ELEMENTAL ANALYSES OF OIKOPLEURIDS AND FACTORS AFFECTING HOUSE PRODUCTION RATE OF *Oikopleura vanhöeffeni* (TUNICATA, APPENDICULARIA) IN COASTAL NEWFOUNDLAND WATERS

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BY

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Abstract

Oikopleura vanhoëffeni and O. labradoriensis are cold-water pelagic tunicates that are abundant in coastal Newfoundland waters. The oikopleurid feeding apparatus or 'house' is a secreted structure which can be discarded and replaced in response to animal disturbance or house clogging. This study examined the energy costs associated with house production and factors that effect the frequency of house abandonment.

The elemental content of O. vanhoëffeni and O. labradoriensis increased at about the third power of trunk and tail length. The mean C:N ratio of O. vanhoëffeni in this study was 3.51 (n=203) and for O. labradoriensis, 3.57 (n=12). These values are similar to others reported in the literature.

Elemental analyses of 11 newly-secreted O. vanhoëffeni houses showed that 'new' houses had a mean carbon content of 8.38 µg C (7.5% of body carbon) and a mean nitrogen content of 2.60 µg N (20.1% of body nitrogen). No relationship was found between carbon measures of 'new' houses and of the animals that produced them; similar-sized animals showed a five-fold variation in house carbon content. Upwards of 88 µg C of natural particulate material were trapped within a single house.

The mean house production rate (HPR) for O. vanhoëffeni was 1.7 houses day^{-1} (n=104), and for the somewhat smaller O. labradoriensis, the average was 2.3 houses day^{-1} (n=8). HPR between species was not significantly different. Food availability was the most important variable affecting HPR. No relationship could be determined for temperature vs HPR, and POC vs HPR.

The O. vanhoëffeni house elemental content and HPR from this study indicated that a mean daily carbon investment of 14.3 µg C (13% of body carbon day^{-1}) was required for the house structure. Additional energy investment would be required for house secretion, expansion and other metabolic processes.

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Chapter 1
General Introduction

1.1 Biology of the Tunicates

Tunicates, sub-phylum Urochordata, possess three morphological characteristics at some stage in their life cycles which are common to the phylum Chordata. These characteristics are gill slits, a nerve cord and a primitive spinal column or notochord. The three classes which comprise the Urochordata are Ascidiacea, Thaliacea and Larvacea (Fig. 1.1).

Ascidiacea include the sea squirts, which are planktonic as larvae and sessile as adults. Thaliacea, which means 'complete muscle rings', is comprised of planktonic tunicates with hoop-like muscle bands around the body. Three orders and about 100 species belong to this class. Individuals of the order Doliolida are barrel-shaped and rarely longer than 25 mm (Fraser 1982). Short pulses of the body muscles provide locomotion, while cilia move water through the mucous net (Alldredge and Madin 1982). Salps (order Salpida) have a cylindrical appearance and jelly-like consistency with lengths up to 250 mm (Fraser 1982). With 20 or more muscle bands in some species (Fraser 1982), muscular peristalsis allows for large volumes of water to be filtered which may account for the fact that salps are the largest thaliaceans (Alldredge and Madin 1982). Colonies of the order Pyrosomida resemble a test-tube with the oral aperture of each individual on the outside of the colony and the anal aperture on the inside (Fraser 1982). With this arrangement, water is pushed out one end of the colony by cilia, giving a slow but continuous motion through the water column (Alldredge and Madin 1982, Fraser 1982).

Thirteen genera and approximately seventy species belong to the class Larvacea or Appendicularia (Alldredge 1976a). This class consists of planktonic individuals, with a trunk and tail, that are associated with an external, renewable, mucous feeding

structure, called the 'house' (Lohmann 1909). The trunk contains all of the organ systems. Highly developed oikoplast cells on the surface of the trunk secrete the mucopolysaccharides that constitute the house (Alldredge 1976a, 1976c). The tail, which is attached to the ventral surface of the trunk, contains a notochord and the muscles used to pump water and food particles through the house structure (Alldredge 1976a).

1.1.1 Features of the Larvaceans

The order Copelata has three families: Kowalevskiidae, Fritillaridae and Oikopleuridae (Fig. 1.2), which have been described by Alldredge (1976a). Though not much has been reported on the family Kowalevskiidae, with its one genus, Kowalevskaia, members of this family have a short trunk with a long, leaf-like tail. The body is surrounded by a house similar to an umbrella but lacks the intricate filters of the Oikopleuridae. Members of the family Fritillaridae have a slender, flat trunk with a broad, short tail. A small house is situated in front of the mouth and resembles a gelatinous bead.

Individuals in the family Oikopleuridae have a spheroid trunk and an elongate tail, entirely enclosed within a mucous house. The anterior section of the trunk is covered by the oikoplast epithelium which secretes the house rudiment. Water is driven through elaborate filters of the house by rhythmic undulations of the tail. House lengths of 6-37 mm have been reported for various oikopleurid species (Alldredge 1976c). Oikopleurid houses are the most complex and best studied of those of the three families of appendicularians.

Oikopleurids are hermaphrodites, except for Oikopleura dioica (Alldredge 1976a). Sperm are released followed by the release of eggs (Alldredge 1976a). The trunk splits open to free the eggs, resulting in the animal's death (Alldredge 1976a). A few species are protandrous, having the ability to produce sperm and eggs from the same gonad at different times (Alldredge 1976a).

1.1.1.1 Oikopleurid Houses: Secretion and Expansion

The oikoplast epithelium, located on the anterior surface of the trunk, secretes the house rudiment (Alldredge 1976a). The rudiment is kept folded against the trunk until a house is discarded (Alldredge 1976c). Once the oikopleurid has begun to feed in a new house, the secretion of another house rudiment begins (Alldredge 1976c).

House expansion behaviour has been described by Fenaux and Hirel (1972) and further documented by Alldredge (1976c). Four phases (IA, IB, II and III) have been detailed for five oikopleurid species (Fig. 1.3). Phase IA is initiated when the animal leaves the house. The animal swims rapidly for 1 to 10 seconds and then drifts motionless in a vertical position. This is followed by quick, jerking motions every few seconds in order for the animal to maintain its position in the water column. Oikopleurids that have not completed the secretion of the house rudiment before the initiation of phase IA will do so during this phase. These individuals will float and swim periodically until the house rudiment is fully secreted. Secretion of a house rudiment may take only 126 seconds, as with Oikopleura cornutogastra (Alldredge 1976c), or as long as four hours in other species (Alldredge 1976a).

Phase IB involves the expansion of the house rudiment. Through a series of three different tail behaviour patterns known as somersaulting, cartwheeling, and nodding, the house rudiment is lifted off the trunk and slightly enlarged. This phase may last for 6 to 66 seconds depending on the species involved.

Once the house rudiment has undergone some initial expansion in phase IB, the tail of the oikopleurid enters the house rudiment through an aperture near the base of the tail. This motion occurs in less than one second (Fenaux and Hirel 1972, Galt 1972) and is referred to as phase II.

The remainder of house rudiment expansion occurs in phase III. Initially, only the base of the tail is able to move inside the expanding house rudiment. However, with time, the sinusoidal wave motion of the tail is able to travel its entire length as the house

is inflated. This phase may last as long as 145 seconds in the field. Q. dioica have been reported to complete phase III in 10 to 30 seconds (Galt 1972) and 24 seconds (Fenaux and Hirel 1972). The initiation of normal filtering behaviour signals the end of phase III.

1.1.1.2 Oikopleurid Houses: Structure and Function

There have been numerous studies on the morphology and function of oikopleurid houses (Lohmann 1909, Körner 1952, Jorgensen 1966, Alldredge 1975, 1976a, 1976b, 1976c, 1977, 1981; Paffenhöfer 1975, Fenaux 1977, 1986; Flood 1978, 1981, 1991a, 1991b; Alldredge and Madin 1982, Deibel and Turner 1985, Deibel *et al.* 1985, Deibel 1986, Deibel and Powell 1987a, 1987b; Flood *et al.* 1990, 1992). Although the specific chemical nature of the house is unknown, it is a complex mucopolysaccharide structure (Körner 1952) and contains three types of filters (Fig. 1.4). They include a pair of inlet filters (Alldredge 1976c, Flood et al. 1990), a pair of food-concentrating filters (Deibel 1986), and a pharyngeal filter (Alldredge 1976a).

The inlet filters, also known as incurrent filters, act as sorting devices which exclude particles such as large dinoflagellates, most diatoms, and large detritus (Alldredge 1977). They have a pore size which is species-specific. Deibel and Turner (1985) found that the inlet filters of Oikopleura vanhoëffeni have an average pore size of 169 x 88 µm. To date, this is the largest pore size reported for any oikopleurid species. It allows large, armoured cells and diatom chains to enter the house. Q. intermedia has a similar trunk length to Q. vanhoëffeni but the inlet filters have a pore size of only 38 x 34 µm (Alldredge 1977). Flood (1991b) measured a pore size of 74 x 13 µm in Q. labradoriensis. The smallest inlet filter pore size reported is of Q. fusiformis, at 13 x 13 µm (Alldredge 1977).

The food-concentrating filters, formerly known as feeding filters (Alldredge 1975), are composed of three layers (Deibel 1986). Seawater flows through the recurved wing-shaped filters, concentrating particulate matter as water leaves the sieving apparatus

(Deibel 1986). Particles that remain inside the filters of Q. vanhoëffeni houses are estimated to be concentrated 100 to 1000 fold (Morris and Deibel, in press). Flood (1991b) calculated a similar concentration of particulate matter by Q. labradoriensis. His estimate was about 900 x the particle density in surrounding seawater.

The mean pore sizes of the food-concentrating filters in several oikopleurids have been measured: Q. vanhoëffeni at 1.04 x 0.22 μm (Deibel *et al.* 1985), Q. labradoriensis at 0.69 x 0.18 μm (upper filter) and 1.43 x 0.24 μm (lower filter) (Flood 1991a), Q. albicans at 0.92 x 0.19 μm (Flood 1981), Q. longicauda (Fig. 1.5) at 0.61 x 0.15 μm (Deibel and Powell 1987a) and Q. dioica at 0.98 x 0.15 μm (Flood 1981).

The pharyngeal filter is secreted by the endostyle near the mouth of the animal (Fenaux 1968). Its structure and function has been well documented by Deibel (1986). The concentrated particulate suspension is transported from the food-concentrating filter, through the buccal tube, to the mouth. The upper lip of the mouth can detach from the buccal tube so that the oikopleurid can regulate the amount of the concentrated particulate suspension that it receives from the buccal tube. An animal may do this when the gut is full or when unsuitable food particles are present. The portion of the suspension that enters the mouth, usually 100%, is sieved through the pharyngeal filter as water is removed from the pharynx by a pair of ciliated spiracles. The pharyngeal filter and the particulate matter associated with it are slowly wound into a thread and transported into the stomach.

Deibel and Powell (1987b) reported a mean pharyngeal filter pore size for Q. vanhoëffeni of 3.26 x 6.35 μm. This pore size is considerably larger than that of the food-concentrating filters (Deibel *et al.* 1985). Consequently, very small particles may not be efficiently retained by the pharyngeal filter (Deibel and Powell 1987b). A recent study on the retention efficiency of the pharyngeal filter of Q. vanhoëffeni indicated an increase in pore size with increasing body size and a corresponding decrease in the retention efficiency of submicrometer particles (Deibel and Lee 1992).

Clogged filters or strong physical disturbance of the house can cause an

oikopleurid to evacuate its house (Alldredge 1976c). When most oikopleurids leave a house, they do so by physically forcing their way through the walls of the house, trunk-first (Alldredge 1976a, 1976c). However, there have been indications of an 'escape passage' or 'escape chamber' in the houses of O. albicans (Alldredge 1976a), O. dioica (Fenaux 1986), O. labradoriensis and O. vanhoëffeni (Flood *et al.* 1990). This passageway may serve as an exit portal for animals when filters become clogged or when escaping from predators (Alldredge 1976c, Hamner *et al.* 1975). Outside of the house, oikopleurids are highly susceptible to planktonic predators. Though the house offers protection from most invertebrate predators, it is not a safe haven from predatory fish which may ingest entire houses and their occupants (Alldredge 1975).

Houses also provide buoyancy for oikopleurids. When not within a house, these animals sink rapidly and must expend considerable amounts of energy to maintain position in the water column (Galt, 1972). Their large, mucous house allows them to stay afloat with minimal effort.

1.1.1.3 Oikopleurid House Production Rates

Although the structure of oikopleurid houses has received much attention, only three studies have observed oikopleurid house production rates (Lohmann 1909, Paffenhöfer 1973, Fenaux 1985). They include limited experimental data with only one oikopleurid species (Oikopleura dioica). These studies and field observations of the abundance of discarded houses and concentrations of particulate organic carbon and nitrogen in surrounding seawater (Alldredge 1976b) suggest that temperature and food abundance are factors which influence house production rate. A more detailed discussion on this topic is presented in Chapter 4.

1.2 Importance of Larvaceans in Marine Planktonic Food Webs

Larvaceans are an important metazoan link in marine food webs (Alldredge 1972, 1977; Paffenhöfer 1973). The house is capable of filtering out a broad size range of particles and making this energy available to larger organisms. While copepods filter-feed on particles > 3 μm (Gauld 1966, Poulet 1974), appendicularians are able to retain particles as small as 0.1 μm (Flood 1978, Fenaux 1986). This enables them to feed on nanoplankton (Alldredge 1972), picoplankton (Paffenhöfer 1973), bacteria (Alldredge and Madin 1982) and even dissolved organic matter (Fenaux 1985, Flood *et al.* 1990, Flood *et al.* 1992). This by-pass of several trophic levels permits an efficient energy transfer and is referred to as the 'larvacean shunt' (Azam *et al.* 1983).

Particulate matter concentrates on the filters and adheres to the internal and external surfaces of a functioning house (Alldredge 1976b). Alldredge (1976a) estimated that upwards of 50,000 phytoplankton cells can be trapped on a single oikopleurid house. With up to sixteen houses discarded daily by some appendicularians (Fenaux 1986), this could be an important mechanism for energy transfer in marine ecosystems (Alldredge 1976b).

