The influence of dietary lipid inclusion and daily ration on growth and smoltification in 1+ Atlantic salmon (Salmo salar) parr.

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Abstract

The effects of diet regime on growth and smoltification in 1+ Atlantic salmon parr were studied. Two groups of approximately 400 salmon parr, in triplicate, were fed diets containing either 25% or 12.5% lipid from first-feeding. Two further treatments were fed either the 25% or 12.5% lipid diet for 98 days, after which they were fed the alternate diet. In a second experiment three groups of 550 parr, in duplicate, were fed at full, two-thirds or one-third of the manufacturers' recommended ration, from first-feeding. All groups were maintained on their respective diet regimes until smoltification approximately one year after first-feeding.

In experiment 1, lipid level had a significant effect on whole body lipid content. However growth and the incidence of smoltification was not affected by dietary lipid inclusion, with upper modal group fish from each treatment achieving a similar smolt status (in terms of condition factor and Na^+, K^+-ATPase) at seawater transfer. In experiment 2, growth and the incidence of smolt transformation increased with ration. Full and two-thirds ration fish maintained similar body lipid contents throughout the experiment, with that of the one-third ration fish lower only during early development, indicating that growth was controlled by the maintenance of a distinct lipid level.

It is concluded that ration, and not dietary lipid inclusion, has a significant effect on growth and the decision to undergo smoltification in salmon parr.

Keywords: Atlantic salmon, parr, growth, smoltification, nutrition

1. Introduction

It is well established that growth in Atlantic salmon parr is an important determinant in life history strategy. In particular, the yearly cycle of growth leads to the development of a bimodal population structure in the first growing season (Thorpe, 1977; Bailey et al., 1980; Kristinsson et al., 1985). Smoltification is thought to be dependant on individuals attaining a particular size threshold (Elson, 1957; Kristinsson et al., 1985; Skilbrei, 1988), prior to winter, which is followed by a period of rapid growth (Kristinsson et al., 1985; Skilbrei, 1991). These individuals typically smolt in the following spring (Kristinsson et al., 1985; Skilbrei, 1988; Duston and Saunders, 1992), with lower modal group fish requiring at least a further year in fresh water before migration is possible (Thorpe, 1977, 1987). Although environmental parameters are known to be important cues for smoltification (Eriksson and Lundqvist, 1982; Duston and Saunders, 1992; Solbakken et al., 1994), their influence on growth may determine which individuals can proceed with smoltification many months prior to seawater transfer.

Energy intake ultimately influences fish growth and development (Jobling, 1994), with such effects mediated through diet quality and quantity. Where feed is unlimited, dietary lipid inclusion has been found to have only negligible effects on growth in juvenile salmonids (Reinitz, 1983; Shearer et al., 1997; Shearer and Swanson, 2000), having a greater influence on whole body lipid levels (Reinitz, 1983; Grisdale-Helland and Helland, 1997; Shearer et al. 1997; Shearer and Swanson, 2000). However, ration influences growth (Storebakken and Austreng, 1987; Stead et al., 1996; Shearer et al., 1997), with body lipid levels only affected by ration when lower rates are applied (Storebakken and Austreng, 1987). Subsequently, it has been

suggested that salmonid feed intake and therefore growth is controlled by a lipostatic mechanism (Silverstein et al., 1997; Jobling and Johansen, 1999; Johansen et al., 2001).

Although such relationships have been proposed with regards to the effects that diet has on growth, the direct effects of feed regime on smoltification are poorly understood. Smoltification results in a distinct reduction in body lipid (Woo et al., 1978; Birt and Green, 1986; Helland and Grisdale-Helland, 1998), although at conflict with these findings it has been suggested that high fat levels are not necessary for smoltification (Saunders et al., 1982). In fact neither dietary lipid level (Redell et al., 1988) nor winter feed restriction (Dickhoff et al., 1989; Larsen et al., 2001) have been shown to directly influence smoltification. However, such studies have focused on nutritional influences during smoltification, rather than the preceding year.

The current study aimed to investigate these interactions, in particular, the effects of long-term diet manipulation on freshwater development.

2. Materials and Methods

2.1. Fish stock and rearing conditions: Experimental fish (Salmo salar) were of Scottish stock, maintained at the Buckieburn Freshwater Research Facility, Scotland (56°N) under ambient water temperatures except during early development in the hatchery, when the ambient water temperatures were artificially elevated (Fig. 1). Flow rates were maintained at 1 $l.s^{-1}$ with oxygen levels >8 $mg.l^{-1}$.

2.1.1. Dietary lipid experiment

From first-feeding on 16th March 2000, 2500 fish were placed into each of two, 1m square tanks and exposed to LD24:0. Each tank was supplied with one of two, separately formulated, experimental diets (EWOS; UK) (Table 1), containing either 12.5% (proximate composition: 51.2±0.8% protein, 12.3±0.3% lipid, 6.6±0.6% moisture; mean±S.E.M) or 25% (proximate composition: 49.8±0.8% protein, 24.6±0.3% lipid, 5.3±0.4% moisture; mean±S.E.M) lipid inclusion. Each diet was fed at the manufacturer's recommended rate for commercial feeds, throughout the light phase of the photoperiod. On 16th May, 400 fish from each diet were placed into each of six, 0.7m diameter, circular tanks. Fish were maintained on their respective diets under LD24:0 until 21st June, when three tanks from each diet regime were transferred onto the alternative diet, creating four experimental treatments in triplicate (Fig. 1) termed the 25/25, 25/12.5, 12.5/25 and 12.5/12.5 groups respectively. From this time groups were exposed to a simulated natural photoperiod.

2.1.2. Ration experiment

From first-feeding on 22nd April 2001, 2500 fish were placed into each of three, 1m square tanks and exposed to LD24:0. Each tank was supplied with a commercial feed (EWOS Micro, EWOS; UK) fed at 100%, 66% or 33% of the manufacturers' recommended daily ration. On 28th June, 550 fish from each group were placed into two $1m^2$ tanks and exposed to a simulated natural photoperiod, with the duplicated treatments maintained on their respective rations.

