

AEROBIC METABOLISM AND DIETARY ECOLOGY OF *OCTOPUS*
RUBESCENS

by

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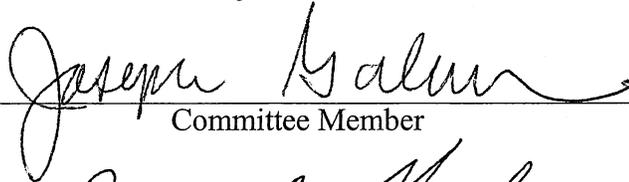
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ABSTRACT

Several lines of evidence suggest that octopuses have a large impact on benthic communities through the octopuses' trophic ecology. Octopuses have a high metabolism and require substantial quantities of food in proportion to their body size. They also can be very abundant where they occur and may be more pervasive than realized due to their cryptic nature. *Octopus rubescens* is the most common shallow water octopus on the west coast of North America, and seems to be a likely candidate to exert considerable influence on lower trophic levels. To begin exploring this ecological role, the aim of this project was to relate prey choice of *O. rubescens* to energy budgeting by the species.

Thirty male *Octopus rubescens* were collected from Admiralty Bay on Whidbey Island, Island County, WA. Energy budgets were constructed for several of these octopuses, prey preference and handling time determined, and metabolic measurements taken for each. In these experiments the prey choices made by *O. rubescens* deviated widely from those expected from a simple model of maximizing caloric intake per unit time. *O. rubescens* chose *Hemigrapsus nudus* over *Nuttallia obscurata* as prey by a ratio of 3 to 1, even though when tissue energy content and handling time are accounted for the octopus could obtain 10 times more calories per unit time from *N. obscurata* than from *H. nudus*. Octopus energy budgets were similar when consuming either of the prey species except that lipid extraction efficiency (ratio of assimilated to consumed lipids, the remainder is defecated) was significantly higher in octopuses

consuming *H. nudus*. This suggests that lipid digestibility may play a considerable role in the prey choice of *O. rubescens*.

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INTRODUCTION

The two near-shore octopuses of the United States Pacific Northwest are the giant Pacific octopus (*Enteroctopus dofleini*) and the red octopus (*Octopus rubescens*). While *E. dofleini* is a relatively well studied octopus (likely second in sheer published volume only to *Octopus vulgaris*) *O. rubescens* is much less well known despite being the most common shallow-water octopus on much of the United States west coast. Especially little is known about *O. rubescens*' physiology and its relationship to the species' ecology. A great deal of basic biology still must be done to understand this species.

Taxonomy

Octopus rubescens is a cephalopod of the superorder Octobrachia, differentiated from the superorder Decabrachia (which includes squid and cuttlefish) primarily by the presence of only eight arms as opposed to eight arms and two feeding tentacles. Until 1953, when the species was described, *O. rubescens* were generally considered to be a juvenile *E. dofleini* when encountered (Hochberg, 1997). As a result many earlier studies asserted to be on the larger giant Pacific octopus actually used in part *O. rubescens* (Berry, 1912; Green, 1973). Synonyms found in the literature can thus be divided into two logical categories, synonyms that are in common with *E. dofleini* and those which are unique. Synonyms in common with *E. dofleini* include *Octopus dofleini*, *Octopus apollyon*, *Octopus punctatus*, and *Polypus hongkongensis*. The only synonym unique to *Octopus rubescens* is *Octopus pricei* (Hochberg, 1997).

The generic placement of *O. rubescens* is still uncertain. The genus *Octopus* is polyphyletic and is in need of revision (Norman and Hochberg, 2005). In the past several years the review of octopus taxonomy has led to the removal of many species from the genus and their placement in resurrected genera such as *Enteroctopus* (Hochberg, 1998) and *Amphioctopus* (Huffard and Hochberg, 2005), or in promoting subgenera to genus status such as *Abdopus* (Norman and Hochberg, 2005). *O. rubescens*' placement has yet to be fully reviewed and may yet be found to fall outside the *Octopus* genus (Norman and Hochberg, 2005).

Natural History

O. rubescens starts life as a planktonic hatchling where it remains until reaching a length of as much as 54 mm (Hochberg *et al.*, 1992). During this stage of their life pelagic cephalopod juveniles are known as “paralarvae” because, while cephalopods exhibit direct development and thus have no true larvae, these young individuals have drastically different size and lifestyle than the adults (Hochberg *et al.*, 1992; Iglesias *et al.*, 2004; Iglesias *et al.*, 2007; Navarro and Villanueva, 2003; Villanueva, 1995; Villanueva *et al.*, 1995; Villanueva *et al.*, 1996). While in the plankton *O. rubescens* paralarvae can form large schools which feed on euphausiids (Norman, 2000). Occasionally these schools will be mixed with juveniles of the squid *Gonatus onyx* (Hunt and Seibel, 2000). Little is known about the duration of this stage of their lives, but it is thought the paralarvae of *O. rubescens* likely settle after two months, possibly at a mantle length (ML) between 4 and 10 mm (Green, 1973), or about one gram in

weight (Dorsey, 1976), though in deep water *O. rubescens* paralarvae have been found as large as 25 mm ML (Green, 1973), making the paralarvae of this species one of the largest known in octopuses (Strugnell *et al.*, 2004). The octopuses will spend the remainder of their lives as primarily benthic creatures and in total live about two years (Dorsey, 1976; Hochberg, 1997). As an adult *O. rubescens* can reach over 400g (Hochberg, 1998).

Breeding may occur year-round but has two peaks, one occurring in April and May and the other in July and August (Dorsey, 1976; Hochberg, 1997). The third right arm of the male octopus is modified into a hectocotylus for deposition of two of the male's eight spermatophores into the female's oviduct during each breeding event (Hanson *et al.*, 1973). The female has a single ovary connected to both the left and right oviducts which open into the mantle cavity near the funnel organ (Winkler and Ashley, 1954). Spawning by the female can take place as long as 42 days after fertilization, and she may brood the eggs from as short as 40 days to as long as six months depending on water temperature (Anderson and Little, 2006; Hochberg, 1997; Osborn, 1995).

Life for the octopuses ends shortly after mating. Females generally spend their last days brooding and guarding their eggs (Anderson *et al.*, 2002). Males, with no eggs to brood, show more marked signs of senescence such as eating less or refusing food altogether, becoming less discriminating in choosing locations to drill clam shells, developing skin lesions and wandering aimlessly in

the open until natural death or predation (Anderson *et al.*, 2002; Anderson *et al.*, 2008).

Ecology

Octopus rubescens as prey

Octopuses' soft bodies without bones make them sought after prey of nearly any predator able to capture them. It is well established that *O. rubescens* is an important dietary component of harbor seals (*Phoca vitulina richardsi*) (Lance and Jeffries, 2007; Pitcher, 1980; Stewart and Yochem, 1999); in some areas composing nearly 40% of the total diet and seasonally representing the single most common prey item (Oxman, 1995). California sea lions (*Zalophus californianus*) are also known to consume *O. rubescens*, but in much smaller quantities (Stewart and Yochem, 1999). During and after El Niño events both harbor seals (Stewart and Yochem, 1994) and California sea lions (Lowry *et al.*, 1990) increase their consumption of *O. rubescens*. This was attributed to a decrease in the abundance of other important prey items during El Niño events, while octopus population likely remained stable, causing the pinnipeds to focus foraging on octopuses (Stewart and Yochem, 1994).

Besides pinnipeds, *O. rubescens* falls prey to a diverse array of other predators including seabirds such as pelagic and Brandt's cormorants (*Phalacrocorax pelagicus* and *P. penicillatus*) (Ainley *et al.*, 1981) and common murrelets (*Uria aalge*) (Ainley *et al.*, 1996), pelagic fishes such as chinook salmon (*Oncorhynchus tshawychtsa*) (Hunt *et al.*, 1999), and benthic fishes such as

sandpaper skate (*Bathyraja kincaidii*) (Rinewalt *et al.*, 2007) and longnose skate (*Raja rhina*) (Robinson *et al.*, 2007).

Octopus rubescens as predator

Currently there is a paucity of data concerning the diet of *O. rubescens*. Early casual observations suggested that *O. rubescens* prefers small crabs and hermit crabs in the wild (Hochberg and Fields, 1980). More recently midden counts have suggested that the red octopus feeds primarily on gastropods, particularly in the genera *Olivella*, *Alia*, *Kurtziella* and *Nassarius*, and perhaps on barnacles (Anderson *et al.*, 1999). In laboratory conditions *O. rubescens* has been known to eat many different species of bivalves and crabs (Dorsey, 1976).

Habitat use

Octopuses tend to spend much of their time sheltered in a den created in crevices in rocks or at the interface of boulders and sandy bottom (Hartwick *et al.*, 1984; Katsanevakis and Verriopoulos, 2004). These dens prove to be especially important to the life of this organism. It is thought that the soft body of octopuses leaves them especially vulnerable to predation and in response they tend to seek shelter or dens (Hartwick *et al.*, 1984). Unless otherwise motivated an octopus will spend nearly all of its time in its den (Anderson and Wood, 2001). *Octopus vulgaris*, for example, has been shown to spend as much as 88% of its time in its den during daylight hours (Mather & O'Dor 1991). *O. rubescens* often use discarded glass bottles as dens (Anderson *et al.*, 1999). Unlike many other octopuses, the middens of discarded prey parts are not left outside their dens.

Instead, red octopuses pull what parts they can inside of the den, possibly to avoid detection by predators (Anderson *et al.*, 1999; Dorsey, 1976).

Octopus Digestion and Metabolic Physiology

Digestive anatomy and function

Food enters the octopus' digestive system through the mouth, which is located at the base of the eight arms (Winkler, 1953). The mouth consists of a parrot-like beak surrounded posteriorly by a muscular mass known as the buccal mass (Figure 1). As with most mollusks, within the mouth lies a radula which the octopus can use to drill very small, distinctive holes (often < 1 mm) in prey shells or exoskeletons. Adjoining the posterior aspect of the buccal mass is the anterior salivary gland. Extending posteriorly from the buccal mass is the esophagus which leads to the expanded crop. The esophagus passes through the donut-shaped brain in the head, meaning the octopus must swallow small enough pieces of prey not to interfere with the brain. The posterior salivary gland empties into the esophagus and is the location of venom production in octopuses. This venom is used to subdue and likely dismantle prey and *Octopus rubescens* has been recognized as venomous nearly as long as it has been recognized as a species (Beltz, 1956). Venom is delivered to the prey via a salivary proboscis that can be everted a short distance from the mouth of the octopus (Ballering *et al.*, 1972). Behind the esophagus and ventral to the posterior salivary glands lies the crop, which is likely primarily used for short-term food storage. The crop extends to the posterior end of the mantle cavity where it

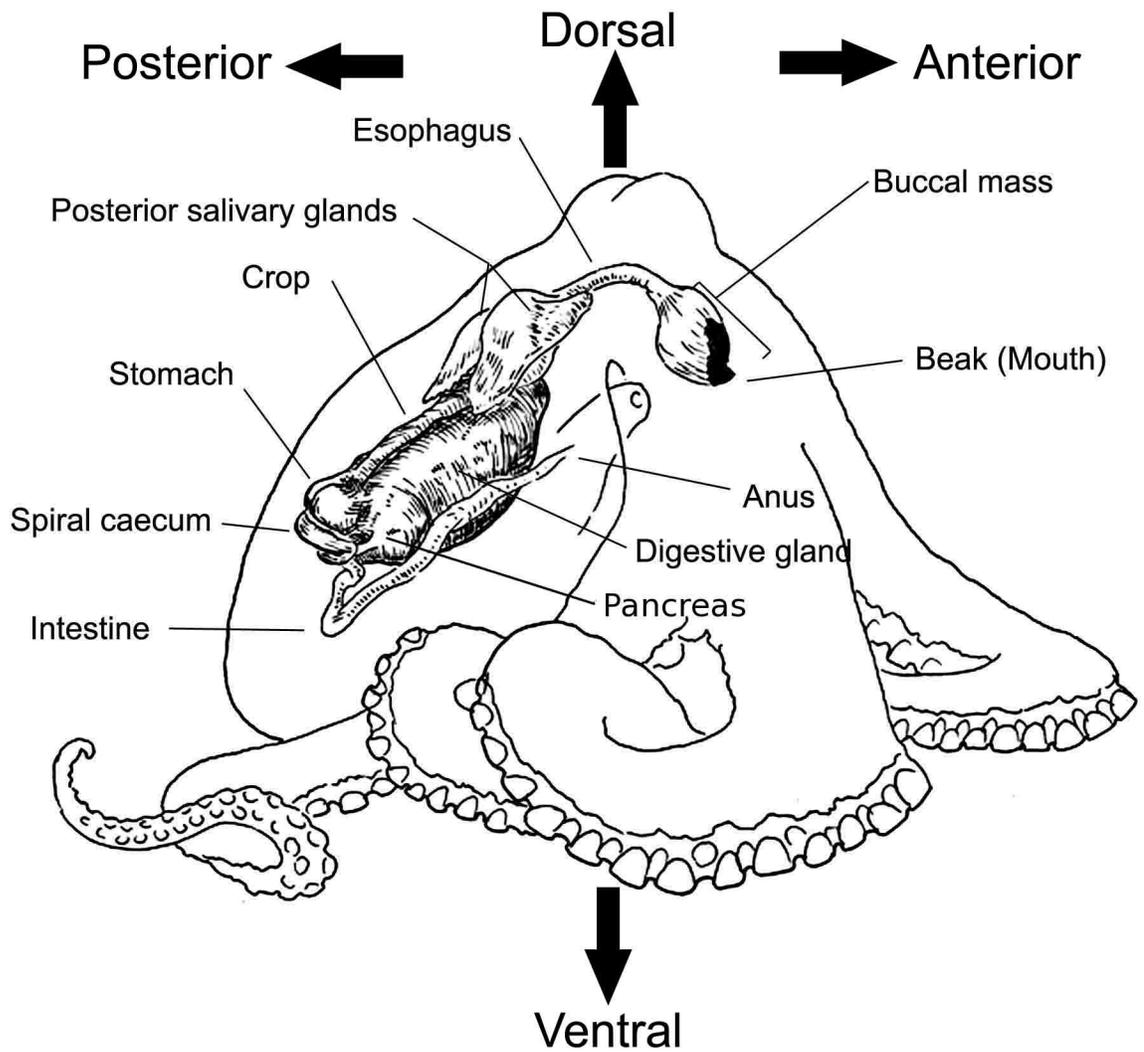


Figure 1: Digestive system of *Octopus rubescens*. Illustration by Nathaniel Johnson

empties into the stomach, a muscular organ likely used, along with the crop, for storing food. Adjacent to the stomach is the spiral caecum (Figure 1). The spiral caecum is one of the primary organs in the absorptive process, an important location for amino acid and fat absorption (Boucher-Rodoni and Mangold, 1977). Anatomically the spiral caecum consists internally of a thickened wall on the exterior of the spiral and longitudinal folds that increase the area of absorptive surfaces on the interior of the spiral (Winkler, 1953). The digestive gland (also known as the liver) empties its secretions by a duct into the spiral caecum (Stachowitsch, 1992). Materials ready for additional absorption are passed by ducts to the digestive gland (Boucher-Rodoni and Mangold, 1977). At the posterior side of the digestive gland lies the pancreas through which food is passed before reaching the digestive gland. In the digestive gland it appears that carbohydrates and amino acids, but not fats, are absorbed (Boucher-Rodoni and Mangold, 1977). The digestive gland consistently has a high lipid content and likely serves as a lipid reservoir.

Beyond the spiral caecum lies the intestine, which loops ventrally and posteriorly, behind the stomach and caecum, before turning anteriorly and extending to the base of the funnel organ (Winkler, 1953). At the funnel organ the anus empties into the ventilatory stream to be expelled from the body.

Macronutrient utilization by octopuses

A useful tool for determining metabolic substrates is to compare oxygen consumption to nitrogenous waste production by the atomic O:N ratio (Mayzaud

and Conover, 1988). Theoretical calculations suggest that O:N ratios between 3 and 16 suggest pure protein catabolism, while ratios between 50 and 60 indicate equal amounts of protein and lipid catabolism (Mayzaud and Conover, 1988). O:N ratios have been used extensively to determine the metabolic substrates for octopuses (Boucher-Rodoni and Mangold, 1985; Boucher-Rodoni and Mangold, 1988; Daly and Peck, 2000; Katsanevakis *et al.*, 2005b; Petza *et al.*, 2006; Rosas *et al.*, 2007). These works have consistently indicated that octopuses have a protein-dominated metabolism, even while on high lipid diets (Katsanevakis *et al.*, 2005b; Petza *et al.*, 2006).

Carbohydrates by contrast are found only in extremely low amounts in cephalopods, mostly as glycogen in mantle muscle (Lee, 1994). As such, carbohydrates are not likely an important nutritional component of *O. rubescens*' diet. What few carbohydrates are ingested are predominantly catabolized rapidly and the remainder are stored in the muscle, likely as glycogen. These muscle carbohydrate reserves are utilized predominately during locomotion and not during starvation (O'Dor *et al.*, 1984).