1.3 Oikopleurids and the Labrador Current

Two oikopleurid species commonly occur in the Labrador Current, a water body of Arctic origin (Bailey and Hachey 1950). Oikopleura vanhoëffeni (Lohmann) is a cold water species and an indicator of the Labrador Current (Thompson and Frost 1935, 1936; Udvardy 1954), preferring the coldest water (< -1°C) and rarely found in waters above +5°C (Deibel 1987). O. labradoriensis (Lohmann) also inhabits this cold ocean current (Thompson and Frost 1935, 1936; Udvardy 1954), but is more commonly found in warmer waters (+4-12°C), where the Labrador Current is mixed with coastal water

(Deibel 1987).

Oikopleurids are seasonally abundant in coastal waters of Newfoundland with recorded peak densities approaching 100 m^{-3} (Mahoney and Buggeln 1983, Knoechel and Steel-Flynn 1989) and up to several hundred m^{-3} during the spring and early summer period (Deibel 1988). Davis (1982, 1986) reports a maximum concentration of 448 oikopleurids m^{-3} during early July in a coastal Newfoundland bay.

Q. vanhoëffeni is one of the largest species of oikopleurids, with an adult trunk length > 6 mm (Deibel *et al.* 1985) and a house length of up to 70 mm (Flood 1991a). The smaller Q. labradoriensis has a mean trunk length of 1.8 mm (Mahoney and Buggeln 1983) and can produce houses with lengths up to 18 mm (Flood 1991a). Little is known of the frequency of house production in these species. However, concentrations of up to 1000 houses m^{-3} have been estimated from SCUBA observations of discarded houses in Newfoundland coastal waters (Mahoney and Buggeln 1983). On occasion, abandoned oikopleurid houses have been found in dense aggregates at discrete depths, measuring up to 20 cm thick, 30-40 m long and several metres wide (Mahoney 1981).

Knoechel and Steel-Flynn (1989) found an average clearance rate of 177 ml per day for Q. vanhoëffeni in coastal waters of Newfoundland. They suggested that the oikopleurids in Conception Bay can clear approximately 1.4% of the water column daily compared to an estimated copepod community clearance rate of 1.1%. Deibel (1988) calculated the removal of upwards of 50% of the ingestible daily phytoplankton production by the Q. vanhoëffeni population in Logy Bay. Although smaller in both size and abundance, Q. labradoriensis is capable of clearing an estimated 840 ml per day (Flood 1991a). These results suggest a substantial grazing impact by oikopleurids in Newfoundland coastal waters, with an importance comparable to that of the copepod community.

1.4 Study Objectives

The house of an oikopleurid is an essential component of the feeding apparatus. However, it is a disposable structure and is frequently discarded and replaced when clogged or when the animal is disturbed. Although formation of a new house requires energy expenditure on a regular basis, little is known of the energy costs associated with the building of these structures and the frequency with which they are produced.

The goal of this study was to estimate the energy invested in the production of houses by Oikopleura vanhoëffeni and the less abundant O. labradoriensis in cold, coastal Newfoundland waters. This estimate requires determinations of the house production rate and the elemental content of oikopleurids and their houses.

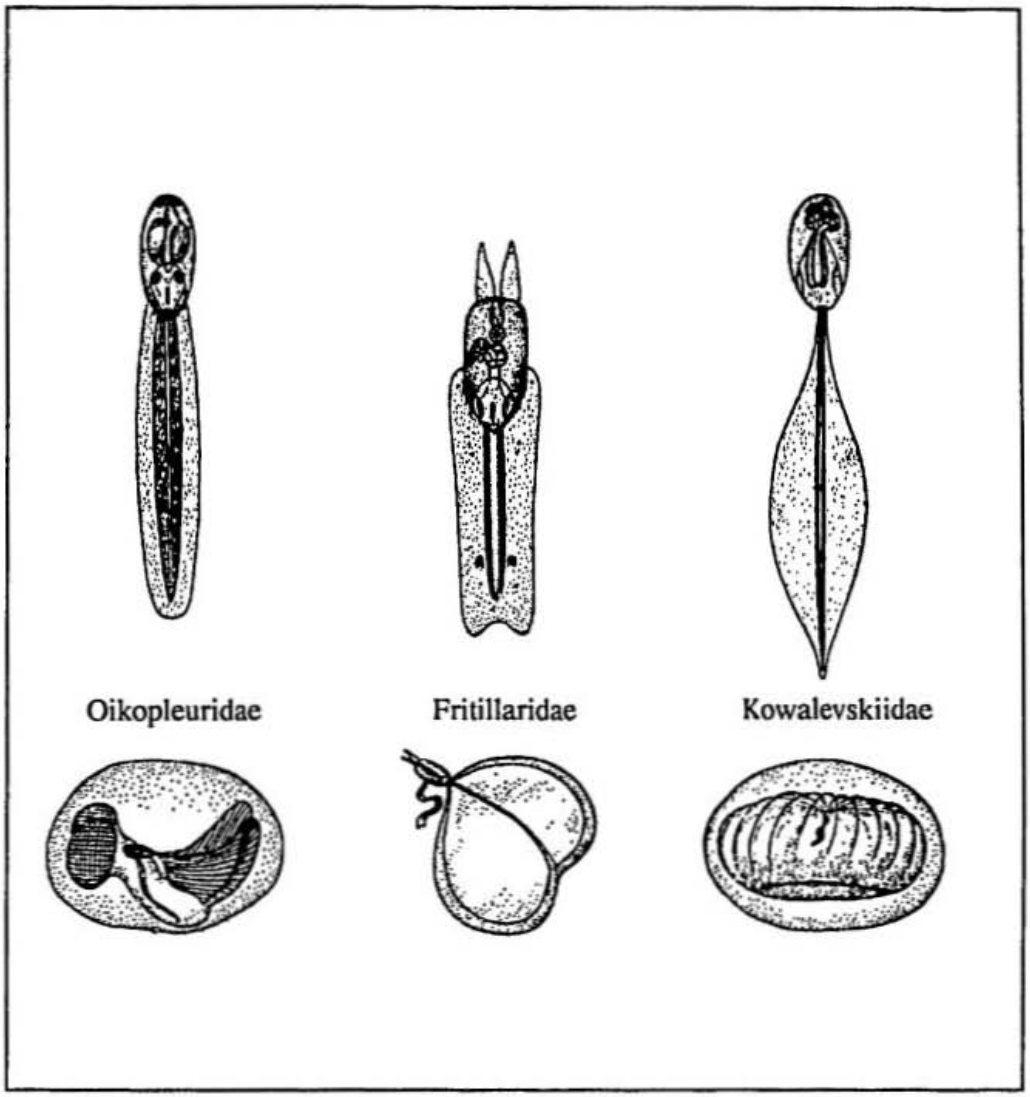
The primary objectives were: 1. to quantify and compare the carbon and nitrogen content in the bodies of O. vanhoëffeni and O. labradoriensis; 2. to quantify the carbon and nitrogen content of newly produced houses; 3. to determine house production rates in relation to oikopleurid body size, temperature, and seston concentration; and 4. to calculate the daily metabolic cost of oikopleurid house production.

Figure 1.1. Classification of the Urochordata. (Parker and Haswell 1962)

Phylum Chordata

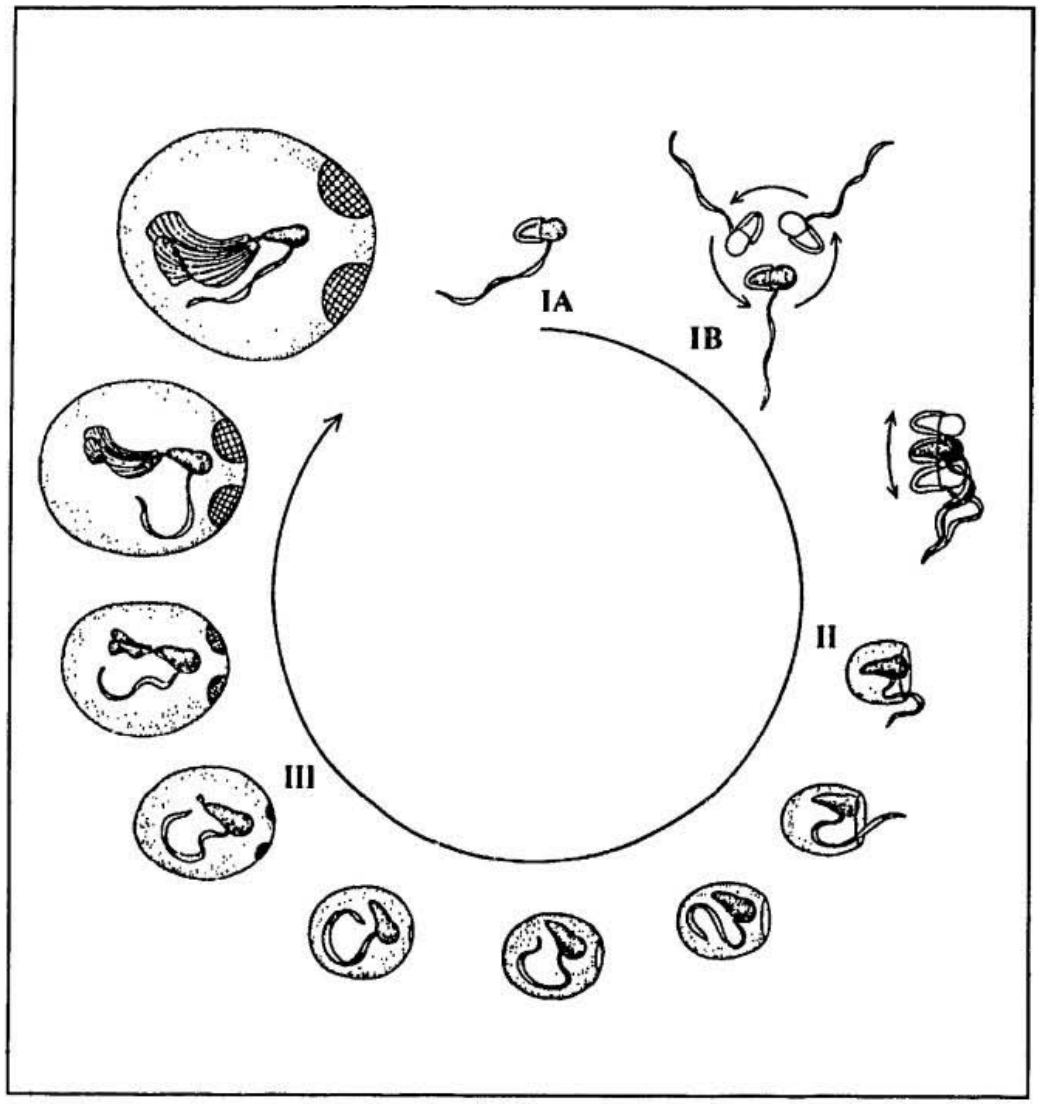
- Sub-Phylum Urochordata
  - Class Ascidiacea
  - Class Thaliacea
    - Order Doliolida
      - Family Doliolidae
    - Order Salpida
      - Family Salpidae
    - Order Pyrosomida
      - Family Pyrosomidae
  - Class Larvacea
    - Order Copelata
      - Family Kowalevskaiidae
      - Family Fritillariidae
      - Family Oikopleuridae
        - Genus Oikopleura

Figure 1.2. The Three Families of the Larvacea.



The animals of the individual families are depicted above their respective houses. Adapted from Alldredge (1976a).

Figure 1.3. Larvacean House Production: Secretion and Expansion.



Schematic depicting the commencement of each of the four phases (IA, IB, II, III) as described in Section 1.1.1.1.

Adapted from Alldredge (1976a).

Figure 1.4. Oikopleura vanhoëffeni House Structure.

