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Effects of food and silt on filtration, respiration and condition of the freshwater mussel *Hyridella menziesi* (Unionacea: Hyridae): implications for bioaccumulation

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Abstract

The effect of exposure to different concentrations of food and suspended silt on filtration, respiration and condition were studied in the freshwater mussel *Hyridella menziesi*. Using a milk solids-based food and kaolin to simulate silt, mussels were maintained at different combinations of food and silt concentrations for 3 weeks. Between treatments mean filtration rates ranged from $0.97-1.66 \ l \ g^{-1} \ h^{-1}$, and respiration from $0.50-1.35 \ mg \ O_2 \ g^{-1}$, h⁻¹. Silt (non-volatile suspended solids up to 35 mg l⁻¹) failed to have a significant effect on filtration rate or condition, but with increasing food levels (volatile suspended solids up to 35 mg l⁻¹) filtration rate was reduced, and condition was reduced at the lowest food concentration (<5 mg l⁻¹). Respiration showed a food × silt interaction between treatment blocks. When food was low respiration increased with increasing silt concentrations, and when silt was low (<5 mg l⁻¹) respiration increased with increasing food concentrations. The observed effects of food and silt on filtration, respiration and condition are discussed in terms of their potential for affecting contaminant bioaccumulation. In low-food situations (i.e., <5 mg l⁻¹), if mussels are pumping large volumes of water, contaminant uptake rates could be enhanced, whereas abundant food would result in lower pumping rates and lower uptake rates. Changes in metabolism with food concentration have implications for contaminant elimination, and changes in biochemical composition associated with changing condition could affect the tissue distribution and retention of contaminants.

Introduction

Freshwater mussels are commonly used for environmental monitoring of metals (e.g., Jones & Walker, 1979; Adams et al., 1981; Millington & Walker, 1983), and organic compounds (e.g., Hartley & Johnston, 1983; Storey & Edward, 1989; Herve, 1991; Kauss & Hamdy, 1991). A basic requirement of species used for monitoring aquatic contamination is that they should exhibit the same correlation between the bioaccumulated concentration of a contaminant and the average contaminant concentration in the surrounding water at all monitoring locations and under all conditions (Phillips, 1980). For mussels this necessitates an understanding of the many abiotic and biotic factors which may determine the relationships between

contaminant levels in the environment compared to those accumulated in the mussel tissue (Widdows & Donkin, 1992). Despite the already widespread and increasing use of freshwater mussels for contaminant monitoring, the many factors influencing bioaccumulation are still poorly understood (Elder & Collins, 1991). Muncaster et al. (1990) considered the relative contribution of water- and sediment-bound contaminants to the total contaminant body burden in Lampsilis radiata and Elliptio complanata, and the effect of enclosure on contaminant uptake. The effects of various heavy metals and their uptake kinetics have been studied in Anodonta cygnea (Salánki & V.-Balogh, 1989, and references therein).

Species of the hyriid mussel *Hyridella* are found throughout Australasia (Walker, 1981a), and the Aus-

tralian species. Hyridella australis, has been used for biomonitoring of the pesticide endrin (Ryan et al., 1972) The New Zealand species Hyridella menziesi has also been recognised as having potential for enviconmental monitoring (Roper & Hickey, 1994), and tissue analyses have revealed accumulations of heavy metals and organic compounds (authors' unpublished data). When monitoring in rivers it is common to encounter downstream gradients in food and suspended silt. Both factors are known to strongly affect bivalve physiology (e.g., Bayne et al., 1976; Grif-1984; Griffiths. 1987; Bricelj & Malouf, 1984; Grant & Thorpe, 1991) and hence have the potential to affect bioaccumulation. To understand better the possible role of these extrinsic factors in contaminant accumulation this study examines the effect of exposure to different concentrations of food and suspended silt on tiltration, respiration and condition of Hyridella mendest.