Lipids are also found in low amounts in cephalopods, except they are often found at relatively high levels in their digestive gland. Due to the lack of evidence that octopuses metabolize lipids for energy, it is assumed that their use of lipids is limited to structural uses such as cellular membranes and perhaps as hormones (Lee, 1994). Lipid may also serve as an energy storage. During starvation the O:N ratio of *Octopus vulgaris* rises, indicating an increased

reliance on non-protein metabolic substrates, which are often assumed to be lipids from the digestive gland (Boucher-Rodoni and Mangold, 1985). During fasting lipid in the digestive gland drops from 0.30% body weight to 0.06% body weight within six days, suggesting that lipids in the digestive gland are indeed the alternate metabolic substrate (O'Dor *et al.*, 1984).

Despite limited use as a metabolic substrate in octopuses, lipids are likely an important dietary component. Lipids have been suggested to be the limiting nutrient for egg production by female *Octopus vulgaris* on a crab diet (O'Dor *et al.*, 1984). Additionally, lipids have been shown to be important dietary components of *O. rubescens* paralarvae (Navarro and Villanueva, 2003). High lipid diets, however, could possibly be detrimental to growth (García García and Aguado Giménez, 2002) and to digestibility of the food (Petza *et al.*, 2006).

Excretion of protein metabolites

Octopuses excrete large amounts of nitrogenous wastes due to their protein-based metabolism. Most octopods which have been tested, such as *E. dofleini* and *O. vulgaris*, are ammonotelic, excreting the large majority of their nitrogenous wastes as ammonia. Neither uric acid nor urea were detected in the urine of *E. dofleini* or *O. vulgaris*. Besides ammonia, the second greatest byproduct of protein metabolism in *E. dofleini* urine appears to be guanine, but this is produced at a concentration of only 0.2% of the total nitrogenous wastes (Boucher-Rodoni and Mangold, 1985; Potts, 1965).

Metabolic scaling

Of the energy budgets estimated thus far for octopods, all with the exception of *Pareledone charcoti* have been of species which attain relatively large sizes (greater than 2 kg as an adult). *Octopus rubescens* grows only to 400 g. This is particularly important because metabolism scales with size, generally to the $\frac{3}{4}$ power of the mass (Kleiber, 1947). In other words, as mass goes up, metabolic rate per unit mass goes down. This principle has been demonstrated in a wide variety of organisms from unicellular organisms to elephants, both homeotherms and poikilotherms (Hemmingsen, 1960). Metabolic scaling by mass bears out particularly well in birds, mammals, amphibians, and fish (Schmidt-Nielsen, 2001). Mass and metabolism can generally be related by the power equation $R_b = aM^b$, where R_b is basal metabolic rate, a is the scaling constant, M is mass, and b is the scaling exponent, generally considered to be $\frac{3}{4}$ (Kleiber, 1947).

Invertebrates do not seem to hold as well to the $\frac{3}{4}$ power of scaling, with b ranging from 0.67 to 1 (Schmidt-Nielsen, 2001). This is also true for octopods. A review of the scaling of cephalopods found an interspecies scaling exponent in the family Octopodidae of 0.73 (Seibel, 2007), but other investigations of scaling within a single species have been widely varied with scaling exponents from 0.90 (Katsanevakis *et al.*, 2005b), to 0.83 (Maginniss and Wells, 1969) to 0.72 (Segawa and Hanlon, 1988).

Temperature is another factor that has a significant effect on metabolism (Schmidt-Nielsen, 2001), especially for aquatic animals. This effect is usually

expressed in terms of Q_{10} , the multiplier by which biological functions change with 10 degrees temperature change. The Q_{10} of octopuses appears to be close to 2.1 (O'Dor and Wells, 1987). Temperature effects have been shown to be considerable for cephalopods, and not always similar across species (Daly and Peck, 2000; Katsanevakis *et al.*, 2005a; Katsanevakis *et al.*, 2005b; Rigby and Sakurai, 2004; Semmens *et al.*, 2004).

Oxygen Consumption

Octopuses are generally efficient at extracting oxygen from their environment. Octopuses take up oxygen primarily through gills inside the mantle cavity (Wells and Wells, 1982), but also a significant portion of their oxygen uptake can occur cutaneously, up to 41% of total oxygen uptake while the animal is at rest (Madan and Wells, 1996). *Octopus vulgaris* and *Octopus cyanea* have been shown to be oxyregulators in oxygen tensions down to 25 mmHg (Maginniss and Wells, 1969; Wells and Wells, 1995), *Octopus bimaculoides* down to 16 mmHg, and *Octopus californicus* down to the limit of detectable oxygen levels (Seibel and Childress, 2000). Octopuses primarily regulate their oxygen uptake by adjusting frequency and volume of mantle ventilations (Wells and Wells, 1985), while slowing heart rate (Wells and Wells, 1983). *Octopus vulgaris* is extremely efficient at extracting oxygen from water removing as much as 76% of oxygen from each ventilation under normoxic conditions (Wells and Wells, 1982).

Growth and Energy Budgets

Measuring the growth of octopuses in the field has been difficult due to the lack of a reliable aging method (Semmens *et al.*, 2004), although methods of aging octopuses using rings in beaks or in stylets (the highly reduced internal shell) may bring promise in time (Doubleday *et al.*, 2006; Hernández-López *et al.*, 2001). In captivity, however, octopuses generally exhibit a two phase growth pattern over their lifetime in which younger individuals show exponential growth, then power growth as they mature (Semmens *et al.*, 2004). Other investigations have shown that *E. dofleini* may actually exhibit three phase growth along with significant differences in aerobic metabolism corresponding to three size classes: <100g, 100g-1kg, and >1kg (Rigby and Sakurai, 2004). In short term studies *E. dofleini* has shown daily growth rates as high as 1.3% in the wild (Hartwick *et al.*, 1981) and 2.7% in captivity (R. Anderson pers. comm.).

Complete energy budgets have been estimated for five other species of octopod: *Octopus maya*, *Octopus cyanea* (Van Heukelem, 1976), *Pareledone charcoti* (Daly and Peck, 2000), *Octopus vulgaris* (Petza *et al.*, 2006) and *Enteroctopus megalocyathus* (Perez *et al.*, 2006) (Table 1). An energy budget was estimated in part for *E. dofleini*, however the thrust of the study was to determine best dietary choices for this species under aquaculture conditions (Rigby and Sakurai, 2004). These energy budgets use some form of

Table 1: Comparisons of extant average energy budgets for octopods along with temperature the budget was measured at and assimilation efficiencies (AE_T %), for definition, see table 5, for definitions of energy budget components see table 4). All energy budget components are reported in $J \cdot g^{-1} \cdot d^{-1}$.

Species	Temp (C)	C	R	E_U	E_F	G	X	AE_T (%)
<i>Pareledone charcoti</i> ¹	0	10.5	7.04	0	0.41	3.13	--	96%
<i>Octopus vulgaris</i> ²	20	67.92	38.57	0.01	9.06	17.38	--	87%
<i>Octopus cyanea</i> ³	20	83.96	40.47	--	3.69	40.8	1.26	96%
<i>Octopus maya</i> ³	20	76.64	27.95	--	3.08	45.61	--	96%
<i>Octopus maya</i> ⁴	?	279.57	63	20	6.00	190.57	--	98%
<i>Enteroctopus megalocyathus</i> ⁵	17	147.72	39.94	1.28	1.18	--	--	99%
<i>Enteroctopus dofleini</i> ⁶	9.5	80.96	21.48	--	4.57	31.63	--	--

¹(Daly and Pack, 2000), ²(Petza et al., 2006), ³(Van Heukelem, 1976), ⁴(Rosas et al., 2007), ⁵(Perez et al., 2006), ⁶(Rigby and Sakurai, 2004)

the generic equation for energy budgeting: $C=R+G+E+X$; where C is the energy in the food consumed, R is the energy used for metabolism, G is the energy expended in growth, E is the energy lost by excreted products (E_U representing urine and E_F representing feces), and X is other processes requiring energy (Lucas, 1996).

As can be seen in Table 1, energy dedicated to aerobic metabolism (R) loosely correlates to the temperature at which the energy budget was obtained. *Octopus vulgaris*, *O. cyanea*, *O. maya*, and *E. megalocyathus* which were all tested at around 20 C, have the highest metabolic rates while metabolism for *E. dofleini* kept at 9.5 C and *P. charcoti* kept at 0 C were sharply lower.

There is also some variation among the species in the ratio of energy used for metabolism and growth. *O. vulgaris* and *P. charcoti* allocated over twice as much energy towards metabolism than towards growth while *O. cyanea* allocated nearly equal amounts of energy to each, and *E. dofleini* and *O. maya* allocated more energy to growth than to metabolism. This does not seem to be closely correlated to relative size and may simply be a variation among species or experimental conditions such as temperature. There are also several other possible explanations for the variations in partitioning including diet of the octopus. Perez *et al.* (2006) demonstrated that diet can dramatically adjust the energy partitioning of an octopus. In that study the authors generated a metric that was the difference between energy consumed and metabolism plus excretion ($=C-[R+E]$) in order to determine the energy available for growth on

various diets. In the case of *E. megalocyathus* a crustacean diet yielded a metric of $105.40 \text{ J}\cdot\text{g}^{-1}\text{d}^{-1}$ indicating plenty of energy available for growth, while a diet of mussels yielded $-40.14 \text{ J}\cdot\text{g}^{-1}\text{d}^{-1}$ suggesting that growth was not taking place. Another possible explanation for the disparity in energy budgets could be the use of different age octopuses since growth rates slow as the octopus ages (Semmens *et al.*, 2004). Petza *et al.* (2006) fed *O. vulgaris* anchovies, a fish very high in lipids, during the duration of their study, likely lowering the assimilation efficiency recorded. Also, the lack of standardization in procedures for these energy budgets has decreased the ability to compare between species.

Purpose

The purpose of this study was to 1) determine the energy budget of *Octopus rubescens* 2) find how this energy budget is altered with varied diet, and 3) relate this budgeting to how *O. rubescens* chooses its prey species.

METHODS

Overview

To determine the relationship between energy budget and prey choice I chose to use a simplified two-prey model system consisting of the purple shore crab *Hemigrapsus nudus* and the recently introduced purple varnish clam *Nuttallia obscurata*. To determine energy budgets I measured food consumption, oxygen consumption, ammonia production, and growth for one week for each octopus on each diet. To determine prey choice I allowed *O. rubescens* to choose between *H. nudus* and *N. obscurata* in captivity and analyzed *O. rubescens* middens collected in the wild. *H. nudus* and *N. obscurata* were chosen because they are locally available, can be obtained in large quantities, and are readily eaten by *O. rubescens*. *H. nudus* is easily collected intertidally in large numbers near Rosario Beach Marine Laboratory and *N. obscurata* is easily dug near the Marine Laboratory and is also available in local grocery stores in Walla Walla. A diet of snail was also attempted, but I was not able to locate any *Olivella baetica*, the dominant prey item found by Anderson *et al* (1999) and the alternative tried, *Nucella lamellosa*, was not readily taken by the octopuses.

Collection of Octopus

Thirty male *Octopus rubescens* ranging in size from 43 g to 353 g were collected by SCUBA from Admiralty Bay on Whidbey Island, Washington (UTM coordinates: 10U N5334525, E526962) between June 2006 and August 2007. Only males were collected to minimize amount of energy invested in

reproduction, particularly eggs. Divers inspected discarded glass bottles that had been overgrown by barnacles and/or anemones for the presence of octopus (Anderson *et al.*, 1999). Bottles containing octopuses were placed in a sealable plastic bag and transported to the Rosario Beach Marine Laboratory or to Walla Walla University for further work. Octopuses were held in 15 liter tanks of aerated seawater at 11C.

After capture the octopuses were given a one week acclimation period before any experimental trials began. During acclimation the octopuses were fed *Nuttallia obscurata* or *Hemigrapsus nudus*. The final two days of this acclimation period the octopuses were not fed so that any previously eaten food could clear the digestive system before respirometry began.

After the study octopuses were released at the location of capture.

Midden Collection

Twenty-one glass bottle dens of *O. rubescens*, with entrance sizes ranging from approximately 2 cm (beer bottle) to 13 cm (mason jars), were collected from Admiralty Bay on Whidbey Island, Washington. Each den collected contained an octopus with its midden of discarded food items. All of the contents of each bottle were removed and sorted. During sorting, barnacles and old items were discarded. Food items were determined to be old if they were stained or had encrustations such as algae or bryozoans on the interior. The minimum number of individuals for each prey species was determined for each taxa in every

midden by counting the body parts represented and recording the highest number of unique body parts.

Prey Preference

Prey preference trials were conducted in a flow-through tank, approximately 58.1 cm x 116.5 cm. Four *H. nudus* and four *N. obscurata* were weighed and marked with a number for identification after the trial. Prey items were placed, one of each species in each corner of the tank, and allowed to settle into the environment for a few minutes. The octopus was then placed in the center and left to choose its preferred prey for 2 hours. After the trial, the octopus and prey items were removed and the remains of prey items chosen were weighed to determine the mass consumed.

Prey Handling Time

Prey handling time for each *H. nudus* and *N. obscurata* were determined by using a time-lapse video camera during several feedings. Handling time was measured from the moment the octopus pulled the prey item under its arms until it dropped the last of the remains. Generally all prey remains (such as all crab pieces) were dropped simultaneously. Prey items were weighed before feeding and remains were weighed after feeding to determine mass consumed.

Determination of Metabolic Rate

Determination of Metabolic Rate

Description of respirometry apparatus

Sealed respirometry chambers were used to determine oxygen consumption (Figure 2). Chambers were approximately 6 liters in volume, in the shape of a cylinder with an inside diameter of 22.86 cm, and had a magnetic stir bar to maintain water mixing. There were two ports in the chamber, one incurrent and one excurrent. Beyond the excurrent port water passed into a smaller chamber 0.17 liters in volume containing an oxygen electrode and a stir bar. The oxygen electrode was connected to a data logging computer to record oxygen concentration every 30 seconds. After the water exited the electrode chamber it passed through an aeration column in which there was an air stone, and then back into the respirometry chamber via a peristaltic pump. An aeration bypass shunt was installed that bypassed the aeration column and closed the respirometry system from gas exchange with the environment during actual respirometry runs. Automated pinch valves placed at the beginning of the aeration bypass shunt, as well as before and after the aeration column, controlled water flow into either the aeration column or the shunt. Respirometry systems were constructed by Dr. David Cowles and myself with the help of Walla Walla University Technical Support Services.