2.2. Sampling regime:

For both experiments, six batch weight measurements per treatment were taken at monthly intervals from first-feeding until late June. Then from late June until the conclusion of the experiments in mid May, individual fork length (±1mm) and weight (±0.1g) measurements were taken at monthly intervals from 90 to 180 individuals per treatment. At each sample point, 6 samples per replicate were taken for the assessment of whole body lipid content, using the Soxhlet extraction method. Samples taken for whole body fat determination were dried to a constant mass at 100^{o}C prior to analysis, with the lipid subsequently extracted using petroleum ether (Fisher Scientific; Loughborough, UK).

In mid February, and then at 14 day intervals from mid March until the conclusion of the experiments, gill samples were taken from 15 to 20 upper modal group (UMG) fish per treatment for the determination of gill Na^+, K^+-ATPase, using the method detailed by McCormick (1993). Modal groups were determined based on size (UMG >130mm fork length) and the presence of body silvering. At the conclusion of the experiments in mid May the remaining fish were culled with the numbers of upper and lower modal group (LMG) fish recorded.

2.3. Calculations and statistical analysis: Condition factor (CF) was calculated as: weight (g).fork length $(cm)^{-3}$.100. Data were analysed using Minitab v13.1. Changes in weight, condition factor, whole body lipid level, gill Na^+, K^+-ATPase and population structure were compared using a General Linear Model. To improve statistical analysis, natural log and arcsine transformations were used for the weight and population structure data respectively. Residual plots were used to confirm

normality and homogeneity of variance. A significance level of 5% was applied to the statistical tests (Zar, 1999).

3. Results

3.1. Growth:

All groups increased in size over the experimental period ($P<0.001$) (Fig. 2). In the dietary lipid experiment no differences were found among the weight of treatment groups at any time during the study (Fig. 2a). For the daily ration experiment, the full and two-thirds ration fish became heavier than the one-third ration fish from late July ($P<0.01$) until the conclusion of the experiment (Fig. 2b), with those fed the full ration heavier than the two-thirds ration fish from mid August ($P<0.01$).

In the dietary lipid experiment, the condition factor of the 12.5/25 and 12.5/12.5 groups increased between mid June and mid September ($P<0.05$) (Fig. 3a), with the CF of all groups then declining to the conclusion of the experiment ($P<0.001$). However, throughout the experiment no consistent differences could be found among treatment groups ($P>0.05$). For the daily ration experiment, the CF of all groups initially increased ($P<0.001$) (Fig. 3b), peaking in late July for the one-third ration fish and then in mid August for the full and two-thirds ration fish. Subsequently, the CF of all groups declined to the conclusion of the experiment ($P<0.001$). The CF of the full and two-thirds ration fish remained similar throughout the experiment ($P>0.05$), with that of the one-third ration fish lower ($P<0.05$) than the full ration fish from mid September until mid October, and lower than the two-thirds ration fish in mid September and mid April (Fig. 3b).

3.2. Whole body lipid levels:

All groups in the dietary lipid experiment showed an overall increase in whole body lipid level until mid October (Fig. 4a) ($P<0.05$), with a subsequent decline to the end of the experiment ($P<0.001$). The lipid content of fish was maintained at levels relative to the dietary lipid inclusion they were being fed. As such, between mid May and mid June, the 25/25 and 25/12.5 fish had higher lipid levels than the 12.5/25 and 12.5/12.5 fish ($P<0.001$). Following the change in diet on 21st June, the lipid level of the 12.5/25 fish increased ($P<0.01$) to similar levels as the 25/25 fish, with that of the 25/12.5 fish becoming similar to the 12.5/12.5 fish. Then from mid August, until the conclusion of the experiment, the 25/25 and 12.5/25 fish maintained similar lipid levels, being significantly higher ($P<0.01$) than those of the 25/12.5 and 12.5/12.5 fish.

All groups in the ration experiment showed an overall increase in lipid level until mid October (Fig. 4b), with a subsequent decrease to the conclusion of the experiment ($P<0.001$). The full ration fish had a higher lipid content than the one-third ration fish from late July until mid January ($P<0.01$) and then from mid April onwards ($P<0.05$), whereas the lipid content of the two-thirds ration fish was only higher from mid July until early December ($P<0.01$). The full and two-thirds ration fish had similar lipid levels throughout the experiment ($P>0.05$).

3.3. Na^+, K^+ -ATPase levels:

The Na^+, K^+-ATPase levels of UMG fish from all groups increased between mid February and mid May ($P<0.01$) (Fig. 5). Furthermore, during both the lipid (Fig. 5a)

and ration (Fig. 5b) experiments the Na^+, K^+-ATPase levels of the treatment groups remained similar.

3.4. UMG/LMG ratio:

In the dietary lipid experiment the 25/25, 25/12.5 and 12.5/25 groups had a higher incidence of UMG fish than LMG fish ($P<0.05$) (Fig. 6), although all treatment groups contained similar numbers of fish from the respective modes (Fig. 6a). In the ration experiment, the incidence of UMG fish increased with ration ($P<0.05$) (Fig. 6b). The full and two-thirds ration fish had a higher incidence of UMG fish, whereas in the one-third ration group LMG fish predominated ($P<0.05$).

4. Discussion

This study has shown that dietary lipid level and daily ration have significant effects on growth and the accumulation of body lipid in Atlantic salmon parr, with subsequent influences on smoltification.

Differences in dietary lipid inclusion did not affect the growth of individuals. Similar results have been documented in juvenile salmonids (Reinitz, 1983; Grisdale-Helland and Helland, 1997; Shearer et al. 1997; Shearer and Swanson, 2000), although in adult salmon dietary lipid inclusion has been shown to effect growth (Hemre and Sandnes, 1999; Torstensen et al., 2001; Refstie et al., 2001). Therefore it is possible that in adults, significant lipid accumulation is directed towards growth, whereas in juveniles lipid is important for early organ development and physiologically demanding processes such as smoltification (Woo et al., 1978, Birt and Green, 1986).