Methods

Hyridetla menziesi were collected using SCUBA from Lake Rotokawau (38 °05'S, 176 °22'E) in March (early autumn) when the lake water temperature was about 18.0 C. This small oligotrophic lake on the central volcanic plateau of the North Island, New Zealand, supports dense populations of mussels (James, 1987). Ninety mussels within the length range of 46.1 -57.8 mm (mean 52.7 mm, standard deviation 2.21 mm) were selected. The experimental design consisted of three food and three suspended silt levels, giving nine separate treatments. Ten mussels were randomly assigned to each treatment. Each treatment was maintained in a 15 1 plastic pail, with an 8 cm deep layer of lake sand (washed to remove fines) and 10.61 of local river water. All the water used in experiments was obtained from the Waikato River and had been flocculated with alum and passed through a sand filter at the Hamilton city water treatment station. Mussels were fed on a diet based on Complan, a proprictary human dietary supplement consisting mainly of milk solids, following the recommendation of Walker (1981b), supplemented with ground alfalfa and yeast in the dry weight ratios of 0.02 alfalfa: 0.03 yeast: 0.05 Complan, and kaolin was used to simulate silt. Food and suspended silt were measured as volatile suspended solids (VSS) and non-volatile suspended solids (NVSS, or fixed solids), respectively, using standard methods (APHA, 1989). The three food levels were

achieved by administering no food ('low food'), or dosing the treatments with the stock food solution to give nominal VSS concentrations of 5 mg l-1 ('medium food') or 35 mg l⁻¹ ('high food'). The three silt levels had no kaolin ('low silt'), or were dosed to give nominal NVSS concentrations of 5 mg l-1 ('medium silt') or 35 mg l⁻¹ ('high silt'). For three weeks treatments were dosed with food and kaolin every 12 h, and kept at 20 °C under a natural light/dark cycle with vigorous aeration. The water in each treatment was replaced every second day, and on five occasions during the maintenance period 1 l samples of the treatment water were collected immediately after feeding and 12 h later to determine the range in suspended solid conditions. After three weeks, filtration and respiration rates, and condition of the mussels were measured.

For filtration rate measurements (i.e., the rate at which particles are removed from suspension) the 10 mussels from a treatment were scrubbed clean and placed individually in dishes containing 500 ml of filtered river water. Gentle aeration in each dish ensured adequate mixing of the water. When all the mussels were open with their siphons extended 10 ml of a 2 g l⁻¹ yeast solution was added to each dish. Subsamples of water were collected after 2 min when the yeast was thoroughly mixed and again 15 min later. Yeast concentrations were determined spectrophotometrically by measuring absorbance at a wave length of 450 nm. The filtration rate (l h⁻¹) was determined (after Coughlan, 1969) as:

filtration rate =
$$\frac{V}{t} \log_e \frac{C_0}{C_t}$$
,

where V is the volume of water and yeast solution in the dish in 1, C_o is the initial yeast concentration, C_t is the final yeast concentration, and t is the time interval between samples in h. Pumping rates were corrected for decreases in yeast concentration observed in 2 control dishes containing no mussels.

To determine respiration rates the 10 mussels from a treatment were scrubbed clean and placed individually in sealed 620 ml chambers containing air-saturated filtered river water. The mussels were supported in wire frames above the chamber floor and magnetic stirrers were used to facilitate mixing. The chambers were kept at 20 °C and after a 15 min equilibration period oxygen uptake was measured for about 30 min by means of YSI oxygen electrodes inserted into the chambers. The voltage signal from each electrode was routed through a scaling amplifier to subtract the background oxygen concentration and amplify the working range to 1 mg

l-1 full scale. This signal was monitored on a chart

At the completion of experiments all mussels were shucked and the flesh and shells dried at 60 °C for 48 hours and weighed. Physical condition was calculated as dry flesh weight (mg) per shell weight (g) following Crosby & Gale (1990), and filtration and respiration were expressed per dry weight. For each of the variables measured (i.e., filtration, respiration, and condition) statistical comparisons between treatments were made using analysis of variance (ANOVA) followed by the Tukey multiple comparison procedure (Zar, 1984). Log transformation of condition values was necessary because of heteroscedasticity of variances.

Results

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Relative levels of food as volatile suspended solids and silt as non-volatile suspended solids achieved in the 9 treatments are shown in Table 1. With 12 hourly dosing of the treatments it was not possible to maintain constant food and silt concentrations, and as a result of mussel filtering and settlement, volatile and non-volatile suspended solid concentrations were substantially lower after 12 h, especially at higher concentrations. Results of pairwise (r-test) comparisons of suspended solid levels are shown in Table 1.

