Condition and Health Indicators of EXPLOITED MARINE FISHES

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WILEY Blackwell

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Cover image: Fish swimming on posidonia meadows. Photo by Toni Font.
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Foreword

Fisheries management is evolving from the conservation of individual living resources to conservation of entire aquatic ecosystems. Maximum sustainable harvest strategies require significant indicators to monitor the status of the ecosystems and their living components and thus to safeguard diversity, stock productivity and food availability. The present book compiles the state of the art in research of fish condition, ranging from basic scientific biochemical and physiological aspects to applied ecological aspects such as multiple exploitation strategies. There are prominent examples where fish condition has been proven to provide useful indicators for representing resource status and production driven by mortality, recruitment and growth, while the required data acquisition and assessment of fish condition were reasonably practicable. Given these facts, growing emphasis will be placed on assessments of fish condition of exploited stocks towards an applied standard monitoring procedure, in particular as regards the implication of varying fish condition on recruitment variation to improve fisheries management advice and measures.

Dr Hans-Joachim Rätz
European Commission, Joint Research Centre, Institute for the Protection and Security of the Citizen, Italy and Johann Heinrich von Thünen-Institut, Federal Research Institute for Rural Areas, Forestry and Fisheries, Institute for Sea Fisheries, Germany

Fish condition is often used as an indicator of the general well-being of the population in stock assessment. Condition also has an important impact on stock status through its influence on processes such as maturity at age and size. Yet, despite this importance there is still much to be learned about factors affecting condition and how decisions about energy allocation, including to the components of condition, are made. *Condition and Health Indicators of Exploited Marine Fishes* is an important contribution to this field. It covers all aspects from a description of the measures of condition, to physiology and biochemistry; the underlying biological mechanisms, to environmental factors affecting condition, and finally to the use of condition indicators in stock assessment. This broad coverage will aid researchers in their thinking about how condition is determined and what changes in condition mean for individuals and populations. Bringing all of these aspects together in one volume makes this book a very valuable reference tool.

Dr Joanne Morgan
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Understanding the causes and consequences of variability in condition, or health, of individuals is of intrinsic interest to all ecologists. Fish have made a disproportionately large contribution to our understanding of how variation in the condition of individuals affects vital rates related to reproduction and mortality. The large literature on the physiological

basis of variation in condition was well covered in the book by Shulman and Love (1999). Since then there has been huge growth in our knowledge of how condition relates to the physiology, biology, and ecology of fish and its relevance to the management of commercial species. We also understand better the problems inherent in the measurement of condition. This new work synthesizes these different aspects, making a useful companion volume to the earlier work. By highlighting applications of the information to contemporary issues, including climate change and aquaculture, the book makes the important point that accurate measurements of condition are central to any investigation into fish population dynamics.

Dr. Tara Marshall
University of Aberdeen, Scotland, United Kingdom

This book is very informative and valuable; it is organized into seven chapters, in which detailed descriptions of the most important measures of fish condition (structural, metabolic and functional) are presented. Methods for measuring and use of these indices are discussed, including examination of their merits and demerits. Selected chapters provide clear applied recommendations. Since the earlier publication of *The Biochemical Ecology of Marine Fishes* by Shulman and Love, new devices and methods have appeared. Numerous data have been collected that needed to be systematized, understood and interpreted properly. Moreover, the conviction has grown of the importance of condition indices from an ecological and fisheries perspective. Use of condition indices seems to have become more frequent, especially in new fields like analysis of population status, stock preservation, and understanding stock–recruitment relationships. The book mainly focuses on wild fish and presents a balanced review of worldwide information on the condition of marine exploited fish with broad geographic/ecological range including different life stages of species. It is truly an international example of a review, in which different literature sources (including Russian, Ukrainian, Scottish and Spanish sources that existed as a "grey literature") have been assembled for a broader international audience. The authors, well known for their excellent research, have provided a substantial and undeniable contribution to fish condition studies. The book should certainly be a "desktop" book for many fishery scientist/ecologists as well as for students and all who are interested in marine life research.

Dr Natalia A. Yaragina
Knipovich Polar Research Institute of Marine Fisheries and Oceanography, Russia

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For over a century, condition indices have been used to assess the health of fish. Today, with the development of new indices (mainly physiological and biochemical), they are increasingly used in fishery science and ecotoxicology. They are used to determine fish condition at different stages of ontogenesis, from larval to juvenile and adult phases, and to better understand the influence of the environment on population dynamics. In recent years, condition indices appear as relevant and essential indicators for analyzing the effects of environmental and human pressures on fish and for measuring the quality of the environment in which they live. The seven chapters in this book provide, for the first time, a detailed and complete analysis of the diversity of the current methods used for analyzing fish condition, providing cutting-edge examples of various applications and recent advances. In light of increasing environmental change and pressure in aquatic ecosystems, this book

improves knowledge on fish condition indices and their applications to fisheries and environmental sciences. It is an essential reference for students, researchers and fishery managers interested in fishery topics.

Professor Rachid Amara
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I am glad to comment on this book on the condition and health indicators of exploited marine fishes. Condition and health indicators are a diverse range of techniques widely used in fish biology and fisheries. The authors are to be applauded for providing an updated review on this topic in a single volume and from a variety of perspectives. We live in a world with an ever-expanding literature, where it is difficult to keep up to date, and where synthesis and critical reviews are more needed than ever. The authors provide a synthesis rather than a critical review but numerous aspects of the book are to be commended: the range of condition indicators reviewed, the inclusion of applied recommendations and emphasis of important points, and the diversity of facets treated in relation to condition and health indicators. One can only hope that the book will contribute to further understanding of fish biology and to more sustainable fisheries.

Dr Emili García-Berthou
University of Girona, Spain

Evaluation of fish health or well-being although central for the determination of the productivity of individuals and populations does not receive all the attention it should. This situation is surprising given the availability of a number of reliable indices of fish condition that are easily measured. The links between fish condition indicators and life history attributes like growth and reproductive potential and the impact of the environment on these life history traits give the possibility of using condition indices as integrators of physiological, biological and environmental effects on fish populations. Simple conditions indices like Fulton's K might prove to be most valuable in using time series of standard and largely available morphometric measurements like length and weight or limited information from data-poor stocks to monitor variations in fish stocks productivity. However, an essential and necessary step before using any conditions indices is to examine advantages, disadvantages, and limitations associated with selected indices as well as a validation of these indices as proxies of fish health status. The present book extensively covers all these aspects related to the measurement, significance and usefulness of condition indicators to study fish populations in their natural environment. It represents a significant reference for fisheries biologists interested in measuring fish health status and productivity.

Dr. Yvan Lambert
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Preface

In fish ecology, condition indicators have great significance for revealing the regularities and features of adaptations of fish to the environment and are a central component in understanding the history, behavior, and productivity of species and populations as well as their role in ecosystem functioning. Furthermore, condition indicators play a significant role in fishery science, where they can contribute to fishing management, as well as in aquaculture and stock preservation. The condition indicators that this book deals with are derived from fundamental biological science, and this is the basis for their main practical use, i.e., in fishery science. The book also combines indicators of energy features of fish with structural indicators because both are closely related to each other.

Condition indicators are closely connected with the health of individuals, stocks and populations. Fish health underlies the degree of general well-being and constitutes a significant component of condition. Health status includes many structural and energy characteristics that may be defined by morphological, physiological, and biochemical parameters. The dynamics of these structural and energy components constitute a key attribute for understanding biological, ecological, functional and metabolic features, and health in general. Sensu stricto, condition indicators measure the magnitude of stored energy reserves in fish, and therefore partly define the health status of an individual. These energy stores, which can be evaluated using several methods, can have large consequences for population success. Low energy reserves may lower the chances of survival, leading to an increase in natural mortality. Starvation due to exhaustion of energy reserves, particularly during the non-feeding and reproductive periods, weakens fish and renders them more susceptible to predation and fishery, diseases, and a variety of environmental stressors. Inadequate reserves have been implicated in the reduced reproductive potential of several fish species through reduced fecundity and quality of eggs and larvae or delayed maturation. Condition not only depends on fish physiological status but also on the environmental and anthropogenic circumstances experienced during some previous period. In this book we emphasize the evaluation of "normal" fish condition, i.e., those values at which all processes occur and provide fish with the ability to lead a successful life. Although in some chapters we consider divergences from this normal condition in connection with infections and toxic substances, these are not the main topics of our book.

The condition of fish may help to evaluate not only the well-being of fish stocks but can be also used as a broad indicator of marine environmental health. This is because a reduction in fish energy reserves does not only affect their own population success but may impact as well on the reproductive output of top predators, such as birds and mammals. Finally, fish condition can be used as a marker of habitat quality (identification of essential fish habitats, evaluation of habitat protection, etc.).

Although it is increasingly clear that fish condition indicators have important biological and ecological links with the productivity of fish stocks and the quality of their habitats, at present very few marine fish stocks and habitats are managed in a way that takes fish condition into consideration. At present most fish stocks are assessed using standard

procedures (VPAS, XSA, etc.) and evaluation of the quality of marine habitats, such as the identification of essential fish habitats (EFHs) or the evaluation of "reserve" effects (i.e., effects of habitat protection on fishery resources through the establishment of marine protected areas), rarely takes into account the condition of fish. Furthermore, analysis of the impact of environmental factors (including climate change) as well as anthropogenic factors (e.g. fisheries, pollution) usually disregards the condition of fish. In all these analyses, carried out within the framework of fisheries science, the variables used are commonly a measurement of population size (abundance, biomass, landings, etc.) and demographic traits (e.g., growth and reproductive potential), whereas fish condition is often not considered.

In this book the basic principles and methods that are central to any study of fish condition from a fisheries perspective are outlined and discussed, using concrete examples from the published literature. This book is intended as an introduction to the study of fish condition that will assist advanced undergraduate and postgraduate students, as well as researchers in the main fisheries topics (biology, ecology and stock assessment) and fishery managers. The book focuses on wild fish rather than on farmed fish as the latter show specific condition characteristics as a consequence of higher food supply, different diet and high fish densities, among other factors. The book does not pretend to be a specialized publication on fish physiology for researchers, neither a seafood chemistry book for aquaculture or fish processing companies. The book recompiles and reviews worldwide information on the condition of exploited marine fish from an ecological and fisheries perspective. Therefore, it concentrates on those condition indicators that require a level of cost and time that is acceptable in practical situations during fisheries surveys and studies, and that best represent the amount of energy stored within individual fish. We are well aware that the condition indicators described in the book omit a number of indicators that can be very important in describing fish health, but whose analysis is excessively costly, technically complicated and time-consuming, rendering them impractical for use in fisheries science. Although we are mindful that the number of studies on the condition of marine fish inhabiting the Atlantic and the Pacific is much greater than those from other oceans and seas, and that the number of studies dealing with the condition of particular taxa (e.g., gadoids and clupeoids) far exceeds the number of studies in other taxa, we attempt here to undertake a broader geographic range that consider as many marine species as possible. Thus, far from considering all studies on fish condition, we have selected examples from different seas and oceans around the world, and consider different life stages (from larvae to adults) from a diverse range of species (demersal and pelagic).

In this book we have reassessed old information on the condition of fish that was published as "grey literature" or in languages other than English (e.g., Russian), and therefore this book brings to the international scientific audience valuable historic and updated information about condition of fish that would otherwise stay inaccessible to researchers. In particular, we have gathered together the great work carried out by Russian, Ukrainian, and Scottish marine and fisheries biologists during the last century in the field. The renaissance of fish condition studies is a worldwide phenomenon that spans marine science in the oceans and seas around the world, and this will continue in the future as applications of these indices are being successfully tested in different domains that are important for society such as fisheries and aquaculture.

Although there is quite a large literature on the condition of freshwater fish (condition indicators are widely used in assessing their health), this book concentrates nearly exclusively on marine fish. We refer to freshwater fish only in a few examples to support particularly

important ideas. Compared with freshwater ecosystems, the analysis of fish condition in marine ecosystems has been largely overlooked and this book attempts to fill this gap by combining old literature with recent advances in this field. Given the move towards the incorporation of greater biological and ecological information into fisheries management, we hope that this book contributes to the ecosystem-based fisheries management approach.

In this book, we have not only described the potential abilities of condition indicators but also provide examples showing the use of these indicators in solving practical problems in fish and fisheries ecology. This book does not consider in detail aquaculture problems, because it is mainly intended for marine ecologists and fisheries biologists.

Key aspects of this book regarding physiological and biochemical indicators are derived from methodologies and examples given in *The Biochemical Ecology of Marine Fishes* (Shulman & Love, 1999). It is necessary here to acknowledge the excellent work carried out by Dr R. Malcolm Love from Scotland on the physiological and biochemical condition of marine fish, and his death was a great sorrow for all his colleagues. He made a valuable contribution to the present book, and thus he is included as a full coauthor. We hope that this book increases our knowledge of fish ecological morphology, physiology and biochemistry and that it will be useful for scientists studying natural populations and stocks in their natural environment, including fisheries biologists and managers.

Josep Lloret, Georgiy Shulman
and R. Malcolm Love

Acknowledgments

1 Description of condition indicators

Summary: Biologists have developed a wide range of morphological, biochemical and physiological metrics to assess fish condition. This chapter introduces all these indicators and analyzes the simple methods and criteria used to assess the condition of fish, from simple morphometric (weight–length) to morphophysiological (liver, gonad and mesenteric fat weights) indicators. Each method has its pros and cons, along with limits in their application, which are here detailed together with practical recommendations. The utility of each method is shown using examples from different marine fish species around the world.

Key words: Fulton, Le Cren, condition factor, relative weight, liver (hepatosomatic) index, perivisceral (mesenteric) fat index, digestive index

Biologists have developed a wide range of morphological, biochemical, and physiological metrics to assess fish condition and health. These metrics were originally used to quantify aspects of human health, but have also proven useful to address questions in life history, ecology, and resource management of game and commercial animals (Stevenson & Woods, 2006). Condition is an important descriptor of fish health. Fish in good condition are assumed to have larger energy reserves than poor-conditioned fish, as well as optimum health. Here, "fish health" refers to the maintenance of homeostasis, including the normal occurrence of life cycles (primarily growth and reproduction) and the preservation of abundance and productivity of populations (Hochachka & Somero, 1973, 1984, 2002; Nemova & Vysotskaya, 2004; Depledge & Galloway, 2005). The condition of fish can be assessed by a variety of criteria, ranging from simple morphometric indicators based on weight–length data and morphophysiological indicators based on liver and gonad weights (Fig. 1.1) or on mesenteric fat, to physiological and biochemical measures such as lipid or protein content, the concentration of hemoglobin in the blood, the concentration of myoglobin in red muscle, the color and volume of the bile, the enzymatic activity of the tissues, the pH of the muscle after death, and the content of other substances such as glycogen, glucose, lactate, and creatine phosphate (Shulman & Love, 1999). Each of these measurements of fish condition
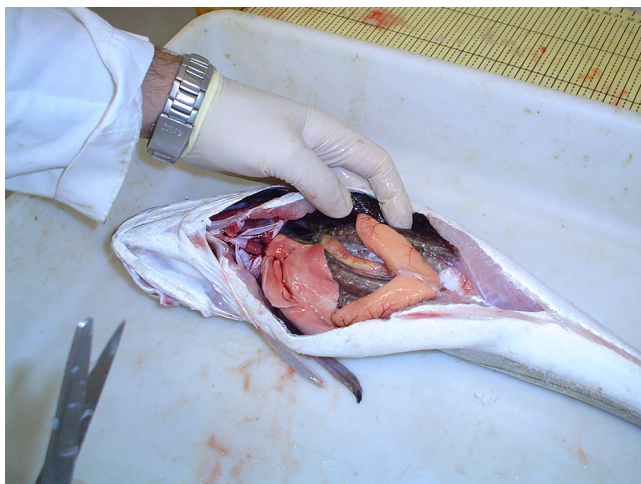
Figure 1.1 Dissection of a female European hake (*Merluccius merluccius*) showing the liver and ovaries. Photo by Dolors Ferrer.

has its own set of advantages and limitations, depending on the objectives of the particular study. In some cases, samples have to be processed and analyzed in the laboratory, which requires varying degrees of time, specialized training, instrumentation and expense, as in the case of biochemical analyses. In other cases the determination of condition indicators may involve only simple fish length and weight measures. Overall, the choice of condition criteria should be based on the objectives of the particular study, the particularities of each species, population and life stage with regard to body distribution and dynamics of energy reserves, as well as an understanding of the different condition techniques, including a detailed examination of the properties of the dataset as well as available laboratory or sampling facilities and budget. In some cases, integration of the information provided by different morphometric, physiological, and biochemical condition indicators may better reflect the overall physiological condition of the fish. In this chapter, the most used condition indicators in fisheries science are explained.