The current study provides further evidence that growth is correlated to ration in juvenile salmon (Storebakken and Austreng, 1987, Stead et al., 1996; Shearer et al., 1997). However, it is possible that environmental factors such as temperature and photoperiod affect an individuals response to different rations, with those receiving high rations maintaining a good physiological condition, allowing energy to be diverted away from growth at seasonally or developmentally critical periods

Both dietary lipid inclusion and ration level affected the accumulation of body lipid, similar to previous findings in juvenile salmonids (Reinitz, 1983; Storebakken and Austreng, 1987; Shearer and Swanson, 2000). However, during the dietary lipid experiment, as well as body fat being maintained at levels relative to those in the diet, when a change in dietary lipid occurred individuals rapidly changed their body fat content. The rapid replenishment of lipid reserves following periods of restricted feeding has been documented in juvenile salmon (Miglavs and Jobling, 1989; Metcalfe and Thorpe, 1992; Morgan and Metcalfe, 2001) and it is likely that the small size of juveniles is of significance in such a rapid physiological change.

Traditionally, compensatory growth following periods of nutritional restriction has been considered in terms of an individuals size (Weatherley and Gill, 1981; Miglavs and Jobling, 1989; Nicieza and Metcalfe, 1997). However, the current study suggests that salmon growth may be under lipostatic control, with increases in size dependant on the maintenance of a distinct body fat content (Silverstein et al., 1997; Jobling and Johansen, 1999; Johansen et al., 2001). The full and two-thirds ration fish maintained a similar lipid level throughout the experiment, regardless of differences in size. Although it may be that these fish had achieved a maximum lipid load, this finding

may indicate a lipostatic mechanism. If this is the case, it seems that during the early stages of the experiment it was not possible for the one-third ration fish to reach the lipid threshold, despite large reductions in growth. However, after mid December similar lipid levels were recorded in all groups of fish. Previously, O'Connor et al. (2000) recorded a reduction in the metabolic rate of food deprived salmon and Elliott (1976) has found that growth and the accumulation of fat are affected by an interaction between ration and temperature. Therefore it is possible that a reduction in metabolic rate, as well as the low winter temperatures, affected lipid deposition relative to that of the full and two-thirds ration fish, thus allowing the one-third ration fish to achieve the lipid level maintained through lipostatic regulation.

Previously, short-term dietary lipid treatment has been shown to have a negligible effect on smoltification (Redell et al., 1988). The current study suggests that this is also the case where long-term dietary lipid regimes are applied. Within the range of lipid treatments used in the current study (12.5% and 25%) all groups displayed a reduction in CF and an increase in Na^+, K^+-ATPase indicative of the parr-smolt transformation (c.f. Solbakken et al., 1994; Duncan and Bromage, 1998; Handeland and Stefansson, 2001). However, although not statistically significant, a levelling of ATPase activity was indicated in the 12.5/12.5 group at the final sample, with this also the case for the one-third ration fish in the second experiment. It is therefore possible that these individuals had passed through the smoltification window more rapidly than the other groups.

Thorpe (1986) and Shearer (1994) suggested that smoltification is dependant upon the attainment of a distinct lipid threshold. However, similar numbers of fish within the

lipid treatments entered the UMG and underwent successful smoltification, suggesting that high fat levels may not be necessary for smoltification (Saunders et al., 1982). In support, the previously documented winter reduction in lipid content (Komourdjian et al., 1976; Saunders and Henderson, 1978; Higgins and Talbot, 1985) occurred at similar rate in all of the treatments. Although Higgins and Talbot (1985) recorded this reduction to occur at similar rates in upper and lower mode fish it has typically been linked to the physiological demands imposed by smoltification (Woo et al., 1978; Birt and Green, 1986; Helland and Grisdale-Helland, 1998). However, if a high lipid threshold regulated smoltification one might expect a differential response for individuals with different body lipid contents. Consequently it may be more appropriate to consider smoltification as requiring only low lipid reserves, with the winter decline in body fat content more a result of a temperature induced reduction in feed intake and activity (Metcalfe and Thorpe, 1992; Nicieza and Thorpe, 1997).

Different rations had a distinct effect on the number of individuals undergoing smoltification, with the full ration group having high recruitment to the UMG and 72% of the one-third ration group remaining in the LMG. It is likely that the differential in recruitment to the UMG was a result of ration related growth that affected the numbers of fish achieving the size threshold for smoltification (Elson, 1957; Skilbrei, 1988).

Interestingly, all groups displayed a reduction in CF, which has previously been linked to the parr-smolt transformation (Solbakken et al., 1994; Duncan and Bromage, 1998). However, ration influenced the incidence of UM fish and in the one-third ration group very low numbers of fish smolted. Duncan and Bromage (1998) found

that smoltification parameters were not necessarily well synchronised, suggesting that the decrease in condition may not be solely due to smoltification. Combined with the findings of the current experiment it may be that CF is not necessarily a good measure of smoltification. Alternatively, it may be that all of the fish in the experiment were stimulated to attempt smoltification by the changing photoperiod (Duston and Saunders, 1992) (as indicated by the reduction in CF) but only those which were able to successfully complete the parr-smolt transformation continued to develop as smolts.

Salmon hatcheries aim to supply high numbers of competent smolts at predetermined times of the year. The current study has highlighted the influence of ration on the incidence of smoltification in Atlantic salmon and as such feed rates should not be compromised during freshwater development. Dietary lipid levels did not greatly affect smoltification and freshwater production may not be impaired by a reduction in dietary lipid content. However, the relationships between juvenile and adult development are not well documented, and such reductions may affect adult growth or maturation rates. Therefore it is important that future investigations consider the longer term effects of dietary variations in salmonids.