Survival of mussels was good with only 1 of the 90 mussels dying before the end of the experiment. Copious pseudofaeces were produced by mussels in the high silt treatment, and to a lesser extent in the medium silt. Results of two-way ANOVA on filtration, respiration and condition are shown in Table 2. Filtration rate and condition both showed no significant effect of silt but were affected by food concentration. With increasing food levels filtration rate was reduced, and condition was highest with medium food, and lowest at the low food concentration (Fig. 1). Two-way ANOVA revealed no significant effect of food or silt on respiration, but there was a significant food × silt interaction. This indicated that the effect of silt and/or food varied between treatment blocks. Respiration data were therefore reanalysed for food and silt effects separately using one-way ANOVA (Table 3). This showed that among the 3 low food treatments there was a significant silt effect, respiration increasing with increasing silt concentrations (Fig. 2). Similarly, in the low silt treatments there was a significant food effect, with increasing respiration associated with increasing food

Table 1. Means (and standard deviations) of volatile and non-volatile suspended solid concentrations in the 9 treatments immediately after dosing (initial) and after 12 h based on five measurements during the maintenance period. Significance of t-test comparisons of initial and 12 h concentrations are shown as ns = not significant at p < 0.05, *= p < 0.05, **= p < 0.01, *** = p < 0.001.

Treatment	Suspended solids (mg l ⁻¹)		
	Initial	12 h	
Volatile			
low food: low silt	0.6 (0.3)	0.9 (0.4)	ns
low food: medium silt	1.1 (0.6)	1.1 (0.6)	ns
low food: high silt	3.4 (2.7)	2.0 (1.5)	пs
medium food: low silt	5.2 (2.0)	0.4 (0.6)	**
medium food: medium silt	5.0 (0.8)	0.7 (0.2)	***
medium food; high silt	7.7 (1.6)	1.8 (0.8)	***
high food: low silt	34.0 (10.7)	14.6 (2.5)	*
high food: medium silt	35.4 (6.3)	11.6 (6.3)	***
high food; high silt	34.7 (7.7)	10.2 (5.3)	***
Non-volatile			
low food: low silt	0.0 (0.0)	0.0 (0.0)	ns
low food: medium silt	6.7 (2.0)	0.2 (0.4)	**
low food: high silt	36.2 (7.5)	2.1 (1.5)	***
medium food: low silt	0.8 (1.8)	0.0 (0.0)	ns
medium food: medium silt	6.0 (2.3)	0.0 (0.0)	**
medium food: high silt	30.5 (8.2)	1.2 (0.9)	**
high food: low silt	0.2 (0.4)	0.0 (0.0)	ns
high food: medium silt	8.1 (4.0)	0.4 (0.9)	*
high food: high silt	33.5 (6.0)	0.5 (1.0)	***

concentrations (Fig. 2). No other significant relations were found for respiration.

Discussion

Rather than using an algal based food the mussels were fed on a diet comprised mainly of milk solids. While this was not a natural food the dissolved and particulate organics, plus the bacteria which would have been present, were sufficient to maintain the mussels for the duration of the experiment. James (1987) has shown that *H. menziesi* in Lake Rotokawau do not rely on algae for food, deriving 95% of their energy from allochthonous organic material.

There are few data available for VSS and NVSS concentrations in New Zealand freshwaters; however, in a study of mining impacts in several streams Davies-Colley et al. (1992) reported suspended solids

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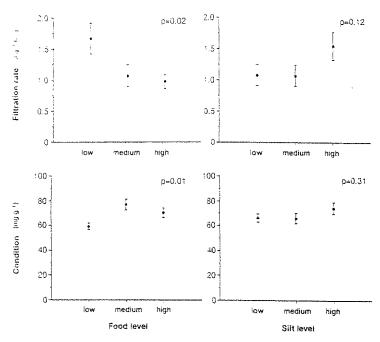


Fig. 1 Means (\pm 1 standard error: n = 29 or 30 for each point) of filtration rate and condition at different food and silt levels.

concentrations (which can be assumed to be nearly all NVSS) of $10.8-275 \text{ mg I}^{-1}$. In the Waikato River system average VSS concentrations at several sites have been found to range between $0.8-16 \text{ mg I}^{-1}$ and NVSS between $0.9-31 \text{ mg I}^{-1}$ (Davies-Colley, unpublished data). Food and silt levels used in this experiment were therefore environmentally realistic, and covered the range of concentrations which mussels might reasonably be exposed to naturally.

