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Changes in the Protein and Lipid Content of Muscle, Liver and Ovaries in Relation to *Diphyllobothrium* spp. (Cestoda) Infection in Powan *(Coregonus lavaretus)* from Loch Lomond, Scotland

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Abstract: The present study was carried out to examine the relationship between helminth infections, nutrient reserves and gonadal status in powan in relation to infection with *Diphyllobothrium* spp. The relationship between fish size and body composition was also investigated. The condition factor increased with increasing length for uninfected fish and decreased for infected fish. This demonstrates the negative effect of infection on the growth of the fish. The mean percentage of protein and lipid content of muscle, liver and gonads were 89 %, 71.7 % and 64.9 % and 5.03 %, 23.3 % and 14.5 % respectively. A statistically significant correlation appeared between the length of the fish and liver protein, gonad protein and the muscle lipid content of the fish. There was no difference between the body composition of male and female fish. There was a positive correlation between length and gonad lipid in uninfected fish. A negative correlation between muscle lipid and gonad lipid and liver lipid and gonad lipid showed that while fish build up gonads they use energy reserves from muscle and the liver. Fish in good condition had a high protein content in their gonads.

Key Words: Infection, *Diphyllobothrium* spp., *Coregonus lavaretus*, lipid, protein, muscle, liver, ovaries.

Lomond Gölü'nden (İskoçya) Avlanan Beyaz Balık *(Coregonus lavaretus)*'da Kas, Karaciğer ve Ovaryum Protein ve Yağ İçeriğinin *Diphyllobothrium* spp. Enfeksiyonuna Bağlı Olarak Değişimi

Özet: Bu çalışma, *Diphyllobothrium* spp. ile enfekte olan beyaz balıkda helmint enfeksiyonu, besin depoları ve gonat durumu arasındaki ilişkiyi ortaya koymak için yapıldı. Ayrıca balık büyüklüğü ve vücut bileşimi arasındaki ilişki de araştırıldı. Balığın kondisyonu enfekte olmayan balıklarda artan uzunluğa bağlı olarak artarken, enfekte olan balıklarda azaldı. Bu sonuç enfeksiyonun balığın gelişimi üzerindeki olumsuz etkisini göstermektedir. Kas, karaciğer ve gonatlardaki protein ve yağ'ın ortalama yüzdesi sırasıyla %89, %71.7, % 64.9 ve %5.03, %23.5, %14.5 bulundu. Balığın karaciğer ve gonatlarındaki protein ve kaslardaki yağ oranı ile balık uzunluğu arasında istatistiksel olarak anlamlı bir korelasyon görüldü. Dişi balık ile erkek balık vücut bileşimi arasında fark yoktu. Enfekte olmayan balıkların uzunluğu ile gonatlarda yağ oranı arasında pozitif bir korelasyon vardı. Kaslarda yağ ve gonatlarda yağ; karaciğerde yağ ve gonatlarda yağ oranları arasındaki negatif korelasyon, balığın gonatları gelişirken kas ve karaciğerden enerji kaynaklarını kullandığını gösterir. Kondisyonu iyi olan balığın gonatlarında yüksek miktarda proteine rastlandı.

Anahtar Sözcükler: Enfeksiyon, *Diphyllobothrium* spp., *Coregonus lavaretus*, yağ, protein, kas, karaciğer, ovaryum.

Introduction

The main sources of energy reserves in fish are protein and lipid, in contrast to mammals in which carbohydrate and lipid are more important. This is perhaps due to the following factors: (I) the diet of fish generally consists of high protein, and the fish metabolism is well adapted to deal with such a diet; (II) unlike mammals, fish have the ability to eliminate nitrogenous waste rapidly and continuously; (III) specific activities of lysosomal enzymes which are involved in protein breakdown are greater in fish than in mammals (5).

