Seasonal changes in haematology and metabolic resources in the tench


Departamento de Fisiología (Fisiología Animal), Facultad de Biología, Universidad Complutense, 28040 Madrid, Spain

(Received 4 March 2002, Accepted 5 February 2003)

Significant variations in the number of white and red blood cells, haematocrit and haemoglobin were found throughout the year in sexually mature male and female tench *Tinca tinca*. In general, the lowest values were observed during autumn–winter and the highest during summer, with males exhibiting higher values than females. Plasma glucose, cholesterol and triglycerides were lower during the winter than during the summer–autumn seasons in both sexes. Gonado-somatic and hepato-somatic indices were inversely correlated in female tench throughout the year. Seasonal patterns in liver metabolic resources were very similar for both sexes. For males and females, liver glycogen and proteins increased during the autumn, whereas the liver stored lipid during spring. Dorsal muscle mainly deposited glycogen, whereas lipid was mainly stored in the ventral muscle. Relations between seasonal changes in environmental factors, such as feeding and temperature are discussed.

Key words: haematology; metabolic resources; seasonal changes; *Tinca tinca*.

INTRODUCTION

The tench *Tinca tinca* (L.) is an economically valuable freshwater fish characterized by high resistance to adverse environmental conditions and low requirements with respect to ecosystem quality (Steffens, 1995). This cyprinid, indigenous to Europe, is becoming popular with consumers and thus, intensive farming of tench is economically important in an increasing number of countries, including Spain, where it is widely distributed (Pérez-Regadera & Velasco, 1998).

It is well known that intensive fish culture is often accompanied by increased incidences of pathologies. Many studies have demonstrated the usefulness of haematology and blood biochemistry in the assessment of fish health (Coles, 1986), and as a biomarker of exposure to pollution (Handy & Depledge, 1999). Previous information on haematology and blood biochemistry in the tench is fragmentary (Svoboda et al., 2001; Collazos et al., 1998; De Pedro et al., 1998).

*Author to whom correspondence should be addressed. Tel.: +34913944984; fax: +34913944935; email: mjdelgad@bio.ucm.es

© 2003 The Fisheries Society of the British Isles
Exposure to environmental seasonal cycles in light, temperature and food availability are likely to affect blood and body composition. Indeed, seasonal changes in body composition, haematology and blood biochemistry have been described in several fish species (Craig, 1977; Rowley et al., 1988; Leamaster et al., 1990; Berg & Bremsæt, 1998; Jonsson & Jonsson, 1998; Leonard & McCormick, 1999), but little is known to date on seasonal variations in body composition, haematology and blood biochemistry in the tench.

Thus, the aim of the present study was to obtain basic information on a species of increasing economic interest by describing the seasonal pattern in haematology, blood biochemistry and metabolic resources for healthy male and female adults.

MATERIALS AND METHODS

FISH

Adult tench (females, 108.9 ± 2.9 g and males, 94.9 ± 2.4 g, mean ± s.e.) were obtained in each season of the year from a commercial hatchery [‘IPESCON’, Salamanca and from Centro de Cyprinicultura, Vegas del Guediana (Badajoz, Spain)]. The fish were then transported to Madrid, where they were maintained for 3–4 weeks in 5 m³ tanks with a continuous filtered freshwater supply under natural photoperiod and temperature. The fish were fed a commercial diet (Mubers) supplemented with rotifers and Daphnia sp. at a daily rate of 1% body mass at 1000–1100 hours, and were fasted for 24 h before sampling. Tench did not feed during the winter. Tench were sacrificed at 3 month intervals, once for each season (n = 64 per season), 1 month after both solstices (winter and summer) and both equinoxes (spring and autumn). The photoperiod and mean water temperature (minimum–maximum) for each season were as follows: winter (9.15L : 14.5D, 5–8°C), spring (13.8L : 10.2D, 15–18°C), summer (14.3L : 9.7D, 22–26°C) and autumn (10.8L : 13.3D, 12–15°C).

HAEMATOLOGY

Individual fish were weighed (M, g) and measured standard length, Lₜ. Blood was withdrawn by cardiac puncture using a 1 cm³ sterile plastic heparinized syringe and a 0.5 mm × 16 mm microlance needle. Blood samples for haematology were mixed immediately with EDTA (5 mg ml⁻¹).

