. All octopuses were kept until they reached senescence (for a description of that phenomenon see Anderson et al. 2003) and stopped eating. Their peak weights were noted. Representative animals were allowed to die of old age and samples of arm tissue and digestive gland were frozen until they could be analyzed for heavy metals and PCBs by the Phoenix Laboratories or the King County Environmental Laboratory. In addition, several dead octopuses from coastal waters (Seaside, OR) were obtained and sampled. The hepatopancreases of six red rock crabs caught at the Seattle Aquarium were also tested for heavy metals.

Results

The octopuses that were not fed live crabs grew to a larger weight before they went into senescence and stopped eating (see Table 1). The larger size of those not eating crabs was significant ($t = 2.3$; $p<0.04$). The octopuses fed crabs grew at a faster rate (mean 1.9%/day body weight) than those not fed crabs (mean 1.2%/day, Table 1). The difference in growth rates was not significant ($t = 2.3$; $p<0.3$; n.s.) but was suggestive.

Table 1. Difference between octopuses fed crabs and those not fed crabs.

Fed crabs	Weight (kg)	Growth rate (%/day)	Not fed crabs	Weight (kg)	Growth rate (%/day)
Dark Avenger	34	1.6	Clyde	32	0.8
Shadow	25	2.5	Bill	42	0.7
Claude	25	0.8	Oscar	32	3.5
Dudley	21	1.9	Big	43	0.5
Scamp	31	2.8	Ahab	32	0.6
Mean	27	1.9		36	1.2

Heavy metals in the octopuses were primarily concentrated in the digestive glands with some exceptions (see Table 2).

Table 2. Heavy metals in octopus arms and digestive glands (means; mg/kg; 0=<mdl).

Metal	Arm	Digestive gland
As	4.8	5.0
Cd	0	34.4
Cu	2.4	230.7
Fe	0.9	95.3
Mn	0.1	0.9
Mo	0	2.9
Pb	0	0.3
Zn	13.6	163.1
N = 8		

Most heavy metals were highest in the digestive glands of those octopuses eating crabs from Seattle's harbor (see Table 3). For example, octopuses fed crabs had higher levels of copper than those not fed crabs. As expected, those crabs from the ocean had the lowest heavy metal concentration.

Table 3. Heavy metals in octopus digestive glands and crab hepatopancreas (mg/kg).

Metal	Ocean	No crabs	Fed crabs	Crab
As	5.4	3.3	4.1	3.0
Cd	10.0	20.1	91.9	5.6
Cu	73.4	69.5	620.2	41.6
Fe	82.5	52.5	112.5	35.6
Mn	0.8	0.7	1.0	1.8
Mo	4.9	9.7	1.2	0
Pb	0.6	1.2	0	0
Zn	138.5	169.0	726.5	46.3
N =	4	2	2	6

Den midden remains suggest that rock crabs are the primary food of octopuses on the Seattle waterfront near the Seattle Aquarium on Elliott Bay (pers. obs.). These crabs had moderately high heavy metal concentration in their hepatopancreases (see Table 3), but generally not as high as the octopuses fed these crabs.

The only PCB found was Aroclor 1254 and this only detected in octopus digestive glands. Other Aroclors were below measurable detectable limits. Aroclor 1254 was higher in octopuses fed crabs than those from the coast (see Table 4). PCBs from octopuses not fed crabs have not been sampled yet.

Table 4. PCBs in octopus tissues and crab hepatopancreas (ppb).

PCB	Ocean	No crabs	Fed crabs	Crab*
Aroclor 1254**	170	?	388	958
N =	2		2	

*from King County Environmental Laboratory data
** all other Aroclors were <mdl

Discussion

It is clear from this data that most heavy metals and PCBs are highly concentrated in the octopus digestive glands. This finding is confirmed by other studies (Miramand and Guary 1980; Miramand and Bentley 1992; Gerpe et al. 2000).

While there is no proof, the heavy metals and PCBs are most likely entering the octopuses via their food. In Elliott Bay octopuses are primarily eating red rock crabs (*Cancer productus*). Red rock crabs spend considerable time buried in the substrate (Jensen 1995) and likely absorb pollutants from the sediments. The buried sediments of Seattle's harbor floor are highly polluted from historic pollution sources with high concentrations of heavy metals and PCBs (West and O'Neill, this volume; Dutch et al., this volume). PCBs in these sediments exceed Washington State Sediment Management Standard values (Dutch et al., ibid.). This data suggests pollutants in crabs are being passed up the food chain to octopuses and bioaccumulated in their digestive glands. This has a consequence for those animals that eat octopuses. Many fish eat octopuses (Hart 1973) as do marine mammals (Haley 1978), which also bioaccumulate pollutants.

These data have implications on the octopus growth model. Mangold (1987) found that underfed octopuses matured earlier than those fed *ad libitum*. Such a strategy may allow octopuses to assure spawning in harsh conditions, thus ensuring survival of the species. This data suggest that male octopuses burdened by a load of pollutants also mature early, regardless of their growth rate. The growth rate of burdened octopuses was higher than those not fed crabs. Octopuses are generalist feeders (Anderson 1996), though it is generally assumed most octopuses prefer to eat crabs but select prey species partly on the basis of availability (Ambrose 1983; Vincent, Scheel and Hough 1998). A steady diet of red rock crabs may not provide all the nutrients necessary for normal octopus growth. Studies in vertebrates show that animals kept underweight live longer (Masoro 2000) but vertebrates and invertebrates may differ in this respect. Their common ancestors diverged more than a billion years ago (Wray et al. 1996), so we cannot necessarily compare the growth strategies between the two.

Pollutants gained by octopuses from eating crabs may be a factor leading to maturity at a smaller size, and earlier senescence and death. Those octopuses fed live crabs matured at a smaller size than those not fed crabs at the Seattle Aquarium. It is likely these smaller males still would be able to successfully reproduce in the wild (see Anderson et al., this volume). At a popular dive site in Elliott Bay female octopuses have been seen to successfully brood eggs to hatching (Williams 2003).

If human pollution causes octopuses to mature at smaller sizes, it may be a reason very large giant Pacific octopuses have rarely been seen in recent times. Several have been reported at over 180 kg (High 1976; Hochberg and Fields 1980; Newman 1994). Modern day pollution may be preventing these octopuses from achieving their full potential size.

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