The relative contributions of lipids and amino acids to energy production in fish depends on a number of factors such as the species involved, nutritional state and environmental temperature. In salmonids, during routine activity, more than 40 % of energy production is considered to be due to amino acid catabolism (6). In healthy fish, protein content (when consider as a percentage of body weight) tends to be relatively constant for a given species. In general, there is no significant seasonal variation in the protein content of fish, but during periods of starvation and gonad development, non-fatty fish draw on their carcass protein. The concentration of lipid varies considerably in different parts of the body of the fish (12). The lipid content in the body of the fish changes depending on the time of the year (5,8), environmental conditions (3, 11), stage of maturity of the gonads (7,23), state of nutrition

(9) and age (17). Lipid is not the major energy store in all fish. For example, in the northern pike, *Esox lucius*, endogenous lipid is not considered to be an important source of energy because of its low concentration in the muscle; instead endogenous protein serves as the main energy substrate in the metabolism.

Germinal tissue can require a significant proportion of the total annual production in sexually mature fishes. In females, mature ovaries that are rich in protein and lipid can reach 15 % total body weight. In many spring-spawning fish, such gonads grow during the winter when food supplies are limited and feeding is reduced. Therefore, nutrients for germinal tissue growth may need to be drawn from somatic tissues (15).

The previous section has demonstrated that gonadal maturation represents a serious drain of energy for many fish. Parasitic worms compete for energy reserves with their fish host (14,20,21) and may also impair foraging in a number of ways (16,20). Meakins (14) found that during the breeding season, the mean gonadosomatic index of stickleback females infected with *Schistocephalus solidus* was depressed to around half of that found in uninfected females. He postulated that this was the result of an energy deficit in infected sticklebacks. Milinski (16) reported the influence of parasites on foraging decisions by altering the competitive ability of fish. Since *Schistocephalus solidus*-infected sticklebacks are often in poorer condition (18) than uninfected sticklebacks, Milinski (16) predicted that they would also be poorer competitors for food. Indeed, he found that parasitized sticklebacks attacked less profitable prey. Tierney (20) argued that if harbouring *S. solidus* does influence stickleback foraging decisions, then field data should highlight dietary differences between infected and uninfected sticklebacks. She found that the stomachs of infected sticklebacks were less full their uninfected counterparts in winter and summer. Therefore, it is likely that parasitic infections may compromise gonadal development. This is indeed the case in some species (14). However, there is clear variation among hosts and parasites in the extent of such effects. This may be due to differences in the extent to which the parasite compromises nutrient reserves and the extent to which gonadal maturation depends on these. The overall aim of the present study was to examine the relationship between helminth infections, nutrient reserves and gonadal status in powan in relation to infection with *Diphyllobothrium* spp.

Materials and Methods

Powan, *Coregonus lavaretus,* were collected on six occasions between 12th of May and 23th of August 1995 from Loch Lomond. In order to eliminate the effects of seasonal changes on the body composition of the fish, the sampling period was kept short. The effect of parasitism on protein concentration was investigated in fish caught on the same sampling date. The parasite status and sex of the fish according to sampling date are given in Table 1. Tissue samples taken from the muscle, liver and gonads of the powan were frozen and dehydrated by freeze-drying for 24 hours in an Edward EF03 freeze drier. The dried tissue was then ground into a powder and used for protein determination.

Protein determination

In the current study, the results were obtained using a simplified and more generally applicable protein assay, modified by Peterson (19) from the method of Lowry *et al*. (13).

Lipid extraction and quantitation

Extraction techniques using organic solvents for a minimum period of 24 h at 4°C can be used for quantitative determination of lipids. A simple method for

	Fish no				
	Infected		Uninfected		Mean intensity
Date	M	F	M	F	of insection
12/5/1995	1	1	–	–	1.5
19/5/1995	4	4	–	2	11.4
22/5/1995	–	1	–	–	7.0
27/5/1995	1	1	3	4	1.5
16/8/1995	2	5	4	2	6.4
23/8/1995	1	–	2	2	3.0

Abbreviations: M: male; F: female.

Table 1. Sex and infection status of powan obtained from Loch Lomond in summer 1995.

the extraction of total lipids is based on homogenizing the tissue with a 2:1 (by volume) chloroform-methanol mixture (10). Lipid values have been obtained by many workers by adapting this basic technique (2,11,15).