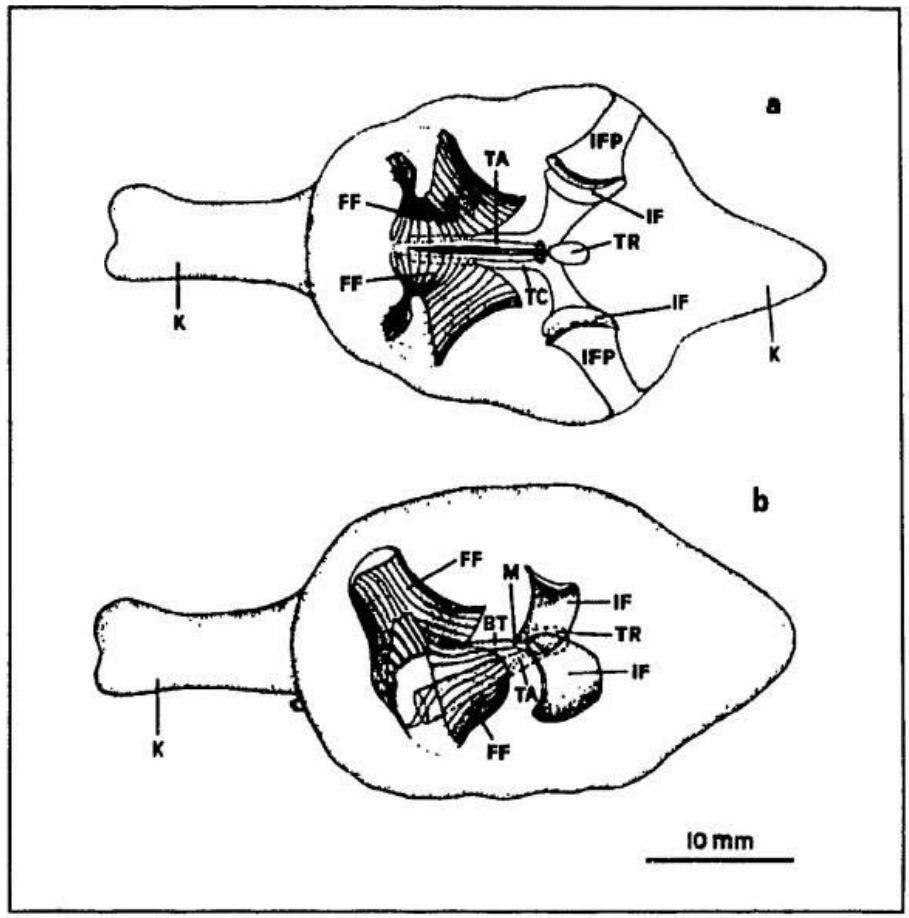
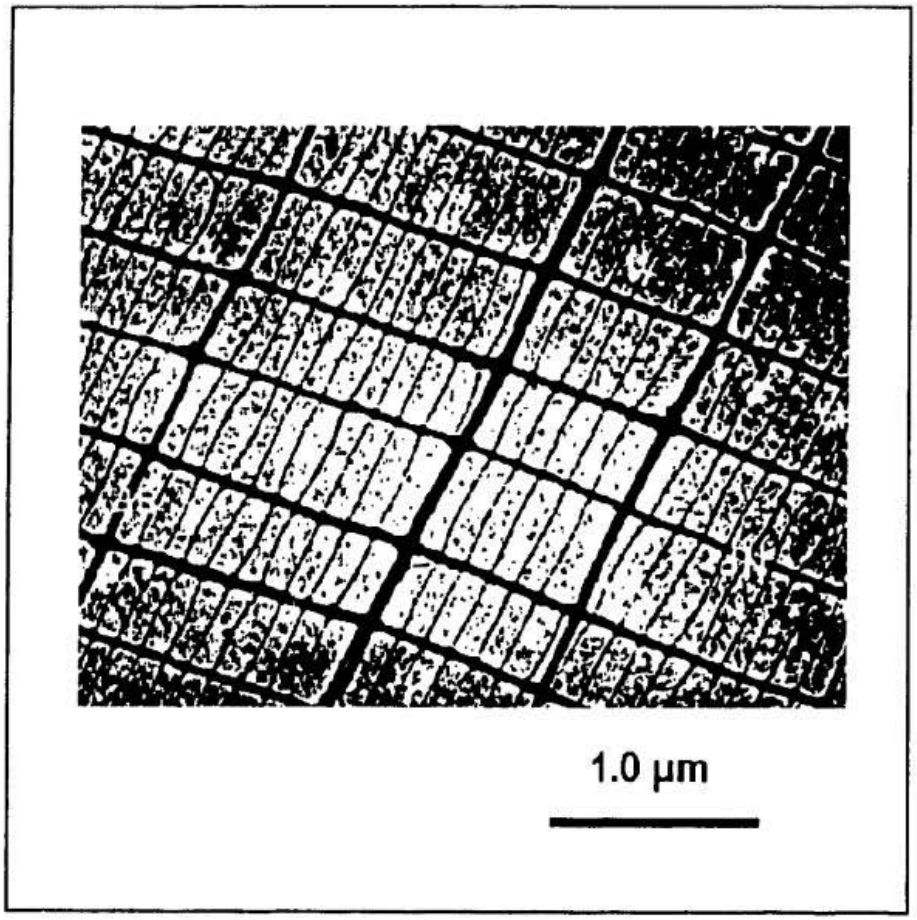


Figure 1.4a. Dorsal view. FF, food-concentrating filter; IF, inlet filter; IFP, inlet filter passageway; K, keel; TA, tail; TC, tail chamber; and TR, trunk.

Figure 1.4b. Lateral view. For simplicity the inlet filter passageways are not shown. Labels are as in Figure 1.4a except: BT, buccal tube; and M, mouth.

Scale bar is an approximation. Adapted from Deibel (1986).

Figure 1.5. Transmission Electron Micrograph of the Food-concentrating Filter of Oikopleura longicauda.



Adapted from Deibel and Powell (1987a).

Chapter 2
General Methodology

2.1 Collection of Animals

Oikopleura vanhoëffeni and O. labradoriensis were collected from cold, coastal Newfoundland waters during January 14 to June 4, 1986 and during January 22 to June 17, 1987. Seven collections were made in 1986 and nineteen in 1987. Each sample consisted of 9 - 25 oikopleurids. The main sampling site was Logy Bay (47°22'N, 52°39'W), with two collections from Bay Bulls (47°18'N, 52°47'W; 4-3-87, 30-3-87), and one each from Tors Cove (47°12'N, 52°49'W; 23-3-87) and Witless Bay (47°16'N, 52°48'W; 25-02-87) (Fig. 2.1).

Oikopleurids were captured individually in 500 and 1000 ml wide-mouth glass jars at a depth of 5 - 20 m by SCUBA divers. Care was taken not to subject the animals to excess vibration, as oikopleurids commonly respond to disturbance by vacating their gelatinous house. Samples were immediately transported to the laboratory. Though attempts were made to maintain animals in the lab through a full generation, these efforts were unsuccessful.

Seawater was also collected in 20 ℓ opaque, plastic containers from the depth of the animals' capture. These seston samples were used to maintain the animals in a natural food environment in the lab for house production observations and experiments.

2.2 Handling of Animals in the Laboratory

Upon arrival at the laboratory, individual jars were placed in a bath of flowing seawater, pumped from Logy Bay. Throughout this study, the ambient temperature of the bath was approximately 0.5 - 1 C° warmer than the seawater from which the animals were captured.

When required for experimental purposes, individual animals were transferred to alternate jars. The following technique allowed the transfer of each animal while still within its house, with minimal disturbance. Under low suction, a 60 cc syringe, with the conical tip removed, was used to remove each animal and house gently. Once inside the syringe, a 60 mm diameter petri dish was held against the syringe opening. The syringe and petri dish were then placed into a new jar previously filled with seston or filtered seawater. The oikopleurid, with house intact, was then slowly expelled from the syringe.

Analyses of the elemental content (carbon, nitrogen) of oikopleurid houses required the separation of a house from the animal. Evacuation of the house was accomplished by gently prodding the house one to three times with a pipet. The vacated house was then removed from the jar with a large bore glass pipet. The house was placed on a watchglass and, using a small glass pipet, seawater was removed from around the collapsed house. Houses analyzed for carbon and nitrogen content were produced in either filtered seawater or a natural seston environment and include both newly inflated houses and houses containing particulate material.

2.3 Analyses Performed

The oikopleurid species were identified following Berrill (1950). All oikopleurids in healthy condition were also examined for trunk and tail length using a Wild M420 macroscope equipped with an ocular micrometer. An oikopleurid was considered healthy when it maintained normal tail beating behaviour. Animals which turned opaque in colour and ceased to move were presumed sick, injured or dead and were subsequently discarded.

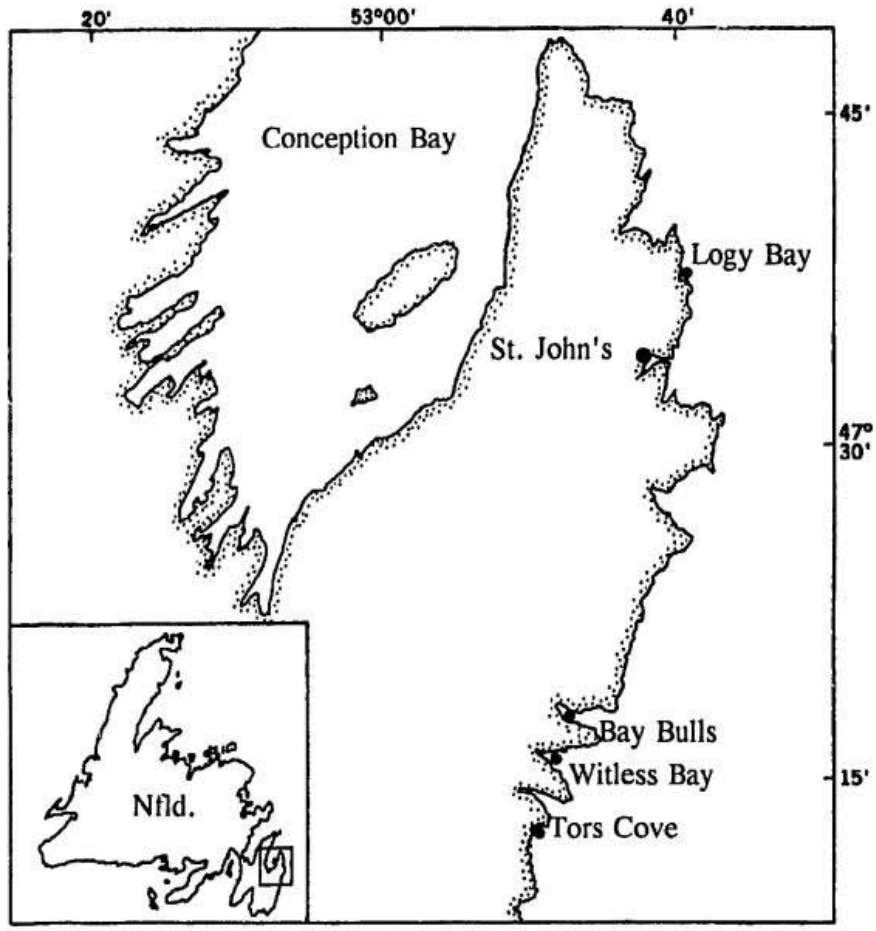
Tail length, the distance between the tail's attachment to the trunk and the tail's tip, was measured to the nearest 0.05 mm. Trunk length may be distorted in mature oikopleurids by enlarged gonads. Therefore, the distance from the mouth to the posterior margin of the stomach was used as the trunk length measure. It was determined to the

nearest 0.01 mm.

Elemental analyses (carbon, nitrogen) were conducted on animals, houses, and samples of naturally-occurring seston. Analyses were performed using a Perkin-Elmer 240-A elemental analyzer with acetanilide as the standard. This method had an empirically-determined accuracy and precision of about 2.4% (Deibel 1986).

Analyses of seston samples also included examination of chloropigment content. Chloropigment concentrations were measured using the standard fluorometric method of Yentsch and Menzel (1963), as modified by Holm-Hanson *et al.* (1965). All samples were extracted overnight in 90% acetone in the dark at 4°C. The extracts were then centrifuged for two minutes and the supernatant read in a Turner Designs Model 10 fluorometer.

Figure 2.1. Map of Newfoundland and Locations of Sampling Sites.



Chapter 3

Carbon - Nitrogen Analyses of Animals and Houses with Relationship to Animal Size

3.1 Introduction

The only published data on the carbon and nitrogen content of oikopleurids are in the work of Alldredge (1976b, 1976c), King *et al.* (1980), Deibel (1986) and Gorsky *et al.* (1988). An analysis of the elemental content of 31 Oikopleura vanhoëffeni, collected from coastal Newfoundland waters, was conducted by Deibel (1986). The smallest animal measured had a trunk length of 0.75 mm and contained 2 µg of carbon and a negligible amount of nitrogen. The largest animal had a trunk length of 5.25 mm and contained 1200 µg of carbon and 350 µg of nitrogen. Log-log transformation of the morphometric and elemental data showed strong relationships between carbon content and trunk length (r^2=0.90) and between nitrogen content and trunk length (r^2=0.74).

The carbon and nitrogen investment into oikopleurid house production has been scarcely documented. Elemental analysis of 'clean' houses has been reported for only two Oikopleura species (Alldredge 1976b, Deibel 1986). Alldredge (1976b) defines a 'clean' house as one which has not been contaminated with external sources of carbon and nitrogen, or been grazed upon, or had any of the elements leached out of it. 'Clean' houses therefore represent the actual amount of carbon and nitrogen invested in house production by the animal. Alldredge (1976b) determined the carbon and nitrogen content of 12 newly-secreted houses of O. rufescens. These houses were produced in a natural seston environment and contained an average (±SD) of 10.0 (± 5.0) µg carbon and 0.04 (± 0.0006) µg nitrogen. In comparison, Deibel (1986) found that 'clean', particle-free houses of nine O. vanhoëffeni contained a mean (±SD) of 20.7 (± 12.2) µg of carbon, representing 23% (± 13) of body carbon.

3.2 Methodology

More than 200 live oikopleurids were examined for measures of tail and trunk length, as described in Chapter 2. During measurement, the trunks of a few animals split open followed by a release of eggs. Consequently, trunk length measures were not determined for these individuals.

Each measured animal and >60 abandoned houses were analyzed for carbon and nitrogen content. All but one of the houses were produced in a natural seston environment and therefore subject to some contamination by components of the seston. Houses were thus visually examined and the level of house contamination qualitatively assessed on a five-point scale (Table 3.1). Individual animals and houses were placed directly, without vacuum, onto Whatman GF/C 25 mm glass fibre filters previously combusted at 450°C for 6 hours in a muffle furnace. The filters were frozen immediately and stored at -30°C. Prior to analysis, the filters were placed in a drying oven for 48 hours at 60°C. Carbon and nitrogen measures were then determined using a Perkin-Elmer 240-A elemental analyzer.

Using the SAS statistical package (version 6.04), the tail and trunk lengths and the carbon and nitrogen data were log-log transformed to obtain power function equation parameters ($Y=aX^b$) for relationships between body size and elemental content. Examination of the residuals showed that log-log transformation of the data was appropriate to stabilize the variance. The values of 'a' and 'b' were estimated using the Model I least-squares linear regression.

3.3 Results

Morphometric and elemental analyses were conducted on a broad size range of oikopleurids. They included 228 Oikopleura vanhoëffeni with a range in trunk length of 0.9 to 6.5 mm and a range in tail length of 4.8 to 32.8 mm. The smaller and less

abundant O. labradoriensis (n=12) had a trunk length range of 1.2 to 4.2 mm and a tail length range of 5.8 to 23.6 mm. The relationships between trunk and tail length for each species are shown in Fig. 3.1. The trunk length of O. labradoriensis is weakly correlated with tail length (r^2=0.31) compared to O. vanhoëffeni (r^2=0.76), presumably as a result of a relatively small sample size.

Carbon vs nitrogen content in both O. vanhoëffeni and O. labradoriensis are plotted in Fig. 3.2. Not included in these plots were four O. vanhoëffeni nitrogen measurements which were unnaturally high relative to carbon (C:N ratios < 1). These apparent inaccuracies are probably due to atmospheric contamination during sample processing. Carbon and nitrogen content in both species were strongly correlated within the animal size ranges measured (r^2=0.99). The mean animal C:N ratios (±SD) of the two oikopleurid species were not significantly different: 3.51 (±0.75) for O. vanhoëffeni (n=203) and 3.57 (±1.14) for O. labradoriensis (n=12).

