LIPID CONTENT AND CONDITION IN AN ESTUARINE TELEOST

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ABSTRACT

The value of lipid content as a measure of the feeding level of fish compared with condition factor was assessed using two populations of juvenile ***Rhabdosargus holubi***, one from the closed West Kleinemond estuary and the other from the open Kowie estuary. Results from feeding experiments conducted in the laboratory showed that total lipid level could be related to feeding level, and was not affected by temperature although food intake at 15°C was half that at 22°C. Condition factor was affected by temperature. ***R. holubi*** from the West Kleinemond estuary showed a seasonal variation in lipid content (6,4% in summer, 0,75% in winter) but those from the Kowie estuary showed little or no variation (1–2%). It is suggested that the difference in the fat content of the fish between the two estuaries in summer was related to the lipid content of the food, which was much less in the Kowie estuary. The condition factor of West Kleinemond fish declined in winter while that of Kowie fish remained stable. It is considered that condition factor is a more sensitive index of the actual feeding level of fish than the lipid content since the latter may be affected by the lipid content of the food.

INTRODUCTION

Since weight but not length of fish usually varies according to the feeding level or reproductive state, assessments of condition, "well-being", or "fatness" of fish have generally employed measurements of the length – weight ratio. These condition factor analyses have long been used in fisheries biology as a relatively rapid means of assessing factors such as the feeding level in one system as opposed to another. More recently Brett *et al.* (1969) showed experimentally, using *Oncorhynchus nerka* Walbaum, that the fat fraction of the body can be used as a measure of the feeding level of a fish without particular reference to temperatures. He went on to state that fat assessment should be of value to the ecologist as a means of assessing the general feeding level of any particular population, providing that metamorphic changes are taken into account. The main function of lipids is as an energy reserve and, according to Tashima & Cahill (1965) lipids in fish are stored at or near the sites of utilization since there is no specialized adipose tissue as in birds and mammals. The oxidation of fatty acids provides for sustained muscular contractions such as are used in normal swimming activity, while glycogen is the major source for violent and sudden muscular activity.

Since lipid levels are not affected by short-term stress (Love 1970), as are for example carbohydrates such as glycogen, it was decided to compare determinations of the total lipid content of the body with condition factor analyses in order to evaluate the feeding level of juvenile *Rhabdo-*

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sargus holubi (Steindachner) (Teleostei: Sparidae) in the closed West Kleinemond estuary (27° 02′E/32° 02′S) in relation to that of similar fish captured in the open Kowie estuary (26° 54′E/33° 36′S). As only juvenile fish were involved in the investigation, the results of lipid analyses and condition factor analyses were not complicated by cyclical changes in lipid level and weight, associated in adults with the formation of gametes.

Experiments to determine the effect of feeding level on the total lipid content and condition of juvenile *R. holubi* were undertaken in the laboratory in order to provide some basis on which to judge results obtained from animals collected from estuaries.

MATERIALS AND METHODS

1. Experimental studies

All experiments were conducted at a salinity of 35‰ in 225ℓ asbestos tanks lined with inert epoxy resin or in a 400ℓ fibreglass tank. Fish between 6 and 14 cm standard length, captured by seining in the West Kleinemond estuary were transported to the laboratory in plastic bags supplied with aeration and transferred directly to the experimental tanks. 'Tetramin' brand tablet fish food was supplied and most newly captured fish began feeding within 24 hours. Experiments were commenced one week after capture. Ten fish, all of the same batch, were used in each experiment. The experiments were performed between March and June 1973.

(a) Starvation experiments: Groups of fish were starved for two, four and eight weeks at 22°C before being killed and analyzed.

(b) Feeding experiments: Two groups of fish held at 22°C were fed to excess twice daily for seven weeks, one group on 'Tetramin' brand tablet fish food and the other on the small shrimp *Palaemon pacificus* Stimps., collected from the east lagoon of the Kowie estuary. Excess uneaten food was removed after one hour. One further group of fish was held at 15°C and fed to excess twice daily for seven weeks on 'Tetramin' brand tablet fish food.

2. Sampling of fish from West Kleinemond and Kowie estuaries

Ten *R. holubi* were collected from each estuary once a month from December 1972 to October 1973 by seining. Fish were immediately placed in crushed ice and subsequently deep frozen to minus 25°C within about eight hours of capture. All fish captured were between 6 and 14 cm standard length.