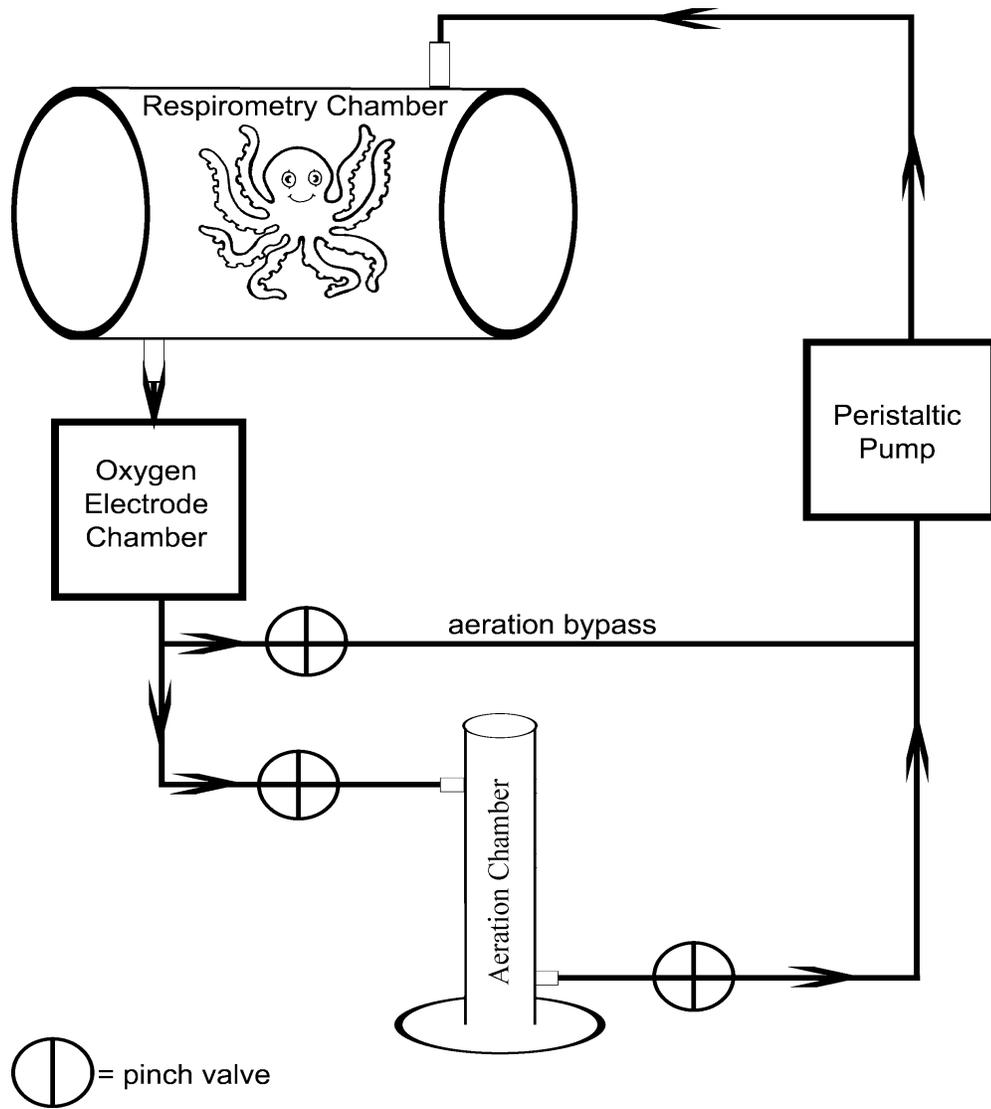


Figure 2: Schematic of respirometry system. Arrows indicate direction of water flow

Respirometry trials

After the one week acclimation period, respirometry trials began on each octopus. These trials were performed while the octopus was on two week-long diets composed each of two species in turn: the clam *Nuttallia obscurata*, and the crab *Hemigrapsus nudus* (Table 2). During respirometry trials octopuses were removed from their holding tanks and placed into the respirometry chambers filled with clean water at approximately 2100 h (Table 3). The octopus then remained in the chambers for 24 h, during which every other hour the automated pinch valves shunted water around the aeration column and the octopus' oxygen consumption rate was measured, while during alternate hours the shunt was closed and the water was re-aerated in order to keep oxygen levels high in the chambers at all times, well above the octopus' critical oxygen pressure (P_C). Octopuses were fed during re-aeration hours while still in the respirometer chamber at either 2300 h or 0700 h the following morning. Prey remains were removed from the respirometer as soon as possible after the octopus dropped them (the next morning if fed at 2300 h and within an hour if fed at 0700 h). Water samples were taken for ammonium analysis during several aeration periods near the end of each respirometry day, but never when prey remains were in the respirometer. These 24-hour respirometry trials occurred three times for each octopus during each week-long diet (Table 2). All metabolic data were measured at 11C.

Table 2: Two week daily schedule of an octopus being tested in a respirometer. Note that crab diets were not always the first diet administered. Octopuses were weighed before respirometry began on both Sundays as well as the Sunday following the last Saturday of the schedule.

Week	Diet	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
1	Clam	Respirometry	Rest	Respirometry	Rest	Respirometry	Rest	Rest
2	Crab	Respirometry	Rest	Respirometry	Rest	Respirometry	Rest	Rest

Table 3: Typical daily schedule of octopus respirometry trials. Note that feeding also occurred alternatively at 7am rather than 11pm in roughly half of the trials

Time	System state	Specific tasks
2100 h	Aeration	Octopus placed in respirometer
2200 h	Respirometry	
2300 h	Aeration	Feeding
0000 h	Respirometry	
0100 h	Aeration	
0200 h	Respirometry	
0300 h	Aeration	
0400 h	Respirometry	
0500 h	Aeration	
0600 h	Respirometry	
0700 h	Aeration	remove remaining food items
0800 h	Respirometry	
0900 h	Aeration	
1000 h	Respirometry	
1100 h	Aeration	
1200 h	Respirometry	
1300 h	Aeration	:59, take water sample for NH ₃
1400 h	Respirometry	
1500 h	Aeration	:01, take 2 nd water sample
1600 h	Respirometry	
1700 h	Aeration	
1800 h	Respirometry	
1900 h	Aeration	
2000 h	Respirometry	
2100 h	Aeration	Octopus removed from resp.

Metabolic rate was compared to other measured factors such as time of day, oxygen saturation, time since feeding and time in respirometer by stepwise multiple regression using SPSS 13.1. Thresholds of $p=0.05$ to enter and a factor $p=0.10$ to remove a factor were used in this analysis.

Octopuses were weighed before the first respirometry trial and after the last respirometry trial of each week. Weighing was accomplished by putting the octopus in a plastic drinking cup and pouring out the excess water. Octopuses reliably expelled the water in their mantle cavity within a few moments of the rest of the water being removed. Excess water in the drinking cup was blotted with a paper towel. After the octopus was weighed on a standard gravimetric scale the octopus was allowed to crawl out of the cup into the respirometer, then the cup was tared. This whole process generally lasted less than 90 seconds.

Ammonia production

Ammonia production was determined using seawater samples collected during respirometry trials. Ammonia concentrations were determined colorimetrically using the reagents found in the Aquarium Pharmaceuticals® aquarium ammonia test kit (product # LR8600) and compared to standards prepared with ammonium chloride in seawater from Rosario Bay. Samples would often become cloudy after addition of the reagents, so the samples were centrifuged for 2 minutes before reading absorbance. Absorbance of reacted samples and standards was determined in a Beckman DU-530 spectrophotometer at 640nm (McCloskey, 2006).

Feeding and Feces collection

All food items were weighed before they were presented to the octopus. After the octopus had eaten the items, any uneaten portions were weighed and subtracted from the mass consumed.

All feces were collected using a small transfer pipette throughout each week-long diet, and were frozen at -20C until nutrient analysis could be performed. Before nutrient analysis feces were dried to constant weight in a room temperature desiccator and weighed. Salt content of dry mass was determined by calculating water lost during dessication and multiplying by 3.0%, the approximate salt content by mass of seawater in Rosario Bay, which was the same as that used in the experiment as determined with a refractometer.

Nutrient Analysis

Lipid content, protein content, gross energy content, dry mass and ash content were determined by the Washington State University Wildlife Nutrition Laboratory, Pullman, WA. Samples analyzed were a homogenate of one whole *Octopus rubescens*, homogenate of the soft tissues of five *Nuttallia obscurata*, soft tissue from inside the carapace and chelipeds (other legs were not generally consumed, but the large lump of muscle extending from each leg into the carapace was included in homogenate) of approximately 45 *Hemigrapsus nudus* except the gills (not usually consumed by *O. rubescens*) a homogenate of all the feces produced by the octopuses while on a *N. obscurata* diet and a homogenate of all the feces produced while on a *H. nudus* diet.

Energy Budget Calculation

All data for energy budgets were collected between June and August 2007. Each component of the energy budget of the octopuses was determined from the measurements described earlier according to the formulas found in Table 4. The ratio of consumed matter minus defecated matter to the whole of consumed matter ($(C-F)/C \times 100\%$, in terms of mass or energy content) is known generally as “Assimilation Efficiency” (Daly and Peck, 2000; O'Dor and Wells, 1987; Petza *et al.*, 2006), but is alternatively known as “Apparent Digestability” in some literature (Lee, 1994). In this study I refer to this ratio as assimilation efficiency (AE). Assimilation efficiencies were calculated from formulas found in Table 5. Atomic O:N ratios were calculated from respirometry data and ammonia production data and were used to determine approximate amounts of protein versus other metabolic substrates catabolized (Table 5).

Table 4: Equations for the determination and descriptions of energy budget components.

Component	Component description	How value is determined
C	Energy consumed	= (Wet mass of food item – wet mass of leftovers)•calorific value of food per wet mass
R	Metabolism	= litersO ₂ •20.083kJ
F	Energy lost in feces	= (dry mass of feces excreted)•(calorific value of feces per dry mass)
U	Energy lost in urine	= (mass of ammonia excreted)•16.91J/g ¹
G	Energy used for growth	= (wet mass of octopus growth)•(calorific value of octopus tissue per wet mass)
X	Energy used by unaccounted for processes	= C-(R+F+U+G)

¹ Calorific value of ammonia from Petza *et al* (2006).

Table 5: Types of assimilation efficiency and how each was determined.

Type	Description	How Determined
AE _L	Assimilation Efficiency of Lipid	$= \frac{[(\text{mass consumed} \cdot \% \text{ Lipid of food}) - (\text{feces excreted} \cdot \% \text{ lipid of feces})]}{(\text{mass consumed} \cdot \% \text{ Lipid of food})}$
AE _P	Assimilation Efficiency of Protein	$= \frac{[(\text{mass consumed} \cdot \% \text{ Protein of food}) - (\text{feces excreted} \cdot \% \text{ protein of feces})]}{(\text{mass consumed} \cdot \% \text{ protein of food})}$
AE _T	Total Assimilation Efficiency	$= \frac{[(\text{mass consumed} \cdot \text{calorific value of food}) - (\text{feces excreted} \cdot \text{calorific value of feces})]}{(\text{mass consumed} \cdot \text{calorific value of food})}$
O:N	Atomic Oxygen to Nitrogen Ratio	$= \frac{(\text{molO}_2/\text{h} \cdot 2)}{(\text{molNH}_3/\text{h})}$

RESULTS

Aerobic metabolism

Specific dynamic action

Aerobic metabolism of *Octopus rubescens* peaked after feeding due to the specific dynamic action (SDA), which is the increase in metabolism due to the cost of processing food (Schmidt-Nielsen, 2001). The SDA of all *O. rubescens* on both diets lasted an average of 14 h (Figure 3) reaching a peak metabolism rate of approximately $2.98 \mu\text{molO}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$, or approximately 9% increase over standard metabolism, three hours after feeding. The typical pattern of SDA for octopuses fed clams (Figure 4) or fed crabs (Figure 5) did not appear to differ markedly from each other either in the height or duration of the peak. SDA for octopus on crab and clam diets were similar with crab diets averaging 14 hours in length while SDA on clam diets averaged 15 hours. For the remainder of this study “standard metabolic rate” refers to rates measured more than 15 hours after feeding and with chamber oxygen saturation values above 50%. “Routine metabolic rate” will refer to all rates, regardless of time since feeding, taken in a chamber with over 50% oxygen saturation.

Individual patterns of SDA following each feeding were too variable, likely due to intermittent bouts of activity by the octopus, to discern the precise end of the SDA, so no formal statistical test could be performed to determine difference in SDA duration between diets. I determined the peak of SDA for individual feedings and compared the means by two-tailed t-test (Table 6). There was no

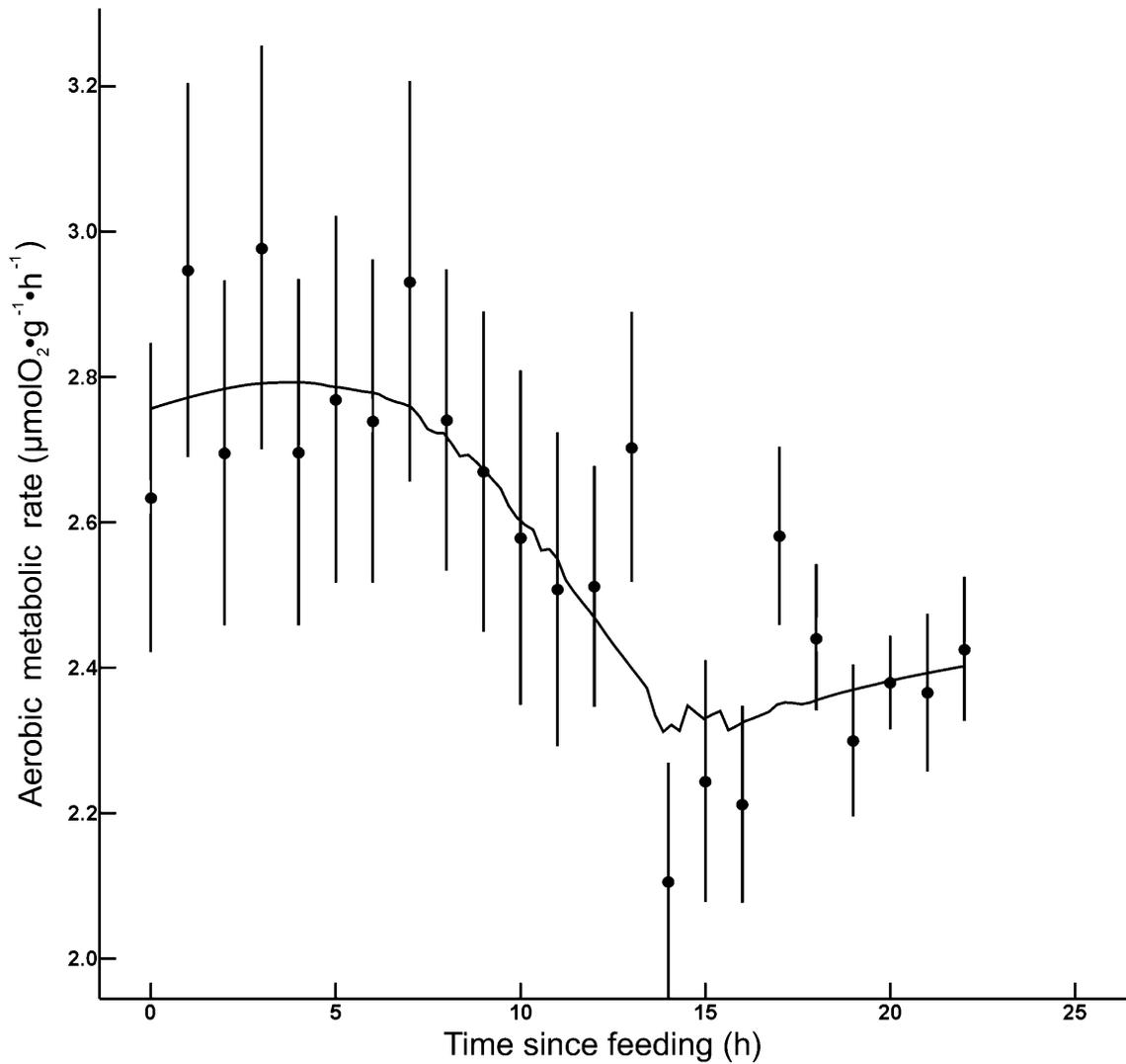


Figure 3: Mean aerobic metabolic rate \pm SE of *Octopus rubescens* against time since last feeding, showing specific dynamic action (SDA). Triweighted LOWESS regression shown (Cleveland and Loader, 1996). Data are hourly means from 15 *O. rubescens* (the mean of each of these individuals) fed both clam and crab diets.

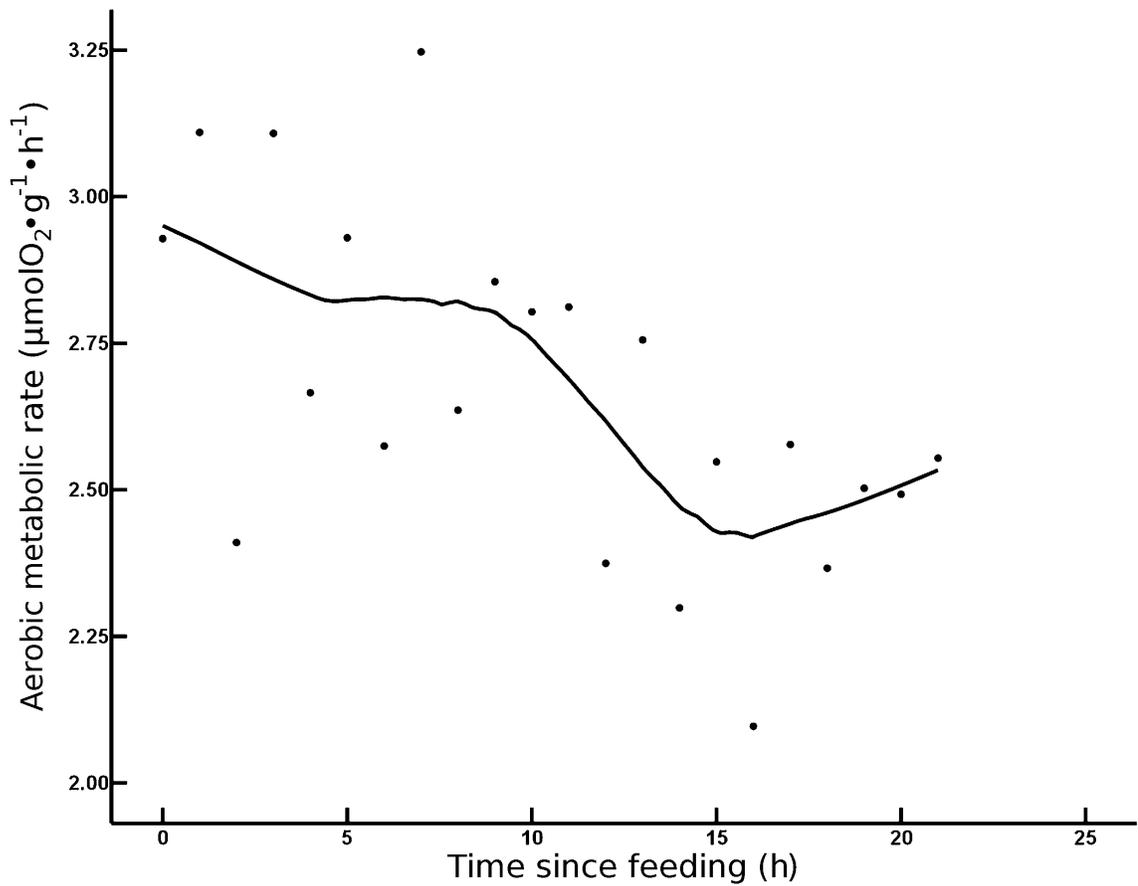


Figure 4: Mean aerobic metabolic rate \pm SE of *Octopus rubescens* against time since last feeding, showing specific dynamic action (SDA). Triweighted LOWESS regression shown (Cleveland and Loader, 1996). Data are hourly means from 9 *O. rubescens* (the mean of each of these individuals) fed only clam diets.