Mean filtration rates of H. menziesi measured in this study ranged between treatments from 0.97-1.661 g⁻¹ h-1, based on mussels with mean dry flesh weights of 0.39 g. These rates are higher than those quoted by James (1987) for *H. menziesi* of $0.20-0.55 \text{ l h}^{-1}$, for a 0.7 g (dry flesh weight) mussel, but are consistent with rates given by Kryger & Riisgård (1988) for other unionacean mussels. Mean respiration rates ranged from 0.50-1.35 mg O₂ g⁻¹ h⁻¹ between treatments, compared with rates quoted by James (1987) of $(0.46-0.51 \text{ mg } O_2 \text{ h}^{-1} \text{ for a } 0.7 \text{ g (dry flesh weight)})$ 11. menziesi. Sheldon & Walker (1989) reported oxygen consumption rates for the Australian hyriid mussels Alathyria jacksoni and Velesunio ambiguus of 0.69 and 0.44 mg O₂ g (dry flesh weight)^{-0.75} h⁻¹, respectively.

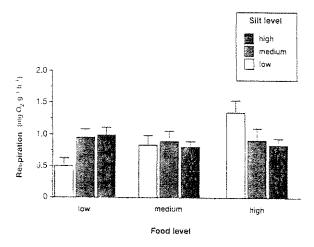


Fig. 2. Mean respiration levels (± 1 standard error; n = 9 or 10 for each bar) measured in the various silt and food treatments.

At the end of the 3 week incubation filtration rates were higher for mussels in the low food treatment compared with the medium and high food treatments, whereas no significant effect of silt was found. Effects of food concentration on bivalve filtration rates have been reviewed by Bayne et al.. (1976), Winter (1978), Bayne & Newell (1983), and Griffiths & Griffiths (1987). Typically, from a low threshold

Table 2. Results of two-way ANOVA of the effect of food and silt on filtration, respiration and condition. There were 10 replicates per treatment, except for low food:low silt which had 9. Comparisons of differences between means were carried out using Tukey's test, with treatments which were not significantly different connected by underlining. (L = low; M = medium: H = high; F = food; ns = not significant at p < 0.05; * = significant at p < 0.05)

Dependent variable	Source of variation	df	F	P	Significance	Comparison of means
Filtration rate	food	2	2.00	0.022	*	LFMFHF
	silt	2	0.06	0.124	ns	
	food \times silt	4	3,52	0.595	ns	
Respiration	food	2	3.98	0.142	ns	
	silt	2	2.14	0.937	ns	
	food \times silt	4	0.70	0.011	*	
Condition	food	2	4.69	0.012	*	$LF\ HFMF$
	silt	2	1.19	0.308	ns	
	food \times silt	4	1.07	0.376	ns	

Table 3. Results of one-way ANOVA of the effect of food and silt on respiration. There were 10 replicates per treatment, except for low food:low silt which had 9. Comparisons of differences between means were carried out using Tukey's test, with treatments which were not significantly different connected by underlining. (L = low: M = medium: H = high: S = silt: F = food: ns = not significant at p < 0.05; *= significant at p < 0.05; *= significant at p < 0.05;

Treatment held constant	Source of variation	df	F	Р	Significance	Comparison of means
low food	silt	2	4.19	0.026	*	LSMSHS
medium food	silt	2	0.11	0.897	ns	
high food	silt	2	2.94	0.070	ns	
low silt	food	2	6.85	0.004	**	$LF\ MFHF$
medium silt	food	2	0.03	0.975	ns	
high silt	food	2	$0.8\dot{5}$	0.437	ns	

as food concentration increases filtration rate rapidly increases and then is kept constant up to a food concentration at which a maximum ingestion rate is reached. From this point filtration rate decreases continuously while the amount of food ingested remains constant (Winter, 1978). Therefore the nature of the filtering rate response (i.e., whether it increases or decreases) depends upon the food concentration. There are two interpretations of the observed filtration rate increase for starved *H. menziesi*. Filtration rate may have increased during the course of the experiment in an attempt to capture what little food may have been present. Although no food was added to the 'low food' treatment it is likely that bacteria and the small amounts

of dissolved and suspended organic material present may have been sufficient to stimulate feeding activity. A variety of suspended particulates, including bacteria, phytoplankton, detritus and microzooplankton, as well as dissolved organic material, can be utilised as food by bivalves (Hawkins & Bayne, 1992), and H. menziesi has been shown to derive more than 95% of its carbon requirements from allochthonous organic material (James, 1987). Although the water used in experiments was passed through a sand filter small amounts of VSS were detected in the low food treatment (Table 1). Griffiths (1980) found that when filtration rate of Choromytilus meridionalis was measured in a range of cell densities, mussels which had been