With the exception of the regression analysis of nitrogen content vs O. vanhoëffeni tail length, all elemental data fit the known cubic function vs trunk and tail length (i.e. the 95% confidence interval of the regression coefficient, 'b', included 3.0 (Tables 3.2 & 3.3, Figs. 3.3 & 3.4)). The regression coefficients in the relationships between animal size (trunk length, tail length) and elemental content (carbon, nitrogen) of O. vanhoëffeni are comparable to those reported by Deibel (1986). There were slight differences in the length-weight regression coefficients for O. labradoriensis and for O. vanhoëffeni. These differences may be due to morphometric characteristics specific to each species or to marked differences in the sample sizes of these species.

Elemental analyses were conducted on 67 houses produced by 45 O. vanhoëffeni animals with a trunk length range of 1.9 to 6.2 mm and a tail length range of 7.1 to 27.2 mm. A summary of the elemental content of the houses, the animals that produced them, and the seston in which they were produced is detailed in Table 3.4.

Out of a total of 24 O. vanhoëffeni animals transferred to jars with GF/C filtered seawater, only one 'clean' house (scale 0) was produced. This single particle-free house

contained 11.7 μg carbon and represented 6.1% of the animal body carbon. An accurate nitrogen measure could not be determined for this house. All other houses were produced in a natural seston environment. However, eight nitrogen measures were excluded from statistical analysis because of apparent nitrogen contamination (C:N ratio <1). Houses that were assessed as 0 (clean), 1 and 2 on the scale of particle loading (Table 3.1) were similar in mean carbon content per house (Table 3.4, Fig. 3.5). Houses scaled as 4 (heavy seston loading) had a significantly higher mean carbon content than all other groups ($p < 0.01$, Wilcoxon Rank Sums test).

The mean (±SD) carbon content of the newly produced houses (scale 1, n=11) was 8.4 (± 3.6) μg and was similar to the carbon content of the 'clean' house (11.7 μg). These 'new' houses represented 7.5% (±3.4%) of animal body carbon and 20.1% (±37.4%) of animal body nitrogen. Because nitrogen measures were highly variable relative to carbon determinations, measures of carbon are preferable to nitrogen in estimates of energy invested in house production.

Interestingly, the mean carbon to nitrogen ratio of newly produced houses was the same as the mean C:N ratio of the oikopleurid animals which produced them. Houses which were seston-contaminated (scales 2, 3 and 4) showed higher C:N ratios (Table 3.4). They were similar to the mean C:N ratio of the filtered seston samples and demonstrate the effect of even low particle loading on C:N ratios of oikopleurid houses.

In addition to the carbon and nitrogen analyses of inflated houses, one Q. vanhoëffeni house rudiment (house development: phase IB, Fig. 1.3) was also analyzed. It was produced by the smallest animal measured: trunk length of 1.2 mm and tail length of 4.8 mm. The rudiment contained 7.72 μg of carbon and 1.74 μg nitrogen. Though the animal was very small, the measures of carbon and nitrogen in the rudiment were within the range shown for 'new' houses produced by larger animals (Scale 1, Table 3.4).

There was no apparent relationship between the amount of carbon contained in houses with little or no seston loading and in the animals which produced them (Fig. 3.6). Relatively particle-free houses (scale 0 and 1, C:N ratio of 3.29), of similarly sized

animals, varied in carbon content by 5 fold; the carbon content of slightly seston-contaminated houses (scale 2, C:N ratio = 5.40) varied by 10 fold.

The contribution of particulate material on houses with heavy particle loading (scale 4) was as high as 80 μg C per house. This estimate is based on a particle-free house carbon content of 8-10 μg. Many of these houses were abandoned without disturbance and may represent typical particle loads of discarded houses in nature.

Table 3.1. Qualitative Assessment of Seston Contamination of Oikopleurid Houses.

Scale	*Status/Visual Appearance of the House*	*Source(s) of Possible Contamination*
0	House produced in filtered seawater	Dissolved organic matter (DOM)
1	House produced in natural seston, not yet functional	Possibility of seston on external surfaces; DOM
2	House produced in natural seston, recently functional	Possibility of seston on external and internal house surfaces; DOM
3	House with some visible particles adhering to the house surfaces	Definite house contamination by seston
4	House with many visible particles adhering to the house surfaces	Heavy house contamination by seston

Table 3.2. Regression Statistics for $\log_{10}$ Animal Carbon (C) Content (µg) vs $\log_{10}$ Trunk (TR) and $\log_{10}$ Tail (TA) Length (mm). Data were fit to the power function, $Y = aX^b$. All constants were significantly different from zero at $p < 0.001$, except where indicated ($^{\dagger} = p < 0.05$) ($^{\dagger\dagger} = p$ not reported). Values shown are: [standard error].

Model	Species	n	Regression coefficient (b)	Intercept (a)	Coefficient of determination (r^2)	Source
log C vs log TR	O. labradoriensis	18*	2.86 [0.45]	7.43 [1.47]	0.71	Present Study
	O. vanhoëffeni	204*	3.10 [0.10]	5.86 [1.13]	0.83	Present Study
	O. vanhoëffeni	25	3.20 [0.22]	4.59 [1.26]	0.90	Deibel (1986)
	O. dioica	~37	2.63††	-7.14††	0.99	King *et al.* (1980)
log C vs log TA	O. labradoriensis	20*	2.59 [0.36]	0.14† [2.45]	0.74	Present Study
	O. vanhoëffeni	212*	3.02 [0.07]	0.05 [1.21]	0.91	Present Study
	O. vanhoëffeni	31	3.29 [0.19]	0.04 [1.62]	0.91	Deibel (1986)

* Data are plotted in Figure 3.3.

Table 3.3. Regression Statistics for $\log_{10}$ Animal Nitrogen (N) Content (μg) vs $\log_{10}$ Trunk (TR) and $\log_{10}$ Tail (TA) Length (mm). Data were fit to the power function, $Y = aX^b$. All constants were significantly different from zero at $p < 0.001$, except where indicated ($^{\dagger} = p < 0.05$). Values shown are: [standard error].

Model	Species	n	Regression coefficient (b)	Intercept (a)	Coefficient of determination (r^2)	Source
log N vs log TR	O. labradoriensis	16*	2.83 [0.48]	2.53† [1.53]	0.71	Present Study
	O. vanhoëffeni	200*	2.85 [0.12]	2.37 [1.16]	0.74	Present Study
	O. vanhoëffeni	19	2.64 [0.38]	2.52 [1.45]	0.74	Deibel (1986)
log N vs log TA	O. labradoriensis	18*	2.40 [0.47]	0.07† [3.31]	0.62	Present Study
	O. vanhoëffeni	208*	2.77 [0.09]	0.03 [1.31]	0.81	Present Study
	O. vanhoëffeni	25	2.92 [0.25]	0.03 [1.89]	0.86	Deibel (1986)

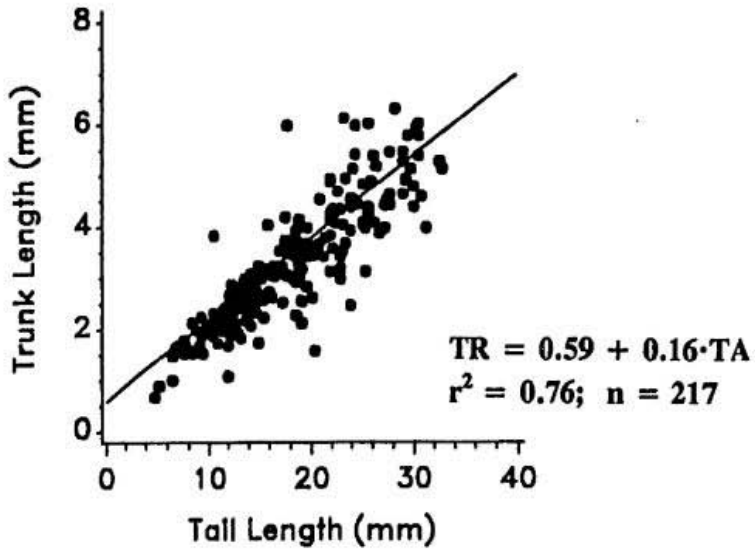
* Data are plotted in Figure 3.4.

Table 3.4. Oikopleura vanhoëffeni House and Animal Elemental Content. House scale was assessed as in Table 3.1. (** = below detection limit)

Sample	Scale	Carbon Range (µg) [n]	Carbon Mean (±SD) (µg)	Nitrogen Range (µg) [n]	Nitrogen Mean (±SD) (µg)	C:N Mean (±SD) [n]
House	0	-- [1]	11.66	**	**	--
	1	3.35 - 14.05 [11]	8.38 (±3.63)	1.04 - 7.04 [8]	2.60 (±1.90)	3.29 (±1.12) [8]
	2	2.21 - 28.05 [28]	12.17 (±6.99)	0.72 - 7.04 [18]	2.86 (±1.47)	5.40 (±2.05) [18]
	3	5.67 - 67.74 [18]	19.51 (±13.16)	1.04 - 8.90 [13]	3.99 (±2.20)	5.62 (±2.06) [13]
	4	10.77 - 88.79 [9]	59.30 (±26.69)	2.14 - 65.12 [9]	20.72 (±24.22)	5.58 (±3.45) [8]
Seston (µg l^{-1})	--	58.4 - 376.3 [9]	146.3 (±107.2)	5.0 - 108.9 [8]	33.0 (±35.2)	6.62 (±2.88) [8]
Animal	--	32 - 1184 [45]	194 (±199)	6 - 350 [43]	59 (±61)	3.47 (±0.92) [42]

Figure 3.1. Animal Trunk (TR) Length (mm) vs Animal Tail (TA) Length (mm) for two Oikopleurid species. Data were fit to a regression line.

<u>O. vanhoëffeni</u>



<u>O. labradoriensis</u>

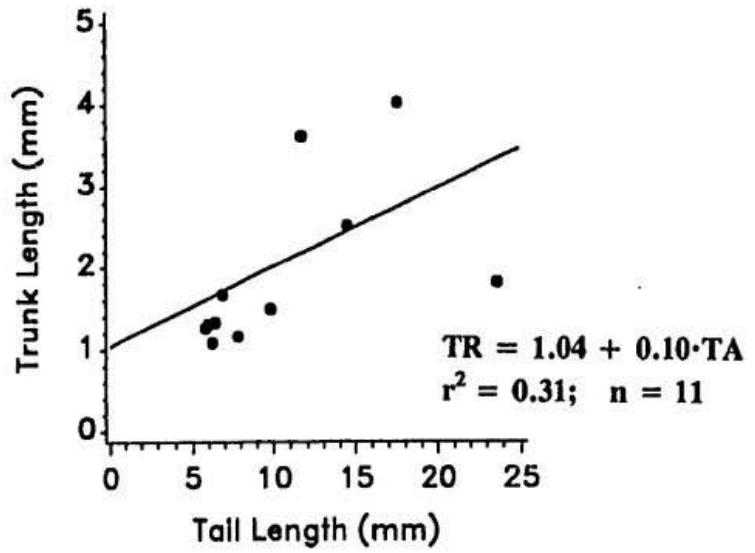
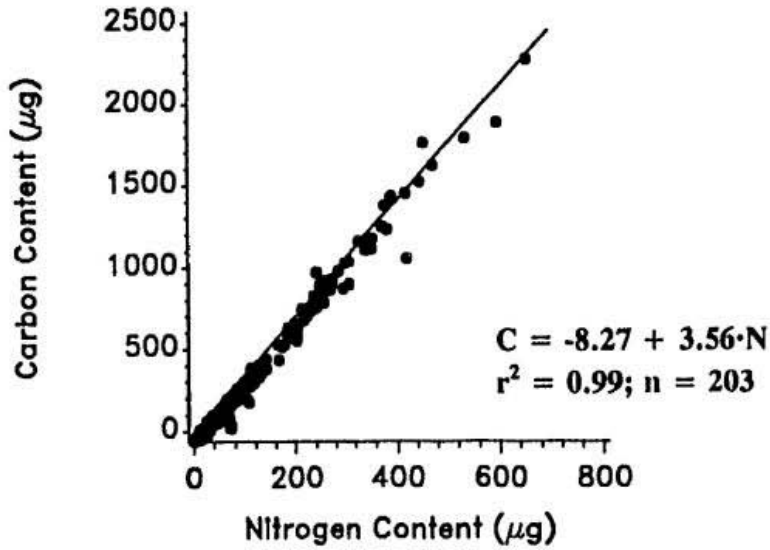


Figure 3.2. Animal Carbon (C) Content (µg) vs Animal Nitrogen (N) Content (µg) for two Oikopleurid species. Data were fit to a regression line.

<u>O. vanhoëffeni</u>



<u>O. labradoriensis</u>

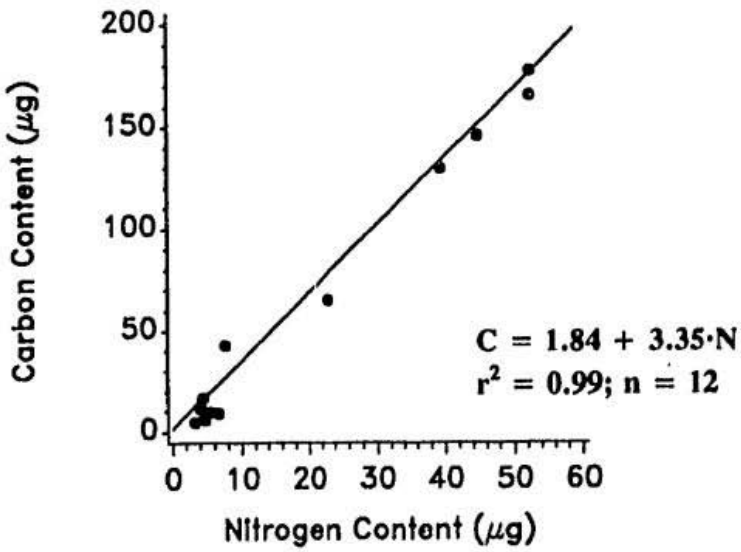


Figure 3.3. Regression Plot of $\log_{10}$ Animal Carbon Content (µg) vs $\log_{10}$ Trunk and $\log_{10}$ Tail Length (mm) for two Oikopleurid species. Data were fit to a regression line (see Table 3.2 for equations).