Day 1. 50 mg muscle tissue and 25 mg gonad or digestive tissue were weighed into 60 ml reagent bottles.

For the calibration curve, 35 mg cholesterol was weighed and 35 ml 2:1 v/v chloroform/methanol was added. 0, 2, 4, 6, 8, 10 ml standard was added to bottles, and then made up to 10 ml with chloroform/methanol mixture. 25 ml C/M mixture was added to each bottle and left for 1 hour. 5 ml NaCl was added and the bottles were stored overnight at 4 °C.

Day 2. Preparation of phosphovanilline reagent: 0.9 g vanillin was dissolved in 450 ml 80 % orthophosphoric acid (ie. 360 ml acid + 90 ml distilled water).

0.5 ml samples were extracted from the bottom layer of the bottles using a Finn-Pipette and placed into numbered test tubes which were then plugged with cotton wool. The samples were evaporated in a vacuum oven at 60 °C maximum using a water pressure-operated vacuum pump. After 3 hours, the dried samples were allowed to cool, 0.5 ml H_2SO_4 (concentrated) was added and the test tubes were plugged. The samples were placed into a bath of boiling water (100 °C) for 10 minutes and then cooled under running water. When cooled, a 0.2 ml sample was transferred to a clean test tube and 5 ml sulphophosphovanillin reagent added. Tubes were stirred using a whirly mix and allowed to stand for 30 minutes (with one more stir half-way through) and then the absorbance of the solution was read at 520 nm using a spectrophotometer.

The formula for determination of the condition factor is as follows:

$$C = \frac{W}{L^3} \times 10^2$$

C = Condition factor

W = Weight of fish in grams and

L = Fork length of fish in centimetres (23).

Statistical analysis

The mean fish size, protein and lipid reserves in the muscle, liver and gonads of powan were calculated. The data were checked for normality and transformed where necessary. For normally distributed variables, parametric statistics were used and for non-normally distributed variables non-parametric statistics were used.

Relationships between various nutrition reserves were examined by means of Product Moment Correlation Coefficient using MINITAB Statistical Software Release 10. Body compositions in male and female fish and between infected and uninfected fish were compared using the T-test. An analysis of covariance (ANCOVA) was used to investigate the relationships between protein and lipid reserves and the body lengths of male versus female and infected versus uninfected fish. The relationships between body composition and length or parasite load were investigated by regression analysis.

Results

Table 2 shows the mean (±SE) values for protein and lipid in the 3 body compartments under investigation. The mean percentage protein in muscle (89.0±1.10) was higher than in the gonads (71.7±0.57) and in the liver (64.9±0.82). Powan liver had a high lipid content (23.3±0.52) compared with the gonads (14.5±0.45) and the muscle (5.03±0.38). Table 3 shows the correlation coefficients for protein and lipid in the three body compartments and the condition factor. In general, the levels of protein and lipid in each compartment were negatively related. While protein content increased in the body parts of powan, lipid content decreased. The condition factor was positively related to gonad protein and liver lipid. Fish in good condition had high protein in the gonads and high lipid in the liver. The percentage of protein in the gonads of the fish was negatively related to the percentage of protein in the muscle of the fish. While the protein level in powan gonads increased, the protein level in the muscle decreased.

The relationship between length, condition factor and the various measures of body composition (protein and lipid) in uninfected fish are given in Table 4. There was only one statistically significant relationship, namely a positive association between the length of the fish and the

Table 2. Mean (±SE) values of protein and lipid of powan from Loch Lomond.

Variables	(%) Mean ±SE
Muscle protein	89.0±1.10
Liver protein	64.9±0.82
Gonad protein	71.7±0.57
Muscle lipid	5.03±0.38
Liver lipid	23.3±0.52
Gonad lipid	14.5±0.45

Table 3. Correlation coefficients and levels of significance between protein and lipid reserves and condition factor of fish (N=40).