Some of the best techniques for analyzing fish condition are time-consuming and/or costly (e.g., lipid analysis with gravimetric methods), whereas other techniques that have lower precision are easy to carry out on board or in the laboratory with minimum equipment and cost (e.g., evaluation of morphometric indices). The expert will need to assess which is the best method to use in each case, taking into account the balance between precision, cost, and time. Indicators of the condition of exploited fish are similar to those used to measure condition in medicine and veterinary sciences. The aim of research in this topic is to identify indicators of fish condition that can define the best possible state of organisms and populations, and which can also signal and quantify deviations from it. Here we focus on these indicators that are most suitable and which have been widely used for the evaluation of fish condition for fisheries ecology and management purposes. We must keep in mind, however, that a series of indicators, rather than just one, gives much more information. For example, the protein content of cod muscle decreases during starvation only after the level of liver lipid has dropped below a certain critical value (Black & Love, 1986), so the extent of depletion can be realistically judged only by measuring both. The determination of muscle protein alone fails to detect the early stages of depletion, while liver lipids do not change further during a long series of subsequent stages.

Some authors have argued that, apart from the morphometric, physiological and biochemical indicators described in this book, it is important to establish autopsy-based assessment of the health and condition of fish (Goede & Barton, 1990; Leamon et al., 2000). Several infectious agents, such as viral, bacterial, fungal and parasitic infections, are known to severely affect fish condition, leading to sublethal or lethal effects (Goede & Barton, 1990) and therefore an empirical autopsy-based system of organ and tissue indicators would improve our knowledge regarding fish condition and also the impact of disease(s) on natural fish populations.

Even though simple condition indicators are not always capable of estimating the health status of a given species, Lloret et al. (2012) proposed that those such as morphometric and organosomatic (biometric) indicators (e.g., Le Cren, Fulton, hepatosomatic), and whenever possible total lipid content, are used as a first step for evaluating the amount of energy reserves in fish. This should not be a substitute for standard stock assessment methods but can provide additional information for determining the status of a given stock. Moreover, simple measures of parasite infection such as prevalence, intensity, and abundance could be evaluated (Lloret et al., 2012). For practical purposes, the authors proposed that monitoring could also include the macroparasites (i.e., those large enough to be seen with the naked eye such as cestodes and nematodes) but not the microparasites (e.g., protozoans, which are more difficult to detect) even though they may also have an impact on the condition and reproduction of fish (see for example Kramer-Schadt et al., 2010; Sitjà-Bobadilla, 2009). The monitoring of parasitism will provide therefore a further index of fish health. While no single measurement of fish health uniquely indicates a source of stress (Buckley, 1985), all these simple related energy reserve and parasitism indicators taken together could be used as an index of fish condition (health).

In particular, the analysis of fish health during critical life periods (e.g., prior to spawning or migration, or in early life stages) is important for detecting the effects on stock productivity and thus their availability to the fisheries (Lloret et al., 2012). Several studies suggest that the condition of spawners at or just before the time of spawning would be a better proxy for reproductive potential (Marshall & Frank, 1999). In addition, knowledge of the lipid content in fish species can further enhance our ability to determine the human health benefits of consuming these fish, particularly with regard to essential fatty acids (fish are often promoted as a healthy component of the human diet because of high levels of essential fatty acids). In the following sections of this chapter we focus on simple condition (morphometric and organosomatic) indicators.

1.1 Morphometric indicators

Morphometric condition indicators are the simplest indicators of energy storage in fishery species. They are constructed with simple weight and length data that can be easily obtained from surveys or commercial landings using minimum and affordable equipment such as an ichthyometer (Fig. 1.2) and scale. These indicators assume that heavier fish of a given length are in better condition (Jones et al., 1999). Therefore, they are based on the premise that a fish of a given species and length should weigh as much as a standard for its length, and variations from the standard are taken as an indication of the relative fitness of an individual. These morphometric condition indicators have been available since the early 1900s and have undergone an evolution in methodology (Murphy et al., 1991). They have remained popular tools because they are inexpensive, simplistic, and mostly non-destructive, and are
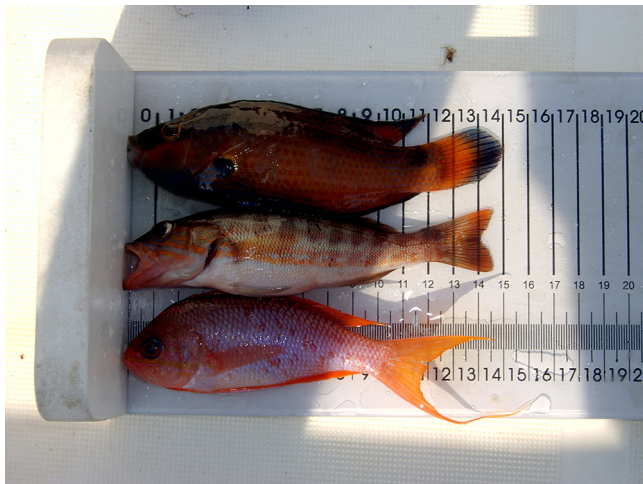
Figure 1.2 Fish length measurement using an ichthyometer. Photo by David Caballero and Núria Zaragoza.

easily calculated from historical datasets that describe the length and weight of individuals (Lambert & Dutil, 1997a; Pope & Kruse, 2001). However, their use has remained sometimes controversial. Blackwell et al. (2000), Pope and Kruse (2001), and Nash et al. (2006) provide thorough reviews of the history of condition factors, together with the controversies surrounding their analysis and interpretation. Stevenson and Woods (2006) argue that morphometric condition factors actually measure the shape (i.e., girth) of a fish rather than being a direct measure of the extent of energy reserves, and several reviews have highlighted the statistical deficiencies of morphometric condition indicators (e.g., Cone, 1989; Hayes & Shonkwiler, 2001). To overcome potential bias and errors, Froese (2006) gives several recommendations for the proper use and presentation of morphometric condition factors, including guidelines for data collection and analysis of weight–length relationships.

For the determination of condition indicators based on length and weight, it is always preferable to use eviscerated weight instead of total weight because the latter is not affected by the viscera and gonad weights. Some authors have even used muscle weight instead of eviscerated weight (e.g., Kurita et al., 2003).

1.1.1 Fulton's *K* condition factor

K (or Fulton's) condition factor (Ricker, 1975) was the first morphometric condition factor used in fisheries science. According to Nash et al. (2006) the origin of this condition factor is attributed to Heincke (1908). *K* is computed using the formula:

$$K = (W/L^3) \times 100 \tag{1.1}$$

where *W* is the weight of the individual and *L* its total length. The index uses 1 as a benchmark for the condition of a standard fish: fish above or below 1 are considered in relatively better or worse condition than a standard fish, respectively, depending on their distance from the benchmark. Nevertheless, it is important to note that the stated formula assumes isometric growth in fish, in other words the *b*-value of the weight–length relationship has to be 3 or

very close to 3. In some species such as cod, this assumption is met and therefore a number of studies have used K to evaluate their condition (e.g., Lambert & Dutil, 1997a; Lloret & Rätz, 2000).

However, in other fish species this is often not the case (b-value is not exactly 3 and not close to that value), and there appear to be correlations between the condition factor and length (Bolger & Connolly, 1989; Cone, 1989). Thus K increases with increasing length (b-value >3.0) and decreases with decreasing length (b-value <3.0). In these cases, this limits the application of K to fish of similar length within the same species. Thus the interpretation of the condition factor is prone to error when the growth of fish is not isometric (when b is above or below 3.0). To use K correctly, the assumption of isometric growth must be checked within each stratum (e.g., sex, population) for which comparisons will be made (Cone, 1989). For example, a difference in mean condition between two populations can be caused by different mean lengths in the respective populations. A way to avoid the problems that this could create is to compare individuals of similar length, or populations with similar length structures only (Blackwell et al., 2000). However, one must consider that bathymetric and spatial distribution of a species is often related to length (see for example Macpherson & Duarte, 1991). In order to solve the problem some authors included length as a continuous predictor variable into the model of K (e.g., Lloret et al., 2002). Nevertheless, other indicators have been widely used to overcome the length dependence with accuracy, and these are described here.

1.1.2 Le Cren's relative condition factor (K_{n})

Le Cren (1951) attempted to solve the deficiencies of Fulton's K condition factor by comparing the actual weight to a standard predicted by the weight–length regression based on the population from which the fish was sampled. Hence, he introduced the relative condition factor (K_{n}), which compensates for changes in condition with increase or decrease in length. It measures the deviation of an individual from the average weight for length in the respective sample. This length-independent measure of condition is calculated with the following formula:

$$K_{n} = W/W_{e} \qquad (1.2)$$

where W is the observed weight of the fish and W_{e} the estimated weight of that fish. K_{n} uses 1 as a benchmark for the condition of a standard fish: fish above or below 1 are considered in relatively better or worse condition than a standard fish, respectively, depending on their distance from the benchmark.

However, a disadvantage of K_{n} is that the mean value of this index is a function of weight–length relationship parameters. Because weight–length relationships can vary among populations and geographic sites, comparisons of K_{n} must be confined to those populations with homogeneous weight–length parameters (Bolger & Connolly, 1989). The result is that different weight–length equations are needed to compute W_{e} for each region or population, making comparisons across water bodies difficult.

To solve this problem, one can derive a single weight–length relationship obtained from all individuals to be considered in the analysis, with the formula:

$$K_{n} = W/W_{e}' \qquad (1.3)$$

where W_e' is computed using the weight–length measurements for all individuals that are to be included in the analysis. This approach is very similar to the analysis of the relative weight (see section 1.1.3) but relies on the availability of the original weight and length data to construct a common weight–length relationship from all samples (populations, regions, sexes, ages, months, years, etc.). Thus, if a single weight–length relationship is estimated for the whole dataset, the K_n will be comparable across all samples in the dataset. The necessity to have original weight–length data from all samples can be a handicap because it is based on cooperation between different agencies/researchers in charge of data collection in order to share weight–length data, and on the need to reevaluate the common weight–length relationship as new data become available. Then, to evaluate interstock differences in fish condition, it would be advantageous to develop stock-level condition indicators from standardized databases on weight and length (Marshall et al., 2004).

Many studies have confirmed the ability of this approach to compare the condition of fish from different samples, always providing that the estimated weights are derived from weight–length relationship representative of all individuals in all samples. K_n has been applied to several species, for example cod (*Gadus morhua*) in the North Atlantic (Bishop & Baird, 1994; Pardoe et al., 2008) and Arctic cod (*Boreogadus saida*) in the Gulf of Alaska (Khan et al., 1997).

1.1.3 Relative weight (*W*$_r$)

The relative weight was first proposed by Wege and Anderson (1978) as a fish condition index, and represents further evolution of the K_n concept by allowing comparisons of condition across the geographic occurrence of a species. The W_r index is calculated as:

$$W_r = W/W_s \times 100 \tag{1.4}$$

where W is individual fish weight and W_s is a length-specific standard weight predicted from a weight–length regression developed to represent the body form of the species across its geographic range (see Blackwell et al., 2000 for a list of developed standard weight equations). This index uses 100 as a benchmark for fish in good condition: fish above or below 100 are considered in relatively better or worse condition than a standard fish, respectively, depending on their distance from the benchmark.

The application of W_r has increased over the last decade and has been commonly used as a condition assessment tool in United States freshwater fish surveys (Blackwell et al., 2000). Relative weight can serve as a surrogate for estimating fish energy reserves, as a measure of fish health, and to assess prey abundance, fish stocks and management actions (Blackwell et al., 2000). The analysis of W_r relies on the availability (prior to the analysis) of standard weights equations (W_s) that cover the entire length structure of the species. If this is not the case, problems may arise when determining condition of small and juvenile fishes for example, because many of these individuals are below the minimum applicable length of W_s equations. Thus, the quality of the W_s found in the literature can underpin the analysis of the dataset. This problem will be solved by using Le Cren's K_n condition factor explained in section 1.1.2.

1.1.4 Other methods based on weights and lengths

Other methods have been used to analyze weight and length data but their use has been rather limited in fisheries science. For example, weight–length regression has been used by

several authors to compare condition. Several methods have been proposed for evaluating the weight–length regression including ordinary least-squares regression (Cone, 1989) and analyses of covariance to test differences in weight–length regression lines (García-Berthou & Moreno-Amich, 1993; Blackwell et al., 2000). Another method is the residual analysis (Fechhelm et al., 1995), which is synonymous with the concepts of K_n and W_r in that all three examine the deviation of predicted weight from some common weight–length relationship.