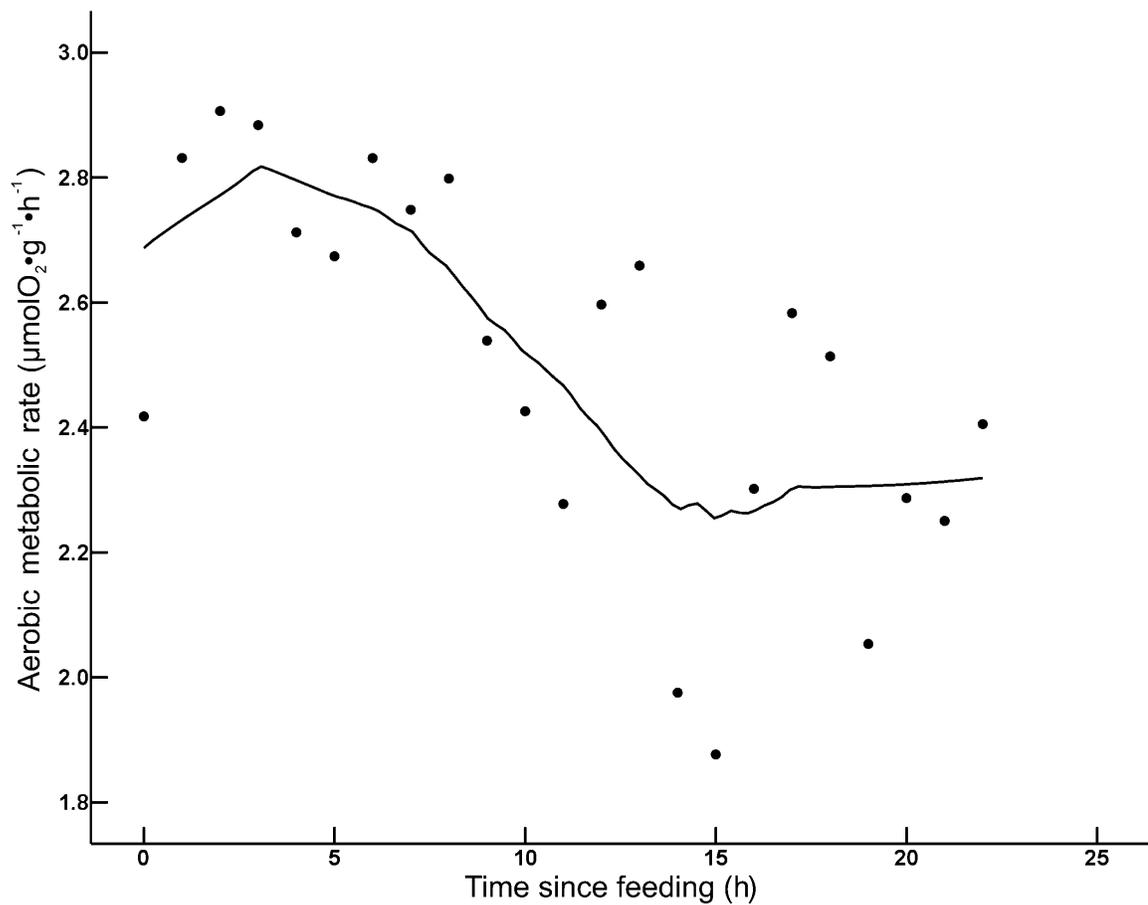


Figure 5: Mean aerobic metabolic rate \pm SE of *Octopus rubescens* against time since last feeding, showing specific dynamic action (SDA). Triweighted LOWESS regression shown (Cleveland and Loader, 1996). Data are hourly means from 14 *O. rubescens* (the mean of each of these individuals) fed only crab diets.

Table 6: Average peak (\pm SD) of standard dynamic action (SDA) expressed as mean metabolic rate 3-12 hours after feeding for octopuses fed clam and crab diets. Peaks are expressed both as mass specific metabolic rate ($\mu\text{molO}_2\cdot\text{g}^{-1}\text{h}^{-1}$) and as a percent of standard metabolism measured for each individual octopus. Means are not significantly different (two tailed t-test, $df = 21$, p-values in table)

Diet	Metabolic rate	% of Standard metabolic rate
Clam	2.67 ± 0.83	$106\% \pm 17\%$
Crab	2.69 ± 0.90	$109\% \pm 25\%$
p-value	0.95	0.68*

* Two-tailed t-test performed on arcsine of $\frac{1}{2}$ the percentage value.

significant difference between diets in peak SDA when expressed either as mass-specific oxygen consumption or as a percent of the standard metabolism of each octopus individually (Table 6).

Early respirometry trials (prior to June 1, 2007), were plagued with gradually dropping oxygen in the respirometry chamber after feeding (down to 45 mmHg) due to an ineffective peristaltic pump. After filtering out oxygen levels from SDA analysis, these trials regularly yielded only 5-7 hours worth of post-feeding metabolic data. Since these octopuses were small (<150 g), with higher metabolic rates than average, inclusion of these data raised the first hours of the SDA, while not affecting later hours, likely distorting the SDA. For this reason data preceding June 1 were dropped from the analysis.

Rates of standard and routine metabolism

The average rate of standard aerobic metabolism of *Octopus rubescens* measured during this investigation was $2.55 \pm 0.81 \mu\text{molO}_2 \cdot \text{g}^{-1} \text{h}^{-1}$ (all error is reported as standard deviation unless otherwise identified). The average routine metabolic rate was not much higher at $2.70 \pm 0.84 \mu\text{molO}_2 \cdot \text{g}^{-1} \text{h}^{-1}$. The highest average metabolic rate for a 24 hour trial was $6.48 \pm 5.69 \mu\text{molO}_2 \cdot \text{g}^{-1} \text{h}^{-1}$ by a 41g octopus and the lowest average metabolic rate for a 24 hour trial was $1.14 \pm 1.02 \mu\text{molO}_2 \cdot \text{g}^{-1} \text{h}^{-1}$ by a 235g octopus. Both of these 24 hour trials occurred on the same day.

Stepwise multiple regression

The relationship between *O. rubescens*' routine rate of aerobic metabolism and other factors was determined by stepwise multiple linear regression using the statistical program SPSS 13.1. In this regression the most significant factor affecting mass-specific metabolism was octopus mass, which had a negative correlation as expected. (Table 7). Weaker, yet still significant correlations were also found with diet, time since feeding, oxygen saturation of the seawater, and hour of day. Significance of each stepwise regression was tested by ANOVA and each factor was significantly different from zero by a p-value of <0.001. This regression model accounted for 24.9% of the variation in observed metabolic rates. Time in respirometer was a factor entered into the regression but was rejected as non-significant.

Metabolic scaling

Total standard aerobic metabolism was significantly correlated to mass by a power curve as expected (Figure 6). A total of 19 octopuses were used for this analysis, and several respirometry runs were made on each over a period of from 2 weeks to 6 months. If the octopus gained less than 25% of its original body mass between experiments all values for that individual were averaged and only one value is reported. However, if the octopus gained 25% of its original body mass or more between experiments, as occurred with 2 individuals, the subsequent respirometry results were recorded separately, resulting in a total of 21 points in the figure. Among these individuals, total metabolism scaled to the

Table 7: Results of stepwise multiple regression of the relationship between various variable and metabolic rate of *Octopus rubescens* in $\mu\text{molO}_2 \cdot \text{g}^{-1} \cdot \text{h}^{-1}$. Significance of each correlation was tested by ANOVA and p-values are shown in table. Analysis based on 147,727 records from 77 24-hour respirometry trials from 22 octopus.

Factor	Unstandardized coefficients	p-value
Octopus mass (g)	-0.012	< 0.001
Diet (Crab=1, Clam=2)	0.147	< 0.001
Time since Feeding (h)	-0.012	< 0.001
Oxygen saturation (%)	0.003	< 0.001
Hour of day (0-23)	-0.022	< 0.001
Variables entered and not found to correlate significantly		
Time in Respirometer (h)		

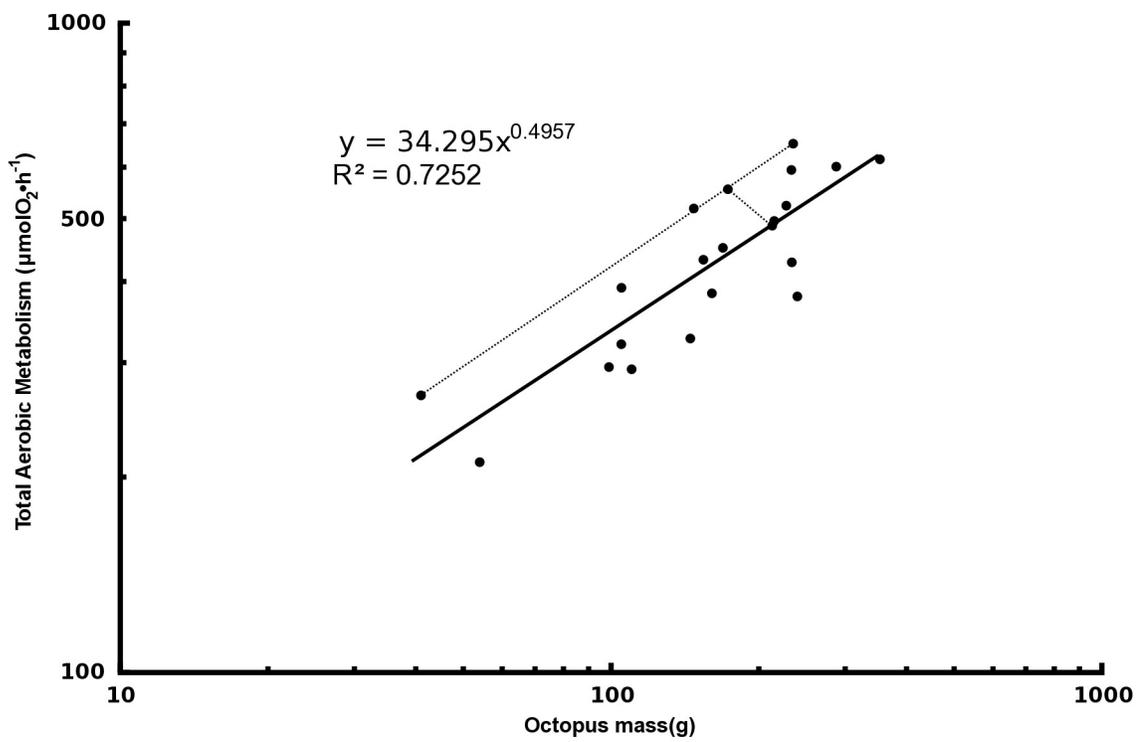


Figure 6: Standard total metabolic rate of *Octopus rubescens* plotted against mass. Dotted lines connect data points from the same octopus, solid line is power regression, which is significant (equation and R^2 on graph, ANOVA, $p < 0.0005$).

0.50 ± 0.07 power, significantly lower than 0.75 as predicted by the Kleiber equation (one-sample t-test, d f= 19, t = 3.628, p < 0.002). Relationship between mass specific metabolism and mass (A relationship that is the inverse of the total metabolism) is shown in Figure 7.

Critical oxygen pressure

On several occasions the automatic valves malfunctioned during respirometry, leaving the chamber sealed and oxygen levels dropping to zero. This led to an accidental test of the octopus' critical oxygen pressure (P_c). P_c was calculated as the oxygen pressure (mmHg) at which a regression of the points well below the inflection point intersected the regression of the points well above the inflection point (Seibel and Childress, 2000). On the first exposure to low pO_2 the mean P_c averaged 31.21 ± 13.53 mmHg (n = 6, range: 14.57 – 53.14).

Two octopuses were each subjected twice to low oxygen events in the respirometry chamber. The first was a 104 g octopus which experienced two low oxygen events about two weeks apart (Figure 8). During the first of these events this octopus survived in relative anoxia (<5mmHg O_2) for 7 hours and 25 minutes, and returned to normal behavior within 24 hours when placed in saturated seawater. The second octopus was a 169 g octopus which experienced two low oxygen events about two days apart (Figure 9). In both cases P_c for the second event was sharply lower than that for the first event. It appears that *O. rubescens*'s critical oxygen pressure can be adjusted as low as 5.95 mmHg O_2 (Figure 7-B) or be as high as 53.14 mmHg O_2 , depending on the individual and

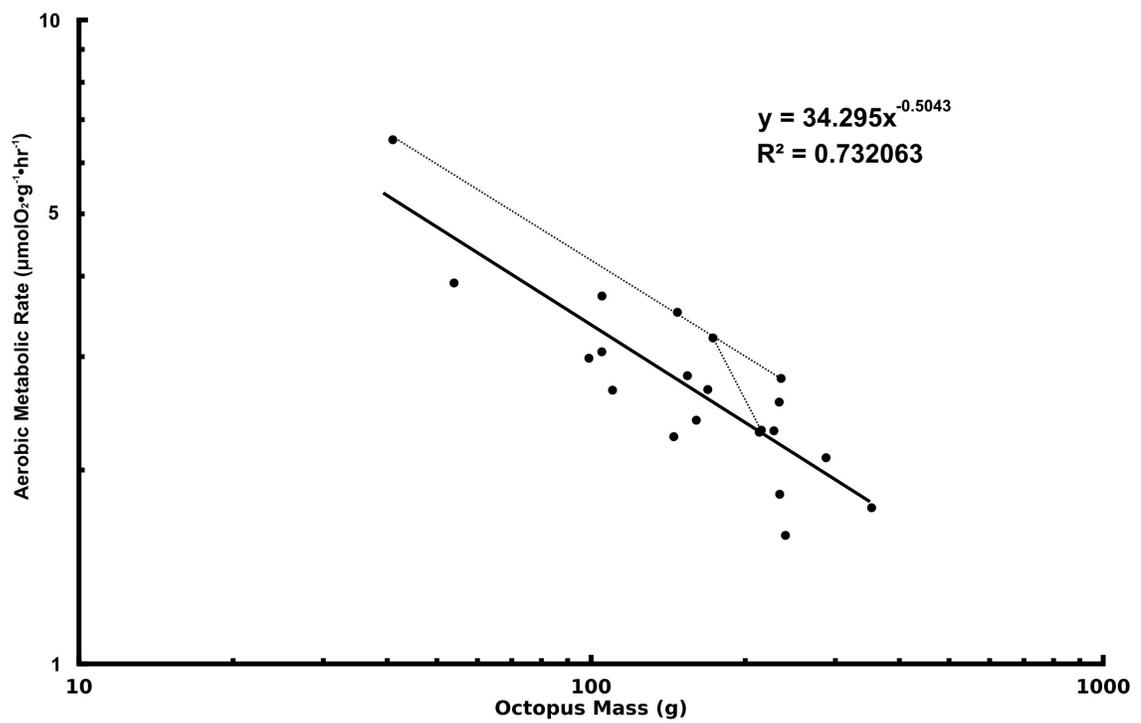


Figure 7: Mass specific standard metabolic rate plotted against octopus mass. Dotted lines connect data points from the same octopus, solid line is power regression, which is significant (equation and R^2 on graph, ANOVA, $p < 0.0005$).