LP	GP	ML	LL	GL	CF	
0.210	-0.384*	-0.831**	-0.060	0.281	-0.029	MP
	-0.410**	-0.035	-0.349*	0.312*	-0.264	LP
		0.203	0.283	-0.523**	0.506**	GP
			0.131	-0.292	-0.157	ML
				-0.207	0.444**	LL
					-0.168	GL

Abbreviations: MP: muscle protein; LP: liver protein; GP: gonad protein; ML: muscle lipid; LL: liver lipid; GL: gonad lipid and CF: condition factor. (**): P<0.01; (*): P<0.05 (Product Moment Correlation).

Table 4. Relationships between length and body composition of uninfected fish.

	Equation	F	df	P
Muscle protein	MP=7.07+0.68 Length	4.34	1,17	0.053
Liver protein	LP=67.0-0.99 Length	0.04	1,17	0.842
Gonad protein	GP=8.95-0.35 Length	0.83	1,17	0.376
Muscle lipid	ML=4.25-0.60 Length	1.46	1,17	0.243
Liver lipid	LL=2.46+0.74 Length	13.03	1,17	0.002
Gonad lipid	GL=3.07+0.13 Length	1.44	1,17	0.247
Condition factor	CF=0.45+0.22 Length	2.85	1,17	0.110

Abbreviations: Equation: regression equation; F: calculated F value; df: degrees of freedom; p: probability value.

lipid content of the liver ($F_{1,17}$=13.03, p<0.05). A marginally significant positive relationship was found between muscle protein and the length of the fish. In other words, the percentage of muscle protein increased with increasing fish length ($F_{1,17}$=4.37, p<0.05). No statistically significant association was found between the length of the fish and protein contents of the liver and gonad and the lipid contents of muscle and gonad and condition factor of fish.

Figure 1 shows a positive correlation between the length of the fish and percentage lipid in the liver of infected fish. There were also highly significant correlations between the length of the fish and lipid in the liver of uninfected fish. 50 % of male powan (9/9) and 54.5 % of females (12/10) were infected with *Diphyllobothrium* spp. These proportions were not statistically different (χ^2=0.775, df=1, p>0.05).

The mean (±SE) for all morphological (condition factor, length and weight) and biochemical measures (protein and lipid content of muscle, liver and gonads) for male and female powan are given in Table 5. No differences were observed in any variables between male and female fish (p>0.05). Table 6 summarises the results of ANCOVA for the indices of body condition and length in males versus females. No statistically significant differences were found in any measurements (p>0.05). Data from the males and females were therefore combined for subsequent analysis. The number of worms increased with the increasing length of the fish.

Table 7 shows the means (±SE) of morphological and biochemical variables in infected and uninfected powan. Infected fish were longer (and heavier, although not significantly so) than uninfected fish. Their condition factor was marginally lower (T=1.92, df=31, p=0.57). The percentage of protein and lipid in gonads of uninfected powan was significantly higher than in infected fish (T=4.35, df=35, p<0.01; T=2.71, df=37, p=0.01). Surprisingly, the percentage of protein in the muscle of powan was high in infected fish (T=2.27, df=26, p<0.05). This finding suggests that the muscle protein was not transferred into the gonads. Infected fish were longer than uninfected fish (T=2.83, df=22, p<0.05).