1.1.5 Limits of use of morphometric condition indicators

Many questions remain whether any weight to length ratio is a valid and interpretable indicator of physiological condition in fish. Ideally, any study using morphometric condition indicators should define formally what is being measured by that condition index and validate it against a suitable benchmark, for example a biochemical index (Davidson & Marshall, 2010; McPherson et al., 2011). This type of validation has been carried out for several species and several studies have found positive relationships between morphometric and organosomatic and biochemical indicators in different fish species (Rose, 1989; Brown & Murphy, 1991; Lambert & Dutil, 1997a,b; Pangle & Sutton, 2005; Kaufman et al., 2007). Thus for example, Fulton's K condition factor was positively correlated with crude-lipid content of juvenile lake herring *Coregonus artedi* (Table 1.1; Pangle & Sutton, 2005) whereas the condition factor correlated with the percentage lipid content of somatic tissue of adult Atlantic salmon (Fig. 1.3; Todd et al., 2008). In this case, the poorest condition salmon, which were about 30% underweight, showed lipid reserves reduced by about 80% compared with the highest condition fish. It is also important to note that the strength of the relationships between various condition factors and biochemical condition indicators can vary

Table 1.1 Relationship of Fulton's K condition factor with proximate composition components of juvenile lake herring *Coregonus artedi**

Day of experiment	Linear-regression equation	MS error	SE of K coefficient	r^2	P
75	Crude lipid $= 0.599 + 9.845\,K$	1.482	1.218	0.612	<0.001
	Crude protein $= 12.059 + 2.208\,K$	1.644	0.669	0.208	0.076
	Gross energy $= 332.8 + 1153.5\,K$	38,766.324	196.892	0.453	0.004
	Ash $= 4.232 + 1.666\,K$	1.152	2.258	0.112	0.642
	Water $= 86.417 - 8.888\,K$	26.169	1.528	0.435	0.005
150	Crude lipid $= 0.305 + 11.222\,K$	1.458	1.208	0.615	<0.001
	Crude protein $= 10.789 + 7.353\,K$	13.995	0.716	0.661	<0.001
	Gross energy $= -339.8 + 2687.9\,K$	47,745.583	218.508	0.737	<0.001
	Ash $= 4.377 + 0.868\,K$	0.154	0.559	0.039	0.496
	Water $= 90.704 - 20.687\,K$	117.246	1.722	0.748	<0.001
225	Crude lipid $= -1.994 + 15.634\,K$	1.781	1.335	0.640	<0.001
	Crude protein $= 8.173 + 13.631\,K$	13.995	0.716	0.674	<0.001
	Gross energy $= -683.2 + 3577.1\,K$	72,530.75	269.315	0.695	<0.001
	Ash $= 2.962 + 4.464\,K$	3.472	0.894	0.250	0.058
	Water $= 93.222 - 27.189\,K$	132.963	2.048	0.697	<0.001

*Linear-regression variables of the relationships for crude lipid, crude protein, gross energy, ash and water content and the Fulton condition factor (K) for juvenile lake herring on days 75, 150 and 225 of the laboratory experiment. Crude lipid, crude protein, ash and water content were expressed as percentages of wet body mass, while gross energy was expressed as J/g wet body mass.
Source: Pangle & Sutton (2005).
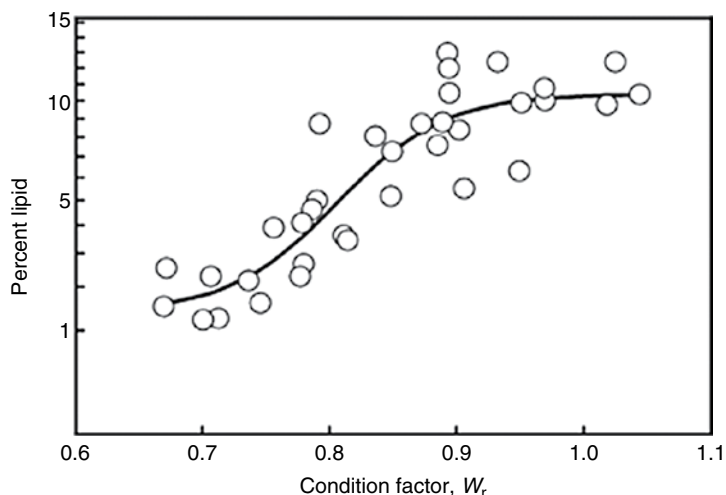
Figure 1.3 Relationship between condition factor and percentage lipid content of somatic tissue of adult Atlantic salmon (*Salmo salar*). From Todd et al. (2008).

considerably among populations, and also between sexes within populations, for example adult walleyes (*Sander vitreus*) in Canadian lakes (Kaufman et al., 2007).

Notwithstanding these examples, the relationship between condition and lipid reserves has not always been observed. Thus for example, the relationships between morphometric condition factors and bioenergetic and biochemical indicators in Atlantic herring (*Clupea harengus*) are inconsistent and often nonexistent, with the correlation dependent on both the maturity stage of the individual fish and the fat depot with which it was being compared (Davidson & Marshall, 2010; McPherson et al., 2011). Whereas Fulton's K was significantly correlated with fatmeter values (Fig. 1.4; Davidson & Marshall, 2010; McPherson et al., 2011), the relationship between K and mesenteric fat was inconsistent and often nonexistent (Fig. 1.5; McPherson et al., 2011). Similar to this, in bluegills (*Lepomis macrochirus*), the relative weight shows an imprecise relationship to body constituents such as lipid and protein (Copeland et al., 2011). It is even possible that the higher weight of a given fish is due to the higher water content in the tissues and not really lipids or any other components of energy stores (Shulman & Love, 1999). Because 60–80% of the fresh weight of a fish consists of water, variations in water content (and not energy reserves) could account for most of the variation in weight. In some cases, the low accuracy of morphometric factors renders them invalid for estimating the impact of factors on fish condition. For example, parasites provoked a twofold to threefold decrease in triacylglycerol in the body of anchovy (Fig. 1.6), while Fulton's condition factor remained unchanged (Shulman & Love, 1999). Furthermore, the relationship between these different indicators may depend on the reproductive stage or the season (Pangle & Sutton, 2005; Copeland et al., 2011; McPherson et al., 2011). For example, Fulton's K was particularly correlated with fatmeter values for fish with inactive gonads (Fig. 1.7; McPherson et al., 2011).

Despite all these facts, many studies use morphometric condition indicators without validation. If morphometric indicators are not validated by being correlated with biochemical indicators, then they should be considered as putative indicators of condition (McPherson et al., 2011). Another aspect to consider is the number of data needed to evaluate these
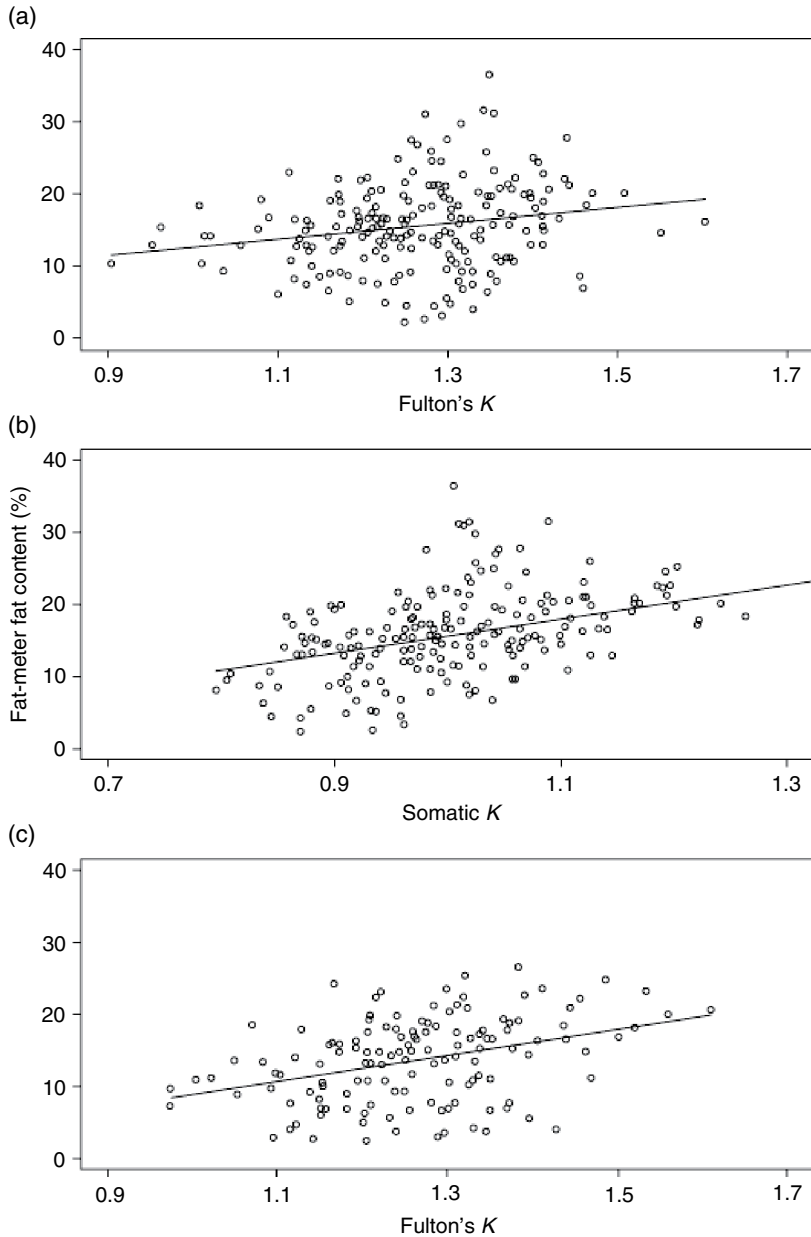
Figure 1.4 Correlation between fatmeter fat content and (a) Fulton's *K* and (b) somatic *K* from the 2004 data, and (c) fatmeter fat content and Fulton's *K* from the 2005 data, in herring *Clupea harengus*. From Davidson & Marshall (2010).

simple condition indices. It only makes sense to use them when there are hundreds or thousands of individuals measured. Indeed, when there are fewer weight–length values, the use of these morphometric condition indices is not recommended, and organosomatic and particularly biochemical indicators must be considered instead.
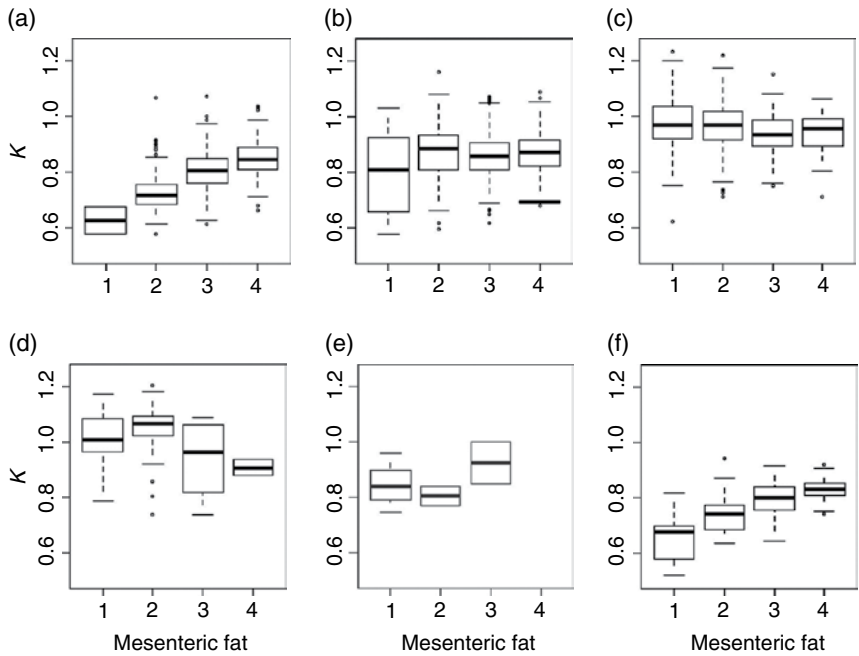
Figure 1.5 Relationship between four mesenteric fat stages and K values across the maturity cycle of Atlantic herring (*Clupea harengus*), including both sexes: (a) immature; (b) maturing 1; (c) maturing 2; (d) maturing 3; (e) spawning; (f) spent/recovering. These six categories (a-f) are related to the degree of gonad development. Boxplots are used to clarify the differences in median and variance between the four mesenteric fat stages. From McPherson et al. (2011).

Figure 1.6 Anchovies (*Engraulis encrasicolus*) captured in the northwest Mediterranean. Photo by Sílvia Vila.

The appropriateness of condition indicators in statistical testing has also been the subject of several reviews. Bolger and Connolly (1989) indicated several statistical difficulties with ratio data such as K_n and W_r, such as increased variability when compared to the variables forming the ratio; biased estimation of the true mean value of the ratio; unusual and non-normal distributions; and a tendency to obscure intervariable relationships. Furthermore,
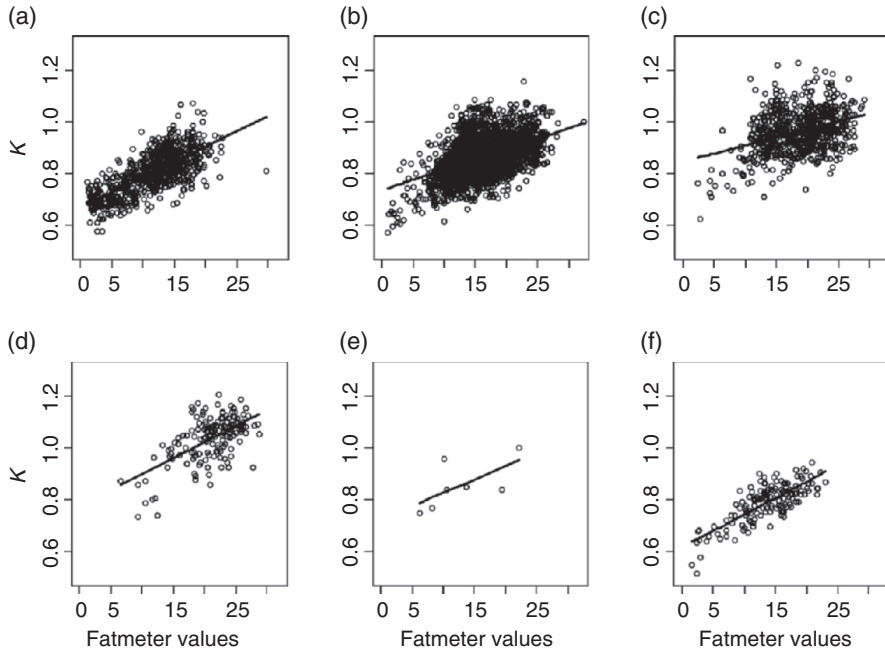
Figure 1.7 Relationship between fatmeter values and *K* across the maturity cycle of Atlantic herring (*Clupea harengus*), including both sexes: (a) immature; (b) maturing 1; (c) maturing 2; (d) maturing 3; (e) spawning; (f) spent/recovering. These six categories (a–f) are related to the degree of gonad development. Lines indicate linear regression. From McPherson et al. (2011).

they indicate that these types of data commonly exhibit heteroscedasticity, which violates the assumptions of common statistical tests (e.g., regression and analysis of variance) and weakens the power of these comparisons. Given these arguments, it is apparent that care should be taken when statistically analyzing condition indicator values to ensure that the assumptions of each statistical test are not violated.

Furthermore, the interpretation of any morphometric condition index based on individual weights is prone to error when total weights are used. For example, a difference in mean condition between two populations can be caused by differences in the development (weight) of gonads or by differences in the stomach content between the two samples/individuals. Therefore, it is preferable to use eviscerated weights instead of total weights because the latter are not affected by the viscera and gonad weights.

Finally, it is important to note that accurate morphometric condition assessments are dependent on correct length and weight measurements. Length measurements are relatively easy to obtain with fish measuring boards and are generally relatively accurate (Blackwell et al., 2000). It is important to clarify if these are total lengths, fork lengths, or standard lengths (Fig. 1.8). Weighing fish with scales of appropriate precision can be more difficult and time-consuming than measuring fish length and there is increased potential for making errors if appropriate scales are not used (Blackwell et al., 2000). The quality of the scales must be taken into account, especially with regard to variables such as precision, calibration, and motion compensation. Scales with motion compensation allow stable and accurate weights to be obtained while at sea. To keep a scale in top condition, calibrations need to be carried out on a regular basis. Furthermore, the user must check the units (g, kg, cm, mm, etc.) of the weight and length data used to compute these morphometric indicators, as well

Common measurements
The measurement is taken flat, not along the curve of the fish.

Total length

Note: Mouth should be shut and tail fin pinched closed.