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starved for 3 weeks showed a marked increase in filtration rate, and that filtration rates were highest when measured in low cell densities. This was taken as an indication that C. meridionalis may adjust its filtration rate when subjected to limited food over a 2-3 week period. An alternative interpretation of the filtration rate increase for starved H. menziesi is that after three weeks of starvation, during which time filtration rate may have declined, filtration was subsequently stimulated at the time of measurement by exposure to the yeast cells. For example, Bayne et al.. (1976) state that Mytilus edulis does not filter in very dilute suspensions, and Bayne & Scullard (1977) showed marked filtration rate increases immediately after feeding starved M. eduits. Whatever the nature of the response of mussels in the low food treatment, it is likely that the filtration rates shown by mussels in the medium and high food treatments were depressed. Based on a number of studies of bivalve filter-feeding, Griffiths & Griffiths (1987) concluded that high food availability leads to decreased filtration rate. Sprung & Rose (1988) showed that for the freshwater mussel Driessena polymorpha filtration rate reduction at high food levels was directly brought about by reduced pumping

In contrast to the effect that food was found to have on filtration rates, no significant differences in filtration were detected after exposure to silt concentrations of up to about 35 mg l^{-1} . As with the food response, however, it may not be reasonable to assume that the response of the mussels during exposure to silt is necessarily the same as that response measured at the end of the experiment; especially as filtration measurements were made in silt-free water. While clearance rate in Spisula subtruncata is unaffected by suspended sediment at concentrations of up to 25 mg l⁻¹ (Møhlenberg & Kiørboe, 1981), increasing suspended solids loads up to 44 mg 1-1 reduced clearance rates in Mercenaria mercenaria (Bricelj & Malouf, 1984). Because of the high correlation between oxygen consumption and filtration rate (Griffiths & Griffiths, 1987), Grant & Thorpe (1991) used respiration as an indicator of ventilation rate. Using this technique they found that exposure to elevated suspended sediments (up to 2000 mg l-1) inhibited the ventilation rate of Mya arenaria. The increase in oxygen uptake of H. menziesi, which was seen with increasing silt concentrations in the absence of food (Table 3, Fig. 2), suggests that filtration rate was being increased. The reason that no response was detected when tiltration rate was measured directly may have been because of the large variability between individuals, or that the incubations had not been run sufficiently long enough to induce a measurable change. Although not significant, mean filtration rate was higher in the high silt treatment (Fig. 1). It is also a possibility that the metabolic cost of pseudofaeces production seen at the high silt concentration was causing increased oxygen consumption.

Filter-feeding bivalves display two main strategies for dealing with suspended sediment: they may reduce clearance rate, or they may eliminate unwanted particles as pseudofaeces (Foster-Smith, 1976). Hyridella menziesi appears to use the latter strategy, as copious pseudofaeces were produced by mussels in the high silt treatments, and to a lesser extent in the medium silt concentrations. Despite this high pseudofaeces production mussels were maintaining food ingestion at an adequate level as condition did not drop in response to increases in silt. This could only have happened if H. menziesi was extremely efficient at selectively rejecting silt particles and/or increasing pumping rate to ensure a sufficient ration was ingested. This supports the conclusion that, based on respiration measurements, filtration rate had been increased in response to exposure to silt. It has been discussed above that in the low food treatments silt appeared to be increasing filtration. Particle selection with preferential ingestion of food particles and rejection of particles without food value as pseudofaeces has been extensively documented for filter-feeding bivalves (Jørgensen, 1990), and is also very likely to occur in H. menziesi. Bricelj & Malouf (1984) suggested that bivalve species which reduced clearance in response to silt will be less successful at exploiting turbid environments, whereas those species which produce copious pseudofaeces and control ingestion primarily by producing pseudofaeces will be better adapted to cope with high suspended sediment loads. Hyridella menziesi appears to be well adapted to living in turbid freshwater environments by utilising the latter strategy.