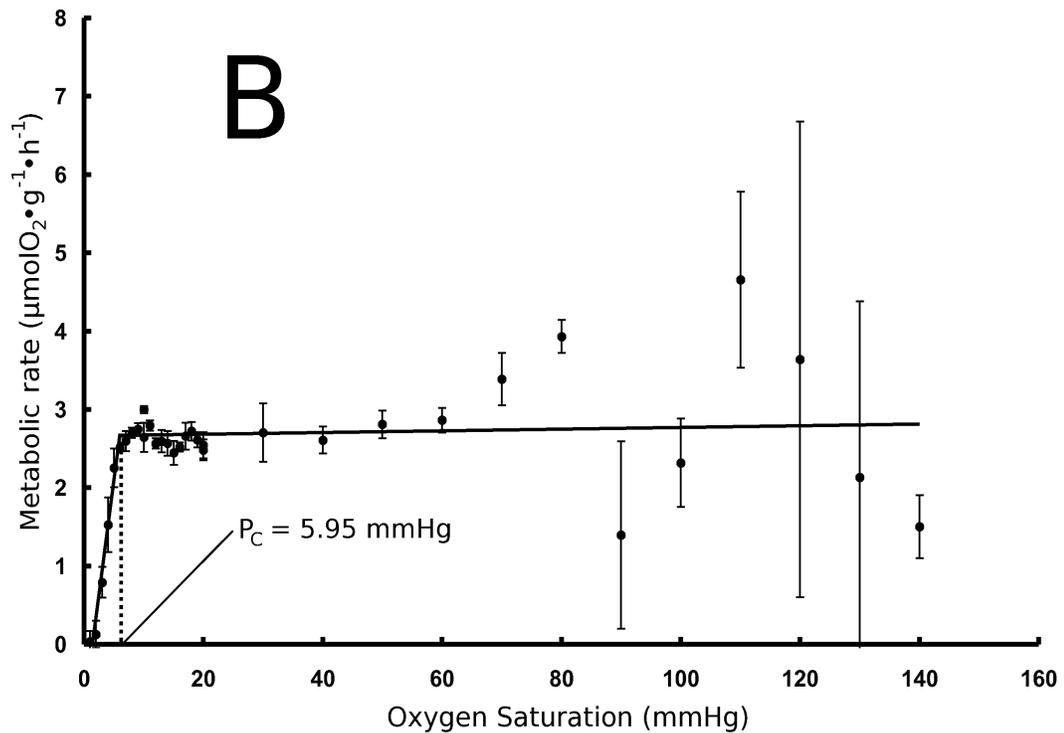
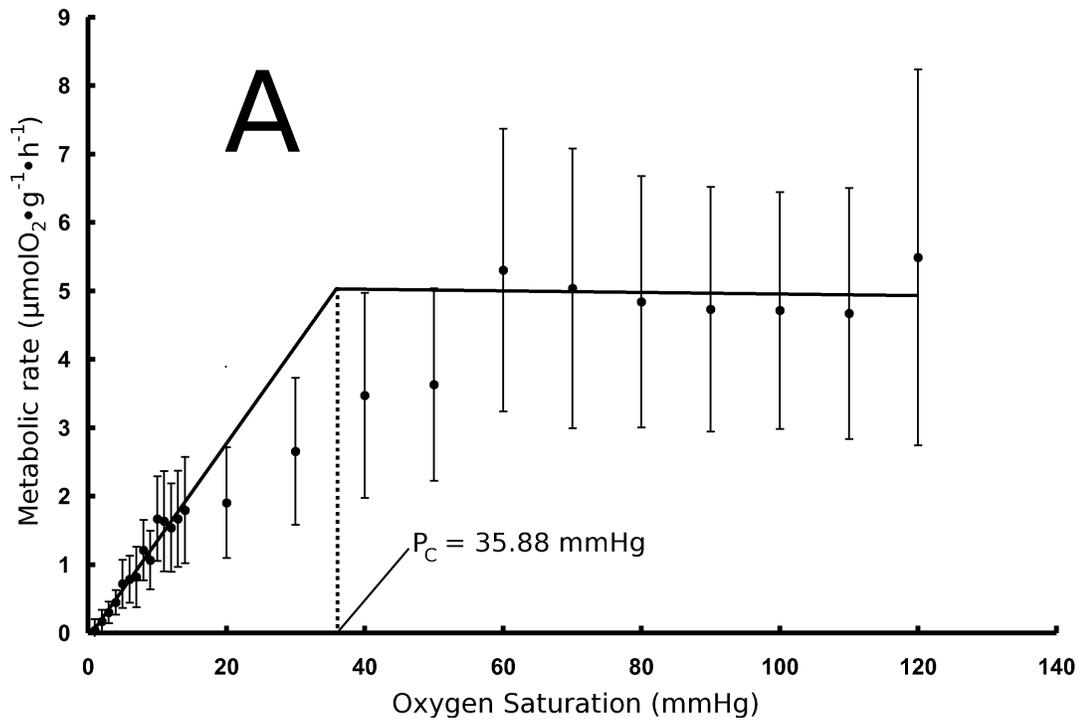


Figure 8: Aerobic metabolism of a single *Octopus rubescens* on two occasions plotted against oxygen saturation: A) 4-18-07, B) 5-01-07. Linear regressions are shown for the flat section at high oxygen saturation and for the sloped sections at low oxygen saturation, as well as x value for the intersections of these lines, which is the critical pressure value (P_C).

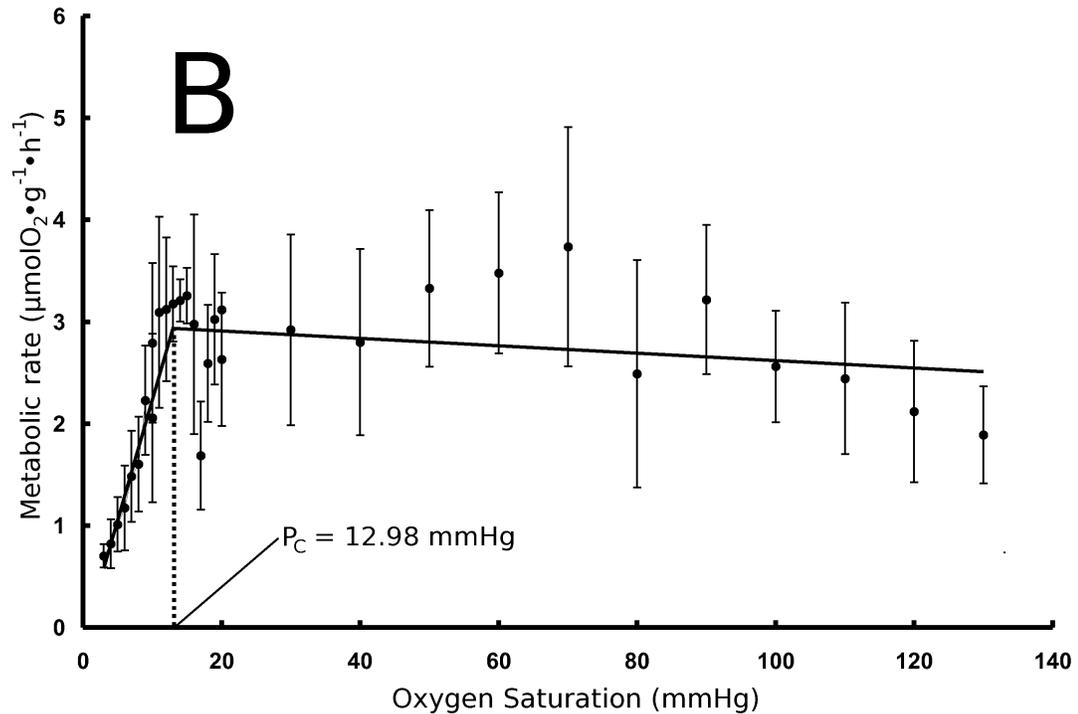
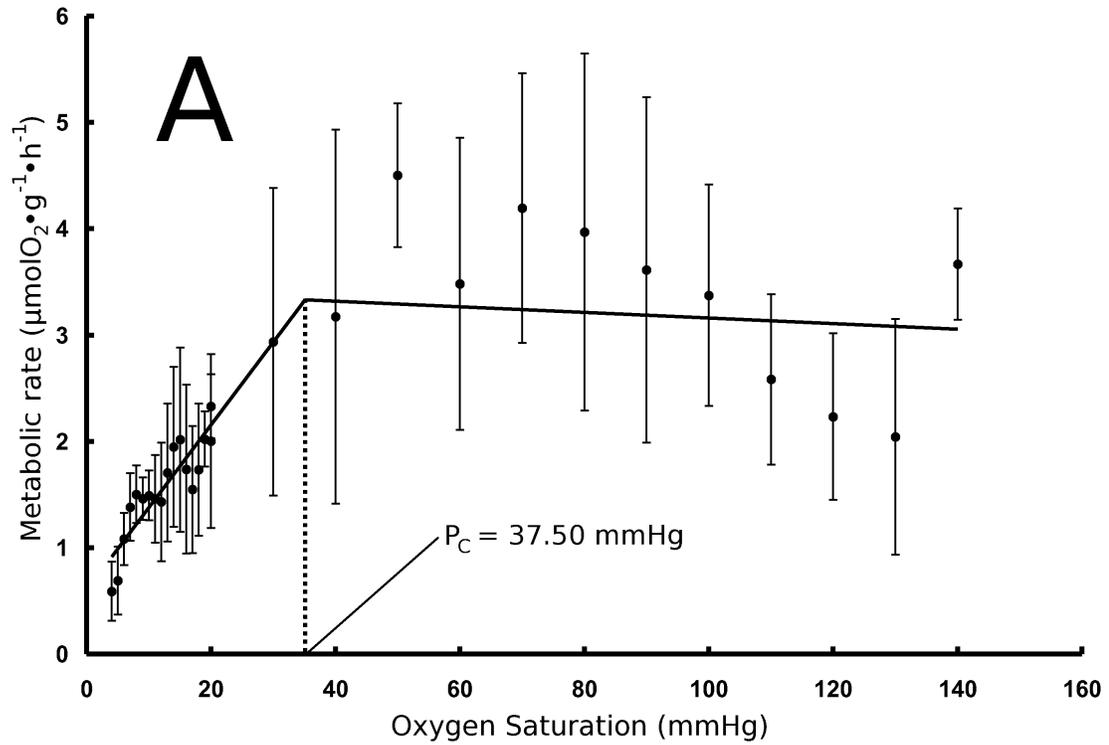


Figure 9: Aerobic metabolism of a single *Octopus rubescens* (octopus #7) on two occasions plotted against oxygen saturation: A) 5-27-07, B) 5-29-07. Linear regressions are shown for the flat section at high oxygen saturation and for the sloped sections at low oxygen saturation, as well as x value for the intersections of these lines, which is the critical pressure value (P_C).

circumstances. Even the highest of these P_C values did not approach the lower cutoff for using my metabolic data of 50% oxygen saturation (78 mmHg), verifying that all the metabolic data used for normal calculations (those in which the effects of oxygen pressure on metabolism were not examined) were within the octopus' normal range of oxyregulation.

Prey choice trials

Prey choices

Nineteen prey choice trials were accomplished with eight different octopuses. Each octopus was used for 1 to 5 trials, depending on the availability of the octopus while not being used for respirometry. These trials yielded a total of 47 prey choices. Numerically, octopuses chose the crab *H. nudus* over three times as often as they did the clam *N. obscurata* (Table 8). Octopuses appeared to have individual preferences, with only 3 of 8 octopuses consuming both clams and crabs, one octopus consuming only clams and four octopuses consuming only crabs. When mass consumed of each prey item is considered (the clams have more consumable tissue), consumption of prey mass was virtually identical (Table 8).

Prey Handling Time

Prey handling times were recorded for nine crab feedings and six clam feedings from six octopuses (four octopuses used for each prey type). *O. rubescens'* prey handling time was significantly longer when consuming *H. nudus* than when consuming *N. obscurata* (Table 9), with *H. nudus* handling time

Table 8: The number of crabs and clams eaten by *Octopus rubescens* during prey choice trials. The numbers of prey items eaten are significantly different (Chi-square with Yate's correction, p-value in table). Since clams weigh more than crabs the mass of clams and crabs eaten was not significantly different (Wilcoxon signed-ranks test, p-value shown).

Octopus #	# of prey consumed		Mass of prey consumed (g)	
	crabs	clams	mass of crabs	mass of clams
2	3	0	6.36	0
3	3	0	5.16	0
11	0	3	0	2.98
13	11	0	8.46	0
15	9	5	11.03	25.36
17	4	0	3.91	0
19	4	2	4.54	4.3
21	2	1	2.61	4.28
Total	36	11	42.07	36.92
Chi-square value	6.127660			
p-value	0.000464		0.5469	

Table 9: Prey handling time and mass consumed per prey item for crab prey (n=9) and clam prey (n=6) by *Octopus rubescens* (mean \pm SD). Calories consumed per minute calculated from $\text{g}\cdot\text{min}^{-1}$ multiplied by calorific values found in table 10. Means compared by Mann-Whitney, p-values shown.

Measure of feeding efficiency	Crab	Clam	p-value
Handling time (min)	123.9 \pm 88.3	38.2 \pm 19.9	0.001
Mass•prey item ⁻¹ (g)	1.9 \pm 0.9	5.3 \pm 2.2	0.002
g consumed•min ⁻¹	0.02 \pm 0.01	0.15 \pm 0.04	0.001
calories obtained•min ⁻¹	72.7 \pm 28.3	669.1 \pm 208.4	0.0004

averaging over two hours while *N. obscurata* handling times were slightly longer than a half hour. Further, the mass of the tissue consumed from each *N. obscurata* was more than double that consumed from *H. nudus* (Table 9). When considered together, the amount of food *O. rubescens* obtained per minute handling time of *N. obscurata* was an order of magnitude greater than that obtained from consuming *H. nudus* (Table 9).

Midden analysis

The contents of twenty-one middens obtained from glass bottle dens were analyzed during the course of this study. The substrate at Admiralty Bay is dominated by broken barnacle shells, including sediments inside glass bottles. For this reason barnacles were not counted as part of the midden. Had that been done, barnacles would have easily composed >99% of each midden, the vast majority of it likely attributable to its background occurrence in the substrate. During this study, octopuses fasting in tanks with barnacles did not eat the barnacles, nor did I find any drilled barnacle parts within the middens.

The most numerous non-barnacle prey item found was *Nucella lamellosa*, a predatory snail, followed by two crabs, *Petrolisthes eriomerus* and *Lophopanopeus bellus* (Figure 10). Over half of all body parts counted and virtually all body parts from the three most common prey items were found to have a distinctive octopus-type drill hole, strongly supporting the assumption that an octopus actually did capture alive and consume these items. Comparatively, the

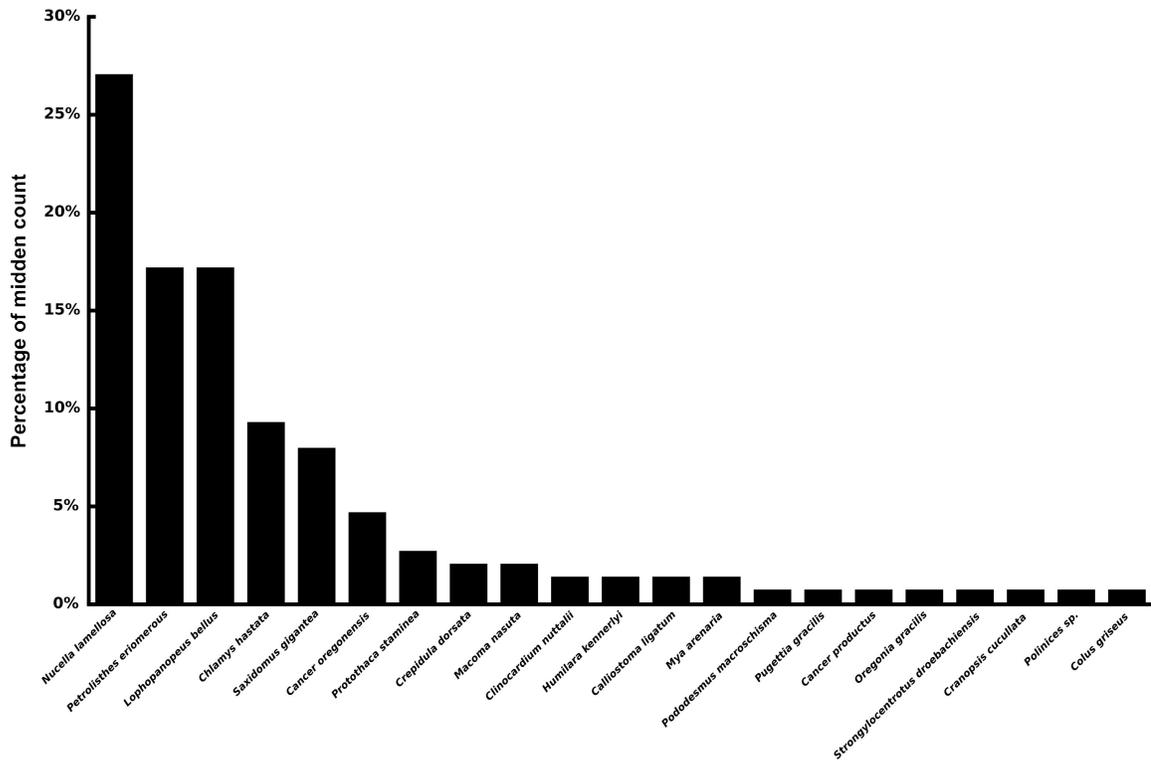


Figure 10: The percentage each prey species found in *O. rubescens* middens in this study composes of total midden count (count of all prey items found in all middens). 152 prey items were recovered from 21 middens. For raw counts and common names see Appendix I.