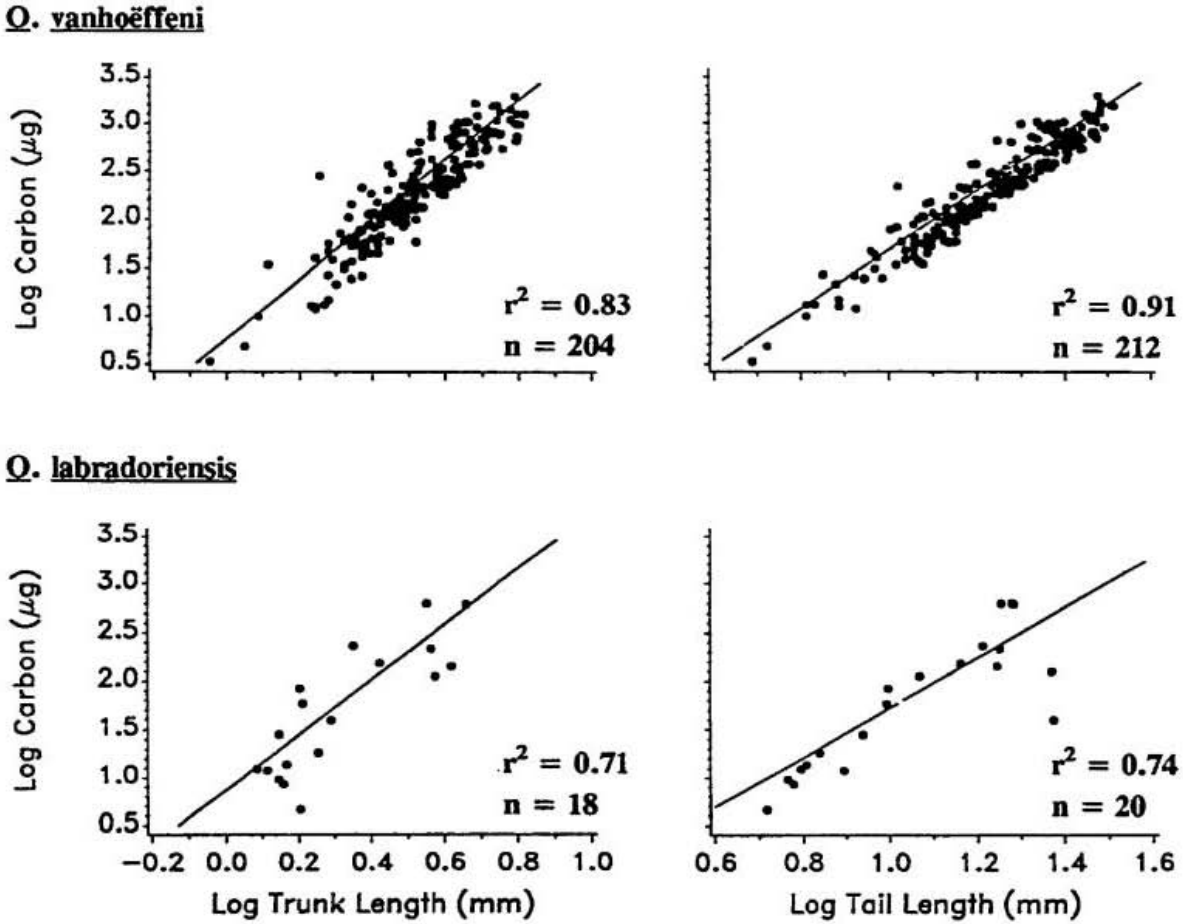


Figure 3.4. Regression Plot of $\log_{10}$ Animal Nitrogen Content (µg) vs $\log_{10}$ Trunk and $\log_{10}$ Tail Length (mm) for two Oikopleurid species. Data were fit to a regression line (see Table 3.3 for equations).

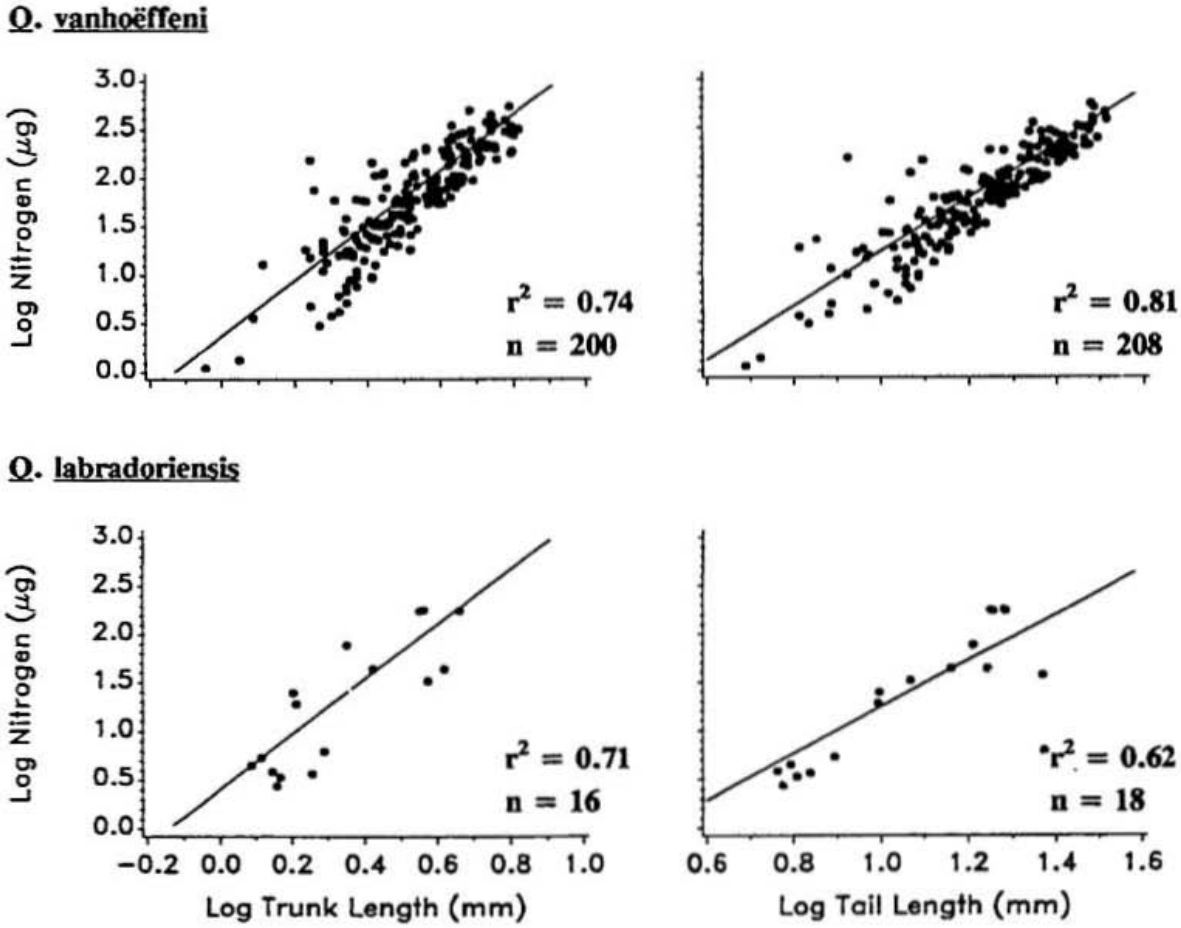


Figure 3.5. The Effect of Relative Particle Load on House Carbon Content. The levels of relative house particle load are detailed in Table 3.1. The number of observations (n) are indicated above the standard deviation bars.

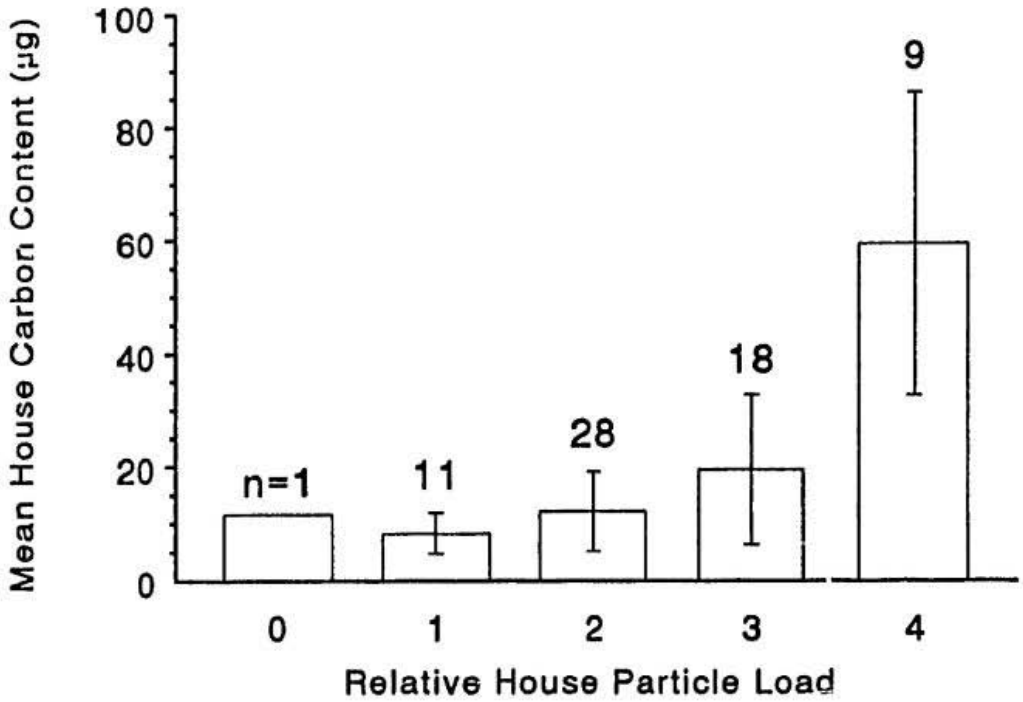
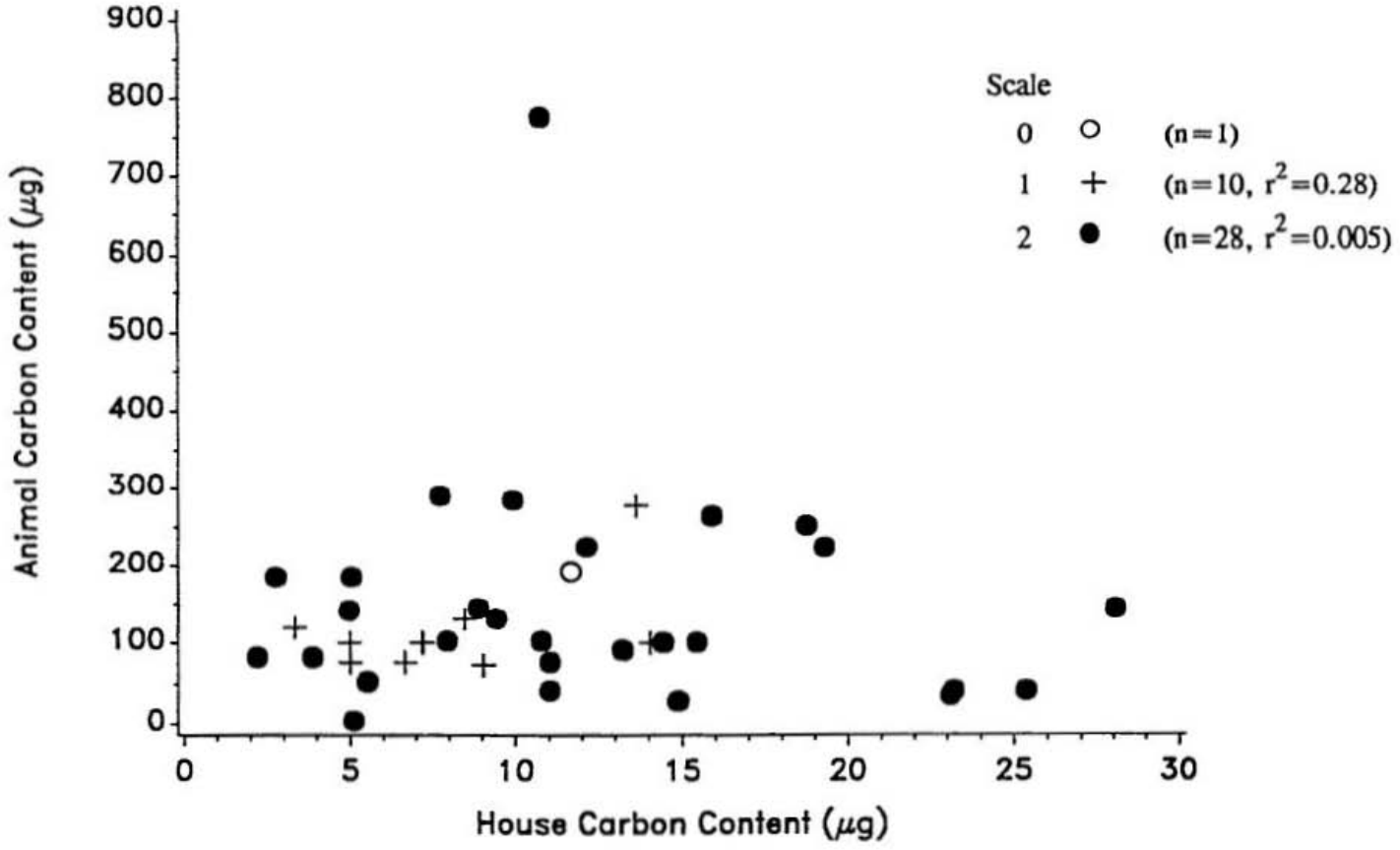


Figure 3.6. Animal Carbon Content (μg) vs House Carbon Content (μg). Houses relatively free of particles. Scale assessed as in Table 3.1.



3.4 Discussion

The carbon and nitrogen content of both oikopleurid species increased as an approximate cubic function of trunk length and tail length. These results are similar to those of Deibel (1986) and King *et al.* (1980) (Tables 3.2, 3.3). In contrast, the weight of salps increased as the second power of the body length (Madin *et al.* 1981, Deibel 1982). Deibel (1986) suggests that these taxonomic differences may be associated with animal body features (i.e. the salp's hollow body compared to the solid trunk and tail of oikopleurids).