The red blood cells (RBC) and white blood cells (WBC) were manually counted after dilution, ×200 and ×50, respectively, in modified Dacie’s fluid as previously reported (Blaxhall & Daisley, 1973). Values were expressed as number of cells mm⁻³. Haematocrit was determined using a microhaematocrit reader and the values were expressed as the percentage of erythrocytes. The haemoglobin concentration (g 100 ml⁻¹) was estimated by spectrophotometry (540 nm) using the cyanomethaemoglobin method with Drabkin’s reagent (Blaxhall & Daisley, 1973). The red cell indices, mean corpuscle volume (MCV), mean corpuscle haemoglobin (MCH) and mean corpuscle haemoglobin concentration (MCHC) were calculated using standard formulae (Coles, 1986). Differential leucocyte counts were made in blood smears dried in air, fixed with methanol and stained with Jenner–Giemsa. The percentage of particular types of leucocytes were determined on randomly selected fields of a stained smear (Ellis, 1977).

PLASMA BIOCHEMISTRY

Blood samples for biochemical analysis were collected in heparinized tubes and centrifuged (5000g, 5 min), and plasma was immediately removed and stored frozen at −80°C until used. Glucose (Glucose Trinder), cholesterol (Cholesterol-Trinder) and
triglycerides (GPO-Trinder) were determined using enzymatic–colorimetric methods by means of commercial kits (Sigma Diagnostics). Total plasma proteins were measured by the Lowry et al. (1951) method using serum bovine albumin as standard.

**METABOLIC RESOURCES**

The condition factor $K$ was calculated as $100 \frac{ML_S}{C_0^3}$. Total liver and gonads were carefully dissected and samples of both dorsal (below the dorsal fin) and ventral (above the pelvic fin) muscle were removed and weighed. Organ indices were calculated according to the formula: organ index (%) = $100 \frac{organ mass}{M^{-1}}$. Protein content in liver and muscle was determined as described by Lowry et al. (1951) using bovine serum albumin as the standard. Total lipids in liver and muscle samples were extracted with chloroform:methanol (2:1) according to Folch’s procedure (Folch et al., 1957), evaporated to dryness and reconstituted in dioxane at 100°C (De la Huerga, 1969). The total amount of lipids was determined by spectrophotometry (505 nm) using triolein as standard. Liver and muscle glycogen content was quantified by spectrophotometry (Dubois et al., 1956) after extraction with ethanol and previous digestion with KOH (Montgomery, 1957).

**STATISTICAL ANALYSIS**

The data were checked for homogeneity of the variances, and logarithmic transformations were made when necessary. Data are expressed as means ± s.e. Differences between means were assessed by one-way ANOVA followed by the Student’s Newman–Keuls (SNK) multiple range test for multi-group comparisons.

**RESULTS**

Male and female tench exhibited a very similar seasonal pattern in haematological values (Table I). The highest count of red and white blood cells, and haematocrit were found in summer ($P < 0.01$ compared with the rest of the seasons). Red and white blood cell counts were lowest in the autumn and winter. The haemoglobin content was significantly higher in spring ($P < 0.01$) for both sexes, and consequently, the highest MCH and MCHC values were also found in spring. Significant differences between sexes were detected only in summer, when red and white blood cell counts, haematocrit and haemoglobin values were significantly higher in males than in females. A high percentage (35–40%) of the white blood cells in the tench were lymphocytes, with very similar values among seasons and both sexes. The relative percentage of thrombocytes reached the highest values in spring. No statistically significant differences were recorded in the percentage of granulocytes either throughout the year or between sexes. Monocytes were the least common cells in the blood of the tench (<6%), and the relative percentage of these cells was significantly lower in spring for both sexes ($P < 0.01$).