3. Determination of total lipid content

Total lipids were determined for each fish by the Groves procedure for dry extraction of fat (Brett *et al.* 1969) using n-hexane.

The fat content of the food items of *R. holubi* was determined in a Sohxlet apparatus using anhydrous diethyl ether extraction. n-Hexane was not used in these determinations as it was found to contain traces of an unknown impurity. This impurity did not affect the results of the Groves method which relied upon the difference in weight between dried fish and fat-free dried fish, but could have influenced the results from the Sohxlet method where the fat itself was collected and weighed.

4. Condition factor analyses

The relationship between length and weight provides the basis on which condition factors may be calculated. The formula used for calculating condition factor was:

$$C.F. = \frac{W \times 100}{L^b}$$

The exponent b was obtained from the relationship between length and weight and in the case of juvenile *R. holubi* has the value 2,8512 (Blaber 1974a). There is no change in the length–weight relationship of *R. holubi* between 5 and 15 cm S.L. (Blaber 1974a) thus the value of 2,8512 could be used to calculate the condition factor of fish from both the West Kleinemond and Kowie estuaries in addition to the fish kept under experimental conditions in the laboratory.

RESULTS

LIPID ANALYSES

1. Experimental studies

(a) Starvation experiments: The total lipid and water content of juvenile *R. holubi* held for two, four and eight weeks without food at a salinity of 35‰ and a temperature of 22°C are shown in Table 1. Although each batch consisted of individuals collected on the same occasion, different batches were used for each of the experiments and as shown in Table 1 their initial fat and water values were different, therefore the percentage change in fat and water content are shown in Table 1. There was a significant inverse relationship between the fat and water content of the body ($r = 0{,}599$; d.f. $= 32$; $P < 0{,}001$), the sum of the two con-

TABLE 1

The total lipid and water content of three groups of *R. holubi* starved for three different periods of time (g/g = gram per gram body weight.)

	LIPID				WATER			
Period of starvation	*x̄ initial lipid g/g*	*x̄ lipid after starvation*	*S.E.*	*% change in lipid*	*x̄ initial water g/g*	*x water after starvation*	*S.E.*	*% change in water*
2 weeks	0,037	0,026	0,003	−31	0,79	0,77	0,004	+2,0
4 weeks	0,050	0,029	0,005	−43	0,80	0,76	0,004	+5,3
8 weeks	0,065	0,043	0,006	−34	0,83	0,77	0,008	+7,2

stituents usually representing about 80% of the total body weight. A relationship between the fat content and the size of the body has been reported in *Anguilla anguilla* L. and *Trachurus trachurus* L. (Lovern 1938; Arevalo 1948), but this relationship did not exist in the size group of *R. holubi* used throughout this investigation. It is possible, however, that it may be present in a wider size range of *R. holubi* than was used in this study.

(b) Feeding experiments: The lipid and water content of fish fed to excess are shown in Table 2. There was no significant difference in total lipid content between 'Tetramin' fed fish kept at 15°C and those at 22°C ($t = 1{,}98$; d.f. $= 14$; $P > 0{,}05$). There was, however, a significant difference between the two groups of *R. holubi* kept at 22°C. Those fed on 'Tetramin' tablets had a significantly higher fat content than those fed on *P. pacificus* ($t = 2{,}92$; d.f. $= 17$; $P < 0{,}01$). The total lipid content of the food items was therefore determined and is also shown in Table 2. The lipid content of 'Tetramin' tablets was found to be 17% of dry weight while the lipid content of *P. pacificus* was only 3% of dry weight. The latter determinations were made on winter *P. pacificus*, but the fish were fed on living summer *P. pacificus* whose fat content may have been somewhat higher.

Both groups of *R. holubi* which were given 'Tetramin' tablets were fed to excess and it is important to note that although those kept at 15°C consumed less than half the amount of food of those kept at 22°C (Table 2), their lipid content after seven weeks was similar. This would appear to support the statement of Brett *et al.* (1969) that the fat fraction reveals the feeding level of the fish irrespective of temperature.

2. Results from the West Kleinemond and Kowie estuaries

Figure 1 shows the total lipid and water content of juvenile *R. holubi* from the two estuaries from December 1972 until October 1973. In the West Kleinemond estuary the lipid levels of

TABLE 2

The total lipid and water content of three groups of fish after feeding to excess twice daily for seven weeks. (g/g = gram per gram body weight; d.w. = dry weight.)