Oxygen consumption was also found to have increased with food concentration in the low silt treatments (Table 3, Fig. 2). Reports of increases in respiration rate with ration are common for many bivalve species (e.g., Bayne et al.., 1976; Griffiths & King, 1979; Schurink & Griffiths, 1992), and have been attributed to accelerations in filtration rate and the increased metabolic costs associated with increased feeding activity (Bayne & Scullard, 1977). By contrast, no effect of ration level on oxygen uptake could be found in the marine mussel Choromytilus meridionalis (Griffiths, 1980) or the bivalve Spisula subtrun-

cata (Møhlenberg & Kiørboe, 1981). It seems likely that for *H. menziesi* filtration rate actually decreases with increasing food concentration (see above), so that the observed increase in respiration is more likely to be associated with the metabolic costs of feeding and growth, rather than pumping activity.

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Measurement of condition provides a good indicator of physiological stress in shellfish (Goldberg, 1980; Widdows, 1985). It is not known how condition may have changed from the start of the experiment, but after 3 weeks condition was significantly lower in mussels which had not been fed, whereas addition of silt had no effect. Sprung & Borcherding (1991) found that starvation for 31 days in the freshwater mussel *Dreissena polymorpha* resulted in a loss of 23–34% of the original dry tissue weight, and in particular loss of lipid and protein. The fact that elevated silt concentration had no effect on condition confirms that *H. menziesi* was able to maintain an adequate ration, despite having to eliminate silt as pseudofaeces.

Where mussels are being used to assess environmental contamination it is important to understand the factors determining the relationships between contaminant concentrations in the environment compared with those in the mussel tissue. The degree to which both organic and inorganic contaminants are accumulated can be influenced by biotic factors, including pumping activity, growth, biochemical composition, reproductive condition and metabolism (Widdows & Donkin, 1992). Uptake of organic compounds is generally by direct diffusion across external membranes, and while the mechanisms involved in metal accumulation are unclear (Viarengo, 1989) direct uptake from the surrounding water is still important (see review in Widdows & Donkin, 1992). For this reason, rates of contaminant accumulation are closely linked to the volume of water a mussel processes, and can be summarised in terms of a bioenergetics-based model (e.g., Boese et al., 1988). In experiments with H. menziesi an indication of the amount of water processed was gained from measurements of filtration rate (based on the removal of particles from solution) rather than pumping rate. Filtration rate is closely related to pumping rate but is influenced by efficiency of particle retention (Griffiths & Griffiths, 1987). It is not known exactly how pumping relates to filtration rate in H. menziesi, although it can be assumed that changes in filtration will to some extent be the result of changes in the amount of water being processed. Dreissena polymorpha has been shown to reduce filtration by reducing pumping rate (Sprung & Rose, 1988). Lack

of food seemed to increase filtration, or conversely satiation reduced filtration and, in starved mussels, silt was probably increasing filtration. It is likely therefore that through their effect on filtration (and presumably pumping rate) food and silt concentrations could influence contaminant accumulation. In low-food situations (i.e., <5 mg l⁻¹) if mussels are pumping large volumes of water to obtain sufficient food, contaminant uptake rates could be enhanced, whereas abundant food would result in lower pumping rates and lower uptake rates. However, as there was no significant difference between filtration rates in medium and high food treatments, the potential effect of filtration on contaminant accumulation may only be important at low food concentrations (i.e., $<5 \text{ mg l}^{-1}$). Also, when food levels are low, increases in silt will stimulate pumping and increase the potential for contaminant uptake.

The marked increase in metabolism seen as higher respiration rates in association with increasing food also has implications for bioaccumulation. Metabolism can affect elimination of some bioaccumulated compounds in bivalves (Widdows & Donkin, 1992).

Finally, the physiological condition and biochemical composition of a mussel can influence bioaccumulation by affecting the tissue distribution and retention of contaminants (Widdows & Donkin, 1992). It is likely therefore that the changes in condition, and presumably biochemical composition, which were seen after exposure to different food levels for 3 weeks would have definite consequences for bioaccumulation. The exact nature of this effect, however, would depend upon the biochemical substrate metabolised during starvation, which may involve carbohydrate, lipid and/or protein depending upon the time of year and length of starvation (see review in Sprung & Borcherding, 1991).

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