The mean C:N ratio of Oikopleura vanhoëffeni in my study was 3.51 ($\pm$0.75, n=203), and for O. labradoriensis, 3.57 ($\pm$1.14, n=12). C:N ratios for both species fall within the range reported by Deibel (1986) for O. vanhoëffeni (C:N = 3.73 $\pm$1.01, n=25). Gorsky *et al.* (1988) documented C:N ratios of 3.6 for O. longicauda (n=2) but report a much higher ratio, 5.3, for O. dioica (n=15). Mean C:N ratios in Deibel (1986), Gorsky *et al.* (1988) and my study are not in agreement with the C:N ratio of 250:1 for O. rufescens reported by Alldredge (1976b). She used the methods of Sharp (1974) for particulate organic carbon and nitrogen analyses. However, Sharp (1974) states that the practical lower limit for this type of nitrogen analysis is 1 μg. The nitrogen figure of 0.04 μg/house reported in Alldredge (1976b) is thus suspect.

Other gelatinous zooplankton have higher C:N values than oikopleurids, with mean ratios ranging from 3.7 to 8.3 for various salps (Madin *et al.* 1981, Purcell 1982), 3.7 for medusae, 3.7 to 4.4 for ctenophores (see literature review in Shenker 1985, Gorsky *et al.* 1988), 4.0 for pyrosomes, and 4.6 for Fritillaria (Gorsky *et al.* 1988). Differences in C:N ratios appear to be taxon-specific but can also occur as a result of variations in gut fullness, with higher ratios measured in grazers with full guts (Madin et al. 1981). As gut fullness was not assessed in my study, the impact of a seston-filled gut on oikopleurid C:N ratios is unknown.

Although, in my study, 'new' houses were produced in a natural seston

environment, the carbon content in these houses overlapped with the one truly 'clean' house produced. The 'clean' house carbon represented 6.1% of the animal body carbon; mean carbon content (±SD) of the 'new' houses was 7.5% (±3.4%) of body carbon. This carbon investment per house is much lower than the estimate of 23% determined by Deibel (1986) for O. vanhoëffeni houses produced in filtered seawater. Using ash-free dry weight measures, Alldredge (1976c) calculated a carbon investment per house of 10 to 20% of body carbon for O. rufescens. The mean (±SD) carbon content of O. rufescens houses was 10.0 (±5.0) µg (Alldredge 1986b), similar to the carbon content of newly produced O. vanhoëffeni houses in my study (8.38 ±3.63 µg).

The range in carbon content of the 11 newly produced O. vanhoëffeni houses was 3.55 to 14.05 µg. The single rudiment that was measured contained 7.7 µg C. These carbon measures fall on the lower end of the range reported by Deibel (1986): 6.0 to 46 µg C per house. The animals that produced 'new' houses in this study were smaller (trunk length range of 2.2-3.3 mm) than the oikopleurids in Deibel (1986). Small sample sizes in combination with different animal trunk ranges may account for the observed differences in measures of carbon content per house.

An overlap in carbon content of scale 0 and scale 1 houses and a low C:N ratio for scale 1 houses suggests that scale 1 or 'new' houses contained little, if any, seston particles. Greater carbon measures and higher C:N ratios were found in all houses with visible particle loading. Similar C:N ratios for discarded houses and ambient seawater were also reported by Taguchi (1982). Based on the carbon content of 'new' houses and those with heavy particle loads, the contribution by natural particulate material trapped within and on the house was as high as 80 µg C per house. For larger O. vanhoëffeni, the carbon content of particles adhering to discarded houses could be much greater.

Surprisingly, no relationship was found between measures of 'new' house carbon and animal carbon. Animal size and body carbon content, however, were highly correlated. Statistical analysis confirmed that carbon content per house was not related to oikopleurid body size. My data, however, may be insufficient for a conclusive

interpretation due to a small sample size of 'new' houses (n=11) and a limited size range of animals. It is also possible that the carbon content per oikopleurid house is naturally variable. A larger data set, including animals of a broad size range and with multiple houses produced by individual animals, may be necessary to examine the variability in animal carbon invested per house.

Chapter 4

Oikopleurid House Production Rates : Effects of Animal Size, Temperature and Seston

4.1 Introduction

Lohmann (1909) was the first to report a daily production rate of oikopleurid houses. His observations included the formation and abandonment of six Oikopleura rufescens houses during a 24 hour period. Since then, there have been only two reports of directly measured oikopleurid house production rates (Paffenhöfer 1973, Fenaux 1985). Both of these investigations were conducted on the same species, the relatively small and short-lived O. dioica.

The observations of Paffenhöfer (1973) showed a mean generation time of 9.5 days and a daily house production rate of 5.1 $\pm$ 0.7 for O. dioica at 13°C. Factors which might influence the frequency of house production, however, were not discussed. *In situ* observations have suggested a positive correlation between the number of discarded houses in the water column and the concentration of particulate organic matter (Alldredge 1976b). These observations, however, were not supplemented by experimental study. The only experiments conducted to examine factors which affect the frequency of house production were reported a decade later by Fenaux (1985).

Fenaux (1985) observed a linear increase in O. dioica house production rate as temperature increased from 14-22°C. An average of 7.4 houses were produced per day at 15°C; at 20°C, the mean was 11.8. These short-lived larvaceans (5 days at 15°C) responded to an increase in temperature with a decrease in maturation time and an increase in house renewal rate (Fenaux 1985). Under constant temperatures, Fenaux also observed an increase in house production rate with increasing food supply. Although Fenaux reported enhanced house production rates at raised temperatures and particle concentrations, he also noted that these oikopleurids secrete a near constant number of

houses per lifetime, independent of external stimuli. He suggested that the periodicity of house secretion by O. dioica was controlled by an internal temporal factor.

At high *in situ* particle concentrations, oikopleurid houses rapidly become clogged and result in an apparent increased rate of house abandonment (Alldredge 1976b). Similar observations under experimental conditions, however, are lacking. Alldredge (1976b) also suggested that different species discard their houses at different rates, in part, because of variable filter pore sizes among species. The pores of the inlet filters limit the size range of particles that can enter the house and thus affect the degree of clogging and, ultimately, frequency of house abandonment.

Jettisoned houses may stay in the water column for an extended period of time. Dense aggregates of discarded houses have been found in discontinuity layers in concentrations of >1100 m^{-3} (Alldredge 1975). Similar aggregates of larvacean houses, occurring at discrete depths, have also been observed in a Newfoundland coastal bay (Mahoney and Buggeln 1983). Mahoney (1981) reported SCUBA observations of highly variable abandoned house concentrations, both spatially and temporally. Aggregates of larvacean houses in Newfoundland waters cause severe fouling of fish nets and are referred to as 'slub'. In cold waters, oikopleurid houses are very resistant to decay (Buggeln 1978, Mahoney 1981, Pomeroy and Deibel 1986) and difficult to remove from fishing gear.

Although abandoned houses can be highly patchy in abundance, concentrations of discarded houses in sediment trap collections have been used to estimate larvacean house production rates. Taguchi (1982) calculated an average annual production of 5.3 ± 3 houses day^{-1} for O. longicauda from the contents of traps suspended in a subtropical inlet. This estimate was determined indirectly but is similar to experimentally determined house production rates of other warm water species, O. dioica (Paffenhöfer 1973, Fenaux 1985) and O. rufescens (Lohmann 1909).

Oikopleurid houses in coastal environments may play a significant role in the vertical and horizontal transport of POM. Particle-laden houses are rich in organic

material and, because mucous houses are sticky, they continue to trap particles long after they have been discarded. They may, at times, be responsible for a substantial fraction of organic material sinking to the benthos. Sediment trap analyses have shown annual average sedimentation rates of discarded larvacean houses of 8.9×10^4 m^{-2} day^{-1} (Taguchi 1982).

House production rates and the factors affecting the frequency of house production in coastal Newfoundland waters are unknown. Oikopleurids in these waters are considerably larger than O. dioica, O. rufescens and O. longicauda and, because they inhabit cold water (-1° to +6°C), growth and maturation are considerably slower. The following study examines some physical and biological factors which may influence the frequency of house production by oikopleurids in cold coastal Newfoundland waters.

4.2 Methodology

A total of 239 oikopleurids, from 26 field collections, were observed for measures of daily house production. Animals were individually transferred to glass incubation jars as outlined in Section **2.2.** Each oikopleurid occupied a glass jar (500 ml or 1000 ml) containing either natural seston collected from the sampling site (n=215) or filtered seawater (n=24). These jars were examined for discarded houses at 12 or 24 hour intervals over a 3 day period. Intervals between observations were chosen so as to minimize any disturbance to the animal which may cause premature evacuation of the house or cessation of normal feeding. During each observation period, the incubation temperature and the number of discarded houses in each jar were recorded. Abandoned houses were also removed from the jar using a glass pipette. House removal reduced the possibility of multiple houses colliding and sticking together.

The particle concentration in about half the jars was supplemented with additional food at each observation interval to examine the effect of particle enrichment on house production rate. This involved either the input of a small volume (1-2 ml) of diluted lab

algae (Isochrysis galbana) or the replacement of approximately 200 ml of seawater. Natural seston, collected at the time of animal capture, was used to replace particulate matter which was partially removed by the house filters. Particle-enriched jars were maintained at near ambient food particle concentrations.

Animals generally survived for at least 3 days and up to a maximum of 10 days in jars supplied with food particles. The longest survival time previously reported for O. vanhoëffeni in similar-sized jars with natural food was 7 days at 1°C; animals lived 5-6 days at 5°C (Mahoney 1981). Collapsed houses, which are difficult to discard, and rudiments which became damaged as a result of collision with the glass surface typically resulted in premature death of the animal. In filtered seawater, survival time was reduced to 2-3 days, presumably as a result of starvation. Due to problems associated with long-term incubation in glass jars, only data collected within the first 48 hours were used in the analysis of house production rates.

Upon termination of house production observations, animals were measured for trunk and tail length, as described in Section **2.3**, and indexed by gonad maturity, as outlined in Table 4.1. The maturation index follows that described for O. labradoriensis in Shiga (1976). Samples of natural seston, collected from the sampling site, were examined for total particulate carbon and nitrogen as detailed in Section **3.2**. The potentially 'ingestible' portion (< 70μm, based on pore size of inlet filters) of the seston was also analyzed and was collected by passing seston samples through a 70 μm Nitex mesh, as in Deibel (1988).

Natural particulate samples were also examined for chloropigment content. Both whole and sieved (< 70 μm) samples were filtered onto combusted GF/C filters using low vacuum (20 cm Hg), frozen immediately and stored at -30°C until analysis. Chlorophyll pigments were analyzed as described in Section **2.3**.

Using the SAS statistical package (version 6.04), Spearman partial correlation and multiple regression analyses were performed on the following independent variables: tail length, particle enrichment (seston and lab algae), temperature (°Kelvin), sampling site,

gonad maturity stage, jar volume and components of the water column samples: total chlorophyll a, POC, and PON, and <70 μm fractions of chlorophyll a, POC and PON (Table 4.2). Partial correlation analyses allowed for the comparison of the independent variables to identify those variables that might account for the variance. Further determination of the most contributory variables accounting for the variance was conducted using multiple regression analysis.

4.3 Results

Of the 24 animals incubated in jars with GF/C filtered seawater, only one produced a house. The survival time of these animals was also short: 2-3 days compared to a maximum of 10 days for animals in a food rich environment. The following results pertain only to animals which produced houses in unfiltered water.

The mean (±SD) house production rate (HPR) was calculated for all animals of each species which produced houses within 48 hours. The HPR for 104 Oikopleura vanhoëffeni was 1.70 (±0.78) houses day^{-1} and for eight O. labradoriensis, the HPR was 2.32 (±1.03) houses day^{-1}. These HPRs were not significantly different ($p > 0.05$, Wilcoxon Rank Sums test). Because of the small number of O. labradoriensis examined in this study, all further statistical analyses relate only to O. vanhoëffeni. O. labradoriensis data, however, were included in various figures to show points in relation to those of O. vanhoëffeni.

Examination of the Spearman partial correlation coefficients of the full model indicates that supplementing the incubation jars with additional lab algae and natural seston accounts for 13% and 8%, respectively, of the variance (r^2_p) of the mean house production rate (Table 4.2). Significant relationships ($p < 0.05$) are also shown for both mean HPR and sampling site (7% of the variance), and mean HPR and one component of the water column seston, 'ingestible' PON (< 70 μm), which also accounted for 7%

of the variance. The effects of animal tail length, maturity stage, jar volume and temperature accounted for very little of the variance and were not significant.

Least-squares regression showed negative, though weak, relationships between mean house production rate vs trunk length and vs tail length (Fig. 4.1, $r^2 = 0.04$ and 0.05, respectively). Both relationships with body size were significant ($p < 0.05$).

The effect of temperature and food particle enrichment on mean O. vanhoëffeni house production rates (±SD) is shown in Table 4.3. The grouping of samples at temperatures $\leq$ 1°C and $\geq$ 3°C was conducted to compare HPR at those temperatures which typify the pre-spring bloom and spring bloom ($\leq$ 1°C) and post-spring bloom ($\geq$ 3°C) periods. Potential differences between these mean HPRs were tested by Wilcoxon Rank Sums (Table 4.4). The mean HPR in jars both with and without additional food showed no significant difference with temperature. Within the entire range of temperatures (-1° to +6°C), however, animals given additional food particles (seston and lab algae) had significantly higher mean HPRs than individuals in jars which were not enriched with additional food.

Non-significant relationships between HPR and temperature are shown in Figure 4.2 for both particle enriched and unenriched treatments. They are plotted only to show the variation in HPRs among individuals from the same sample collection. There are also no apparent relationships between mean HPR and water column chlorophyll a concentration (Fig. 4.3) and mean HPR and water column POC concentration (Fig. 4.4). The most remarkable feature in Figures 4.2, 4.3 and 4.4 is the high between-individual variance, 2-3 fold in most cases. It should also be noted that observed HPRs for O. labradoriensis, though few in number, fell within the range of those of O. vanhoëffeni for all variables plotted (Figs. 4.1 - 4.4).