Temperature °C	*Food*	*$\bar{x}$ ration g/g d.w.*	*$\bar{x}$ lipid content of food g/g d.w.*	*$\bar{x}$ lipid content of fish*	*S.E.*	*$\bar{x}$ water content g/g*	*S.E.*
15	Tetramin	0,05	0,17	0,069	0,008	0,71	0,009
22	Tetramin	0,13	0,17	0,084	0,004	0,69	0,004
22	*Palaemon*	0,10	(0,03)*	0,065	0,005	0,70	0,004

* Determined from winter captured *P. pacificus* – fish were fed summer individuals.

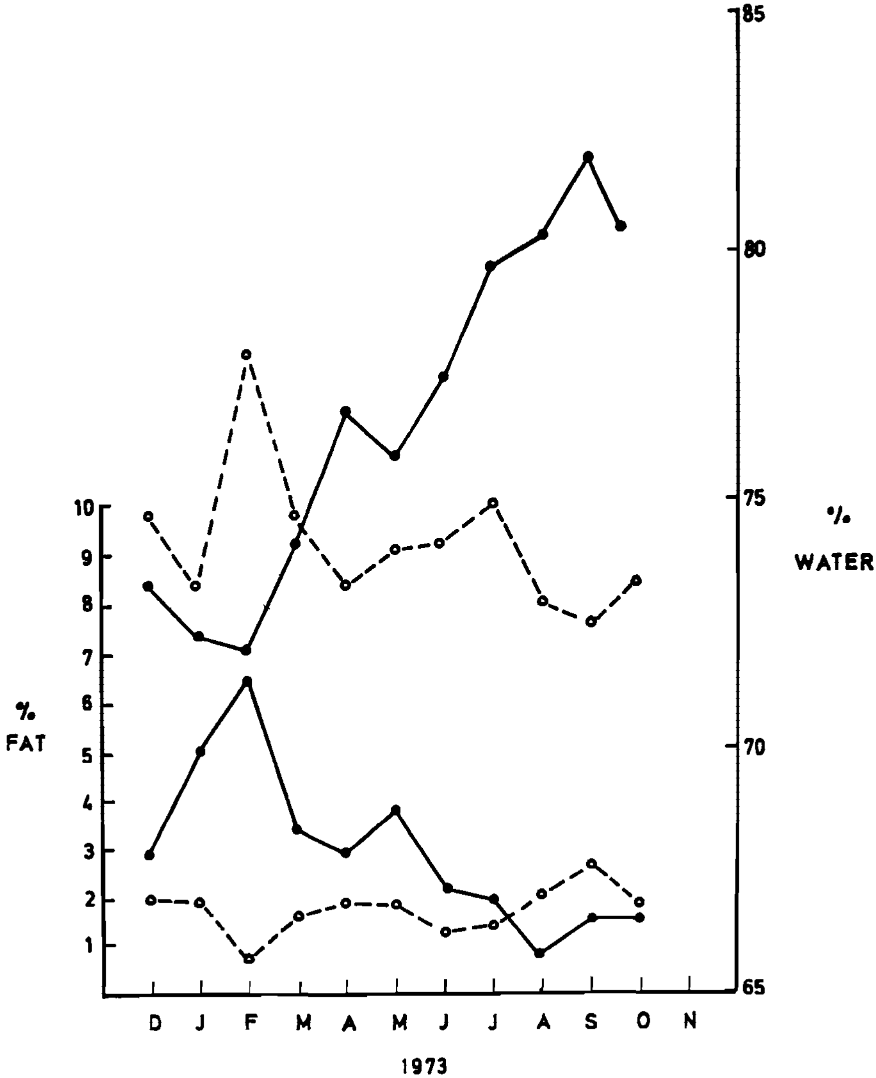

FIGURE 1
Mean monthly lipid content (below) and water content (above) of *Rhabdosargus holubi* during 1973 from the West Kleinemond (——) and Kowie estuaries (- - -).