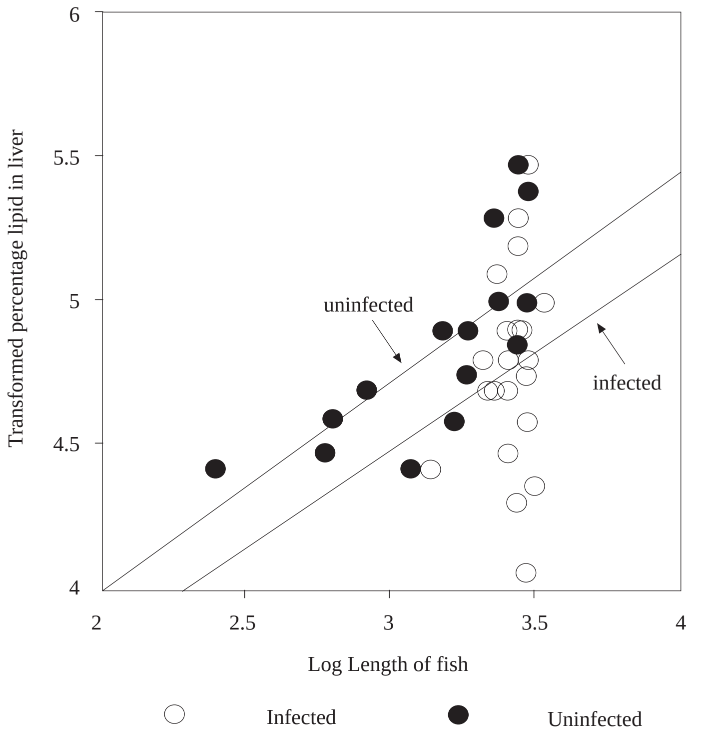

Figure 1. Relationship between length of fish and pergentage lipid in the liver of infected and uninfected fish.

Table 5. Results of T-test between protein and lipid contents of muscle, liver and gonads of male and female fish.

	Mean±SE				
	M	F	T	df	P
Muscle protein	89.53±1.47	88.66±1.62	0.42	37	0.68
Liver protein	65.03±1.55	64.95±0.83	0.04	26	0.97
Gonad protein	55.33±2.74	54.32±2.09	0.20	31	0.84
Muscle lipid	5.08±0.54	5.00±0.54	0.16	37	0.88
Liver lipid	23.19±0.91	23.38±0.60	0.24	30	0.81
Gonad lipid	13.91±0.71	15.00±0.56	1.26	37	0.21
Condition factor	1.12±0.03	1.11±0.03	0.29	35	0.78
Length	27.81±1.20	28.72±1.15	0.37	37	0.71
Weight	268.1±29.7	285.6±22.9	0.31	37	0.76

Abbreviations: M: male; F: female; T: calculated T value; df: degrees of freedom; p: probability value.

The results of the Analysis of Covariance (ANCOVA) for lipid and protein contents and body condition for infected versus uninfected fish are given in Table 8. There were statistically significant differences only in the slopes of gonad protein between infected and uninfected fish ($F_{1,36}$=9.13, p<0.05). There were also differences in the elevation of liver lipid and condition factor between infected and uninfected fish. Marginally significant differences were found in the elevation of gonad lipid between infected and uninfected fish.

Differences were found in elevations of lipid in the liver of the fish (Figure 1) against fish length in infected and uninfected fish. Thus, for uninfected fish, gonad protein decreased slightly with the length of the fish, whereas it increased with length in infected fish. A positive correlation occurred when the length of the fish was plotted against the number of worms (up to 7 worms).

Table 6. Results from Analysis of Covariance (ANCOVA) between protein and lipid contents of male and female fish.

	df	F	P
Muscle protein			
Slopes	1,36	0.11	0.741
Elevation	1,37	0.46	0.502
Liver protein			
Slopes	1,36	0.13	0.723
Elevation	1,37	0.00	0.958
Gonad protein			
Slopes	1,36	1.52	0.226
Elevation	1,37	0.03	0.931
Muscle lipid			
Slopes	1,36	2.82	0.102
Elevation	1,37	0.00	0.958
Liver lipid			
Slopes	1,36	0.05	0.828
Elevation	1,37	0.01	0.904
Gonad lipid			
Slopes	1,36	0.08	0.773
Elevation	1,37	1.38	0.248
Condition factor			
Slopes	1,36	0.43	0.517
Elevation	1,37	0.71	0.481

Abbreviations: F: calculated F value; df: degrees of freedom; p: probability value.