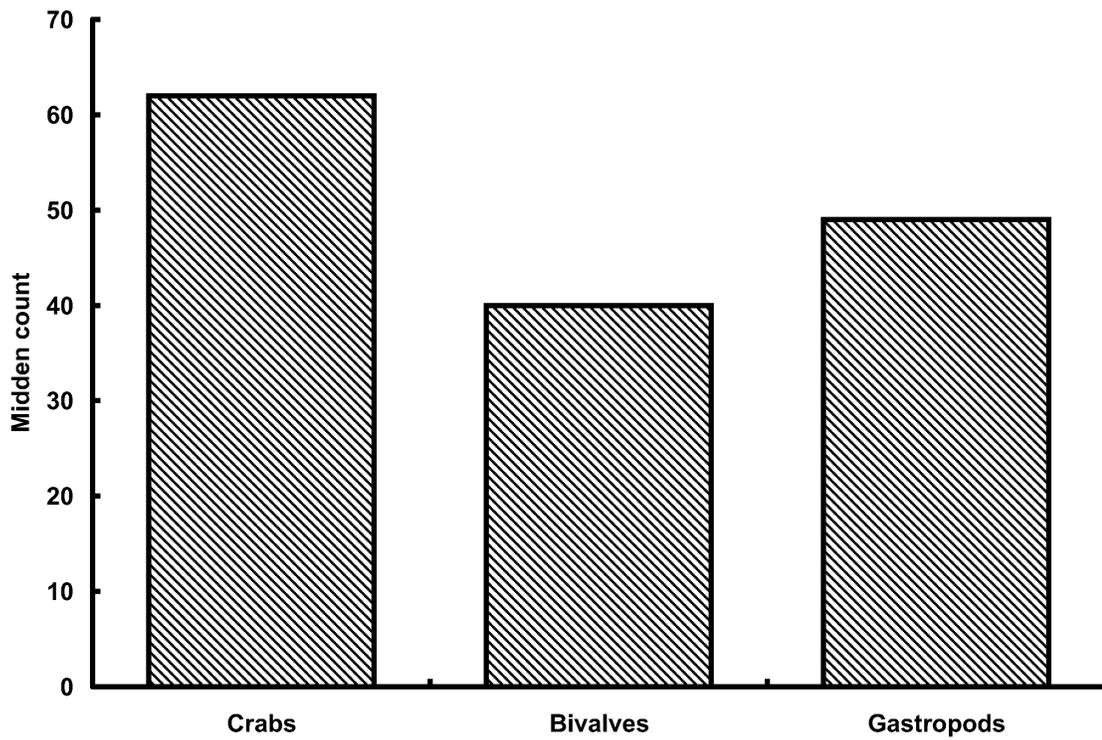


Figure 11: The count of each of the three most common prey groups found in *O. rubescens* middens in this study. 152 prey items were recovered from 21 middens.

middens examined in this investigation had a relatively diverse range of species (Figure 10) and nearly equal numbers of gastropods, crabs and bivalves (Figure 11).

Energy Budgeting

The amount of lipid (fat), protein and energy content of crab tissue, clam tissue and octopus tissue are shown on Table 10.

Complete energy budgets for *O. rubescens* were determined for eight individuals based on a diet of *Nuttallia obscurata* and for nine individuals based on a diet of *Hemigrapsus nudus*. Values for each component of the energy budgets were quite variable between individuals (Table 11). Average X components of energy budgets for both diets were negative, indicating that energy expenditures on average exceeded energy consumption. Each component of the energy budget was compared pairwise between the two diets by a two-tailed t-test. Only energy excreted as feces was statistically different between the two budgets with a greater amount of excretion by octopus on a clam diet by a factor of three. Food consumption was very nearly significantly different between diets (two-tailed t-test, $p=0.06$).

Growth was extremely variable between individuals (Figure 12), with several losing weight during the trial week rather than gaining. On *Nuttallia* diets one of eight octopuses lost weight whereas four of nine octopuses on a *Hemigrapsus* diet lost weight.

Table 10: Nutrient values for soft body tissues of *Octopus rubescens* and its prey items, as well as *O. rubescens* feces while on both diets. Other than % dry matter, all values are reported as a function of dry matter.

Material	% dry matter	% ash	% crude fat	% crude protein	gross energy content (cal•g ⁻¹)
<i>Nuttallia obscurata</i>	20.14	9.34	4.09	51.78	4452
<i>Hemigrapsus nudus</i>	19.88	24.64	9.53	45.36	4099
<i>Octopus rubescens</i>	18.87	11.01	2.68	75.88	4557
<i>Nuttallia</i> diet feces	--	59.15	7.62	12.53	1817
<i>Hemigrapsus</i> diet feces	--	70.24	2.76	11.54	966

Table 11: Average energy budget (\pm SD) of *Octopus rubescens* fed a diet of *Nuttallia obscurata* and of *Hemigrapsus nudus* in joules•g of octopus⁻¹•day⁻¹. Each energy budget component was compared pairwise between diets by the two tailed t-test; p-values are shown in table. For abbreviations see table 4.

Diet	C	R	G	E _U	E _F	X
<i>Nuttallia</i>	63.89 \pm 36.57	30.57 \pm 18.26	31.20 \pm 36.13	0.02 \pm 0.02	2.94 \pm 1.29	-0.84 \pm 48.56
<i>Hemigrapsus</i>	33.03 \pm 25.98	27.45 \pm 10.00	9.49 \pm 41.50	0.01 \pm 0.01	0.96 \pm 0.82	-4.88 \pm 39.77
T-test p-values	0.06	0.37	0.27	0.58	0.002	0.99

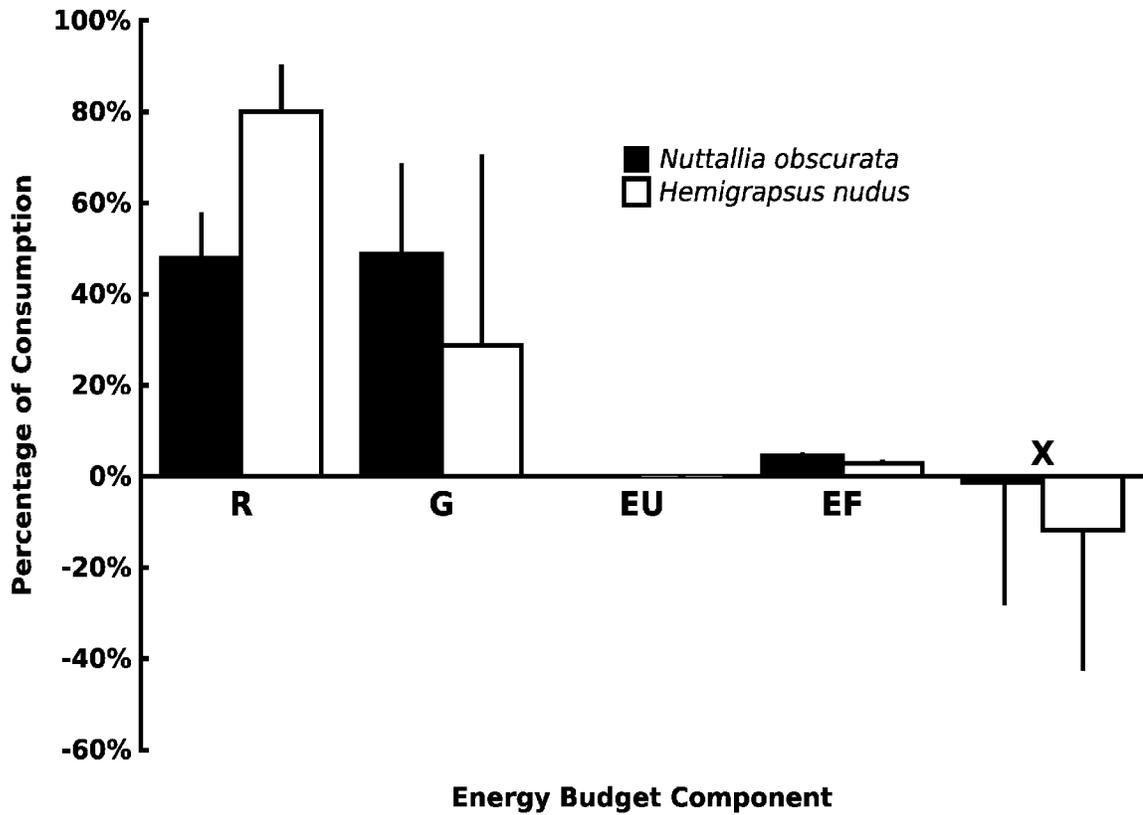


Figure 12: Energy expenditures of *Octopus rubescens* expressed as a percentage of energy consumed (C). For definitions of abbreviations see Table 4.

Energetic efficiencies

Assimilation efficiencies were very high for octopus both in terms of energetics and of mass, and were very similar between diets. Total assimilation efficiency in terms of energy was $94.4\% \pm 2.7\%$ on the *N. obscurata* diet and $95.8\% \pm 3.2\%$ on the *H. nudus* diet. Similarly, protein assimilation efficiencies (in terms of mass) were also very high averaging $96.7\% \pm 1.6\%$ on the *N. obscurata* diet and $95.4\% \pm 3.6\%$ on the *H. nudus* diet. Interestingly, lipid assimilation efficiencies were quite different between the diets with *N. obscurata* diets yielding only a $74.6\% \pm 12.4\%$ assimilation efficiency while *H. nudus* diets yielded $94.9\% \pm 4.0\%$ extraction efficiency (Table 12). These lipid extraction efficiencies were significantly different (Mann-Whitney test, $p = 0.0003$, $df = 15$).

Atomic oxygen to nitrogen ratios

Atomic oxygen to nitrogen ratios (O:N) for both energy budgets were well within the range consistent with pure amino acid metabolism (below 15), with a single outlier of 60.41 obtained during a crab diet, which is suggestive of equal portions of lipid and protein metabolism (Table 12). Discounting this outlier, O:N ratios are strikingly similar for octopuses on both diets, with *Nuttallia* fed octopuses averaging 5.70 ± 5.48 and *Hemigrapsus* fed octopuses averaging 6.85 ± 3.25 . These means are not significantly different (two-tailed t-test, $p=0.6$, $df = 15$)

Table 12: Assimilation efficiencies (AE, mean \pm SD, defined in table 5) for *Octopus rubescens* on a diet of the clam *Nuttallia obscurata* and the crab *Hemigrapsus nudus*. Total AE is expressed as a function of energetic content of the food, while protein and lipid AEs are expressed as a function of mass because energetic content of protein and lipid fraction of food items is unknown. P-values for two-tailed t-tests shown in table (df = 15). O:N ratios (mean \pm SD, df = 14) for each diet are also included.

Species	Total AE*	Protein AE*	Lipid AE**	O:N ratios
<i>Nuttallia</i>	94.4% \pm 2.7%	96.7% \pm 1.6%	74.6% \pm 12.4%	5.7 \pm 5.5
<i>Hemigrapsus</i>	95.8% \pm 3.2%	95.4% \pm 3.6%	94.9% \pm 4.0%	6.9 \pm 3.3
p-values	0.260	0.431	0.0002	0.641

*T-test performed on arcsine transformation of percentages

**Welch's T-test performed due to heteroscedastic data.

DISCUSSION

Predation habits

Prey preference and handling

O. rubescens' numerical preference for *H. nudus* despite a considerable calorific advantage gained by consuming *N. obscurata* suggests that there is more included in the octopus' prey choice than a simple optimal foraging model would imply. In these experiments the prey choices made by *O. rubescens* deviated widely from those expected from a simple model of maximizing caloric intake per unit time. *O. rubescens* consumed nearly an order of magnitude higher calories per unit time when consuming the clam *N. obscurata* rather than the crab *H. nudus* (Table 9). However, despite this fact, *O. rubescens* chose *H. nudus* over *N. obscurata* as prey by a ratio of 3 to 1 (Table 8). Interestingly, even with this disparity in choices between *H. nudus* and *N. obscurata*, *O. rubescens* consumed nearly equal mass of each prey item over the course of the experiment.

Increased handling time for the comparatively smaller crabs could be a result of several factors. Crabs have a complex exoskeleton when compared with the external shell of a clam, which likely takes longer for the octopus to extract edible tissues especially from the legs and chelae of the crab. Increased handling time could also be a function of prey preference, with octopuses investing more time to consume their preferred prey more thoroughly. Alternatively *O. rubescens*' numerical prey choice for *H. nudus* may be a result of

deeper instinctual motivations or a learned behavioral search image for crabs in general that do not accurately reflect any sort of preference for *H. nudus* in particular (Curio, 1976). This, however, seems unlikely due to the flexibility of behavior conferred on octopuses by their high degree of intelligence (Mather and Anderson, 1993; Mather and Anderson, 1999; Mather and Anderson, 2007).

Midden analysis

My findings contrast sharply with those of Anderson *et al* (1999) from the southern Puget Sound, who found that nearly 80% of the non-barnacle contents of the midden was accounted for by a single species, *Olivella baetica* (Figure 14), and 98% by a single group, gastropods (Figure 15). The most common midden item in both my study and Anderson *et al* (1999) was a gastropod species, contrasting with quantifications of middens from other octopus species which were generally found to primarily focus on crabs and to a lesser extent, bivalves (Cosgrove, 1989; Dodge and Scheel, 1999; Hartwick *et al.*, 1981; Smith, 2003). In the early stages of this study a third diet of *Nucella lamellosa* was attempted but, despite the prevalence of this species in *O. rubescens* middens, was dropped because of the great difficulty of getting the octopuses to eat this species of snail in captivity. This suggests that at Admiralty Bay the high prevalence of *N. lamellosa* in *O. rubescens*' middens is more a function of the snails' abundance rather than a preference over crab or bivalve species. Studies with *Octopus bimaculatus* have found in this species' middens in the field a high

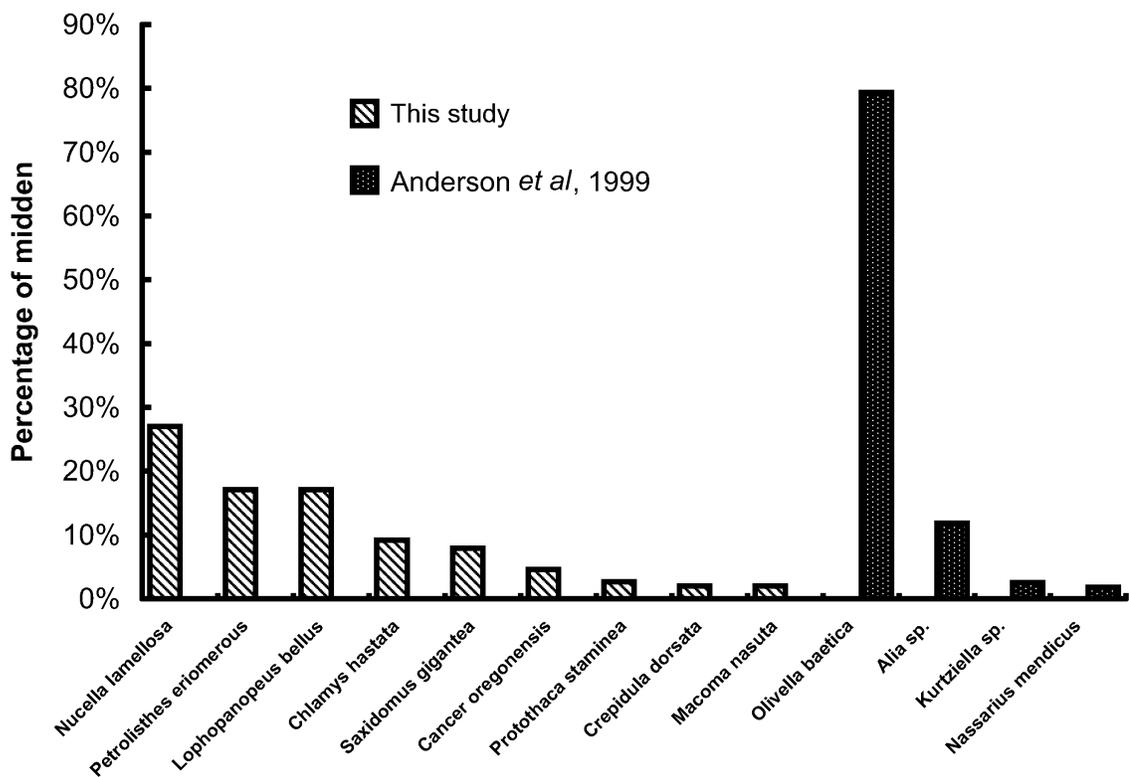


Figure 13: Percentage of midden count for this study and for the only other published quantification *Octopus rubescens* middens from Anderson *et al* (1999). Only prey items composing more than 2% of the midden count are shown. Additionally barnacles are discounted from Anderson *et al* for sake of comparability.