R. holubi were at a maximum in summer (6,4 %) and a minimum in winter (0,75 %). Fish from the Kowie estuary showed little seasonal variation, their lipid level fluctuating between 1 % and 2 %. The results from the West Kleinemond estuary are similar to those obtained by Arevalo (1948) working on *Trachurus trachurus* L. in which there was a maximum lipid level (9 %) reached at the end of summer and a minimum lipid level (1,5 %) at the end of winter. The inverse relationship between fat and water which was present in the laboratory fish also occurred in the *R. holubi* from the two estuaries. Lipid analyses of epiphytic diatoms, a major food item of juvenile *R. holubi* (Blaber 1974b), taken from *Ruppia spiralis* from the West Kleinemond estuary showed they had a mean lipid content of 9 % dry weight. Epiphytic diatoms from red algae from the Kowie estuary had a mean lipid content of only 1,36 % dry weight.

CONDITION FACTOR ANALYSES

1. Experimental studies

Condition factor reflects the feeding level of the various groups (Table 3). Starved fish held at 22°C had a very reduced condition factor when compared with fish fed to excess at 22°C. The group which were fed to excess at 15°C showed a lower condition factor than those fed to excess at 22°C, reflecting perhaps the lower food intake of the 15°C fish, although as previously stated the two groups had similar lipid levels.

2. Results from the West Kleinemond and Kowie estuaries

The mean condition factors of *R. holubi* captured in the West Kleinemond estuary from 1971 until 1973, and in the Kowie estuary in 1973 are shown in Figure 2. There was a seasonal fluctuation in the condition factor of West Kleinemond fish: their condition factor fell off in winter to a minimum value in August and was at a maximum in summer (December–January). *R. holubi* from the open Kowie estuary showed little seasonal change in condition factor which remained at a similar level to that of summer fish from the West Kleinemond estuary.

TABLE 3

The condition factor of the three groups of starved fish and the three groups of fish fed to excess.

Treatment	*Temperature °C*	*Duration of experiment*	$\bar{x}$ *Condition factor*	*Standard error*
Starved	22	2 weeks	52	2,0
Starved	22	4 weeks	56	1,0
Starved	22	8 weeks	51	1,0
Fed 'Tetramin'	22	7 weeks	70	1,0
Fed 'Tetramin'	15	7 weeks	59	2,0
Fed *Palaemon*	22	7 weeks	64	1,0