Multiple regression analysis was used to assess four of the independent variables (seston enrichment, lab algae enrichment, tail length, and chlorophyll a) to determine whether a statistically sound equation for predicting HPR could explain a greater portion of the variability than an equation with only one independent variable. The full model

explained 48% of the variance in HPR (Table 4.5). Adjusted-r^2 values were reported as they are better estimates of the variance than r^2. Adjusted-r^2 values take into account the reduction in the degrees of freedom as well as the error sum of squares when a variable is entered into the model (Deibel 1988). The model of seston, lab algae and tail length was statistically sound (adjusted-r^2 = 0.21) and had predictive power similar to more complex models.

During this study, house abandonment and subsequent rudiment expansion were observed in nine animals. These houses were discarded naturally and not as a result of physical disturbance. The duration between jettisoning of the house and the initiation of filtering behaviour in a new house ranged from 13 to 48 minutes, with a mean ($\pm$SD) of 23 ($\pm$10) minutes (n=11 houses).

Table 4.1. Qualitative Assessment of Oikopleurid Maturity Stage. Adapted from Shiga (1976).

Stage	*Visual Appearance and Location of the Gonadal Tissue*
1	Gonad absent. Posterior part of the trunk is taken up by the stomach and the intestine.
2	A small thin gonad appears at the posterior margin of the intestine.
3	The gonad increases in height along the posterior margin of the alimentary canal.
4	The gonad mainly increases in thickness and continues to lengthen and expand to the posterior.
5	Fully mature oikopleurid. Well developed gonad occupies the entire posterior part of the trunk.

Table 4.2. Spearman Partial Correlation Coefficients of Mean Daily House Production for Oikopleura vanhoëffeni. Values shown are: ('$p <$' value) and [r^2_p].

Tail Length	Seston Added	Lab Algae Added	Temp.	Sampling Site	Maturity Stage	Jar Volume
-0.03 (0.83) [<.1]	0.29 (0.03) [8]	0.37 (0.006) [13]	0.14 (0.31) [2]	0.27 (0.04) [7]	0.005 (0.98) [<0.1]	0.11 (0.42) [1]

Components of the seston:

Chl a	Chl a < 70 µm	POC	POC < 70µm	PON	PON < 70 µm
-0.16 (0.26) [2]	0.05 (0.7) [.2]	-0.07 (0.59) [0.5]	0.21 (0.12) [5]	0.03 (0.81) [0.1]	-0.27 (0.05) [7]

Table 4.3. Mean Daily Oikopleura vanhoëffeni House Production Rate as affected by Temperature (°C) and Food Particle Enrichment. Values shown are: (±SD) and [n]. (* = temperature ≤ 1°C only)

Temperature (°C)	No Particle Enrichment	Seston Added	Lab Algae Added	Combined Enrichment
≤ 1°	1.27 (0.70) [30]	2.10 (0.49) [15]	1.87 (0.74) [37]	1.94 (0.68) [52]
≥ 3°	1.54 (0.74) [18]	2.65 (0.89) [4]	no data	2.65 (0.89) [4]
All Temps: -0.6° to +5.8°	1.37 (0.72) [48]	2.21 (0.61) [19]	1.87* (0.74) [37]	1.99 (0.71) [56]

Table 4.4. Wilcoxon Rank Sum Probability Values of Mean Daily Oikopleura vanhoëffeni House Production Rate: Relationships with Temperature (°C) and Food Particle Enrichment. Results of statistical analyses are in Table 4.3. NS = not significant ($p > 0.05$).

Treatment	Comparison	p Values
$\leq$ 1°C	No Enrichment vs Seston Enrichment	$p < 0.001$
	No Enrichment vs Algae Enrichment	$p < 0.001$
	No Enrichment vs Combined Enrichment	$p < 0.001$
	Seston Enrichment vs Algae Enrichment	NS
$\geq$ 3°C	No Enrichment vs Seston Enrichment	$p < 0.037$
All Temp. (-0.6° to +5.8°C)	No Enrichment vs Seston Enrichment	$p < 0.001$
	No Enrichment vs Algae Enrichment	$p < 0.001$
	No Enrichment vs Combined Enrichment	$p < 0.001$
	Seston Enrichment vs Algae Enrichment	$p < 0.05$
No Particle Enrichment	Temp $\leq$ 1° vs Temp $\geq$ 3°C	NS
Particle Enriched (Seston)	Temp $\leq$ 1° vs Temp $\geq$ 3°C	NS

Table 4.5 Multiple Regression Statistics for several models of Oikopleura vanhoëffeni House Production Rate. The independent variables in the full model are tail length, seston added, algae added, temperature, sampling site, maturity stage, jar time, chloropyll a, POC, PON, and chlorophyll a, POC, PON sieved through a 70 μm mesh. Adjusted-r^2 values are a better estimate of the variance then r^2 as they take into account the reduction of the degrees of freedom as well as the error sum of squares when adding a new independent variable. The *p* values indicate the significance of 'F', which is testing the null hypothesis that all regression coefficients are equal to zero.

Model	r^2	adjusted-r^2	F value	*p* value
Full	0.48	0.35	3.73	0.001
Seston+Algae+ Tail+Chlor	0.23	0.19	5.92	0.001
Seston+Algae+Tail	0.24	0.21	9.95	0.001
Chlor+Algae+Tail	0.19	0.16	6.36	0.001
Seston+Algae+Chlor	0.18	0.15	6.06	0.001
Seston+Tail+Chlor	0.15	0.11	4.65	0.01
Seston+Algae	0.18	0.17	11.4	0.001
Tail+Seston	0.15	0.13	8.63	0.001
Algae+Chlor	0.13	0.11	6.18	0.01
Tail+Chlor	0.10	0.08	4.45	0.02
Seston+Chlor	0.09	0.07	4.50	0.02
Tail+Algae	0.08	0.06	4.35	0.02
Seston	0.10	0.09	11.1	0.01
Tail	0.05	0.04	5.57	0.02
Lab Algae	0.03	0.02	2.80	0.1
Chlor	0.03	0.02	2.60	0.11

Figure 4.1. Mean Daily House Production Rate (Rate) vs Trunk (TR) and Tail Length (TA) (mm). Oikopleura vanhoëffeni data are fitted to a regression line.

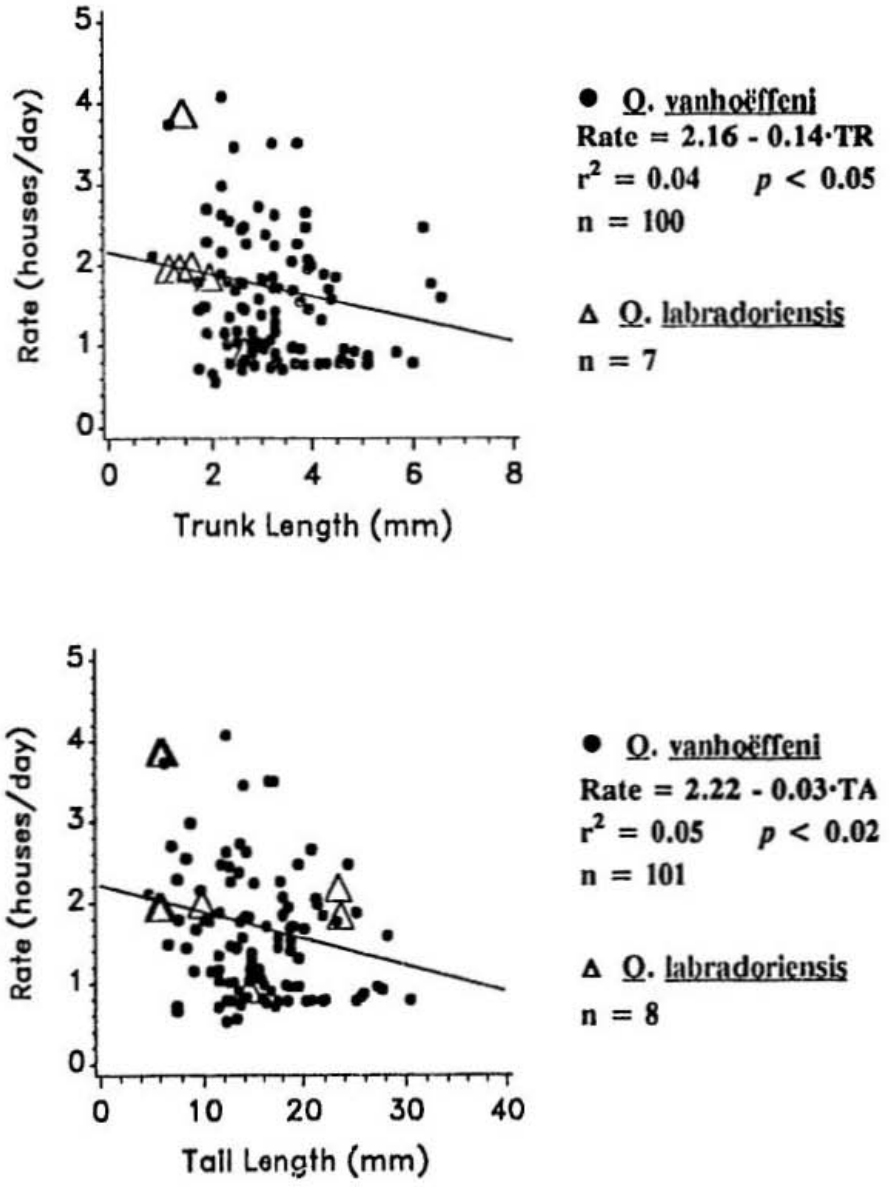


Figure 4.2. Mean Daily House Production Rate (Rate) vs Temperature (Temp) (°C) without ('a') and with ('b') Food Particle Enrichment. Oikopleura vanhoëffeni data are fitted to a regression line.

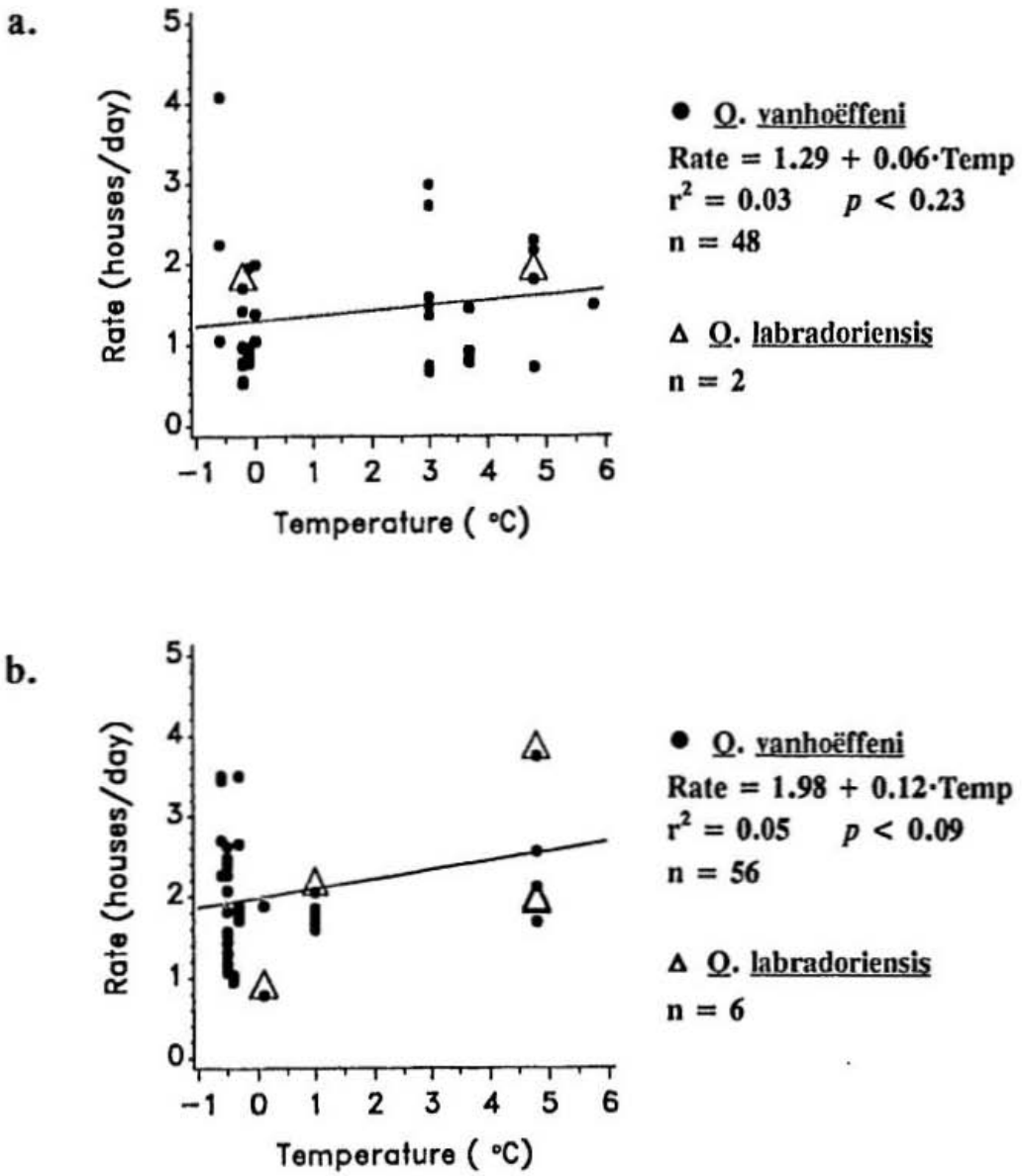
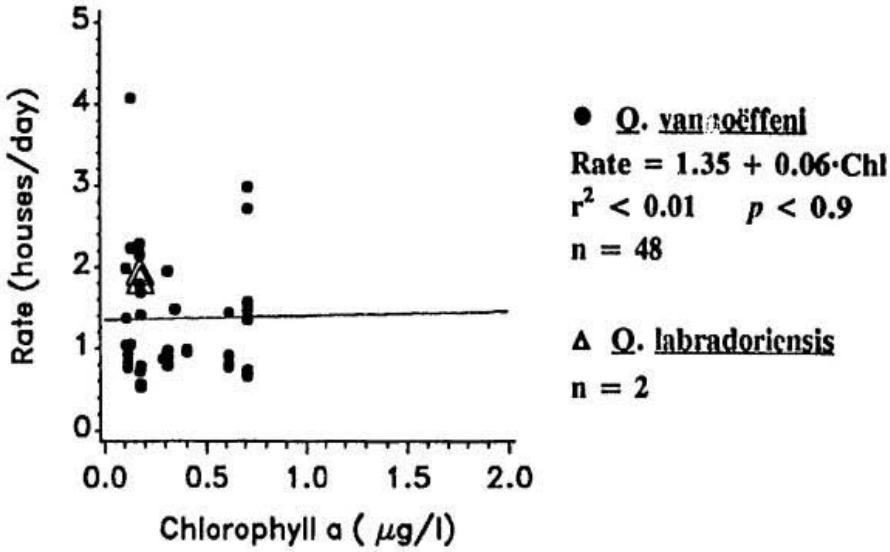


Figure 4.3. Mean Daily House Production Rate (Rate) vs Total Chlorophyll a (Chl) (µg/l) without ('a') and with ('b') Food Particle Enrichment. Oikopleura vanhoëffeni data are fitted to a regression line.

a.



b.