Discussion

It is well known that salmonid fish muscle is rich in protein reserves and poor in fat reserves (9,22,23). This study also showed that the percentage of protein in powan (which is a salmonid fish) muscle is higher than in other compartments of the fish body, and that powan muscle has a lower lipid content than the liver and gonad. Longer fish, which are usually older, contain storage lipid in the liver. Bigger fish also have more protein in the muscle. There are no gender effects on the body composition of powan from Loch Lomond. The effects of parasites on the growth and reproduction of fish have been demonstrated on several occasion (1,20). Powan infected with *Diphyllobothrium* spp. are stunted. The existence of less protein and lipid in the gonads shows that *Diphyllobothrium* spp. infection has an effect on the reproductive system of powan in Loch Lomond.

The main energy sources for fish are protein and lipid. As mentioned by Chellappa (5), this may be due to the fact that the fish metabolism is well adapted to deal with diet in which protein and lipid are high. An other factor is the ability of fish to eliminate nitrogenous wastes rapidly and continuously. Fish also have a high level of enzymes for breaking down protein.

Protein content within a fish population tends to be relatively constant, but during periods of starvation and gonad development, non-fatty fish especially draw on their carcass protein. For example, a study carried out by Dawson and Grimm (8) showed that in female plaice during development of the ovaries, the breakdown of body protein occurred and concentrations of plasma cortisol were elevated.

It was observed that the body length of powan has some influence on the protein content of the liver and gonads and lipid content of the muscle. Powan is a winter spawner and gonad growth occurs during the summer (4). As good condition might imply good health, the positive correlation between fish condition and

Table 7. Mean (±SE) for morphological and biochemical variables in infected and uninfected powan.

	Mean±SE				
	Infected	Uninfected	T	df	P
Muscle protein	91.3±0.97	86.5±1.91	2.27	26	0.032
Liver protein	66.0±0.90	63.8±1.40	1.31	31	0.20
Gonad protein	48.9±2.11	61.2±1.68	4.35	35	0.0001
Muscle lipid	5.7±0.66	4.4±0.37	1.53	29	0.14
Liver lipid	23.7±0.77	22.8±0.71	0.90	37	0.38
Gonad lipid	13.4±0.68	15.4±0.52	2.71	37	0.01
Condition factor	1.07±0.02	1.16±0.04	1.98	31	0.057
Length	30.4±0.51	26.0±1.48	2.83	22	0.009
Weight	306.3±14.3	246.2±33.9	1.63	24	0.12

Abbreviations: T: calculated T value; df: degrees of freedom; p: probability value.

percentage protein in gonads shows that healthy fish are likely to be more successful in breeding. In other words, a positive correlation between condition factor and gonad protein shows that fish in good condition may have well-developed gonads, and fish with well-developed gonads have lower than average levels of muscle and liver protein. This is in accordance with the findings of Love (12), Medford and Mackay (15), who thought that nutrients for germinal tissue growth may be drawn from somatic tissue. The fact that larger fish have more protein in the muscle and more lipid in the liver and in the gonads reveals that older fish build up more energy reserves than younger fish.

The concentration of lipid varies considerably in different parts of the fish. Similar to protein, the lipid content in the body of the fish also changes depending on the time of the year, environmental conditions, stage of maturity of the gonads, state of nutrition and age of the fish (3,5,7,8,9,11,17,22). The liver stored large quantities of lipid (15-65 % liver weight) and this was later utilised during gonad maturation and spawning. Medford and Mackay (15) also noticed utilisation of protein and lipid in the muscle of northern pike during the spawning season. The decrease in muscle protein concentration in both sexes was smaller than the decrease in the lipid.