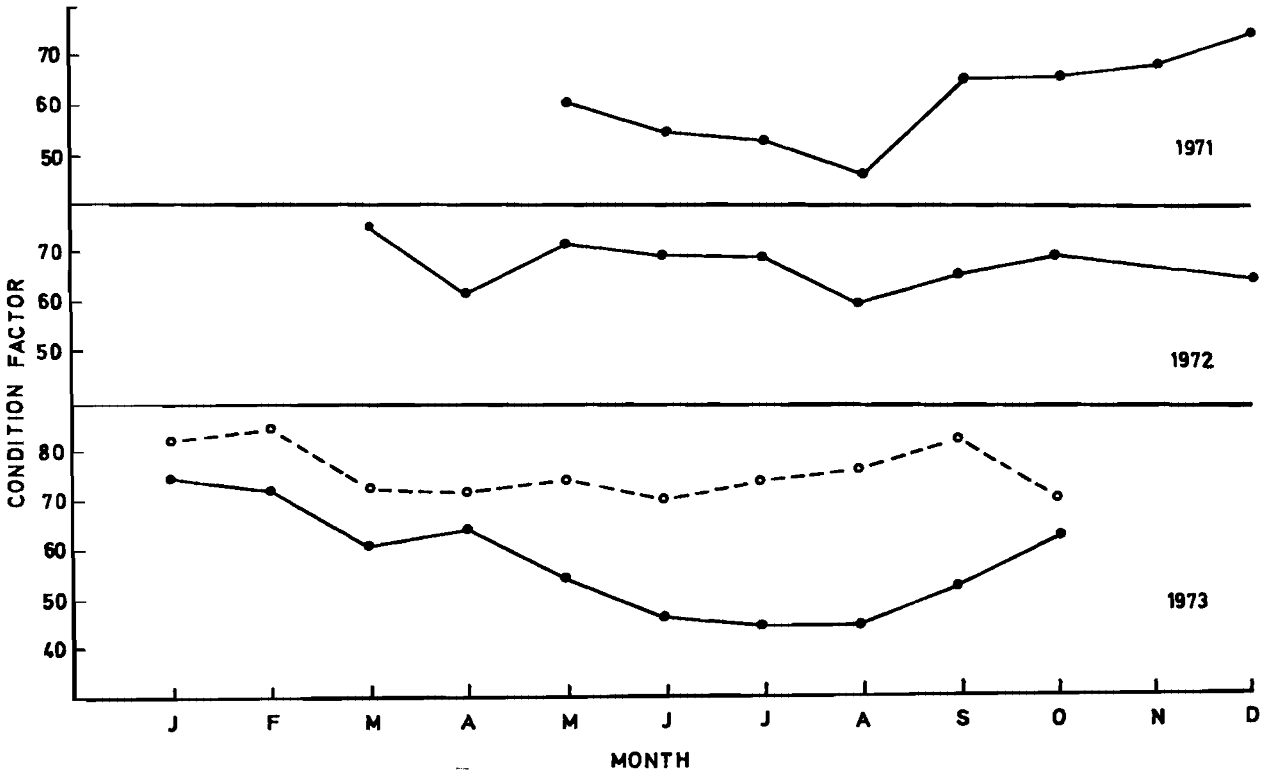

FIGURE 2

Condition factor of *Rhabdosargus holubi* in the West Kleinemond estuary (——) from 1971 to 1973 and in the Kowie estuary in 1973 (– – –). Mean value for each month shown. (Standard error between 1,0 and 2,0 in all months.)

DISCUSSION

The striking inverse relationship between the lipid and water fractions of the body of *R. holubi* has been demonstrated in other species by several workers (Brandes & Dietrich 1956; Herrera & Munoz 1957; Coppini 1967). As the lipid falls so the amount of water apparently increases and *vice versa*. Blaxter & Holliday (1963) have shown that the reason for this relationship is that most measurements have been based on percentage changes, as was the case in this investigation. Where there is an absolute fall in the fat content the percentage of other substances, such as water, must rise relative to total body weight. In *R. holubi* the fat and water together make up about 80% of the body weight, a value similar to that reported by Iles & Wood (1965) for *Clupea harengus* L. Although percentage changes in the water content of the body of fish may be misleading, the water content can be analyzed relatively quickly, and provides a useful check on the accuracy of lipid analyses if the total of the two constituents is known.

The analyses presented here show that the lowest lipid levels were reached after four weeks of starvation. After eight weeks the proportion of lipids present had risen. The percentage water content, however, reached a maximum at eight weeks when it appeared that the lipid content had also risen. It is likely that the increase in the proportion of lipids at eight weeks was due to the decrease in some other constituent such as protein, this suggestion is supported by the fact that the amount of water continued to increase. Starvation thus seems to reduce the total lipid content of the body, but only up to a certain point, beyond which other body components, possibly protein, may be utilized. Wilkins (1967) found that when *C. harengus* were starved, all the neutral triglyceride fat was metabolized but a residual amount of fat was always present in the body, representing structural and functional elements essential for survival. It appears possible from the experimental part of this study that a similar situation may have existed in *R. holubi* kept under starvation conditions.

R. holubi which were fed to excess in the laboratory showed a markedly higher lipid content than the individuals which were starved. Edwards *et al.* (1972) found that the relative proportions of lipid and protein did not change in relation to feeding level in *Gadus morhua* L. since energy is apparently not stored as lipid in the cod. It does seem, however, that in the experimental *R. holubi* the lipid content was dependent upon feeding level, but not upon temperature. Although the amount of food consumed decreased with temperature, the lipid content remained the same (Table 2).