Male and female tench exhibited identical seasonal changes in plasma glucose levels (Fig. 1). The glucose levels were lower in winter ($P < 0.01$) and spring ($P < 0.01$) than in summer and autumn. Plasma cholesterol levels in both sexes showed the highest value during the autumn and decreased markedly in winter ($P < 0.01$). These seasonal changes were very similar in male and female tench, except for summer when plasma cholesterol levels were lower in females than in males ($P < 0.05$). This sex variation in summer was also observed for the plasma triglyceride levels, which showed a significant decline throughout autumn to
<table>
<thead>
<tr>
<th></th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>611 ± 40</td>
<td>680 ± 30</td>
<td>1280 ± 80##</td>
<td>970 ± 70##</td>
</tr>
<tr>
<td></td>
<td>(10^12 cells mm^-3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>21.35 ± 0.47</td>
<td>21.25 ± 0.50</td>
<td>41.60 ± 2.41##</td>
<td>28.00 ± 1.26##</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12.92 ± 0.54*</td>
<td>7.68 ± 0.46</td>
</tr>
<tr>
<td>Haemoglobin (g/100 ml^-1)</td>
<td>19.48 ± 0.79##</td>
<td>19.74 ± 0.82##</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCV (μm^3)</td>
<td>372.32 ± 31.05</td>
<td>313.20 ± 13.53</td>
<td>330.45 ± 19.40</td>
<td>295.33 ± 14.19</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>341.95 ± 21.30##</td>
<td>302.74 ± 16.37##</td>
<td>102.96 ± 5.17**&amp; &amp;</td>
<td>79.96 ± 2.24</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>94.16 ± 3.11##</td>
<td>95.34 ± 3.22##</td>
<td>31.74 ± 1.82&amp; &amp;</td>
<td>27.38 ± 0.90&amp; &amp;</td>
</tr>
<tr>
<td>White blood cells</td>
<td>6.13 ± 0.49##</td>
<td>6.25 ± 0.63##</td>
<td>19.27 ± 2.29##</td>
<td>13.04 ± 1.84##</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>35.00 ± 1.50</td>
<td>35.65 ± 2.04</td>
<td>40.98 ± 3.37</td>
<td>42.71 ± 2.68##</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>36.17 ± 1.57</td>
<td>35.56 ± 1.16</td>
</tr>
<tr>
<td>Thrombocytes (%)</td>
<td>38.88 ± 1.48##</td>
<td>34.50 ± 1.23</td>
<td>29.01 ± 1.62</td>
<td>27.58 ± 2.51</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28.53 ± 1.24</td>
<td>26.35 ± 1.32</td>
</tr>
<tr>
<td>Granulocytes (%)</td>
<td>23.48 ± 1.27</td>
<td>23.94 ± 1.07</td>
<td>21.66 ± 2.92</td>
<td>26.85 ± 2.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28.40 ± 1.74</td>
<td>28.80 ± 1.55</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>3.17 ± 0.35##</td>
<td>2.67 ± 0.35##</td>
<td>7.05 ± 0.86</td>
<td>5.98 ± 0.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.24 ± 0.69</td>
<td>5.51 ± 0.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4.38 ± 0.49</td>
<td>5.05 ± 0.48</td>
</tr>
</tbody>
</table>

#, P < 0.05, ##, P < 0.01 cf. other seasons; #&, P < 0.01 cf. spring and winter; #*, P < 0.05, ***, P < 0.01 cf. males.

* P < 0.05, **, P < 0.01 cf. females.
Fig. 1. Seasonal changes in (a) plasma glucose, (b) cholesterol, (c) triglycerides and (d) total protein content of adult male (□) and female (■) Tinca tinca. Data are mean ± s.e. (n = 32 per group). Statistical differences (SNK test): ##, P < 0.01, cf. the remainder of the seasons; $$, P < 0.01, cf. spring and winter; &, P < 0.05, cf. spring; *, P < 0.05 and **, P < 0.01, males cf. females.

reach the lowest values for both sexes during winter. Plasma total protein (Fig. 1) varied little throughout the year in male tench, unlike females, where the highest values occurred in winter and the lowest in summer.

The highest mass–length relationships \((K)\) was found in spring for both sexes \((P < 0.01, \text{Fig. 2})\). Liver mass was always significantly higher in female than in male tench \((P < 0.01)\), but the seasonal profile of the hepato-somatic index \((I_H)\) was similar for both sexes. The lowest \(I_H\) was found in summer, and a significant increase occurred in the autumn, reaching a maximum level in winter \((P < 0.01)\). Pronounced seasonal differences were found in the gonado-somatic index \((I_G)\), particularly in female tench \((\text{Fig. 2})\). Spring spawning initiated a significant decline in \(I_G\).

The seasonal patterns in liver metabolic resources were very similar for both sexes \((\text{Fig. 3})\). Total protein content in the liver was higher in autumn and winter than in spring and summer, with more hepatic protein in males compared with females \((P < 0.05)\) during these warm seasons. Liver lipid content was highest in summer and was depleted in winter in males, and in autumn in females \((P < 0.01)\). Glycogen content in liver exhibited similar seasonal changes in males and females, with a continuous and significant increase from spring to autumn.