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Table 1. The formulations used to create two experimental diets (EWOS; UK) containing either 12.5% or 25% lipid inclusion. a) crumble diet formulation, b) pellet diet formulation. The fish oil (Cargill; Liverpool, UK) used was primarily herring oil.

a)

Constituent	Percentage inclusion	
	12.5% diet	25% diet
Low temperature fish meal (LT94)	50	65
Wheat	15	14
Soybean meal	17	-
Rapeseed meal	9	-
Fry vitamin/mineral	1	1
Finnstim	1	1
Fish oil	7	19

b)

Constituent	Percentage inclusion	
	12.5% diet	25% diet
Low temperature fish meal (LT94)	50	67
Wheat	15	13
Soybean meal	17	-
Rapeseed meal	10	-
Fry vitamin/mineral	1	1
Finnstim	1	1
Fish oil	6	18

Fig. 1. Ambient water temperatures experienced during experiments in which Atlantic salmon parr were reared using different dietary lipid inclusions (a) or ration levels (b). Between ‘x’ and ‘y’ water temperatures were artificially elevated to improve early rearing. The percentage lipid inclusions used during the dietary lipid experiment are shown relative to the temperature profile; i) 25% throughout development, ii) 25% until 21st June, 12.5% thereafter, iii) 12.5% until 21st June 25% thereafter, iv) 12.5% throughout development.

Fig. 2. Changes in weight of Atlantic salmon parr reared using different dietary lipid inclusions (a) or ration levels (b) (mean ± S.E.M., n=3 for the lipid experiment, n=2 for the ration experiment). Figure legends denote the dietary lipid inclusions and daily rations experienced in the respective experiments. Differences in lettering denotes statistical differences ($P<0.05$). Where lettering has been stacked it is displayed in the same order as the graph lines.

Fig. 3. Changes in condition factor of Atlantic salmon parr reared using different dietary lipid inclusions (a) or ration levels (b) (mean ± S.E.M., n=3 for the lipid experiment, n=2 for the ration experiment). Figure legends denote the dietary lipid inclusions and daily rations experienced in the respective experiments. Differences in lettering denotes statistical differences ($P<0.05$). Where lettering has been stacked it is displayed in the same order as the graph lines.

Fig. 4. Changes in the whole body lipid content of Atlantic salmon parr reared using different dietary lipid inclusions (a) or ration levels (b) (mean ± S.E.M., n=3 for the lipid experiment, n=2 for the ration experiment). Figure legends denote the dietary

lipid inclusions and daily rations experienced in the respective experiments. Differences in lettering denotes statistical differences ($P<0.05$). Where lettering has been stacked it is displayed in the same order as the graph lines.

Fig. 5. Changes in the gill Na^+, K^+-ATPase activities of upper modal group Atlantic salmon parr reared using different dietary lipid inclusions (a) or ration levels (b) (mean ± S.E.M., n=3 for the lipid experiment, n=2 for the ration experiment). Figure legends denote the dietary lipid inclusions and daily rations experienced in the respective experiments. Differences in lettering denotes statistical differences ($P<0.05$).

Fig. 6 The structure of Atlantic salmon parr populations recorded at the conclusion of experiments (mid May) in which groups of fish were reared using different dietary lipid inclusions (a) or ration levels (b). Figure legends denote the dietary lipid inclusions and daily rations experienced in the respective experiments. Closed bars denote the length-frequency structure of a population sample (n=180 for the lipid experiment, n=100 for the ration experiment), open bars denote the percentage of UMG and LMG fish from the entire population (mean ± S.E.M., n=3 for the lipid experiment, n=2 for the ration experiment). Differences in lettering denotes statistical differences ($P<0.05$). Capital lettering denotes between treatment differences in either UM or LM groups, lower case lettering denotes within treatment differences between UM and LM groups.