The seasonal trend in lipid content in *R. holubi* from the West Kleinemond estuary appears to be absent from Kowie fish. The fat content of fish from both estuaries fell below that of laboratory fish which had been starved. Evidently the Kowie fish which had a consistently low lipid content were not starving throughout the year since their condition factor was always relatively high, at a similar level in fact to fish which had been fed to excess in the laboratory at 22°C (Table 3). The reasons for the all-year-round low lipid levels in the Kowie estuary may be connected with the food supply, which although not deficient in quantity may have been low in lipid content. It has been shown that epiphytic diatoms are important food items of *R. holubi* (Blaber 1974b): the diatoms from the West Kleinemond estuary contained 9% fat while those from the Kowie estuary contained only 1,36% fat. As shown in Table 2, the lipid content of *R. holubi* is directly related to the lipid content of the diet. If Kowie fish were feeding predominantly on food containing only 1,36% fat while the West Kleinemond fish were consuming diatoms with a relatively high fat content of 9% it would be expected that there would be a difference in lipid content of the fish. The reasons for the lipid content of the diatoms in the two estuaries being different are not easily explained. It has been suggested, however, that the amount of oil present in diatoms may reflect their nutritional state (Morris 1967). It thus appears possible that there was a relationship between the total lipid content of the food and the total lipid content of the body of *R. holubi*. This does not explain, however, why the lipid levels of Kowie fish (throughout the year), and West Kleinemond fish (in winter) should be lower than those of fish which had been starved for up to two months in the laboratory. Love (1970) states that the greatest care must be used in interpreting values obtained from fish kept in the laboratory, a number of changes in the various constituents of the body may take place after capture, for example the proportions of proteins may change in newly caught fish. The condition factor analyses show a more uniform and constant picture than the lipid analyses. As with the lipid content the condition factor of Kowie fish remained relatively stable throughout the year, at a level similar to that of fish fed to excess at 22°C. During summer the condition factor of West Kleinemond fish was also at a similar high level, but during winter declined to the level reached by fish fed to excess at 15°C. Evidently the actual amount of food eaten affects the condition factor, and since the temperature also affects the amount of food eaten then it too will influence the condition factor. These results therefore indicate that West Kleinemond fish carried on feeding throughout the winter but at a considerably reduced level, while the *R. holubi* from the open Kowie estuary fed at a similar level all year round.

The condition factor shows little correlation with the lipid content of the body which may perhaps be controlled far more by the lipid content of the food than the amount of food. Thus lipid content may perhaps be used as an index of the amount of lipid in the food while the condition factor may reflect the quantity of food eaten. The fall in condition and lipid level in the fish from the West Kleinemond estuary in winter suggests both a reduction in the quantity of food eaten and a reduction in the lipid content of the food. The former is consistent with the laboratory findings that a decline in temperature causes a decline in food consumption and a decline in condition. The latter suggests that the oil content of the diatoms may be lower in winter.

The winter temperatures in the West Kleinemond estuary remain consistently as low as 11–12°C for at least one month, with a daily fluctuation of only 1–2°C (Blaber 1973). It is possible that these low temperatures in the West Kleinemond estuary, which are only a few degrees above the lethal limit of juvenile *R. holubi* may perhaps reduce feeding to a level low enough to cause a drop in the condition factor as well as total lipid level. *R. holubi* in the open Kowie estuary do not lose condition or suffer a drop in their total lipid content during winter. Daily temperatures in the Kowie estuary in winter range between 11 and 16°C, the high tide causing a rise in temperature to at least 15°C (Hill & Allanson 1971). Since at least one temperature peak always occurs in daylight hours, the fish may be able to feed at an increased level during the time the water is at a relatively high temperature. This would create the situation where feeding would occur at least once every 24 hours, but for much of the remainder of the time, when the temperature falls to around 11°C, metabolic activity would be very low. Thus the amount of food consumed during the warmer part of the tidal cycle may be sufficient to maintain the condition factor and lipid level, taking into account the fact that metabolic activity would fall very low for half the day.

Thus, although it is possible to relate lipid changes to changes in the quantity of food eaten under laboratory conditions, it is evident that the lipid content of *R. holubi* under natural conditions cannot be used to assess the level of feeding without prior knowledge of such factors as the lipid content of the food and environmental temperatures. In this study it was not possible to relate the amount of lipid in the body with the feeding level of fish from the two estuaries. In fact, condition factor analyses provided more information on the probable level of feeding. The use of total lipid content alone to compare the feeding level of the fish from the two estuaries would have indicated for example that the Kowie fish were continually starving. Although lipid analyses proved useful in showing up the possible differences in the foods from the two estuaries, condition factor analyses showed at what level feeding was taking place.

Lipid analyses may provide useful information on feeding level in fish kept under controlled conditions in the laboratory, where all environmental parameters are known, but their value in field studies is extremely limited. It is not practical to monitor all the continuously variable factors which are likely to affect the lipid content of fish in a natural system, especially a marine or estuarine one. The complexity of lipid analyses compared with condition factor analyses, together with the difficulty of interpreting lipid content, indicate that lipid analyses are not a practical means of assessing the feeding level of fish populations.

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