Girth
The distance around the fattest part of the fish

Fork length
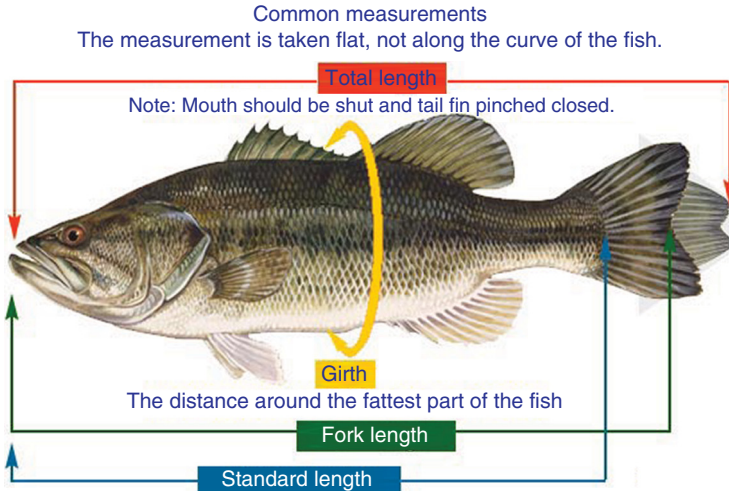
Standard length

Figure 1.8 Common measures of fish length: standard length, fork length, and total length. Original picture from Duane Raver, United States Fish and Wildlife Service. Modified by Bob Wattendorf, MyFWC.com.

as the transformations (e.g., logarithmic) that are sometimes needed prior to the development of statistical analysis when data do not meet the standard criteria (e.g., normality).

1.2 Organosomatic (bioenergetic or morphophysiological) indicators

Whereas condition indicators attempt to indirectly approximate the energetic fitness based on individual whole body mass, other measures of condition relate directly to the physiological composition of body tissues, providing a more precise measure of actual fitness in terms of stored energy. Organosomatic indicators of condition use an index (ratio) of tissue weights where individuals store energy. Among the available indicators, the liver somatic index (also called hepatosomatic index, liver index, or ratio of liver weight), the mesenteric fat index, and the digestivosomatic index are the most common ones. However, the evaluation of these organosomatic indicators is more time-consuming than the analysis of morphometric indicators because individuals need to be dissected in order to remove and weigh their livers or mesenteric fat.

1.2.1 Hepatosomatic index (liver index or relative liver condition)

The liver (Fig. 1.9) is an important organ for energy storage and is usually the first site for lipid (energy) storage in a number of benthic and demersal species such as gadoids (e.g., Kjesbu et al., 1991; Lambert & Dutil, 1997b; Lloret et al., 2008) and sharks (e.g., Rossouw, 1987; Hoffmayer et al., 2006) as well as deep-sea fish such as macrourids (Drazen, 2002). For example, lipids normally constitute more than 50% of the liver wet weight of cod (Lambert & Dutil, 1997b), up to 66% wet mass (or 82% dry mass) in haddock (Hiddink et al., 2005), nearly 70% of the dry liver of hake (Lloret et al., 2008), and up to 56% lipid (wet liver mass) of the common macrourids *Coryphaenoides armatus* and *Coryphaenoides*

Figure 1.9 The liver, an important organ for energy stores in gadoid species such as hake *Merluccius merluccius*. Photo by Josep Lloret.

acrolepis (Drazen, 2002), confirming the important role of the liver in energy storage in these species. In contrast, only about 1% of the wet weight of the muscle tissue of cod consists of lipids (Yaragina & Marshall, 2000) whereas lipids constituted on average only 3% of the dry muscle of hake (Lloret et al., 2008). For all these species, a liver or hepatosomatic index would more accurately measure the condition of such fish and therefore the periodic evaluation of a liver index would be a more reliable measure of condition than simple morphometric indicators. The hepatosomatic index (HSI) can be calculated as:

$$HSI = (LW/EW) \times 100 \tag{1.5}$$

where LW is liver weight and EW the eviscerated weight of the individual.

For other species the liver is still a key organ because it is the principal site of lipogenesis and in some species (e.g., small pelagics such as sardines) it seems that during feeding periods, excess dietary lipids are exported from the liver and are accumulated and stored in specific long-term storage sites such as mesenteric fat, the fat within the white muscle and between skin and muscle (Tocher, 2003). The relationship between biochemical composition (lipid and energy content) and liver index in gadoid species indicates that the liver condition index is a good indicator of the energetic condition of these species (Lambert & Dutil, 1997b; Hiddink et al., 2005; Lloret et al., 2008). For example, in haddock the hepatosomatic index was strongly correlated with the liver oil content of the liver ($r = 0.9$), the energy density of the whole fish ($r = 0.9$), and total energy stores per fish ($r = 0.8$), whereas liver energy stores correlated with muscle energy stores (Fig. 1.10; Hiddink et al., 2005). In particular, it seems that the liver plays a key role in oogenesis and ovarian development of teleosts (Nicolas, 1999), as indicated by the numerous studies that have linked liver indicators and reproductive activity of fish (e.g., sardine; Ganias et al., 2007).

Among the species for which a liver index has been used as an indicator of fish condition are hake *Merluccius merluccius* in the Mediterranean (e.g., Lloret et al., 2008); cod *Gadus morhua* (e.g., Lambert & Dutil, 1997b; Yaragina & Marshall, 2000; Marshall et al., 2004; Pardoe et al., 2008) and haddock *Melanogrammus aefleginus* (Hiddink et al., 2005) in the North Atlantic; Arctic cod *Boreogadus saida* in the Gulf of Alaska (e.g., Khan et al., 1997);
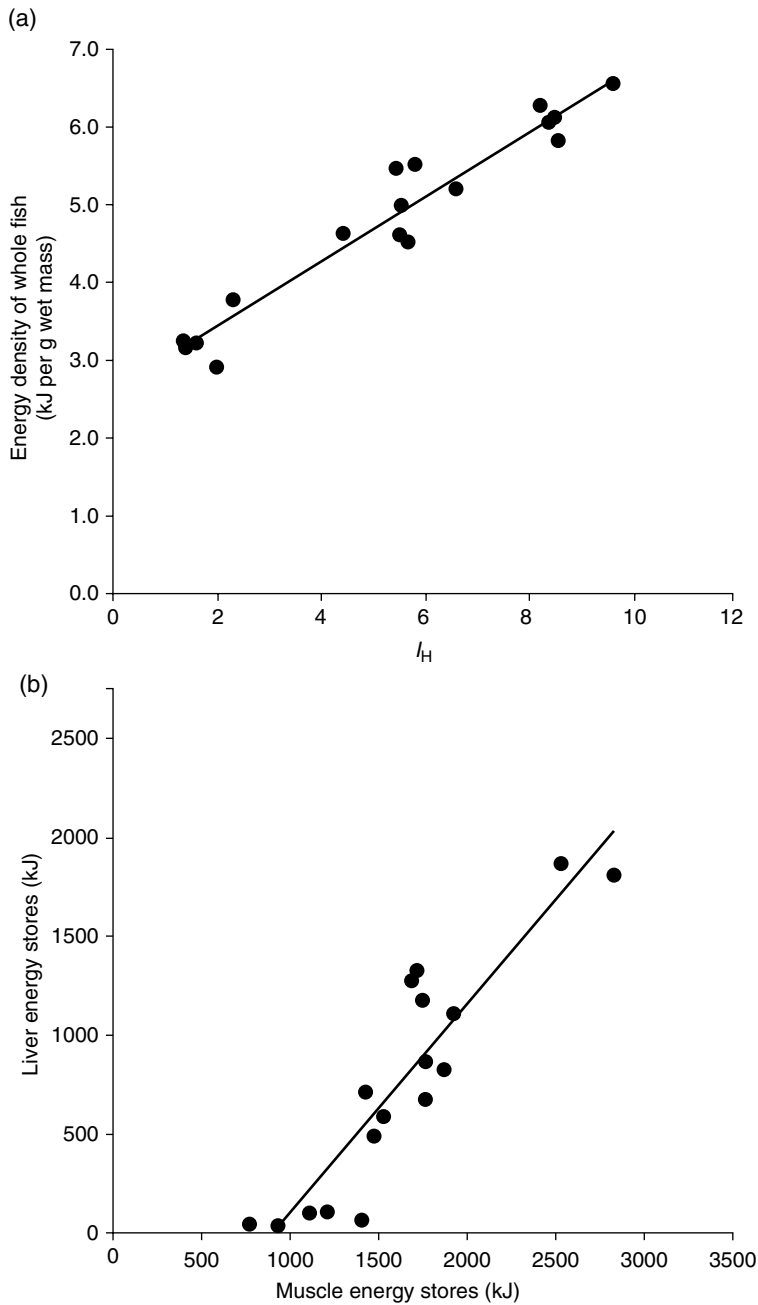
Figure 1.10 The relationship between haddock *Melanogrammus aeglefinus* (a) hepatosomatic index and energy density of whole fish and (b) muscle and liver energy content. From Hiddink et al. (2005).

common macrourids (*Coryphaenoides armatus* and *C. yaquinae*) in the Pacific (e.g. Drazen, 2002); snapper *Pagrus auratus* in New Zealand coastal waters (e.g. Francis, 1997); lesser sand shark *Rhinobatos annulatus* in South African waters (Rossouw, 1987); and pollock *Pollachius virens* in the Pacific (Jensen, 1979).

1.2.2 Mesenteric (adipose or perivisceral) fat index

In some fish species, the perivisceral fat (i.e., mesenteric fat or the adipose tissue surrounding the gastrointestinal tract; Fig. 1.11) develops in particular seasons, constituting important lipid storage. In particular, it seems that mesenteric fat reserves play a key role in the reproductive process of some fish such as bluemouth *Helycolenus dactylopterus* (Muñoz et al., 2010), several small pelagics including *Sardinella aurita* (Ter Hofstede et al., 2007; Mustac & Sinovcic, 2012) and sardine *Sardina pilchardus* (Ganias et al., 2007), and several sparids including red porgy *Pagrus pagrus* (Aristizabal, 2007), blackspot seabream *Pagellus bogaraveo* (Costanzo et al., 2011) and white seabream *Diplodus sargus* (Martínez-Pastor & Villegas-Cuadras, 1996). Mesenteric fat is much more labile than other fat stores, such as muscle fat (Slotte, 1999), and therefore mesenteric fat is likely to be the first fat store to become depleted during gonad maturation, migration, or overwintering, and the first fat store to respond to increased food intake.

From the mesenteric fat stores, a perivisceral fat index (PFI) can be calculated as:

$$PFI = (PF / EW) \times 100 \tag{1.6}$$

where PF is the perivisceral fat weight and EW is the eviscerated weight of the individual. Alternatively, visual assessments of the mesenteric fat can be carried out as a gross measure of the magnitude of fat deposited in the mesentery and has been applied routinely to several species of small pelagic fish such as anchovy (*Engraulis encrasicolus*) and sardine (*Sardinops sagax*) in South African waters (van der Lingen & Hutchings, 2005), *Sardinella aurita* in the Adriatic (Mustac & Sinovcic, 2012) and northwest Africa (Ter Hofstede et al., 2007), herring (*Clupea harengus*) from the North Sea (Slotte 1999), *Helicolenus dactylopterus* in the Mediterranean (Muñoz et al., 2010), and red porgy (*Pagrus pagrus*) in the southwestern Atlantic coast (Aristizabal, 2007).

This method consists of allocation to a number of fat stages depending on the amount of fat associated with the intestine and stomach (for example in *Sardinella aurita*, Table 1.2; Ter Hofstede et al., 2007). This technique has the advantages of being quick and easy to apply, requires no specialized equipment and is cheap, and is therefore well suited for use at sea for those species that accumulate mesenteric fat (van der Lingen & Hutchings, 2005).

Figure 1.11   Dissected anchovy *Engraulis encrasicolus* showing the mesenteric fat. Photo by Dolors Ferrer.

Table 1.2 Classification of fat content (categories 0–3) of round sardinella (*Sardinella aurita*)

Category	Fat content
0	No fat at all
1	Small chains of fat along intestines
2	Chains of fat cover half of intestines
3	Intestines completely covered with fat

Source: Ter Hofstede et al. (2007).

These factors, together with good reproducibility and accuracy, demonstrate its efficacy as a method for assessing the condition of pelagic fish.

1.2.3 Digestivosomatic index or digestive index

Some authors have evaluated a digestivosomatic index (DSI), which expresses the size of the gut relative to the mass of the body of the animal, as a condition indicator. This index has been computed for example in sea cucumber *Apostichopus japonicus*, a commercially important marine species for aquaculture in China (Gao et al., 2008), and in several demersal fish in the Mediterranean including *Mullus barbatus*, *M. surmuletus*, *Pagellus acarne*, *P. erythrinus* and *Diplodus sargus* (Lloret et al., 2002; Lloret & Planes, 2003). The index can be expressed as:

$$DSI = 100\,(DW/W) \times 100 \tag{1.7}$$

where DW is the weight of the digestive tract (stomach plus intestine) and *W* the weight of the whole individual (better eviscerated weight). In some cases the digestive tract has been weighed with food items inside.

1.2.4 Limits of applicability of organosomatic indicators

As was the case with the morphometric condition indicators, ideally any study using bioenergetic condition indicators should formally define what is being measured by that condition index and validate it against a suitable benchmark, such as a biochemical index (McPherson et al., 2011). Although this type of validation has been carried out for several species (reviewed by McPherson et al., 2011), many studies use bioenergetic condition indicators without validation. If bioenergetic indicators are not validated by being correlated with biochemical indicators, then they should be considered as putative indicators of condition (McPherson et al., 2011).

2 Physiological and biochemical condition indicators: their relevance in fish metabolism

Summary: This chapter defines the physiological and biochemical condition indicators used to assess the condition of fish. The relevance of these indicators in several key aspects of fish metabolism is described, including energy expenditure, transport of energy, energy catabolism, and energy anabolism (accumulation of energy). The analysis of lipids (total lipid and lipid classes) along with other parameters that are important for fish condition, and the metabolism of carbohydrates, proteins, enzymes, nucleic acid-based indicators, and hormones are described. Simple options for evaluating lipid content, such as the analysis of water content and the use of electronic portable devices, are also described.

Key words: anabolism, energy, structures, catabolism, lipids, carbohydrates, proteins, enzymes, hormones, fatty acids, RNA/DNA ratio

Physiological and biochemical indicators (biomarkers) are proxies for the functional condition and metabolic state of different biological systems: species, populations, organisms, and at sub-organism levels (tissues, cells, sub-cells, organelles, membranes, and molecules). Their study and practice has been widely developed since the second half of the last century, when it became realistic to apply the traditional approaches of physiology and biochemistry to companion sciences such as medicine, livestock science and ecology. The development of fundamental and applied problems in ecology (adaptations, strategies of biological development and features of biodiversity, life cycles, behavior and distribution, substance and energy transformation, estimation of species and population conditions and their place in communities, ecosystems and the biosphere as a whole) would have been impossible without the application of both these classical sciences (Prosser & Brown, 1962; Hochachka & Somero, 1973, 1984, 2002; Prosser, 1991). All this is undoubtedly related to ichthyology, where the application of these methodologies has become especially urgent, for example in the exploitation of natural stocks and their preservation, and in intensive aquaculture.

Table 2.1 Effect of infection by parasites (nematode larvae) on Black Sea anchovy

Months	N	Strength of infection	Condition factor	Triacylglycerols in muscle (%)
January	12	Weak	0.70	2.654 ± 0.75
	12	Strong	0.78	1.144 ± 0.31
March	12	Weak	0.87	1.823 ± 0.25
	10	Strong	0.88	0.322 ± 0.20
May	14	Weak	0.83	0.363 ± 0.07
	14	Strong	0.83	0.111 ± 0.03
October	10	Weak	0.86	3.867 ± 0.40
	10	Strong	0.89	2.554 ± 0.35

Source: after Shulman & Shchepkina (1983) and Shulman & Love (1999).