Fig. 1

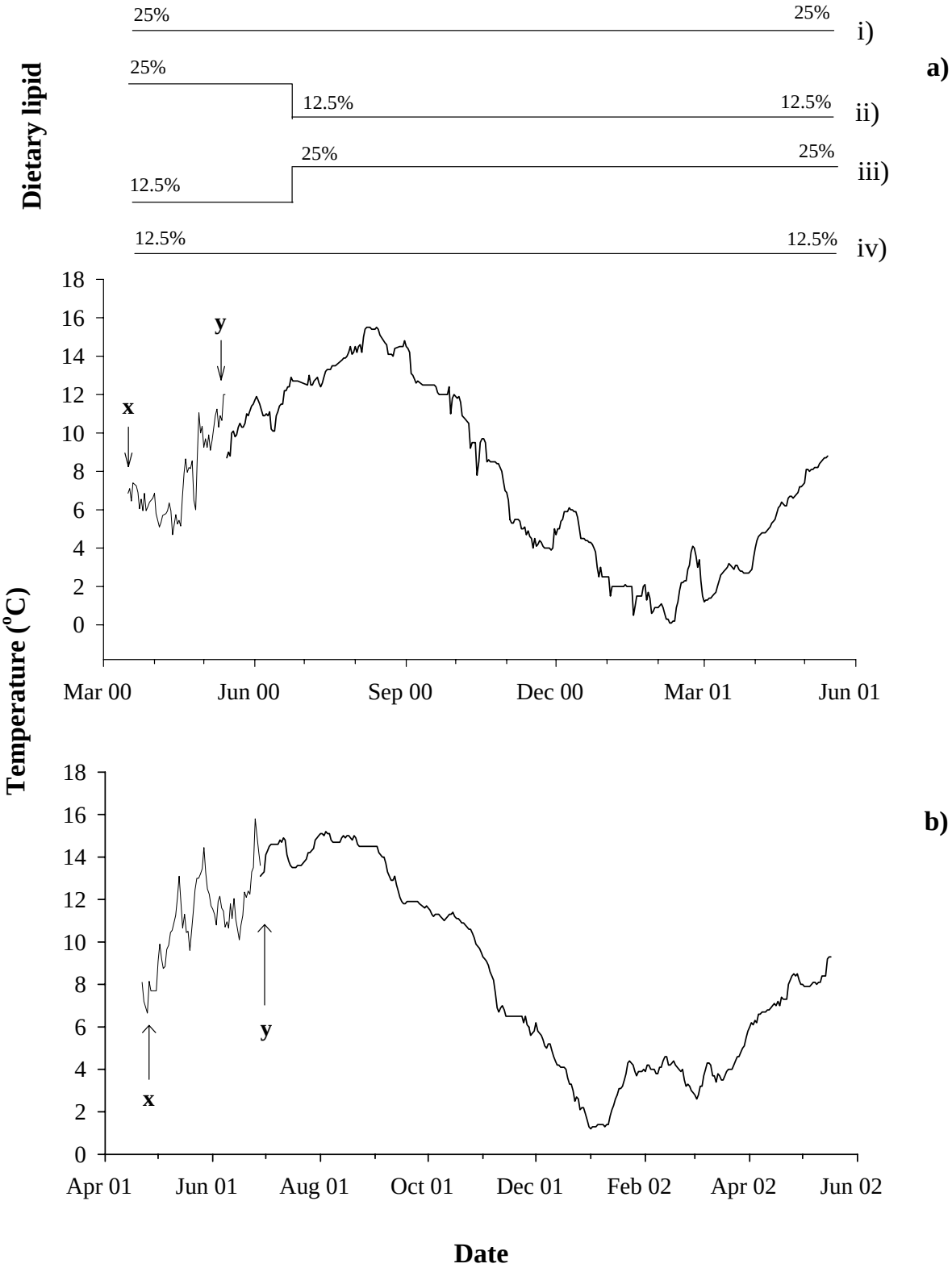

a)
Dietary lipid
25%
25%
i)
25%
12.5%
12.5%
ii)
25%
25%
iii)
12.5%
12.5%
12.5%
iv)
Temperature (°C)
x
y
Mar 00
Jun 00
Sep 00
Dec 00
Mar 01
Jun 01
b)
x
y
Apr 01
Jun 01
Aug 01
Oct 01
Dec 01
Feb 02
Apr 02
Jun 02
Date

Fig. 2

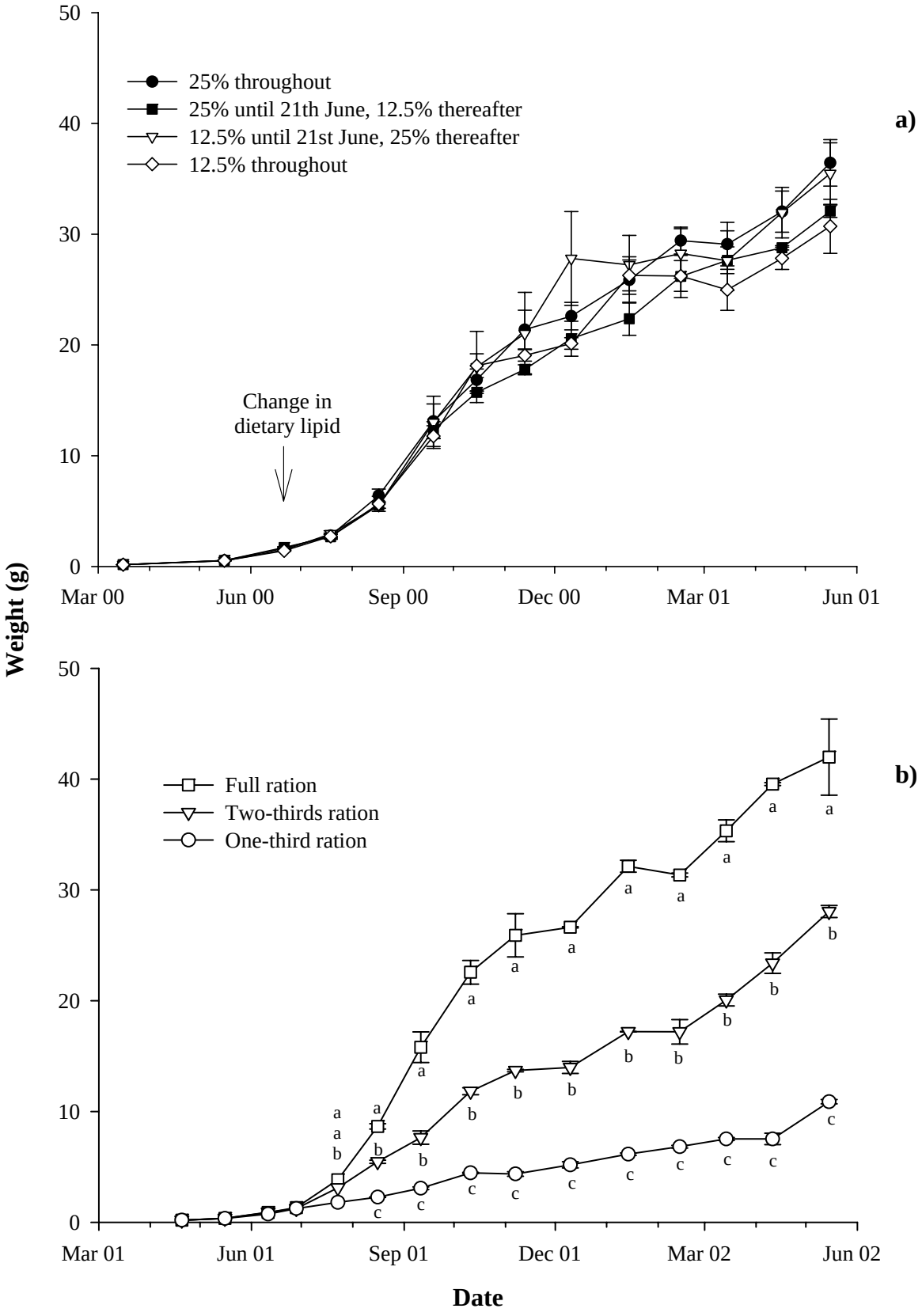

a)
25% throughout
25% until 21th June, 12.5% thereafter
12.5% until 21st June, 25% thereafter
12.5% throughout
Change in
dietary lipid
Weight (g)
0
10
20
30
40
50
Mar 00
Jun 00
Sep 00
Dec 00
Mar 01
Jun 01
b)
Full ration
Two-thirds ration
One-third ration
Mar 01
Jun 01
Sep 01
Dec 01
Mar 02
Jun 02
Date