The total amount of protein was similar in ventral and dorsal muscle, and varied little throughout the year \((\text{Fig. 4})\). The amount of lipid in the ventral muscle of tench was significantly higher than in the dorsal muscle, and conversely the glycogen content was significantly higher in dorsal than in ventral muscle. Both energy reserves in dorsal muscle showed high values in summer, with a very low glycogen content during the autumn and winter. This general pattern was also observed in the ventral muscle, the highest levels of lipid and glycogen were found during the spring and a very low content of these metabolic resources was found in autumn and winter \((\text{Fig. 4})\).

**DISCUSSION**

The haematological values reported in this work provide basic information on a species of increasing economic interest. Significant variations in the number of red blood cells and haematocrit were found throughout the year, with the lowest values during autumn and winter and the highest during summer. These results agree with the only previous study on male tench \((\text{Collazos et al., 1998})\).

This seasonal response probably compensates for the effect of high temperature reducing oxygen availability. Some reports argue that this haematological mechanism works as respiratory compensation in fishes. Thus, water temperature influences the blood parameters in rainbow trout *Oncorhynchus mykiss* \((\text{Walbaum})\) \((\text{Martinez et al., 1994})\) and Arctic charr *Salvelinus alpinus* \((\text{L.})\) \((\text{Olsen et al., 1999})\), and causes a reduction in the quantity of circulating erythrocytes induced by low temperature in the cyprinid, *Phreatichthys andruzzii* Vinciguerra \((\text{Frangioni et al., 1997})\). Haemoglobin values for the tench in the present study are slightly higher than previous reports in different species of fishes \((\text{Blaxhall & Daisley, 1973})\). In addition to endogenous factors, age and general condition of the fish, other factors such as handling and transport, sampling conditions, and even the variety of methods used make comparisons of results difficult. Total leucocyte counts were found to be lower in the present
Fig. 2. Seasonal changes in (a) the condition factor, (b) hepato-somatic index and (c) gonado-somatic index of adult male (◇) and female (◼) Tinca tinca. Data are mean ± s.e. (n = 32 per group). Statistical differences (SNK test): ###, $P < 0.01$, cf. the remainder of the seasons; §, $P < 0.05$ and §§, $P < 0.01$, cf. summer and winter; *, $P < 0.05$ and **, $P < 0.01$, males cf. females.
Fig. 3. Seasonal changes in (a) total proteins, (b) lipids and (c) glycogen in the liver of male (■) and female (□) *Tinca tinca*. Data are mean ± s.e. (n = 32 per group). Statistical differences (SNK test): **, $P < 0.01$, cf. the remainder of the seasons; §§, $P < 0.01$, cf. spring and summer; $\$, $P < 0.05$ and $$, $P < 0.01$, cf. summer; *, $P < 0.05$ and **, $P < 0.01$, males cf. females.

study than those previously presented for the same species (Collazos et al., 1998). The fact that the two tench populations came from different habitats could explain the difference. Indeed, the number of white blood cells clearly depends on the quality of the aquatic environment (LeaMaster et al., 1990; Vijayamohanan et al., 2000). Accordingly, a significant increase in total leucocytes during the summer was found, when apparently the quality of the water in the pond decreased. This result corroborates previous reports suggesting that tench haematology is a useful model to detect pollution in aquatic environments (Svobodová et al., 1995).

Fig. 4. Seasonal changes (a), (d) in total proteins, (b), (e) lipids and (c), (f) glycogen in dorsal (left column) and ventral (right column) muscle of male (□) and female (■) Tinca tinca. Data are mean ± S.E. (n = 32 per group). Statistical differences (SNK test): ##, P < 0.01, cf. the remainder of the seasons; §§, P < 0.01, cf. spring and autumn; §§, P < 0.05 and §§, P < 0.01, cf. autumn and winter; *, P < 0.05 and **, P < 0.01, males cf. females.
Many studies have investigated the effects of different diets on body composition, growth and energy utilization in fishes, but information available on seasonal pattern in metabolic resources is limited to a small number of teleost species. In the present study pronounced seasonal changes in lipids, glycogen and total proteins content in the liver and muscle were found, which were probably related to the reproductive cycle and overwintering. The highest condition factor was observed in the spring, and coincident with the largest increase in $I_G$, due to sexual maturation during the prespawning season. Moreover, the inversely related seasonal cycles of $I_G$ and $I_H$ suggest that hepatic reserves were being used for gonadal development (M.L. Pinillos, M.J. Delgado & A.P. Scott, pers. data). Similar results have been published for the yellowtail rockfish *Sebastes flavidus* (Ayers) (Norton & MacFarlane, 1999). The liver gains mass during postspawning, when intensive food intake generally occurs in male and female tench, similar to the pattern observed in Arctic charr (Jobling et al., 1998). Diet also affects the chemical composition of the liver in tench (De Pedro et al., 2001). Thus, the hepatic energy reserves (proteins and glycogen) in the tench are clearly stimulated during autumn and winter. This seasonal profile in liver mass and metabolism is similar for both sexes in the tench, but the change in total protein is more pronounced in females. This result may in part be explained by hepatic vitellogenin production and confirms the well-known role played by this organ in vitellogenesis. Thus, the liver of female tench starts protein synthesis several months before the final maturation of the ovary at the end of spring (M.L. Pinillos, M.J. Delgado & A.P. Scott, pers. data). The high plasma protein levels in female tench during winter also support this conclusion.