The development of physiological and biochemical indicators of fish condition was based on the morphophysiological indicators that appeared in the first half of the last century and which are considered in Chapter 1. In comparison with morphophysiological indicators, physiological and biochemical indicators allow:

1. the characterization and estimation of significant processes in organisms and populations of fish;
2. the use of relatively small samples, because (surprisingly) physiological and biochemical indicators are considerably less variable than morphological and morphophysiological ones;
3. the use of new modified "express" research methods not only in laboratories but also in field situations as well as during fishing.

In some cases physiological and biochemical indicators reveal the significance of processes that morphophysiological ones are powerless to answer correctly. In this book we will often deal with such examples, but here we will consider only one. While studying the influence of the nematode *Contacaecum aduncum* larvae on the content of triacylglycerol (triglyceride) stores in muscles of the Black Sea anchovy *Engraulis encrasicolus ponticus* (Table 2.1), we showed that this influence is very strong: parasites decrease the level of accumulated lipid reserves several fold (Shulman & Shchepkina, 1983; Shulman & Love, 1999). However, at the same time, one of the favorite morphophysiological indices used by ichthyologists, Fulton's *K* condition factor, differs only slightly between strongly and weakly infested fish. Earlier comparison between a condition factor and fat content in many Black Sea fish showed that a positive correlation is observed only when fat accumulation occurs on a background of stable fish growth (Shulman, 1974).

The aim of this chapter is not to describe a variety of functional and metabolic processes in fish but to show the possible (and in some cases necessary) use of certain physiological and biochemical indicators for qualitative and quantitative estimation of fish condition in the environment. We therefore describe the potential functionality of selected indicators and show examples of their use in the solution of problems in fish ecology and fisheries management. This book focuses on revealing indicators of fish health (i.e., estimations of their optimal condition). In line with other authors (Hochachka & Somero, 1973, 1984, 2002; Nemova & Vysotskaya, 2004; Depledge & Galloway, 2005), we consider that organisms that are healthy are able to support and maintain homeostasis, allowing the

normal occurrence of life cycles (primarily growth and reproduction) and preserving the abundance and productivity of populations. In this monograph, we have attempted to characterize only those indicators that have already been used successfully to estimate the condition of marine fish.

2.1   Basic concepts

All condition indicators in fish and animals in general are characterized by the processes of anabolism (biosynthesis) and catabolism (destruction) of substance (biochemical substrates) and energy. Generally speaking, the balance (budget) of substance and energy in organisms and populations is shown by the following equation (Ivlev, 1939; Winberg, 1956, 1960; Brett & Groves, 1979; Shulman & Love, 1999):

$$C = A + F = Q + P + F \tag{2.1}$$

where C represents substance and energy consumption, A the part that is assimilated (i.e., converted), Q the expenditures for metabolism, P the part used for growth and production, and F the unconverted part. The terms "balance" and "growth" are usually used for organisms, whereas "budget" and "production" are used for populations.

2.1.1   Energy expenditures

The connection between the organism/population and the environment is shown in Fig. 2.1. Food and oxygen are the two main channels connecting biological systems in the biosphere. The oxidation of food liberates free energy, which powers all the processes vital to the organism (self-renewal, biosynthesis, mechanical work, etc.). Oxidation allows the resynthesis of ATP (adenosine triphosphate), without which metabolism is impossible. The substrates that are oxidized in catabolism and which liberate energy comprise the main organic components of the organism: proteins, lipids, carbohydrates, and products of their destruction (nucleic acids are not considered here). Therefore, the level of energy expenditure (its range, rate and intensity) is an indicator of the functional activity of organisms and populations.
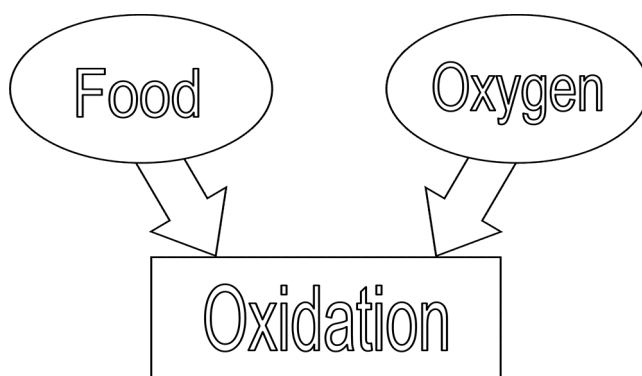
Figure 2.1   The connection between organism (population) and the environment.

In fish and most other animals, energy expenditure is commonly determined by oxygen consumption (respiration) via "indirect calorimetry" which, in contrast to direct calorimtery (i.e., determination of heat production), is carried out under laboratory conditions relatively easily. The disadvantage of indirect calorimetry is the underestimation of anaerobic pathways in energy catabolism (glycolysis and pentose phosphate pathway). Quantitative estimation of total heat production is often unknown. Another problem of indirect calorimetry is that it determines only total oxygen consumption, of which a considerable part is not available for ATP resynthesis but is expended uselessly in heat production (which dissipates by heating the water that surrounds the fish). It is known from biochemistry that oxidative phosphorylation (measured by the relation of ATP resynthesis with oxygen consumption) is extremely dependent on different organism condition and cannot represent more than 40–60% of total oxygen consumption. Nevertheless, indicators of oxygen consumption (oxidative metabolism of total organism) are considered by physiologists as the most adequate indicators of functional activity level. Yet another method for determining the energy expenditure for metabolism is to measure the decrease of "energy equivalent" of fish during starvation in experimental or field conditions. This "equivalent" is defined by the formula:

$$Q = P + L + G \qquad (2.2)$$

where P represents the physiological (not chemical) calorie content of protein (4.1 kcal or 17.18 kJ), L the calorie content of lipids (9.3 kcal or 38.97 kJ), and G the calorie content of glycogen (4.1 kcal or 17.18 kJ). All values are expressed per gram wet body mass (weight). For exact calculations it is necessary to either determine the proximate chemical composition or incinerate the body in an oxygen bomb calorimeter. This last method is only practical for objects of small size. The equipment used to analyze energy expenditures during metabolism of the whole organism using oxygen consumption is manifold and well described in detail in the literature (e.g., Fry, 1957; Brett, 1973).

To analyze oxygen consumption, several requirements are needed. First, information regarding the dependence of rate (or intensity) of oxygen consumption (Q) on body mass (W, weight) is shown by the equation:

$$Q = aW^k \qquad (2.3)$$

where a and k are coefficients (the latter usually ranges between 0.6 and 0.8). Because fish size can be extremely variable, these data are usually shown in the form of a logarithmic graph (Fig. 2.2):

$$\log Q = \log a + k \log W \qquad (2.4)$$

Second, fish inhabit water bodies at different temperatures (between −2°C and more than 30°C), and oxygen consumption depends strongly on temperature. Therefore, for comparison of results it is accepted to refer them to 20°C. This is accomplished by using the van 't Hoff coefficient, which determines the dependence of the rate of metabolic reactions on temperature. In a modified form it is expressed by:

$$\log Q_{10} = 10(\log Q_2 - \log Q_1)/(T_2 - T_1) \qquad (2.5)$$
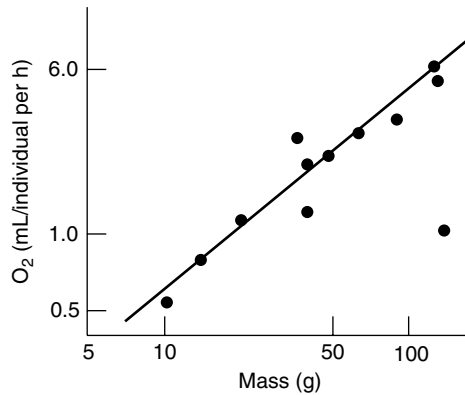
Figure 2.2 Dependence of oxygen consumption on body weight (mass) in scorpionfish *Scorpaena porcus*. After Belokopytin (1993).

where Q_2 is oxygen consumption at 20°C and Q_1 oxygen consumption at the actual temperature, and T_2 is temperature of 20°C and T_1 the actual temperature. At present it is accepted that $Q_{10}=2.2$ for fish (Winberg, 1956, 1960).

Third, the standardization of oxygen consumption at 20°C allows comparison of the energy catabolism of different fish species with considerable precision even if they differ greatly by size (for this, the coefficient k in equation 2.3 must range from 0.6 to 0.8). However, it is always better to compare fish of similar mass. When fish body mass is extrapolated to be equal to unity (e.g., 1 mg, 1 g, 1 kg), then $Q=a$. It is better to make such comparisons at a standard metabolism (SM). SM is determined by oxygen consumption under laboratory conditions in a closed respirometer where fish are able to move freely. Because of the difficulty of obtaining a "motionless" fish, the determination of basal metabolism is not possible without narcotization (Belokopytin, 1993). Hence, the standard metabolic rate (SMR) of fish is calculated as the energy consumed by an unfed "immobile" fish in a postabsorptive state and with no oxygen debt associated with the previous exhaustive activity (Armstrong et al., 2011). SMR is generally considered to represent a baseline level above which metabolism increases when the animal undertakes physiological activities such as swimming, processing of food, and mobilization of somatic tissue to form gonads (Armstrong et al., 2011). Conversely, active metabolism represents the situation when the locomotion of fish is not limited (Ivlev, 1959), and is often called "scope of activity." Total active metabolism is twofold to fourfold higher than SMR (Winberg, 1956, 1960; Belokopytin, 1993; Shulman & Love, 1999). Belokopytin showed that energy expenditure in active metabolism (Q) depends on the swimming rate (v) as:

$$Q = Q_0 b^v \qquad (2.6)$$

where Q_0 represents basal metabolism (i.e., metabolism at "zero" rate) and b is a coefficient.

It is necessary to remember that oxygen consumption also depends on the rate and duration of swimming (Belokopytin, 1993; Shulman & Love, 1999), food consumption (so-called "specific-dynamic effect of food"; Brett & Groves, 1979), "group effect" (in the school, oxygen consumption decreased in comparison with a single fish; Alekseeva, 1959), diurnal rhythms and some other factors. Therefore it is necessary to take into account all these

factors in order to estimate the real value of oxygen consumption of fish as the indicator of their functional activity. Further, determination of metabolic level has to be carried out on acclimated fish, a topic that is an important element of investigation (Khlebovich, 1986).

2.1.2   Sub-organism metabolism

In order to characterize and estimate the condition of organisms and populations, it is also possible to use (as an approximation) a wide set of sub-organism indicators, namely tissue, cell, subcellular and molecular catabolism. Each of these indicators can be used either independently or in combination. Thus studying tissue respiration is used to characterize the level of oxidative metabolism in white and red muscles, liver, gills and some other organs. In order to calculate the unit of studied tissue and the total mass of every tissue, it is necessary to know the mass of the tissue or organ and its relation to fish body mass. Because the mitochondria are the "energetic factories" of a cell that "burn" oxygen, respiration of these subcellular components and their number in a cell are important indicators of the level of oxidative catabolism. Key metabolites and the enzymes of the respiratory chain and Krebs (tricarboxylic acid) cycle – cytochromes and cytochrome oxidase – are indicators of intermediate catabolism. They transfer the electrons that are the main source of the energy for resynthesis of ATP through the respiratory chain. The content of ATP, adenosine diphosphate (ADP), and adenosine monophosphate (AMP) is a significant indicator of the "energetic armament" of an organism. By "energetic armament" we mean the total energy status that allows realization of functional processes in the organism at the necessary level. Resynthesis (oxidative phosphorylation) is simply the restoration of ATP by the two other nucleotides (especially ADP). The energy accumulated in ATP is liberated during its destruction and becomes available for metabolic processes. Measurement of this accumulation is named the adenylate charge (or Atkinson's charge), which is equal to:

$$\frac{[ATP]+\frac{1}{2}[ADP]}{[ATP]+[ADP]+[AMP]} \tag{2.7}$$

We have mentioned indicators of the level (intensity) of energy catabolism before. However, an important indicator of energy metabolism is not only intensity but also its efficiency. Oxidative phosphorylation (so-called respiratory control) and Atkinson's charge characterize this efficiency, which does not always correspond to the intensity of energy expenditure, and are often inversely dependent. A significant indicator of the transformation of adenyl nucleotides is the activity of ATPase, the enzyme that hydrolyzes ATP, releasing large amounts of energy. Creatine phosphate, which also accumulates and releases large amounts of energy, can also be used to characterize energy status of an organism.

2.1.3   Blood: transport of energy

Transport of molecular oxygen to tissues and cells from the gills (and partly through the skin) is carried out by the erythrocytes (red blood cells). Antarctic fish, which lack red blood cells, transport oxygen dissolved in blood plasma. Erythrocytes contain hemoglobin, the colored protein that erythrocytes use to transport oxygen. The concentration (number) of erythrocytes and their hemoglobin content are the most favored physiological indicators, as their determination is not difficult and the obtained results show clearly the level of functional activity of the organism. The indicator often used is hematocrit, namely the percentage of

erythrocytes in total blood volume. Korzhuev (1964) is right to recommend to calculate hematocrit not only per unit of blood volume but also per total mass, because it can vary strongly. This author proposed naming the ratio of absolute erythrocyte number and hemoglobin to fish body mass as "organism supply by blood pigments." An important characteristic of hemoglobin is its degree of affinity to oxygen. This is found by measuring the P_{95} and P_{50} of hemoglobin: the P_{95} is the partial pressure where hemoglobin is 95% saturated with oxygen (i.e., where hemoglobin is mostly in the form HbO_2); the P_{50} is the partial pressure where hemoglobin is 50% saturated with oxygen (i.e., where oxyhemoglobin has dissociated into Hb and O_2). Another condition indicator is the oxygen capacity, namely the quantity of oxygen bound to 100 mL of blood. The muscle analog of hemoglobin is myoglobin, which is found in red muscles and the heart (giving them a red color). It is a reserve respiratory pigment when there is a deficiency of hemoglobin in the blood. Another morphological indicator that has a direct relation to oxygen transport is the number of capillaries, which have huge importance in this transport function.

2.1.4 Alternative pathways of energy catabolism

Oxidative metabolism under normal conditions is carried out by aerobic respiration. However, in some cases (considered later), aerobic respiration is replaced to a large extent or completely by anaerobic pathways of energy utilization comprising anaerobic glycolysis and the pentose phosphate pathway. The terminal product of glycolysis is lactate, which because of its toxicity for fish is changed partly into alanine. The indicators of anaerobic glycolysis and the synthetic pathway that eliminates lactate include the key enzymes of these processes, namely lactate dehydrogenase (LDH) and alanine transaminase. The pentose phosphate shunt connected with glycolysis is determined by isotope analysis (Hochachka & Somero, 1973), but the estimation of fish condition is quite difficult.

2.1.5 Energy anabolism: accumulation of energy

Energy accumulates in an organism to compensate for its expenditure and to form stores (reserves) used in provision of energy necessary for vital processes, such as maturation, spawning, migration, and wintering (the last two are not observed in fish). Calculation of accumulated energy is accomplished using eqn. 2.2. Approximate calculations assume that 1 g of fish dry mass is equal to 1000 kcal (Winberg, 1956, 1960). The calculation of energy destruction in fish under natural conditions is also accomplished using this formula but not by oxygen consumption. To determine oxygen consumption is possible only under laboratory conditions, which as a rule is difficult compared with measurement under natural conditions. In balance experiments on food consumption (nutritive rations, intensity and efficiency of utilization), the energy method has an advantage compared with the substance one as it is independent of transformation processes. The energy method also makes interpretation of the obtained data easier (Ivlev, 1939; Winberg, 1956, 1960). The disadvantages of such methods will be considered later. Values of accumulated energy are the most significant indicators of fish condition (in both single organisms and populations as a whole). Therefore, as distinct to energy catabolism, where indicators of fish condition include enzyme reactions and metabolites, such detailed biochemical methods for indication of anabolic processes are not necessary (strictly speaking, "ATP resynthesis from precursors" is a significant process in energy anabolism but is related to the sub-organism or molecular level and we have examined this previously).