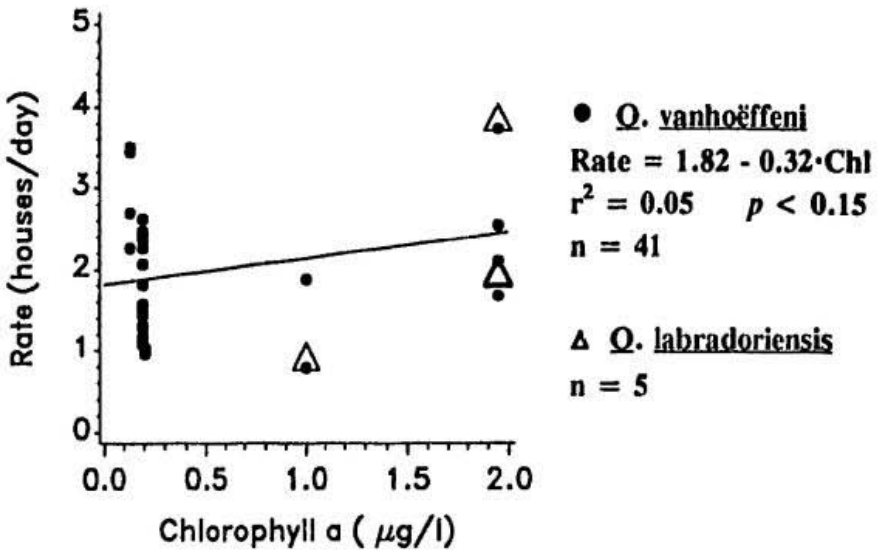
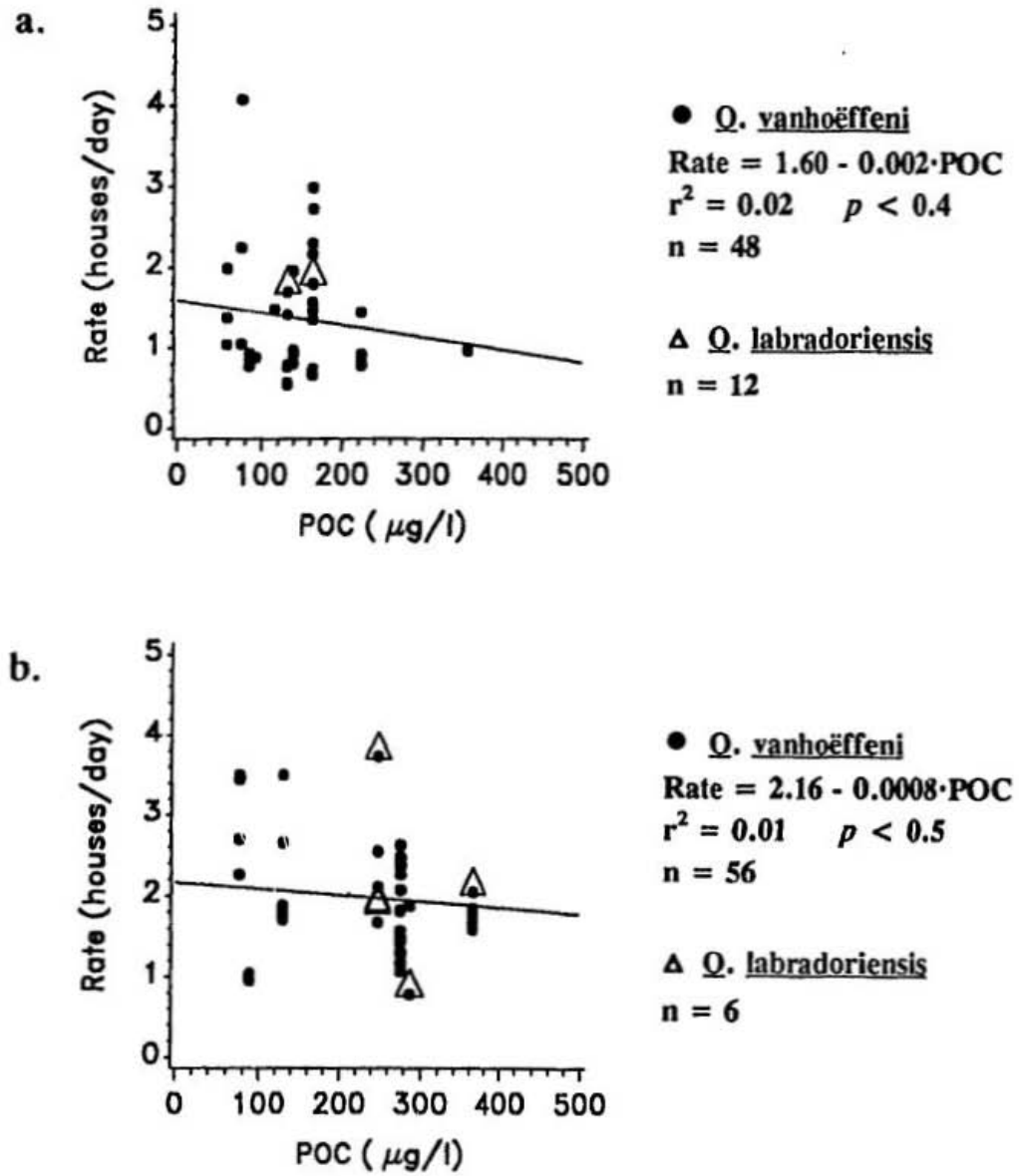


Figure 4.4. Mean Daily House Production Rate (Rate) vs Particulate Organic Carbon (POC) (µg/l) without ('a') and with ('b') Food Particle Enrichment. Oikopleura vanhoëffeni data are fitted to a regression line.



4.4 Discussion

Previous studies of oikopleurid house production rates (HPR) have been conducted on relatively small (trunk length < 1.5 mm), short-lived, warm water species: Oikopleura dioica (Paffenhöfer 1973, Fenaux 1985), O. rufescens (Lohmann 1909), and O. longicauda (Taguchi 1982, HPR indirectly measured). Mean rates of house production reported for these species are >5 and up to 11 houses day^{-1}. Oikopleurids in this study were relatively large, cold water species. The mean house production rate determined for O. vanhoëffeni was 1.7 houses day^{-1} and for the somewhat smaller O. labradoriesis, the average was 2.3 houses day^{-1}.

Fenaux (1985) is the only report which includes an examination of factors which influence the rate of house renewal. He found that both temperature and particle abundance were correlated positively with HPR of O. dioica. However, animals with increased HPRs experienced a decrease in maturation time and lifespan, suggesting some control by an internal temporal factor. In my study, houses were not produced by oikopleurids living in a particle-free environment (filtered seawater). No internal mechanism controlling house production was evident.

Other factors which may influence the rate of house abandonment and renewal include pore size of the house filters. The concept is that smaller pores clog faster, reducing the filtering efficiency of the house and increasing the rate of house abandonment (Alldredge 1977). The inlet and feeding filter pore sizes have been measured for several oikopluerids (see Section 1.1.1.2). The largest pore sizes have been found in O. vanhoëffeni, the largest oikopleurid examined (Deibel 1985). There appears to be a trend of increasing pore size with increasing body size of adult oikopleurids (Deibel and Powell 1987a). Warm water species generally exhibit higher metabolic rates, faster maturation and a shorter lifespan than species inhabiting colder waters. Because body size and metabolic rates are influenced by ambient temperature, higher rates of house production could be expected in oikopleurids of warmer waters. This may account

for the higher HPRs observed in small, warm water species as compared to low HPRs of the much larger cold water species examined in my study.

Within species, body size may also influence rate processes. Periodicity of Q. vanhoëffeni house production decreased slightly, though significantly, with increasing body size (i.e. tail length). The size range of animals examined, however, was narrow compared to the full range reported for this species. An increase in the body size of Q. vanhoëffeni is associated with an increase in house size and filter pore size (Deibel *et al.* 1985), and higher clearance rates (Knoechel and Steel-Flynn 1988). Large animals, feeding on the same particulate suspension as small animals, may experience less clogging of the filters and therefore lower rates of house renewal. A study which examines the full size spectrum may uncover the effects, if any, of body size on HPR.

The effects of temperature and food particle concentration on HPR have been examined for a small, warm water oikopleurid (Fenaux 1985). The same variables were examined in my study but for a cold water species, Q. vanhoëffeni. Changes in temperature, over a range of -1°C to 6°C, showed no effect on the HPR of Q. vanhoëffeni. This temperature range does not exceed that typically experienced by this species. Frequent wind-induced mixing in Newfoundland coastal waters causes temperatures to change abruptly within this range (Deibel *et al.* 1992).

Particle enhancement with natural seston and with lab algae significantly increased the rate of house abandonment and renewal. A similar relationship was reported by Fenaux (1985) and, indirectly, by Alldredge (1976b). Surprisingly, no significant relationships were found between HPR and the water column components, chlorophyll a and POC. Relationships may be lacking due to the narrow range of chlorophyll and POC concentrations included in my study. One of the most interesting features of the data set is the 2-3 fold range in HPR between individuals of similar sizes in similar conditions. Q. vanhoëffeni house production rates appear to be naturally highly variable. The observed time interval between house abandonment and expansion of a new house was also highly variable.

The ecological significance of the accumulation of large numbers of discarded oikopleurid houses in Newfoundland waters is not limited to problems associated with 'slub' production and fouling of fishing gear. Larvacean houses are relatively large and effective particle collectors which remove and trap large amounts of organic material from the surrounding environment. Their impact on particle removal and flux to the benthos may be highly significant in coastal Newfoundland waters, especially when natural particle concentrations are low.

Chapter 5
General Discussion

The cold water larvaceans Oikopleura vanhoëffeni and O. labradoriensis are seasonally abundant in coastal waters of Newfoundland (Davis 1982). These animals have the capacity to filter large volumes of seawater through their mucous house structure. When the house becomes clogged with particulate matter and no longer efficient, it is abandoned and quickly replaced.

House production represents a considerable metabolic cost to the animal, both in carbon and other elements invested in each house, and in respiratory energy expended during house secretion and expansion. The costs of house replacement must be outweighed by the food energy gained with an efficient, particle-collecting house.

The mean carbon content (± SD) invested per house was 8.4 (±3.6) µg C and represented an average of 7.5% of the animal body carbon. This estimate is considerably lower than the estimate of 23% reported by Deibel (1986) for newly produced houses of O. vanhoëffeni. Applying an average HPR of 1.7 houses day^{-1}, the mean daily carbon investment is 14.3 (±6.1) µg C and this represents about 13% of body carbon day^{-1}. Relatively low C:N ratios in newly secreted houses were comparable to C:N ratios in oikopleurid bodies, confirming that 'new' houses in this study were relatively particle-free. Surprisingly, there was no relationship between 'new' house carbon content and animal body carbon content. Carbon content per house showed a five-fold variation for similar-sized animals.

Oikopleurid house production showed a daily range of up to four houses day^{-1}. Mean O. vanhoëffeni HPR was 1.7 (±0.8) houses day^{-1} and for O. labradoriensis, average HPR was 2.3 (±1.0). There was no significant difference in HPR between the two species. Within O. vanhoëffeni individuals, there were no significant relationships with HPR and body size, ambient temperature (< 1°C to +6°C), and seston components (POC and chlorophyll a). However, the addition of both natural seston and lab algae to

the incubation jars, significantly increased the rate of house renewal. Particle enrichment produced about a 1.5 fold increase in HPR. Based on these effects, somewhat higher rates of house abandonment and renewal should be expected at high particle densities characteristic of spring phytoplankton bloom conditions. House production rates appear to be naturally highly variable under the various conditions examined, with a 2-3 fold range in HPR. The interval between house abandonment and completion of new house inflation was also highly variable, ranging from 13-48 minutes and with a mean of 23 ($\pm$10) minutes.

The contribution of carbon in abandoned houses is primarily of particulate origin. In this study, up to 80 µg C, in the form of particulate material and faecal pellets, was measured in the houses of animals with a trunk length range of 1.9 to 6.2 mm. Slightly lower carbon measures were reported by Alldredge (1977) for field-collected, discarded houses, produced by smaller oikopleurids. O. vanhoëffeni is one of the largest larvaceans and 80 µg C per house is probably a very conservative estimate of the carbon content contributed by trapped particles and faecal material.

The highest concentration of discarded oikopleurid houses observed in Newfoundland coastal waters was about 1000 houses m^{-3} (Mahoney and Buggeln 1983). Abundance estimates of O. vanhoëffeni animals in the same waters include concentrations ranging from about 30-40 m^{-3} (Mahoney and Buggeln 1983) up to a maximum of 450 m^{-3} (Davis 1982). Applying a mean HPR of 1.7 houses day^{-1}, a concentration of 1000 houses m^{-3} could be attained in 1-2 days at maximum oikopleurid abundance, in 6 days at concentrations of 100 animals m^{-3} and in 15 days at densities of 30-40 animals m^{-3}. Mahoney (1981) found that an oikopleurid house is still recognizable after 18 days at 0°C and after 11 days at 4°C. However, these houses become somewhat less distinct in appearance following three days at 5°C. Considering the seasonal variation in temperature (-1° to 6°C), as it affects house decay, a discarded oikopleurid house should remain visible and recognizable for about one week. House production rates determined in this study appear to be in agreement with estimates of animals and discarded houses

found *in situ*.

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