The seasonal pattern in total protein content is clearly different in tench muscle and liver. Temperature, oxygen and salinity are important factors governing protein turnover rates in active tissues (e.g. the liver), but protein synthesis in muscle is clearly correlated to feeding (Fauconneau, 1985; Peragón et al., 1994). Such a differential regulation of protein turnover rates would justify the higher protein content in the tench ventral muscle during summer, when food is abundant.

Proteins represent the main fuel during sustained swimming in fishes (Weber & Haman, 1996), the white muscle being the major source of protein during spawning migrations (Ando et al., 1986). The tench, however, does not migrate and therefore, proteins from the muscle are probably mobilized for energy requirements in overwintering, when food availability is low. This has been suggested for brown trout *Salmo trutta* L. (Arévalo & Duran, 1988).

Lipids provide the most economical form of energy storage, and in fact the greatest change in body composition is usually produced in the lipid fraction. Lipids in fishes are stored in several organs. One of the most important lipid storage sites in the female tench is the ventral muscle (about 20-fold greater than the dorsal muscle and liver), which also exhibited the greatest seasonal changes. Similarly, the skeletal muscle in *S. Alpinus* also contains the major lipid store (Jorgensen et al., 1997; Jobling et al., 1998). During spawning, female tench lose a significant fraction of the lipid stores; those in the ventral muscle are fully depleted postspawning. A similar depletion during autumn and winter was reported for the liver of the yellowtail rockfish (Norton & MacFarlane, 1999). Such mobilization of lipid stores could be attributed to gonadal
recrudescence and to meet metabolic demands during cold periods when there is low food abundance (Jobling et al., 1998; Hutchings et al., 1999).

The contribution of carbohydrates to body mass and caloric resources of fishes is very limited compared to lipids and proteins. Nevertheless, absorbed carbohydrates that are not used to provide energy can be deposited in the liver as both glycogen and lipid after conversion (Brauge et al., 1994). The liver in the tench represents an important depot of glycogen, and exhibits significant seasonal changes. The high food availability during summer presumably allowed replenishment of this depot after winter depletion. Supporting this assumption is the finding that fasting induces a decrease in glycogen content in Oncorhynchus kisutch (Walbaum) (Sheridan & Mommsen, 1991) and S. trutta (Navarro et al., 1992). Glycogen depots in the tench liver are quantitatively higher than in muscle, and seasonal variations occur in both tissues. While glycogen content is reduced by 50% in the liver during autumn and winter, it is almost exhausted in muscle. This is similar to the findings of Sherstneva & Shabalina (1971) who studied the effect of temperature on rainbow trout muscle glycogen stores.

One interesting result from the present study is the distinct contribution of dorsal and ventral muscles to metabolic storage in tench. The dorsal muscle was mainly a deposit for glycogen, whereas the ventral muscle was the main storage depot of lipids.

Plasma glucose levels can vary due to different factors, diet being the most important. Also relevant are the stress of capture and handling, and obviously pathological state. Mean glucose levels for the tench are similar to those described in other teleost species, and the seasonal changes relate to food availability. The decline in plasma glucose during winter and spring probably reflects reduced food intake and the increase in tissue uptake mediated by pancreatic hormones (Sheridan & Mommsen, 1991).

The authors wish to thank the staff of the ‘Centro Nacional de Ciprinicultura Vegas del Guadiana’ (Badajoz, Spain) for kindly providing the tench, and to F. Bermejo (ETSIA, Univ. Politécnica Madrid) for taking care of the animals. This work was partly supported by a grant from the Spanish MCYT (AGL2001-0593) and by predoctoral fellowships from the UCM to A.I.G., and from the MEC to M.A.L.P., M.L.P. and E.I.

References