2.2 Lipids

2.2.1 Lipids as energy substrates

Although lipids constitute a smaller proportion of fish body mass (in comparison to proteins), they are a much stronger indicator of the condition of organisms and populations. Another advantage of using lipids is that total lipid content varies greatly from year to year, from one area to another, and from one population to another, thus being a good indicator of fish condition. Conversely, the dynamics of protein content in fish is quite stable and does not have such indicative power as lipids. Glycogen content is also not a good indicator of fish condition because its quantity is usually low (with the exception of some species) and has frequent fluctuations related to short-term changes in fish condition.

Lipids, along with proteins and carbohydrates, are the main energy stores in teleosts and are the first energy reserves to be catabolized during non-feeding and reproductive periods. In juvenile and adult cod, for example, there is clear evidence of lipid depletion first followed by protein (Grant & Brown, 1999). Lipids are a good indicator of stored energy because their energy density is high and labile. Lipids are a key constituent of eggs, and are correlated with the level of protein reserves (McPherson et al., 2011).

Neutral lipids constitute the stores with the highest accumulated energy (total neutral lipids are termed "fat"). Several different families of neutral lipids are present in all cells, including triacylglycerols (triglycerides or TAGs), free fatty acids, and esters of both cholesterol and waxes (the latter are observed only in some species). TAGs represent 80–90% of total neutral lipids and are the predominant storage form of chemical energy.

A considerable advantage of evaluating neutral lipids (including total fat content and TAGs) is that these compounds are present in a large number of fish species and exhibit high variability in fish life-history and annual cycles. They play an important role in the accumulation and expenditure of energy reserves. This dynamic build-up and decline of energy is connected with all stages of ontogenesis, the ecophysiological features of fish species and populations, and fish behavior and distribution. Fat is used for reproductive purposes during breeding, with subsequently levels as low as 1–3% of wet mass. Fat is then accumulated during feeding periods, often up to levels of 20% or more in some species. Fat content can also vary from year to year, from one population to another, and from one habitat to another, and this is mainly attributed to changes in TAG content.

The distribution of fat stores in different organs and tissues provides significant information about the ecophysiological features of fish. The determination of fat utilization as an energy substrate in fish is carried out in a simple way, by taking into account the decreasing fat content or its absolute quantity in the body or in separate tissues and organs. The proximate body composition (including lipids) is usually evaluated as a percentage of wet weight as it corresponds to real lipid content in an organism. In some cases calculations are presented in the form of percentage of dry weight, which only provides a relative parameter as such values depend on several variables. The relationship between fat content and dry and wet mass of fish is shown in Fig. 2.3 (Shulman, 1971).

2.2.2 Lipids as structural metabolic substrates

Structural lipids (basically phospholipids and cholesterol) play an important role in the functioning of organisms. They are components of basal membranes of cells and subcellular organelles, forming a bilayer with the phospholipid "head" toward the outside and fatty acid
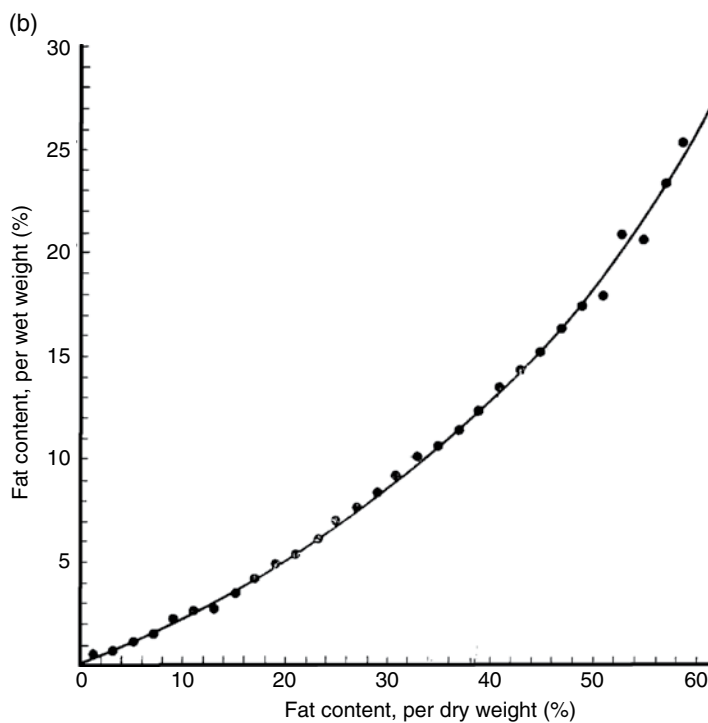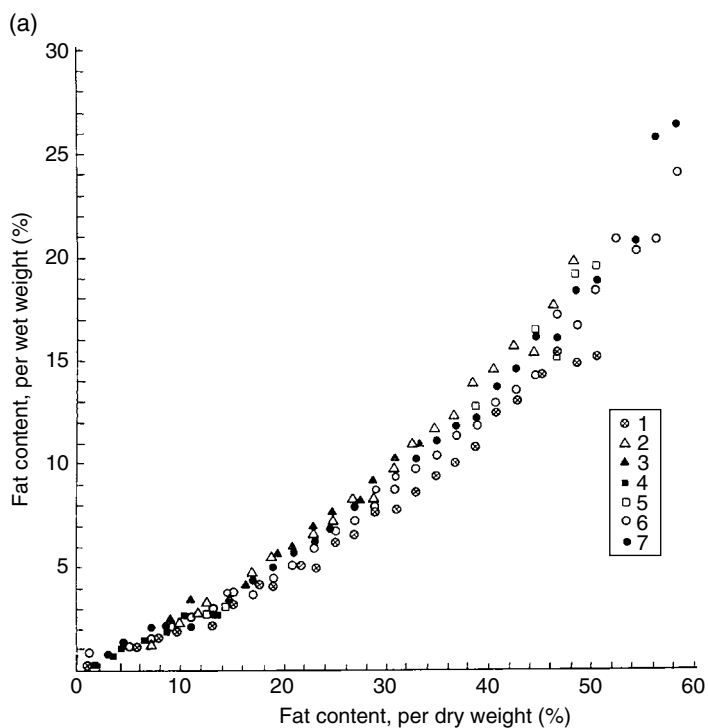
Figure 2.3 (a) Relationship between fat content per wet and dry weight in some Black sea fish: 1, sprat; 2, red mullet; 3, pickerel; 4, whiting; 5, anchovy; 6, Black Sea anchovy; 7, horse-mackerel. (b) Summary curve. After Shulman (1971).

"tail" toward the inside. Phospholipids are abundant in membranes and form the framework in which other components of the membrane (e.g., proteins) are embedded. Together with their structural significance as the membrane's "skeleton," phospholipids are involved in the metabolic activity of cells, regulating their viscosity and permeability and playing a role in the transport of substances across the bilayer. Phospholipid structure is very sensitive to different factors, particularly temperature. Phospholipid content is relatively stable and consists of about 0.5–1.5% of wet mass. The main phospholipid fractions include phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine. These constitute up to 70% of total phospholipids. Other fractions include phosphatidylinositol, sphingomyelin, cardiolipin and some others, which constitute the remaining 30%. Apart from phospholipids, cholesterol is an important component of cell membranes and serves many other functions as well. Cholesterol helps to "pack" phospholipids in the membranes, thus giving more rigidity to the membranes.

2.2.3 Fatty acids

Fatty acids, and more specifically their radicals (glycerol ethers), are the most important components of lipids, defining their energy value (in the case of triglycerides) and forming the structural–metabolic "skeleton" of cellular and subcellular membranes (phospholipid content). Fatty acids can be divided into saturated, monounsaturated, diunsaturated, and polyunsaturated (polyenic) acids depending on the number of bonds in the carbon chain. Two families can be distinguished: linolenic acids (omega-3, ω3, or n-3) acids; and linoleic acids (omega-6, ω6, or n-6). The differences between these families are based on the chemical structure of the fatty acid and location of the chemical double bonds in their structure. Omega-3 fatty acids have at least three double bonds starting from the third carbon from the methyl end of the fatty acid structure, whereas the omega-6 fatty acids have their first double bond located at the sixth carbon in the carbon chain from the methyl end.

Omega-3 fatty acids are relatively more abundant in marine fish, whereas omega-6 fatty acids are relatively more abundant in freshwater fish, and therefore the n-3/n-6 ratio in marine fish is substantially higher than that of freshwater fish (Lovern, 1964; Sargent, 1976; Ackman, 1989). This difference seems to be caused by the different feeding regimes: marine primary plankton and benthos have more lipids with omega-3 acids, whereas freshwater ones have more lipids with omega-6 acids. Anadromous and catadromous fish have a similar amount of fatty acids of both classes.

There is a great variety of fatty acids contained in fish (more than 20 in total). However, only some of them are represented in noticeable number (more than 2–5% of total of all fatty acids). These include myristic (14:0), palmitic (16:0), stearic (18:0), palmitoleic (16:1), oleic (18:1), arachidonic (20:4 ω6), eicosapentaenoic (EPA, 20:5 ω3), 20:5 ω6, and docosahexaenoic (DHA, 22:6 ω3) acids. Saturated and monounsaturated acids are used mainly as sources of energy during lipolysis (especially palmitic and oleic acids). Polyenic acids are available primarily in phospholipids. Food is the source of all fatty acids in fish. Fatty acids are accumulated in TAGs mainly unchanged, whereas in phospholipids of freshwater fish linoleic acid is transformed into polyenic acids by elongation of the carbon "tail" and desaturation (increasing the number of double bonds) (Lovern, 1964; Ackman, 1967). However, as shown by Sargent (1976) and Sargent et al. (1987, 1995), marine fish have lost the ability to produce polyenic acids from linolenic acid; therefore, EPA and DHA are essential fatty acids (EFAs) for marine fish. These acids are important structural components of membranes and, particularly EPA, have a considerable role as precursors of important biologically active substances such as prostaglandins and leukotrienes, while

DHA is important for metabolism of neural tissues (Tocher, 2003). Restriction of EFAs results in a variety of ill effects on fish, including reduced fecundity and embryonic viability, disturbed growth and decreased survival at early life-history stages, improper pigmentation, impaired vision and inability to feed at low light intensity, impaired ontogeny of behavior such as schooling, and decreased membrane function at low temperatures (Tocher & Sargent, 1984). EFAs are produced in appreciable quantities in pelagic ecosystems only by phytoplankton and are transferred up food webs, and therefore EFA availability to fish is strongly regulated by bottom-up processes (Litzow et al., 2006).

Fatty acid composition of fishes and their tissues as well as their dynamics are significant indicators of the metabolic features of individuals organism and populations of fish. These indicators allow estimation of the level of functional activity in fish and many other aspects of fish biology related to the environment. The content of each fatty acid in lipids is usually expressed as the percentage of the total content of all fatty acids. However, this informative index can also be calculated as the quantity of each fatty acid per absolute content of lipids. Unfortunately, such calculations are very rarely made. We do not discuss the enzymes involved in lipid and fatty acid metabolism for estimating the condition of individual fish and fish populations because the indicators described above are thought to be sufficient for use in ecology and fisheries biology.

2.2.4 Lipid analysis

The determination of fat content is known to be one of the best biochemical parameters for evaluating the energy reserves of fishes (Adams, 1999; Shulman & Love, 1999; Morris & Culkin, 2000). It is well accepted that the evaluation of total lipid reserves in whole fish or in particular tissues and organs where fat is stored would be an accurate way to monitor the condition of fish (at least during pre-spawning). Before choosing the tissue/organ where lipid is to be analyzed, one must first look at the principal site of lipid storage, which depends on the fish, as even species of the same genus may have quite different fat depots. In general, fat is concentrated in the liver in benthic and demersal species and the muscle in pelagic species. Abdominal (adipose or perivisceral) fat can also be an important lipid compartment for both demersal and pelagic species. Nevertheless, total lipid extraction and quantification is more costly and time-consuming than the evaluation of simple morphometric and morphophysiological indicators (chapter 1) because it has to be performed with special methods in the laboratory.

The high variation in lipid reserves between individuals of the same species is a common feature in wild fish and may reflect the large differences in nutritional status and reproductive potential of individuals within a given population. This strong intrapopulation variability of lipid reserves probably explains the low correlation values between the different variables studied. Thus, for example, total lipid content in the liver of European hake in the northwest Mediterranean can vary from 2 to 67% in wet weight, whereas in the liver of cod total lipid concentration varies between 30 and 78% wet weight (Falch et al., 2006). This strong intrapopulation variability in lipid concentration must be taken into account in the sampling design.

In some fish, including several reef species from the Great Barrier Reef, the densities of hepatocyte vacuoles were quantified with histological techniques and were used as an indicator of lipid and glycogen stores within the liver (see for example Pratchett et al., 2001, 2004). Hepatocyte vacuoles are bodies for short-term lipid storage, representing energetic resources that are immediately available to the fish.

In other cases, the lipid content in a particular organ and the weight of that organ relative to the total weight of the fish has been combined to produce condition indices. Thus for

example, a lipid hepatosomatic index (LHSI) and a lipid gonadosomatic index (LGSI) can be computed with the following formulas:

$$LHSI = ABSL / EW \times 100 \tag{2.8}$$

$$LGSI = ABSG / EW \times 100 \tag{2.9}$$

where EW is the eviscerated wet weight and ABSL and ABSG are the absolute lipid content in the liver and ovary, respectively, computed by multiplying the respective lipid contents (in percent wet weight) by total liver and ovary wet weights. These indices have been used, for example, with hake in the Mediterranean (Lloret et al., 2008).

Apart from the total lipid content in adult and juvenile fish, lipid content of larvae has also been analyzed. In a study by Sabatés et al. (2003), changes in lipid concentration during early ontogenetic stages were determined in three mesopelagic fish species, the authors demonstrating that total lipid content as a proportion of dry mass per larva increased over the course of development (Fig. 2.4). Furthermore, total lipid concentrations increased significantly from the larval to the juvenile stages in *Benthosema glaciale* and *Maurolicus muelleri*. This study allowed comparison of the ability of early developmental stages of deep-sea fish to accumulate energy reserves, both within and among species. Furthermore, the study revealed that high lipid content in their larvae was associated with high wax esters percentage for buoyancy requirements during vertical migrations (Sabatés et al., 2003). In this case, the discrimination of lipid classes is essential for identifying the biological roles of lipids associated with energy demands of particular life stages. Furthermore, differences in lipid composition related to different life strategies have been observed for five notothenioid fish species from high-Antarctic seas (Hagen et al., 2000).

Ultimately, the separation and evaluation of lipid classes (particularly the evaluation of triglycerides, the most important lipid classes for the energy stores of fish) would provide the best measurement of fish condition, even though the specific methodology for lipid class separation (e.g., high-pressure liquid chromatography) is much more time-consuming than the previous indicators (e.g., analysis of total lipid with Soxhlet apparatus; Fig. 2.5) and therefore is probably impractical for regular fishery assessment and management purposes. Historically, most research on fish condition indicators related to total fat, because even by the first half of the last century the extraction of total fat from fish tissues was done using a simple procedure (with ethyl ether), from which information on the level and dynamics of fat content in fish body was obtained. Although at present a number of scientists studying fish condition have turned to the determination of triacylglycerols instead of total lipid, data on total lipid content must not lose their value because ethyl ether and similar procedures already extract neutral lipids (mainly triglycerides), and because are easy to obtain in the laboratory.