Fig. 3

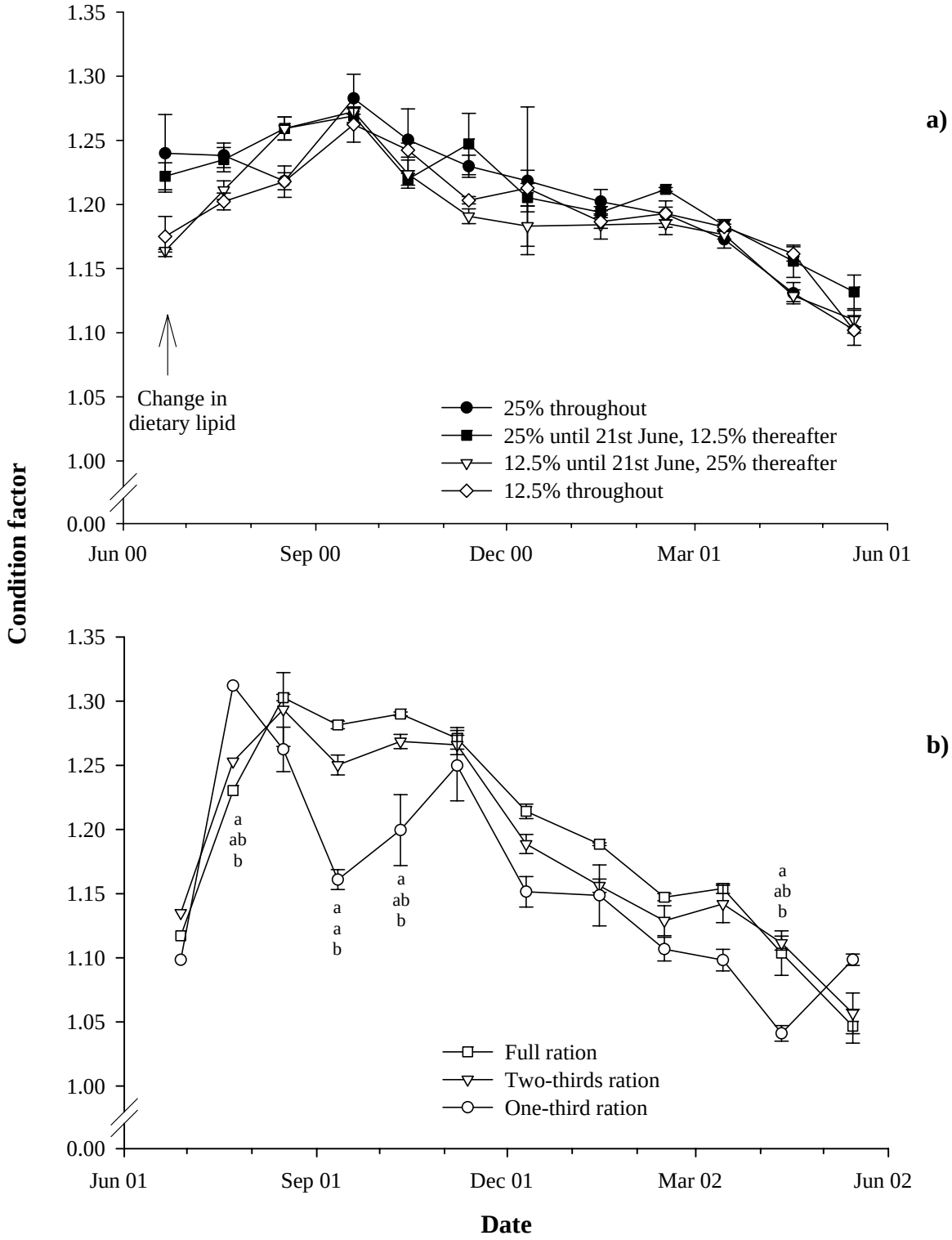

a)
b)
Condition factor
1.35
1.30
1.25
1.20
1.15
1.10
1.05
1.00
0.00
Change in
dietary lipid
25% throughout
25% until 21st June, 12.5% thereafter
12.5% until 21st June, 25% thereafter
12.5% throughout
Jun 00
Sep 00
Dec 00
Mar 01
Jun 01
a
ab
b
a
a
b
a
ab
b
a
ab
b
Full ration
Two-thirds ration
One-third ration
Jun 01
Sep 01
Dec 01
Mar 02
Jun 02
Date