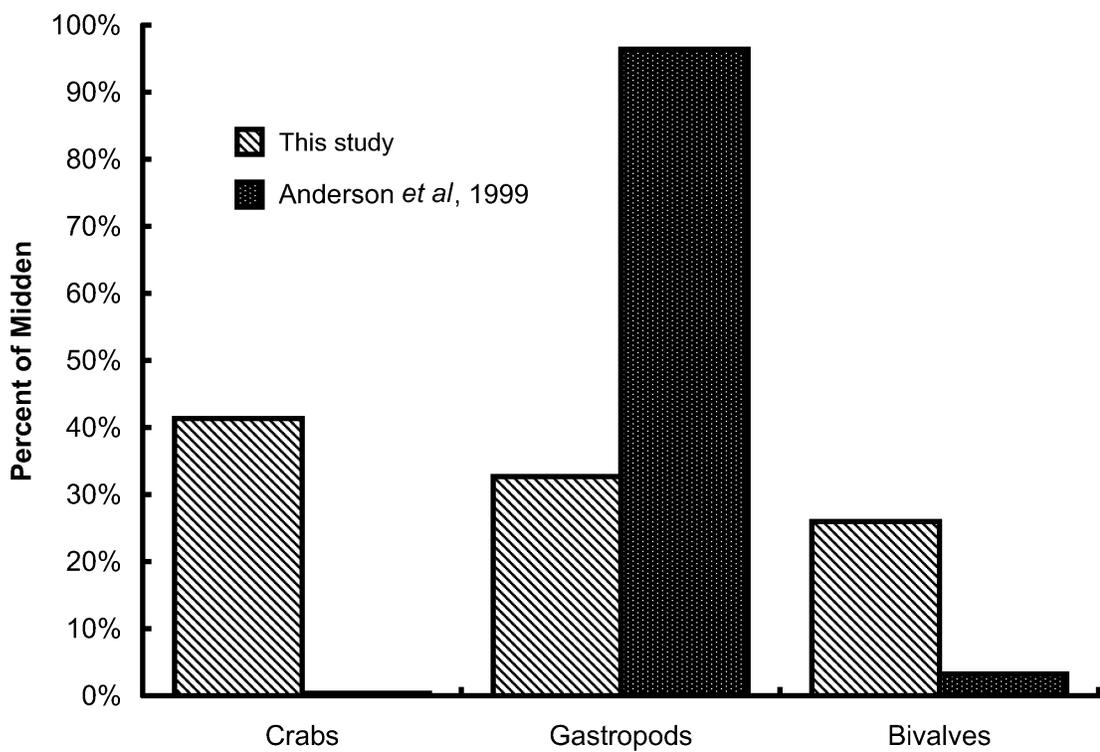


Figure 14: Percentage of *Octopus rubescens* midden count represented by major prey groups for both this study and the values found by Anderson et al, 1999

number of snails, but this species preferentially consume other prey in the lab (Ambrose, 1984). Alternatively, *N. lamellosa* shells in the midden could represent consumption of hermit crabs inhabiting the shells, which are also abundant at Admiralty Bay and inhabit *N. lamellosa* shells, rather than the snails themselves. This, however, seems unlikely since no body parts attributable to hermit crabs were found in the middens. On at least one occasion I observed *O. rubescens* predation on a hermit crab (*Pagurus dalli*) in the lab, however.

Despite the high numbers of the snail *N. lamellosa*, this study found a higher minimum number of crabs in the middens than either bivalves or gastropods. This is in agreement both with counts of prey in other octopus diets (Cosgrove, 1989; Dodge and Scheel, 1999; Smith, 2003) as well as the preference trials conducted in this study.

Octopus midden analysis is fraught with potential bias, and investigation of middens found in glass bottles only compounds these biases. Middens will over-represent hard bodied and shelled prey items such as mollusks and crabs. Softer bodied prey such as shrimp, which are readily accepted in lab and very abundant at Admiralty Bay, and amphipods and fishes, whose soft exoskeletons and bones would not persist in the middens nearly as long as the heavy shells of *N. lamellosa* or the hard chelipeds of crabs, would be undercounted in a midden analysis. Even crab carapaces that would logically be associated with the chelipeds found in the middens were rarely found. A second bias would be associated with the percentage of prey items *O. rubescens* consumes away from

the den as opposed to those brought back to the den for consumption. To date no study has quantified this behavior in the wild for this species. Additionally prey items found in bottle middens are necessarily limited to prey items which can fit into the often narrow bottle mouth. In all, midden analyses, including this study, likely underestimate the diversity in the diet of the animal investigated.

There have been several investigations into octopus midden stability and how accurately the midden contents reflect diet. In low current areas, such as the one in Admiralty Bay, mussel and snail shells in artificially constructed middens near occupied *Octopus bimaculatus* dens were removed from middens at a rate of 0.31 and 0.23 shells per day, respectively (Ambrose, 1983). Snail shells were even more quickly removed in areas with high abundances of hermit crabs. Comparisons of stomach contents of octopuses to midden counts have had differing results, even in similar populations. Workers have found stomach contents and midden counts in populations of *Octopus vulgaris* in South Africa to be comparable (Smale and Buchan, 1981), while later investigations, also on South African *Octopus vulgaris*, have found significant differences in the frequencies of dietary items in stomach contents and in middens (Smith, 2003). Middens contained in glass bottles are likely exposed to markedly different biotic and abiotic biasing factors than middens left at the mouth of a den, greatly reducing the comparability of these studies to *O. rubescens*.

Metabolic scaling

The very low scaling factor (0.50) I found in this study is strikingly different from the high scaling factors found by other workers for *O. vulgaris*, *O. maya* and *O. cyanea* (Katsanevakis *et al.*, 2005b; Maginniss and Wells, 1969), or even the lower interspecies scaling factor found for octopods by Seibel (Seibel, 2007) of 0.73. This low scaling factor may be due to small animals being more active in the respirometer than their larger counterparts, artificially raising the lower end of the regression, though such behavior was not observed. The more likely reason is that this low scaling factor is an artifact of the very limited range of size sampled in this study, under a single order of magnitude. metabolic rates have also been measured for *O. rubescens* paralarva approximately three orders of magnitude smaller than the smallest octopus used in this study (Seibel *et al.*, 1997). Integrating these data points with my data yields a scaling factor of 0.79, much more similar to the previously measured inter- and intraspecies metabolic scaling measurements published to date (Figure 16). This does, however, leave a large gap between the two data points introduced and the rest of the data. Additionally, these paralarva are pelagic with a very different lifestyle than the benthic adults. Until the gap is filled and there are data regarding possible metabolic changes during settlement, it is difficult to assess the comparability of these data. It is reasonable to expect that the metabolic rate for pelagic paralarvae would not be lower than would be expected for scaled benthic adults, so it is unlikely that any correction of systematic errors would yield a scaling factor of 0.52.

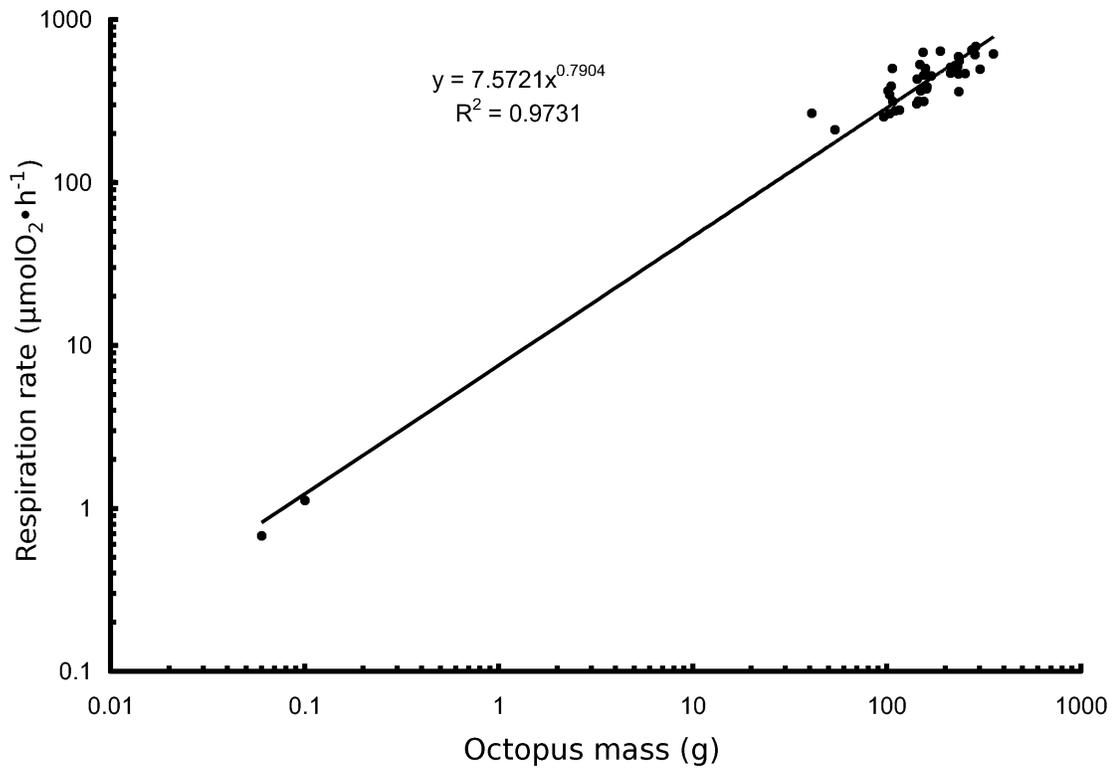


Figure 15: Standard metabolic rate of *Octopus rubescens* plotted against mass, including two data points from Seibel *et al*, 1997. These two data points have been corrected from 5C to a temperature of 11C assuming a Q_{10} of 2.0.

Aerobic metabolism

Comparative metabolism

Compared with other major benthic predators occurring in the Salish Sea area with *O. rubescens*, this octopus species has a high mass specific metabolic rate (Figure 17). The metabolic rates of *O. rubescens* are comparable to fishes in the genus *Sebastes* (rockfishes), an active benthic-pelagic predator, but much higher than that of cottids (sculpins) a less active benthic family of fishes. *O. rubescens* has a metabolic rate approximately an order of magnitude greater than that of asteroids of similar size.

Critical oxygen pressure

The average critical oxygen pressure of 31 mmHg (as low as 14 mmHg) for octopuses first exposed to low oxygen is consistent with that measured in *O. vulgaris* (25-50 mmHg ;Wells and Wells, 1995) and *O. bimaculoides* (16-28 mmHg; Seibel and Childress, 2000). *O. californicus*, an octopus that regularly resides in hypoxic conditions, can regulate its oxygen uptake well below 10mmHg (Seibel and Childress, 2000). Survival of octopuses as long as 7.5 hours in anoxia is also not unprecedented in the published literature. *O. californicus* has been found to survive up to 8 hours in anoxia and *O. bimaculoides* up to 4 hours (Seibel and Childress, 2000).

O. rubescens showed a lower critical oxygen pressure (P_c) when an octopus had been exposed to previous low oxygen pressure. Because these were unplanned trials, enough data were not collected to test this relationship.

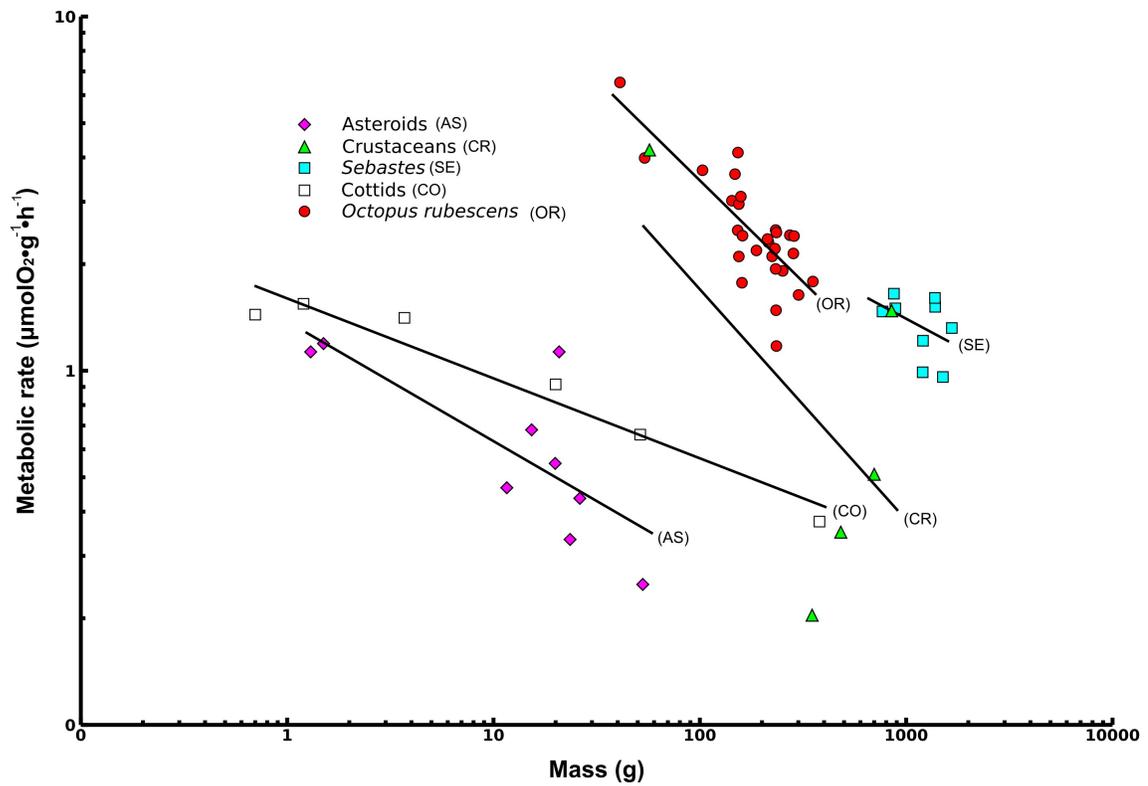


Figure 16: Routine metabolic rate plotted against mass of *Octopus rubescens* and three other classes of benthic marine predators: Fishes (*Sebastes* and *Cottids*), Crustaceans and Asteroids. All values corrected to 11C assuming a Q_{10} of 2.0
 References for:
 Asteroids (Webster, 1975)
 Crustaceans (Bradford and Taylor, 1982; Holsman *et al.*, 2003; McMahon *et al.*, 1979)
Sebastes (Hopkins *et al.*, 1995)
Cottids cited in (Seibel and Drazen, 2007)

Such a relationship would suggest that this species has a long-term adaptive physiological response to low oxygen environments. Octopuses have been shown to have several short-term adaptive responses to hypoxia. Octopuses increase the ventilation frequency and volume of their mantle cavity during hypoxic conditions (Wells and Wells, 1985), while heart rate slows and blood pressure drops (Wells and Wells, 1986). Cephalopod hemocyanin is primarily modulated by temperature and pH rather than by small accessory molecules as in vertebrates and crustaceans (Miller, 1985; Pörtner, 1994). During periods of hypoxia both arterial and venous blood pH rises, coinciding with a rise in hemocyanin oxygen affinity (Houlihan *et al.*, 1982). These mechanisms alone, however, cannot satisfactorily explain a possible long-term adaptive response to low O₂ since each of them appears to be employed to their physiological limit during short-term adaptation, unless octopuses can endure severe alkalosis.

Even the highest critical oxygen pressures (53 mmHg) measured in these unplanned trials were well below the minimum oxygen pressures (78 mmHg) used for any respirometry analyses, ruling out oxygen limitation as a confounding factor.

Energy budgeting

O. rubescens' energy consumption seems somewhat low when compared with the energy budgets found for other octopuses (Table 12), except for that of the antarctic octopus *Pareledone charcoti* and *E. megalocyathus* fed a diet exclusively of mussels. Perhaps this can be accounted for by difference of a

Table 13: Comparisons of average energy budgets for octopods along with the temperature the budget was measured at and total assimilation efficiencies (AE_T%). All energy budget components are reported in Joules•gram(wet wt)⁻¹•day⁻¹.

Species	Temp (C)	C	R	E _U	E _F	G	X	AE _T %
<i>Pareledone charcoti</i> ¹	0	10.5	7.04	0	0.41	3.13	--	96%
<i>Octopus vulgaris</i> ²	20	67.92	38.57	0.01	9.06	17.38	--	87%
<i>O. cyanea</i> ³	20	83.96	40.47	--	3.69	40.8	1.26	96%
<i>O. maya</i> ³	20	76.64	27.95	--	3.08	45.61	--	96%
<i>O. maya</i> ⁴	?	279.57	63	20	6.00	190.57	--	98%
<i>Enteroctopus dofleini</i> ⁵ (mixed diet)	9.5	80.96	21.48	--	4.57	31.63	--	--
<i>E. megalocyathus</i> ⁶ (crab)	17	147.72	39.94	1.28	1.18	6.07	--	99%
<i>E. megalocyathus</i> ⁶ (mussel)	17	2.08	37.82	1.43	2.97	-1.99	--	-43%
<i>O. rubescens</i> ⁷ (clam)	11	63.89	30.57	0.02	2.94	31.20	-0.84	94%
<i>O. rubescens</i> ⁷ (crabs)	11	33.03	26.46	0.01	0.96	9.49	-3.89	96%

¹(Daly and Peck, 2000) ²(Petza et al., 2006) ³(Van Heukelem, 1976) ⁴(Rosas et al., 2007) ⁵(Rigby and Sakurai, 2004) ⁶(Perez et al., 2006), Values for *E. megalocyathus* were obtained at using a 16% wet weight to dry weight conversion and energetic content of flesh is 4144J/g dry wt, both published value for *E. dofleini* (USDA, 2008). ⁷This study

monotypic vs. mixed diet (*E. dofleini*) or lower temperature (all remaining energy budgets) (Table 12). All other values seem to be comparable to other published energy budgets.