Methods of lipid analysis (Folch et al., 1951; Bligh & Dyer, 1959) are well described in several papers and books and we will therefore not compile a list of the different techniques that can be used. For example, the book by Christie (2003) provides a good compendium about isolation, separation, identification, and structural analysis of lipids, including instructions for storage of tissues and sample handing. We recommend reading carefully the particular instructions for lipid analysis, including the preliminary treatments prior to extraction. For example, if frozen storage extends too long, lipids from fish tissues undergo lipolytic changes (enzymatic) resulting in an accumulations of free fatty acids in most marine species (Zhang & Lee, 1997). In fact, these free fatty acids have been long recognized as a seafood quality index as an alternative to sensory assessments (see for example Zhang & Lee, 1997).
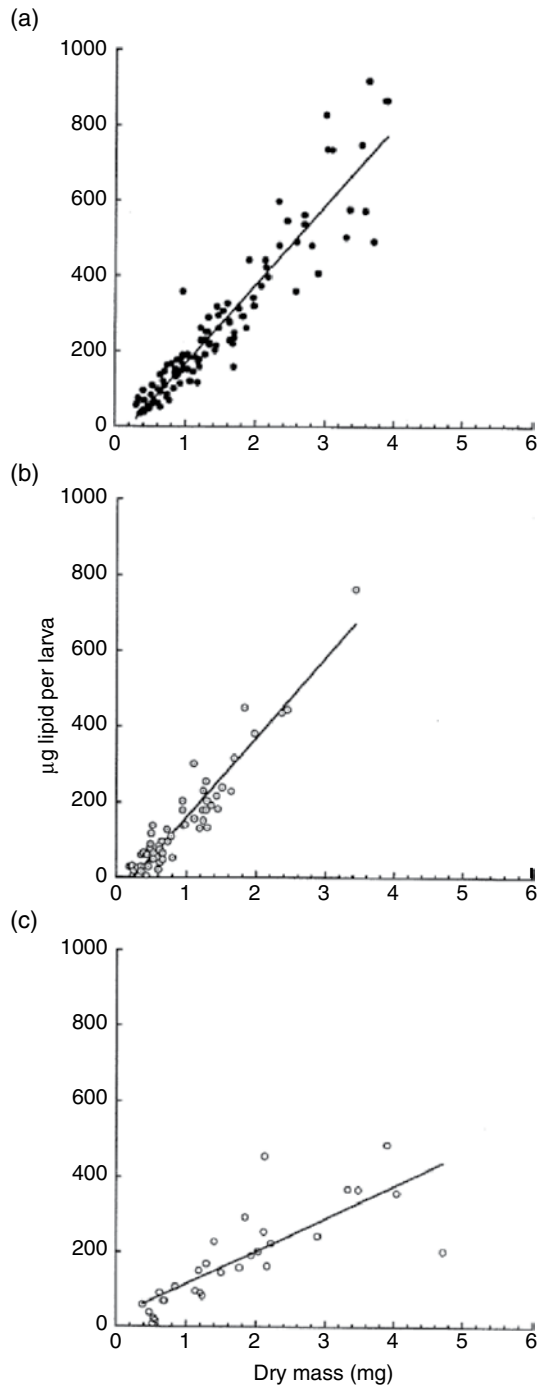
Figure 2.4   The relationship between total lipid content and dry mass in the larvae of (a) *Benthosema glaciale*, (b) *Hygophum benoiti* and (c) *Maurolicus muelleri*. From Sabatés et al. (2003).

Figure 2.5 Soxhlet apparatus used to extract and evaluate total lipid content in fish samples. Photo by Josep Sánchez-Pardo.
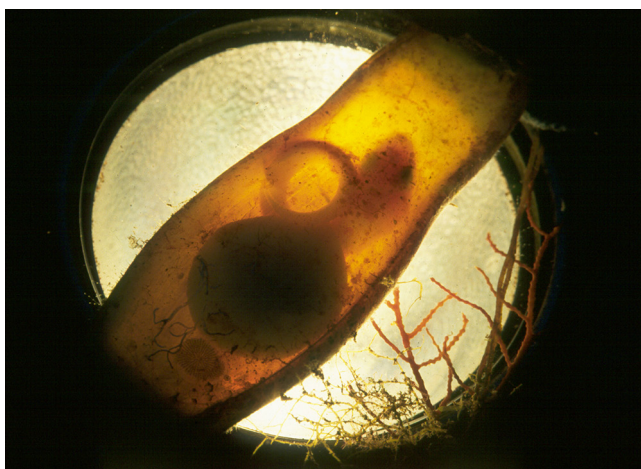
Figure 2.6 Embryo of a catshark (*Scyliorhynus canicula*). Photo by Bernd Morker.

It is also worth noting that total lipid content is often well correlated with better measures of energy reserves such as EFAs, TAGs, and phospholipids, which are the dominant lipid classes, and energy density. TAGs are the major energy storage form in fish and have important ecophysiological relevance as indicators of growth potential and survival (Sogard & Olla, 2000). EFAs are nutritionally critical lipid components, particularly EPA and DHA, which are most commonly identified as limiting in fish (Tocher, 2003). Restriction of EFAs results in a variety of ill effects in fish, including decreased fecundity and reduced embryonic viability (Fig. 2.6), impaired growth and decreased survival of early life-history stages, improper pigmentation, impaired vision and inability to feed at low light intensity, impaired ontogeny of behavior such as schooling, and decreased membrane function at low temperatures (Tocher, 2003). For example, there is a positive significant relationship between total lipid content and content of EPA and DHA in different species of fish from the northeast Pacific (Fig. 2.7c and d; Litzow et al., 2006). In fact, differences among species in total lipid content
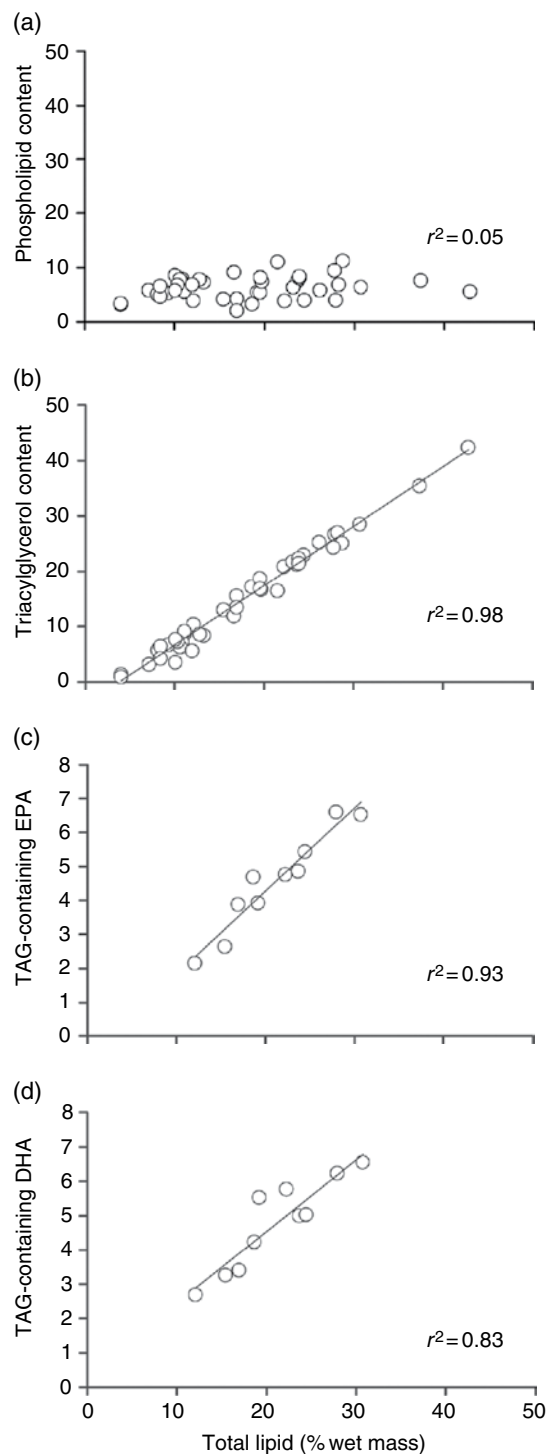
Figure 2.7 Increases in triacylglycerols (TAG) and TAG-containing essential fatty acids with increasing lipid content for 29 species of myctophids, showing the relationship between total lipid content and (a) structural phospholipids (PL), (b) neutral TAG, (c) TAG-containing eicosapentaenoic acid (EPA), and (d) TAG-containing docosahexaenoic acid (DHA). Plotted data were arcsine transformed. From Litzow et al. (2006).

are expected to be expressed almost entirely by differences in TAGs (Olsen, 1999). Also, Litzow et al. (2006) reviewed the positive relationship that exists between total lipid content, phospholipids, and triacylglycerol content for different myctophids (Fig. 2.7). Finally, there is a positive relationship between total lipid content and energy density, making it possible to estimate energy density from lipid using energy equivalents (e.g., energy density can be estimated by converting lipid to energy based on an assumed energy equivalent of 9.5 kcal/g; Winberg, 1956, 1960; Rosa et al., 2010).

2.2.5 Rapid methods for fat measurement in fish

Apart from the standard methods for determination of total fat, there are several methods for rapid fat measurement in fish.

2.2.5.1 *Evaluation of water content*

An easy way to estimate total lipid content in a given tissue is from the tissue water content, which is often (but not always) inversely related to total lipid content in many species. Analysis of lipid and water content in different tissues of fish indicates that these constituents are negatively correlated, so that accurate predictions of each component can be obtained from the measurement of any of the others. Thus for example, the relative liver and ovary lipid contents (as percent of dry weight) in European hake are inversely and significantly and positively related to the water content in these tissues (Lloret et al., 2008), even though the relationship is better in the liver ($r=-0.80$) than in the ovary ($r=-0.40$; Fig. 2.8). Similar results are found in cod, in which seasonal changes in water and energy content of muscle and liver followed an inverse pattern (Lambert & Dutil, 1997a), as well as in juvenile *Coregonus artedi* (Table 2.2; Pangle & Sutton, 2005) and in *Sardinella aurita* from the Tunisian coast (Ben Rebah et al., 2010). Also, in red mullet (*Mullus barbatus*) from the northwest Mediterranean there is a good inverse relationship between water lipid content in the muscle (Fig. 2.9; Lloret et al., 2007). Thus, a simple condition index based on tissue water content could be considered as a rapid indicator of tissue lipid reserve of some species. Nevertheless, the relationship between water content and lipid content must first be validated because it may be that the utilization of lipid reserves can actually result in an increase in water content and consequently body weight (i.e., water replaces lipids). The estimation of water content can be done by drying wet samples of fish tissues. This procedure can be carried out in the laboratory or even on board ship using oven-drying or more rapidly by lyophilization. Since total weight = dry weight + water weight:

$$\text{Fat}\,(\%) = k \times \text{Dry weight}\,(\%) \tag{2.10}$$

where k is a coefficient. See, for example, our example on the Black Sea sprat (Fig. 2.10; Minyuk et al., 1997).

2.2.5.2 *Electronic portable devices*

On the basis of this inverse relationship between water and fat, electronic portable devices have been constructed to non-invasively measure the water and hence the fat content in fish
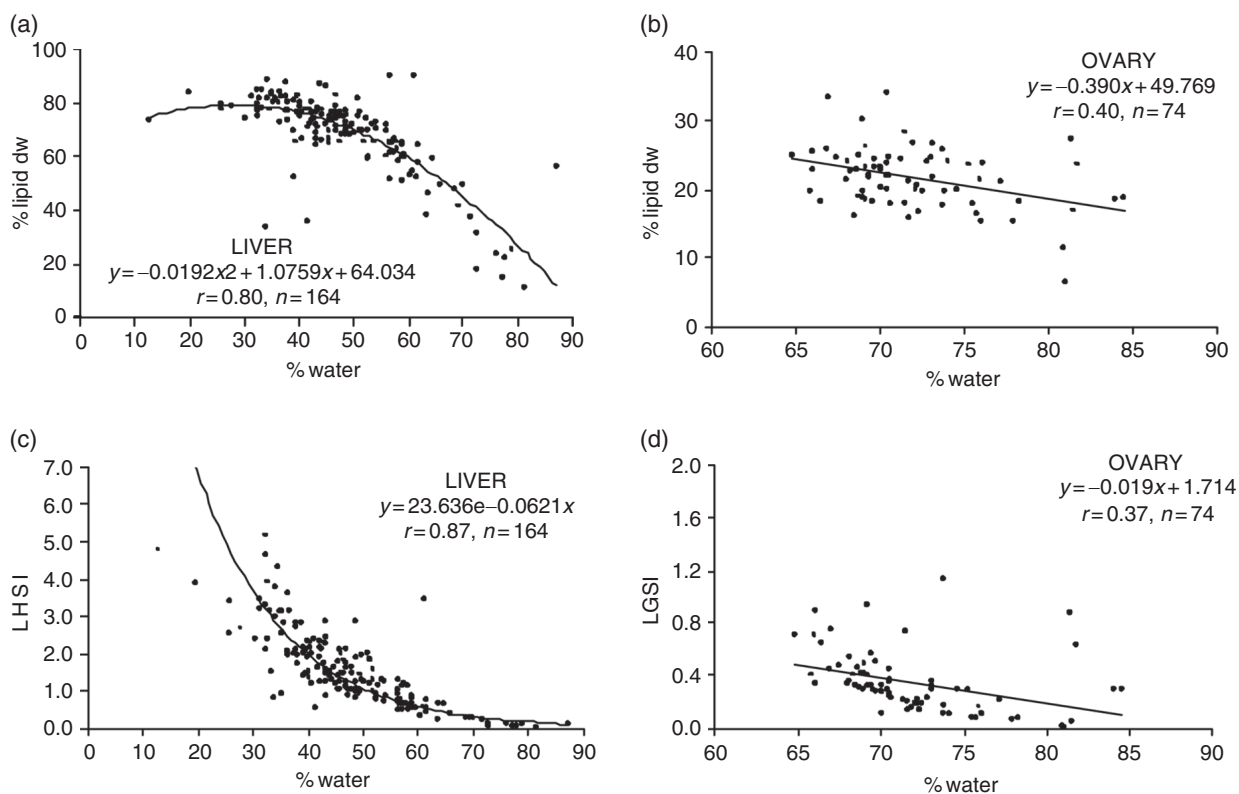
Figure 2.8 Relationship between lipid content (% dry weight) and water content in liver (a, polynomial relation) and ovary (b, linear relation), between lipid hepatosomatic index (LHSI) and lipid water content (c, exponential relation), and between lipid gonadosomatic index (LGSI) and ovary water content (d, linear relation) in European hake. Liver lipid content and LHSI consider all individuals while ovary lipid content and LGSI consider female pre-spawners only. From Lloret et al. (2008).