Fig. 4

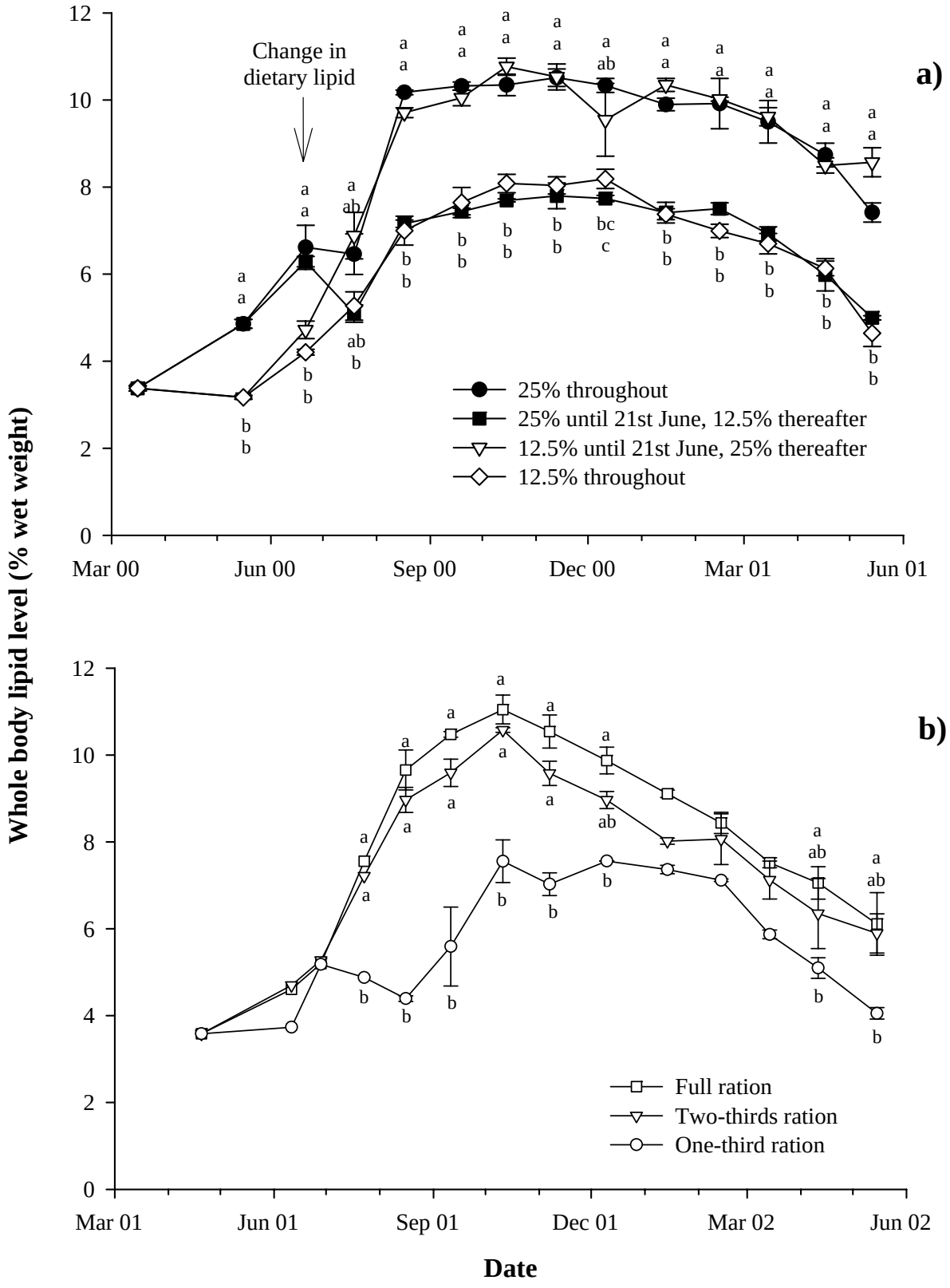

a)
b)
Change in dietary lipid
25% throughout
25% until 21st June, 12.5% thereafter
12.5% until 21st June, 25% thereafter
12.5% throughout
Full ration
Two-thirds ration
One-third ration
Whole body lipid level (% wet weight)
Date
Mar 00
Jun 00
Sep 00
Dec 00
Mar 01
Jun 01
Mar 01
Jun 01
Sep 01
Dec 01
Mar 02
Jun 02
0
2
4
6
8
10
12