References

1. Arme, C., Bridges, J.F. and Hoole, D. (1983). Pathology of cestode infections in the vertebrate host In: Arme, C. and Pappas, P.W. (Eds.). Biology of the Eucestoda II. Academic Press, London, pp. 499-538.
2. Barnes, H. and Blackstock, J. (1973). Estimation of lipids in marine animals and tissues: Detailed investigation of the sulphophosphovanillin method for total lipid. *Journal of Experimental Marine Biology and Ecology* 12, 103-118.
3. Brett, J.R., Shelbourn, J.E. and Shoop, C.T. (1969). Growth rate and body composition of fingerling Sockeye salmon, *Onchorhynchus nerka*, in relation to temperature and ration size. *Journal of Fisheries Research Board Canada* 26, 2363-2394.
4. Brown, E.A.R. and Scott, D.B. (1994). Life histories of the powan, *Coregonus lavaretus* (L.) (Salmonidae: Coregoninae) of Loch Lomond and Loch Eck. *Hydrobiologia* 290, 121-133.
5. Chellappa, S. (1988). Energy reserves in male three-spined stickleback, *Gasterosteus aculeatus* L. (Pisces, Gasterosteidae): annual variation and relation to reproductive aggression. PhD Thesis, University of Glasgow.
6. Cho, C.Y. and Kaushik, S.J. (1985). Effects of protein intake on metabolizable and net energy values of fish diets. In: Nutrition and Feeding in Fish (Eds. Cowey, C.B., Mackie, A.M. and Bell, J.G.) 95-117, Academic Press, London.
7. Craig, J.F. (1977). The body composition of adult perch, *Perca fluviatilis*, in Windermere, with reference to seasonal changes and reproduction. *Journal of Animal Ecology* 46, 617-632.
8. Dawson, A.S. and Grimm, A.S. (1980). Quantitative seasonal changes in the protein, lipid and energy content of the carcass, ovaries and liver of adult female plaice, *Pleuronectes platessa* L. *Journal of Fish Biology* 16:493-504.
9. Elliot, J.M. (1976). Body composition of brown trout (*Salmo trutta*) in relation to temperature and ration size. *Journal of Animal Ecology* 45, 273-289.
10. Folch, J., Lees, M. and Stanley G.H.S. (1956). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 266: 497-509.
11. Gill, H.S. and Weatherly, A.H. (1984). Protein, lipid and caloric contents of bluntnose minnow, *Pimephales notatus* Rafinesque, during growth at different temperatures. *Journal of Fish Biology* 25, 491-500.
12. Love, R.M. (1970). The Chemical Biology of Fishes. Academic Press, Inc., London. 547 p.
13. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* 193, 265-275.
14. Meakins, R.H. (1974). A qualitative approach to the effects of the plerocercoid of *Schistocephalus solidus* on the ovarian maturation of the three-spined stickleback, *Gasterosteus aculeatus. Zeitschrift fur Parasitenkunde* 44, 73-79.
15. Medford, B.A. and Mackay, W.C. (1978). Protein and lipid contents of gonads, liver and muscle of northern pike (*Esox lucius*) in relation to gonad growth. *Journal of Fisheries Research Board of Canada* 35,213-219.
16. Milinski, M. (1984). Parasites determine a predator's optimal feeding strategy. *Behavioural Ecology and Sociobiology* 15, 35-37.
17. Parker, R.R. and Vanstone, W.E. (1966). Changes in the chemical composition of central British Columbia pink salmon durung early sea life. *Journal of Fisheries Research Board of Canada* 23, 1353-1384.

18. Pennycuick, L. (1971). Frequency distribution of a parasite in a population of three-spined sticklebacks, *Gasterosteus aculeatus* L., with particular reference to the negative binomial distribution. *Parasitology* 63, 389-406.

19. Peterson, G.L. (1977). A simplification of the protein assay method of Lowry et al. which is more generally applicable. *Analytical Biochemistry* 83: 346-356.

20. Tierney, J.F. (1991). Studies on the life history of *Schistocephalus solidus*: field observation and laboratory experiments. PhD thesis. University of Glasgow, Glasgow.

21. Walkey, M. and Meakins, R.H. (1970). An attempt to balance the energy budget of a host-parasite system. *Journal of Fish Biology* 2, 261-262.

22. Weatherley, A.H. and Gill, H.S. (1983). Protein, lipid and water and caloric contents of immature rainbow trout, *Oncorhynchus mykiss*, growing at different rates. *Journal of Fish Biology* 23, 653-673.

23. Weatherley, A.H. and Gill, H.S. (1987). The Biology of Fish Growth. Academic Press. London. pp 443.