Energy budgeting was extremely variable among individuals, and most components were not significantly different between diets. This trend was exemplified by growth. Growth was poor and inconsistent on both diets, and was slightly but not significantly lower on a *Hemigrapsus* diet. There are several likely contributing factors to this. The first is the short time span in which growth was measured. In retrospect one week is much too short a time interval to obtain reliable growth measurements. An additional factor contributing to poor growth could be the use of monotypic diets. *Enteroctopus dofleini* has been shown to exhibit poorer growth on monotypic diets than on mixed diets (Rigby and Sakurai, 2004). *Octopus vulgaris* has been found to have more robust growth on a diet of equal portions crab (*Carcinus mediterraneus*) and bogue (*Boops boops*) than on monotypic diets of either or on unequal diets (Garcia Garcia and Cerezo Valverde, 2006). Additionally, *Enteroctopus megalocyathus* fed a monotypic diet of mussels showed very low consumption and a consistent loss of weight (Perez *et al.*, 2006). It has been suggested that when fed prepared diets cephalopod growth may be limited because ingestion ceases when adequate energy is consumed but a proper amino acid balance has not yet been achieved (Lee, 1994). This could also be problematic in monotypic diets and together with the diverse nature of octopus diets, particularly that of *O. rubescens*, suggests that

octopuses have complex nutritional needs that may not be met by a monotypic diet. It is plausible that the diverse observed diet could be symptomatic of *O. rubescens* engaging in nutrient-specific foraging to achieve a proper dietary balance of amino acids and lipids. Such behavior has been observed in other invertebrate predators such as spiders and beetles (Mayntz *et al.*, 2005).

Due to the high variability between energy budget measurements very little difference between the energy budgets on the two diets can be discerned. The most divergent energy budget components were consumption (C) and feces production (E_F), with octopuses consuming only about half the energy per gram per day on a crab diet than on a clam diet and eliminating only about one-third the energy as feces on a crab diet. Clearly these are linked: the less an organism eats, the less it defecates. This could be related to the disparity in energy gained per handling time on each diet or could be an artifact of a monotypic diet.

Perhaps the most interesting difference in dietary energy budgeting was the difference in lipid assimilation efficiency (definition in Table 5, efficiencies found in Table 12). Assimilation efficiencies for individual macronutrients are rare in published literature although lipid assimilation efficiencies for *Octopus vulgaris* have been reported to range from 77% (O'Dor and Wells, 1987) to 46% (Lee, 1994). The values found in this study therefore are on the high end or exceed this range. *H. nudus* was nearly twice as lipid-rich as *N. obscurata*, and lipid assimilation efficiencies on a diet of *H. nudus* were also significantly higher. *O.*

rubescens appears to retain nearly three times as much lipid from crab tissue than it does from clam tissue. This may explain why *O. rubescens* persists in consuming a higher number of *H. nudus* in preference trials despite gaining less energy per unit time. There is no substantial change in the low O:N ratios between diets, however, suggesting that the increase in lipid assimilation efficiency in *H. nudus* diets is not connected to a rise in lipid catabolism for energy. The ultimate fate of these lipids is unknown, but it can be assumed that they are being retained for at least a time in the digestive gland of the octopus as has been shown for other octopus species (O'Dor *et al.*, 1984). The specific fatty acid profile of dietary items can have a considerable impact on the health of *O. vulgaris* paralarvae (Navarro and Villanueva, 2003). While adult octopuses have a markedly lower lipid composition than do paralarvae, sensitivity of octopus health to fatty acid profile of dietary items could persist into adulthood, especially in regards to lipid limited physiological processes such as egg production (O'Dor *et al.*, 1984).

As discussed earlier, this suggests that *O. rubescens* chooses prey to address very specific and complex nutritional needs rather than maximizing energy intake over time.

Ecological Implications

Generalist octopuses have been suggested to be “switching predators” (Vincent *et al.*, 1998). Optimal foraging theory predicts that once a prey item falls below a threshold density an optimally foraging predator will switch to alternative

prey items (van Baalen *et al.*, 2001; Curio, 1976). Switching predators likely stabilize prey population by predating preferentially on the most abundant species (Murdoch, 1969). *Octopus rubescens*, however, does not seem to forage optimally, but rather in a nutrient specific manner, but nevertheless has a generalist diet. Work has been done to connect optimal foraging theory with nutrient uptake by modeling nutrient uptake in a fitness (as defined by growth rate) landscape and showing that some organisms, especially herbivores, will regulate intake to coincide with local maxima in the fitness landscape (Simpson *et al.*, 2004). Predators have been also shown to regulate nutrient uptake in this way and will choose prey that rectify nutritional deficiencies (Mayntz *et al.*, 2005). Perhaps this mechanism could drive “switching” in octopuses, but if switching is not density-dependent as with an optimally foraging predator, it is unclear what effect this would have on population dynamics of prey species.

There have been mixed results concerning optimal foraging behavior in other species of octopuses. In *Enteroctopus dofleini*, for example, Vincent *et al* (1998) predicted that the crab *Telmessus cheiragonus* would be a preferred prey in an optimal foraging framework because of its large size and short handling as time evidenced by the lack of drill holes in midden remains. However, no such preference was evident from midden analysis. In contrast, Anderson and Mather (2007) found that *E. dofleini* preferred the clam *Protothaca staminea* over the mussel *Mytilus trossulus* when both were presented opened to the octopus. However, if the prey were presented closed *E. dofleini* preferred *M. trossulus*,

which has a thinner shell and required less handling time than *P. staminea*, a clear reflection of an optimal foraging strategy.

Conclusions and directions for further research

In these experiments the prey choices made by *O. rubescens* deviated widely from those expected from a simple model of maximizing caloric intake per unit time. *O. rubescens* chose *H. nudus* over *N. obscurata* as prey by a ratio of 3 to 1, even though when consumable tissue mass and handling time are accounted for the octopus could obtain 10 times more energy per unit time from *N. obscurata* than from *H. nudus*. Octopus energy budgeting is similar when consuming either of the prey species except that lipid extraction efficiency (ratio of assimilated to consumed lipids, the remainder is defecated) was significantly higher in octopuses consuming *H. nudus*. These data gathered in the lab suggests that lipid digestibility may play a considerable role in the prey choice of *O. rubescens*.

Future research should examine the role that ratios of specific amino acids and lipids play in prey choice of *O. rubescens*. Additionally, investigations of the ultimate fate and use of individual lipids, and their importance to the overall health of the octopus could shed considerable light on the ecological physiology of octopuses.

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APPENDIX I – RAW MIDDEN COUNT

Table A1- 1: Count of prey items found in middens of 21 glass bottle *Octopus rubescens* dens along with common names and number of each item that was found with a distinctive drill hole. Barnacles were not counted

Prey Item	Common Name	Midden Count	# Drilled
<i>Nucella lamellosa</i>	Frilled dogwhelk	41	32
<i>Lophopanopeus bellus</i>	Black-clawed crab	26	1
<i>Petrolisthes eriomerus</i>	Porcelain crab	26	12
<i>Chlamys hastata</i>	Pacific spiny scallop	14	1
<i>Saxidomus gigantea</i>	Washington butter clam	12	2
<i>Cancer oregonensis</i>	Pygmy rock crab	7	0
<i>Protothaca staminea</i>	Pacific littleneck clam	4	2
<i>Crepidula dorsata</i>	Slipper shell	3	0
<i>Macoma nasuta</i>	Bent-nosed clam	3	0
<i>Calliostoma ligatum</i>	Blue top snail	2	0
<i>Clinocardium nuttallii</i>	Nuttall's cockle	2	0
<i>Humilaria kennerlyi</i>	Corrugated clam	2	0
<i>Mya arenaria</i>	Eastern softshell clam	2	0
<i>Cancer productus</i>	Red rock crab	1	0
<i>Colus griseus</i>	Gray whelk	1	1
<i>Oregonia gracilis</i>	Graceful decorator crab	1	0
<i>Pododesmus macroschisma</i>	False jingle shell	1	0
<i>Polinices</i> sp.	Moon snail	1	1
<i>Pugettia gracilis</i>	Graceful kelp crab	1	0
<i>Cranopsis cucullata</i>	Hooded puncturella	1	0
<i>Strongylocentrotus droebachiensis</i>	Green urchin	1	0

APPENDIX II – PICTURES OF SELECT PREY PARTS



Figure A2 - 1: Chelae of *Petrolisthes eriomerus* and *Lophopanopeus bellus* showing clearly evidence of octopus predation in the form of characteristic drill holes. The chelae in a line on the left are of *Petrolisthes* and the sole, small chelae on the right with a black tip on the claw is from a *Lophopanopeus*. Ruler on left is in cm.



Figure A2 - 2: shell of *Nucella lamellosa* collected from a midden of *Octopus rubescens*. On the last whorl of the spire is a drill hole.



Figure A2 - 3: *Chlamys hastata* shell found in a midden of *Octopus rubescens*. A drill hole can be seen on the right valve (on the left in this photo).



Figure A2 - 4: Valve of *Nuttallia obscurata* fed to *Octopus rubescens* in captivity. A drill hole can be seen near the umbo. Ruler scale is in cm.



Figure A2 - 5: Right and left valves of *Mercenaria mercenaria*, fed to *Octopus rubescens* in captivity. Four drill holes are present, marked by white arrows, but none penetrate to the interior of the shell. Also present is a large notch presumably chewed on the anterior end of the shells.



Figure A2 - 6: Close-up photo of notch in the left valve of valve shown in the previous figure. Many grooves can be seen in notch, presumably made by the beak of *Octopus rubescens* while gnawing the shell.

APPENDIX III – DORSAL VIEW OF THE DIGESTIVE SYSTEM

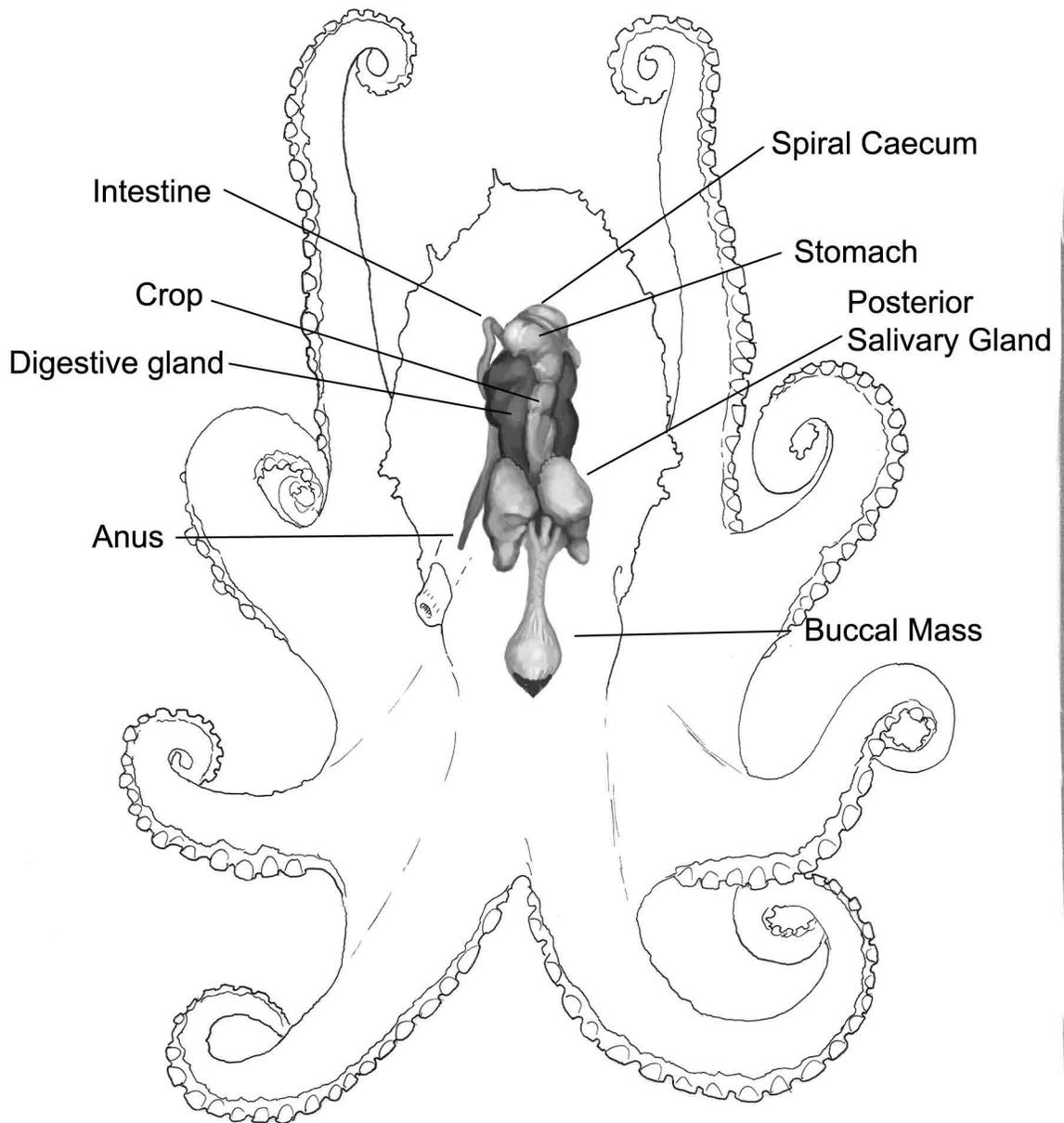


Figure A3 - 1: Dorsal view of *Octopus rubescens* digestive system. Illustration by Nathaniel Johnson.

APPENDIX IV – INDIVIDUAL ENERGY BUDGETS

Table A4 - 1: Complete table of energy budgets constructed for *Octopus rubescens* fed two diets (components defined in Table 4, component values expressed as $\text{j}\cdot\text{g}^{-1}\text{d}^{-1}$), one of crab (*Hemigrapsus nudus*) and one of clam (*Nuttallia obscurata*), along with assimilation efficiencies (AE, defined in Table 5) and atomic oxygen to nitrogen ratios (O:N).

Octopus #	Diet	C	R	G	E_U	E_F	X	AE _T %	AE _P %	AE _L %	O:N
11	Clam	73.60	32.84	-3.90	0.00	1.88	42.78	97.45%	98.49%	88.35%	3.29
16	Clam	26.12	27.52	97.59	0.01	1.70	-100.70	93.48%	96.13%	70.24%	1.01
17	Clam	76.12	26.50	38.31	0.02	3.55	7.75	95.34%	97.24%	78.73%	5.46
19	Clam	136.75	73.83	71.10	0.07	4.81	-13.06	96.48%	97.91%	83.94%	2.72
20	Clam	64.74	17.43	2.04	0.00	1.88	43.39	97.10%	98.28%	86.77%	15.93
21	Clam	59.07	23.77	4.59	0.01	4.09	26.62	93.08%	95.90%	68.42%	10.13
22	Clam	59.16	25.96	24.48	0.01	3.94	4.78	93.34%	96.05%	69.59%	1.35
23	Clam	15.56	16.74	15.38	0.00	1.67	-18.23	89.26%	93.63%	50.96%	5.71
4	Crab	20.37	35.36	-11.25	0.00	0.45	-4.18	97.81%	97.56%	97.30%	6.84
16	Crab	25.53	22.13	-13.67	0.02	0.62	16.42	97.56%	97.28%	97.00%	4.08
17	Crab	41.99	20.02	3.21	0.02	0.86	17.88	97.95%	97.72%	97.48%	3.05
20	Crab	25.00	20.79	37.18	0.01	1.31	-34.30	94.76%	94.17%	93.56%	4.15
21	Crab	21.63	24.99	-15.58	0.00	0.69	11.53	96.79%	96.43%	96.06%	60.41
22	Crab	15.99	16.98	-47.81	0.01	0.69	46.13	95.69%	95.21%	94.71%	11.98
23	Crab	3.09	21.90	2.21	0.01	0.36	-21.39	88.38%	87.06%	85.72%	7.75
24	Crab	52.95	45.06	95.18	0.01	3.03	-90.33	94.28%	93.63%	92.97%	9.33
25	Crab	90.71	39.81	35.94	0.01	0.60	14.36	99.34%	99.27%	99.19%	7.62