Fig. 5

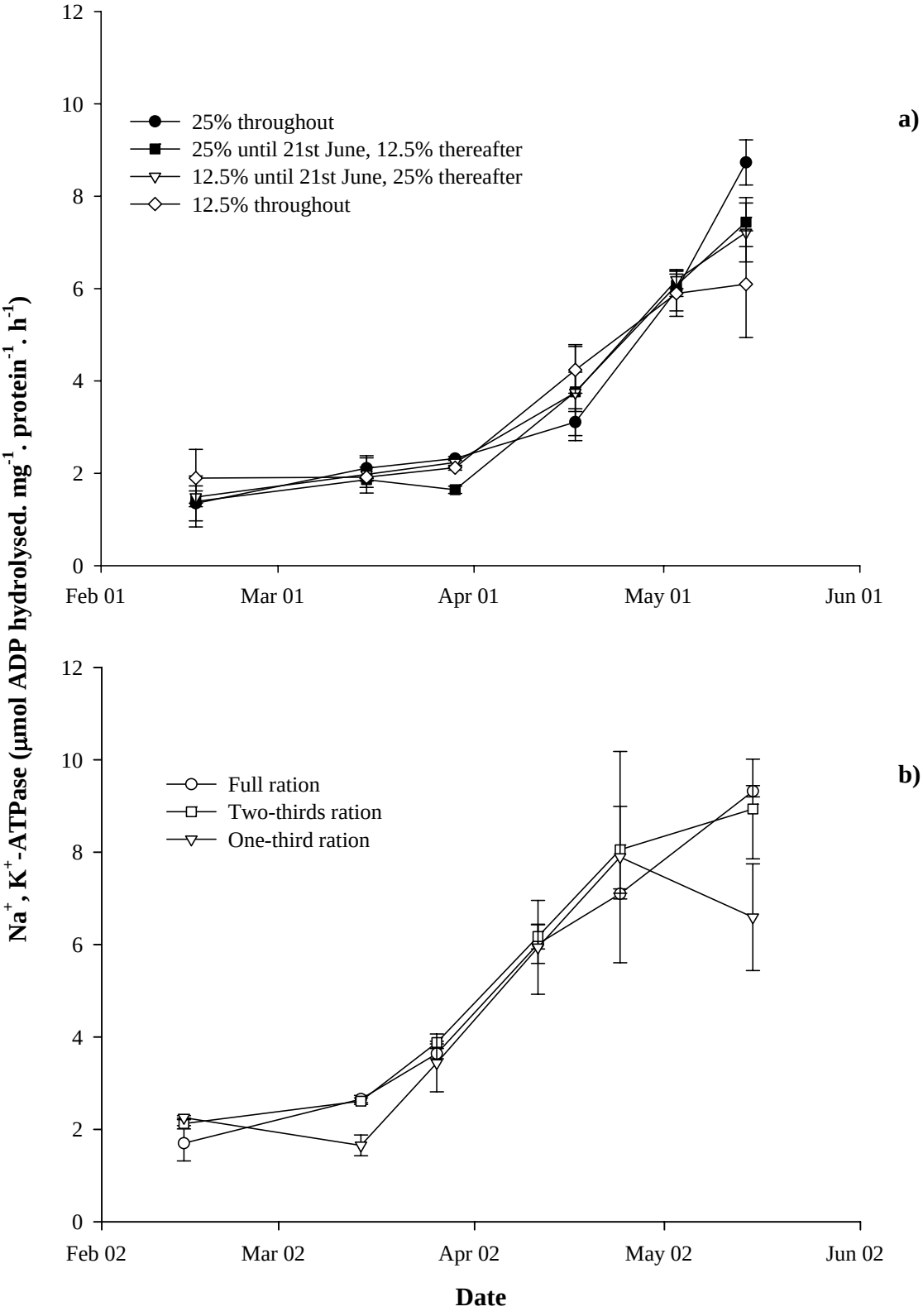
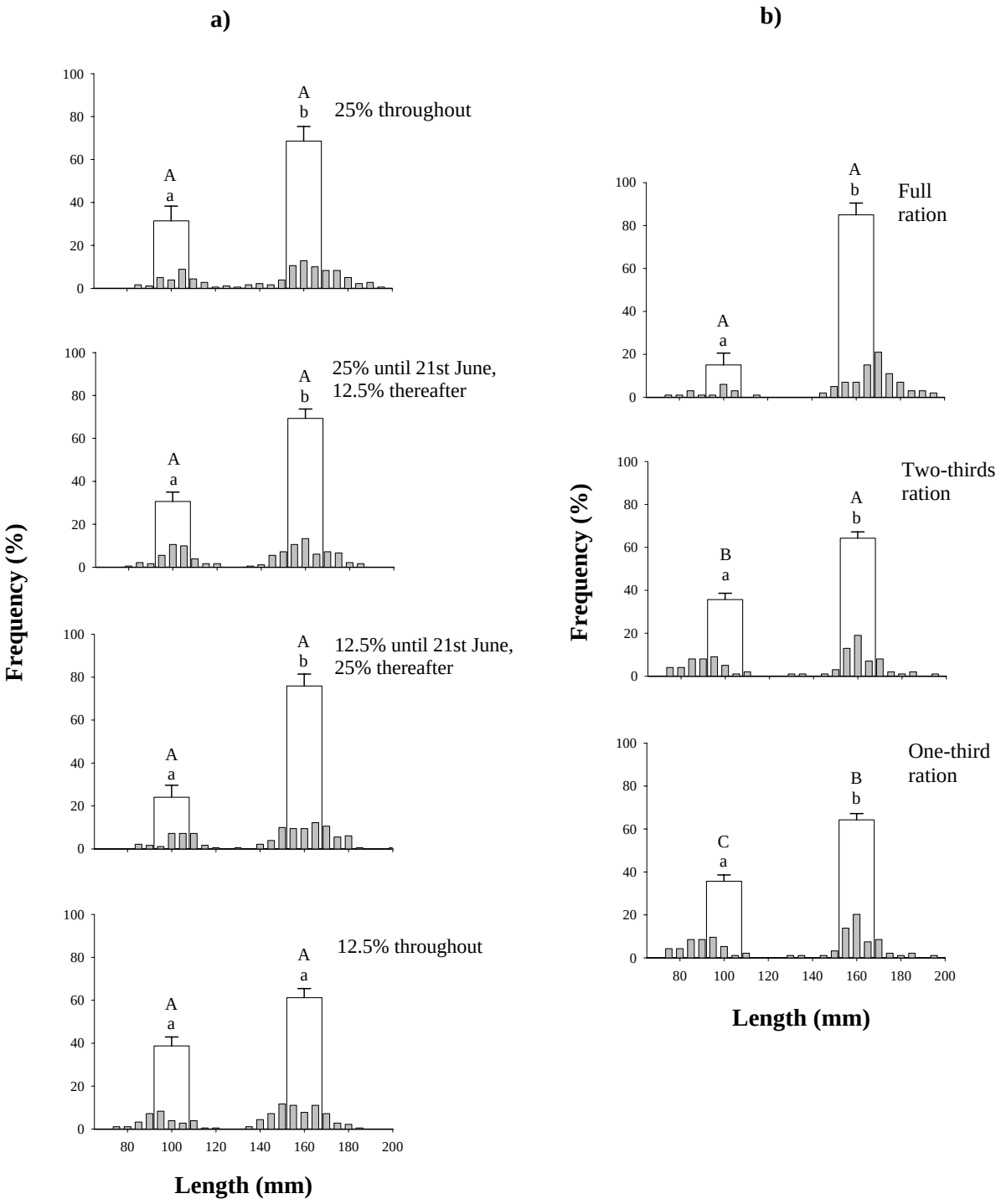

a)
25% throughout
25% until 21st June, 12.5% thereafter
12.5% until 21st June, 25% thereafter
12.5% throughout
b)
Full ration
Two-thirds ration
One-third ration
Na+, K+-ATPase (µmol ADP hydrolysed. mg-1. protein-1. h-1)
0
2
4
6
8
10
12
Feb 01
Mar 01
Apr 01
May 01
Jun 01
Feb 02
Mar 02
Apr 02
May 02
Jun 02
Date

Fig. 6


a)
b)
25% throughout
25% until 21st June, 12.5% thereafter
12.5% until 21st June, 25% thereafter
12.5% throughout
Full ration
Two-thirds ration
One-third ration
Frequency (%)
Length (mm)
A a
A b
B a
B b
C a