Table 2.2 Relationship of water to proximate composition components of juvenile lake herring *Coregonus artedi**

Day of experiment	Linear-regression equation	MS error	SE of W	r^2	P
75	Crude lipid$=46.661-0.478\,W$	14.34	1.671	0.262	0.039
	Crude protein$=31.0924-0.228\,W$	2.961	0.594	0.403	0.012
	Gross energy$=9493.5-103.74\,W$	23,876.5	154.526	0.665	<0.001
	Ash$=23.176-0.223\,W$	6.446	2.289	0.579	0.285
150	Crude lipid$=49.698-0.532\,W$	41.492	0.907	0.791	<0.001
	Crude protein$=41.164-0.331\,W$	16.949	0.550	0.766	<0.001
	Gross energy$=11,358.4-128.79\,W$	10,241.612	101.201	0.968	<0.001
	Ash$=11.672-0.087\,W$	1.125	0.463	0.274	0.038
225	Crude lipid$=49.032-0.542\,W$	56.34	0.960	0.815	<0.001
	Crude protein$=53.699-0.486\,W$	45.421	0.571	0.907	<0.001
	Gross energy$=11,386.9-129.06\,W$	9695.38	98.465	0.960	<0.001
	Ash$=15.914-0.134\,W$	3.465	0.895	0.246	0.048

*Linear-regression variables of the relationships for crude lipid, crude protein, gross energy and ash content, and whole-body water content (W, %) for juvenile lake herring on days 75, 150 and 225 of the laboratory experiment. Crude lipid, crude protein, ash and water content were expressed as percentages of wet body mass, while gross energy was expressed as J/g wet body mass.
Source: Pangle & Sutton (2005).
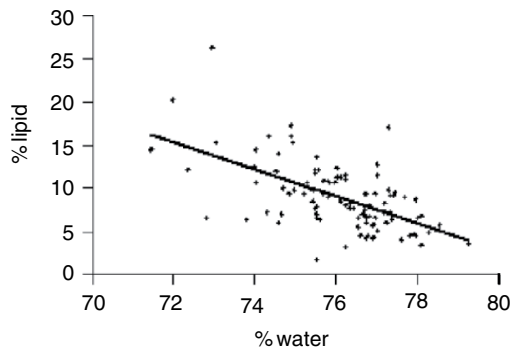
Figure 2.9 Linear relationship between lipid content (% dry weight) and water content in muscle of red mullet (*Mullus barbatus*) in the Mediterranean. $y=-1.553x+127.14$, $r^2=0.34$, $P<0.05$, $n=98$. From Lloret et al. (2007).

muscle (see for example Kent, 1990; Kent et al., 1993). However, in both cases we need to validate (on a species basis) the assumption that the water content is inversely related to the lipid content. These electronic instruments are particularly useful for measuring the muscle fat content of oily fish and therefore these instruments can facilitate routine collection of fish condition data at the individual level. Although a number of techniques exist, the Distell fish fatmeter (Fig. 2.11; Kent et al., 1993), which estimates muscle fat content using microwave technology, has been applied to a number of pelagic species including herring (Vogt et al., 2002; Davidson & Marshall, 2010) and tuna *Thuna thynnus* (Goñi & Arrizabalaga, 2010). Compared with the other indicators, which require sacrifice of the organism, the fatmeter is a non-destructive method that is easy and rapid to use. The accuracy of this method has

$$F = -15.62 + 0.99DW$$
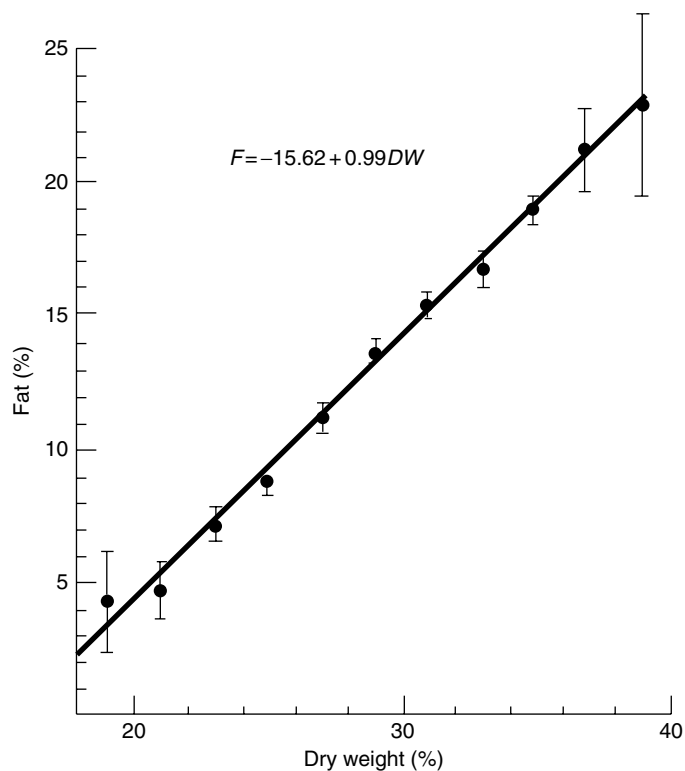
Figure 2.10   Relationship between fat and dry matter content in the Black Sea sprat. After Minyuk et al. (1997).

Figure 2.11   Fish fatmeter. Photo by J.A. Litster (www.distell.com).
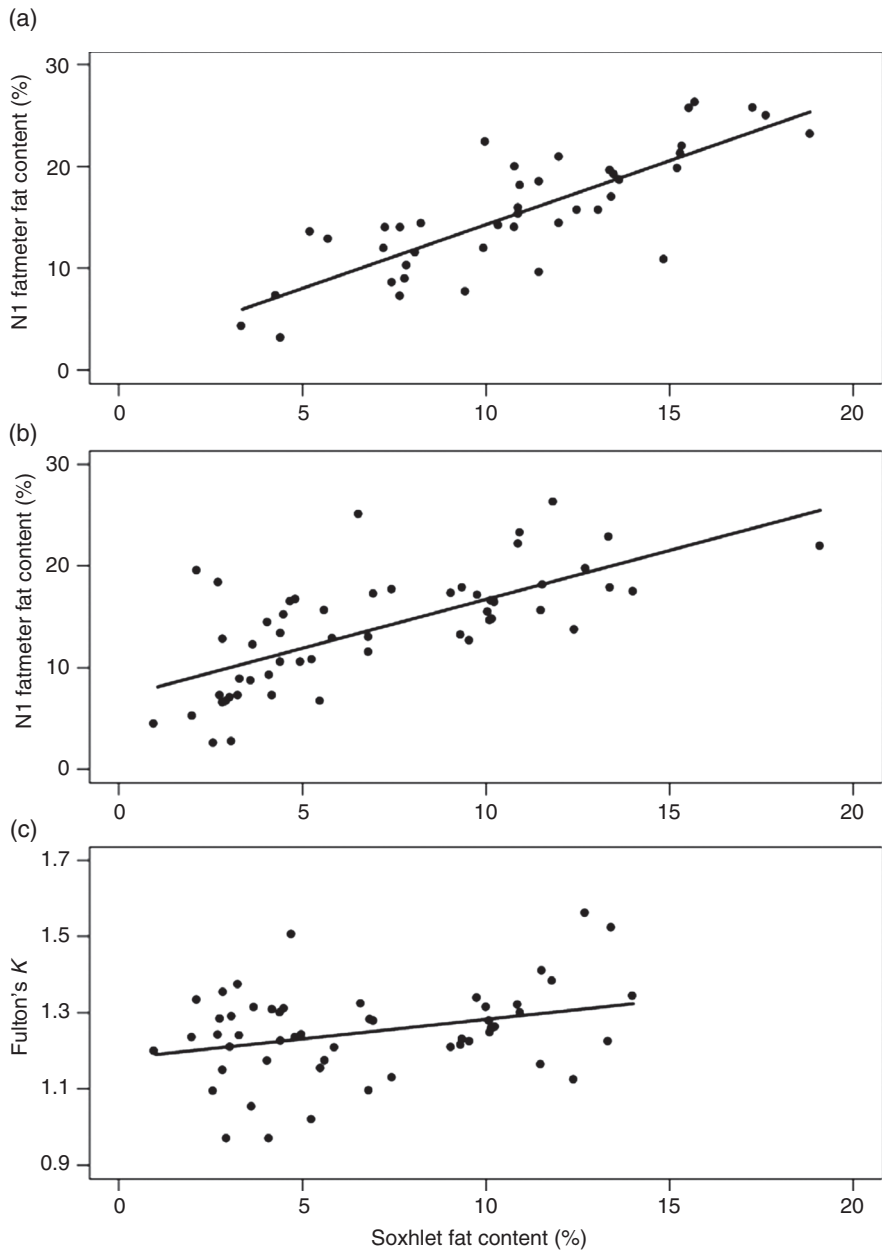
Figure 2.12 Correlation between Soxhlet fat content of North Atlantic herring *Clupea harengus* and (a) N1 fatmeter readings from the 2004 study, (b) N2 fatmeter readings from whole fish from the 2005 study, and (c) Fulton's *K* from the 2005 study. From Davidson & Marshall (2010).

been tested against traditional reference methods of lipid determination such as the Soxhlet procedure (Vogt et al., 2002; Davidson & Marshall, 2010) and the Bligh and Dyer method (Goñi & Arrizabalaga, 2010). For example, there is a positive relationship between fatmeter values and total lipid content estimated with Soxhlet (Fig. 2.12; Davidson & Marshall, 2010). Impressive data for the relationship between standard methods of fat

determinations and Kent's method are also demonstrated by Kaga et al. (2009) carried out on Pacific salmon of genus *Oncorhynchus*.

Because the fatmeter actually evaluates the water content in the tissue (which is supposed to be inversely related to lipid content), the fatmeter must be calibrated by the manufacturer according to the species to be sampled and the type of fillet. Ideally, the fatmeter should be routinely calibrated against chemical analyses of fat content for the specific study species (Davidson & Marshall, 2010). For example, the relationship between the fatmeter and Soxhlet estimates of muscle fat content in herring is quite strong ($r^2 = 0.65$, $n = 42$, $P < 0.001$; Davidson & Marshall, 2010). Although the fatmeter can be used on a whole fish, the logistics involved in using the fatmeter in the field (e.g., on-board a research vessel) might require that the fatmeter be used on a gutted carcass rather than a whole fish in order to cause little disruption to the established sampling protocol (Davidson & Marshall, 2010). Because of the reliance of the fatmeter on water content of the fish muscle, fatmeter readings are considerably influenced by factors affecting the water content of fish, such as icing (increases water content) or desiccation, and therefore the fillet should not be allowed to become desiccated or to take on water as both will affect the reading (Vogt et al., 2002). Further, it is important to check the characteristics of the fatmeter used, for example to ensure the appropriate fatmeter sensor head according to the depth of the fish muscle (Davidson & Marshall, 2010).

2.3 Carbohydrates

The most sensitive carbohydrate indicators of fish condition are glycogen and glucose. Glucose is a terminal product of glycogenolysis, and mobilization of glucose (glycolysis) is involved in the Krebs cycle. While neutral lipids (fats) and free fatty acids are "strategic" stores, glycogen and glucose are used for "tactical" requirements – carbohydrates are easily mobilized, providing a rapid supply of energy for locomotion during attack on prey or escape from predators. Glycogen and glucose are also used by fish in environments where oxygen is deficient, as glycolysis can proceed under anaerobic conditions. The main stores of glycogen in fish are concentrated in the liver, from which it is easily mobilized due to the high metabolic activity of this organ. In addition, glycogen is also mobilized from white muscle and this is responsible for "burst work." Its content in fish is, as a rule, lower than that of fat (1–5% of wet mass). Fluctuations in glycogen content, by virtue of its physiological activities, are less well ordered. When identifying glycogen content in fish, one can face serious technical difficulties resulting from the extreme overexcitement of fish during catching and handling, and this can result in the almost total disappearance of glycogen from the tissues (Love, 1970). In order to identify the true glycogen content in tissues, it is necessary to acclimate the fish by keeping them for some time (often rather a long time) under experimental conditions during which glycogen content can be restored. Love (1970) found an original indirect way for determining glycogen content in gadoids: based on the relation between glycogen content and pH, he estimated the level of glycogen with an indirect index.

The terminal product of glycolysis (glucose destruction) in aquatic animals including fish is lactate, which due to protective synthesis is transformed partly into alanine. The enzymes that participate in this process are lactate dehydrogenase and alanine aminotransferase. These enzymes and products of glucose catabolism (metabolites) can give important information for estimating fish condition.

The main reason why carbohydrates are not widely used as condition indicators in fish is because these compounds, particularly liver and muscle glycogen, are important sources of

energy for intensive activity of fish, but stores are usually relatively small and cannot sustain endurance over the long term (Busacker et al., 1990).

2.4   Proteins and products of protein and nitrogenous metabolism

Proteins are some of the most significant organic components of organisms, being part of the structural foundation of cells and the molecular structure of enzymes, and participating in energy metabolism. It was no accident that until the second part of the last century (before the discovery of the special significance of nucleic acids), life was perceived as "protein bodies." Compared with lipids, protein content in animals, including fish, is relatively stable (about 20% of wet mass) with no great fluctuations (18–22%). Total protein content (including mainly structural proteins) is determined using the Kjeldahl apparatus. This measures total nitrogen content, and use of a conversion coefficient (6.25) allows total nitrogen to be converted to total protein content. More specifically, Kjeldahl identifies all nitrogen components of organic substances, which are conventionally named "raw protein." However, for determination of functional proteins actively involved in metabolism, the method designed by Lowry et al. (1951) has been used, which does not consider the large mass of structural proteins. However, while carrying out balance investigations it is necessary to know total protein content when protein growth and productivity are identified. The expression "protein accumulation" is incorrect as structural and metabolically active proteins are not accumulated but assimilated (or converted), i.e., are involved in active metabolism. The term "accumulation" is used when protein is stored (i.e., to form reserves). However, proteins also have other more significant functions. When calculating the energy equivalent of protein, its calorie content is considered to be equal to (i) 5.6 or (ii) 4.1 kcal/g wet mass. In case (i), the total energy equivalent is the quantity of energy in 1 g of protein; case (ii) shows the useful energy released during protein catabolism (proteolysis). In comparison with fats and carbohydrates, proteins are metabolized not to H_2O and CO_2, but to H_2O and NH_3 (which contains energy that is not involved in metabolism). During proteolysis, intermediate products of protein catabolism are formed (polypeptides, dipeptides, and oligopeptides) whose energy is released by protease enzymes. At this stage it is necessary to refer not to protein but nitrogenous catabolism, since the processes observed are connected not with proteins but with derived products (which all contain nitrogen).

For many ecological investigations where approximate data on "raw protein" content in fish are sufficient, the estimation of protein content can be expressed by the following formulae (Shulman & Kokoz, 1971):

$$RP \approx UDM = DM - (F + G) \text{ or} \tag{2.11}$$

$$RP \approx UOM = DM - (F + G + MM) \tag{2.12}$$

where RP represents "raw protein," UDM unfatted dry matter, UOM unfatted organic matter, F fat, G glycogen, and MM total mineral matter determined as "ash."

There is a direct relation between "raw protein," determined by total nitrogen in Kjeldahl apparatus, and UDM and UOM calculated with high correlation coefficient ($r=0.9$): RP$=98.9+1.01$UDM and RP$=51.9+1.05$UOM (Shulman & Kokoz, 1971). At the same

time it is necessary to remember that "raw protein" is calculated by multiplying the total nitrogen content by the conversion coefficient of 6.25; however, it cannot be considered absolutely exact as it corresponds only with average content of nitrogenous components in protein. If we consider the content of glycogen and total mineral matter equal to about 10% of dry matter, it is sufficient only to determine dry matter content and to calculate fat content by the formula connecting dry matter with fat content (see equation 2.10) in order to obtain approximate values of "raw protein" content in fish.

The terminal process of nitrogenous catabolism is the formation of free (desaminated) amino acids, which are involved in the Krebs cycle. All proteins contain 20 amino acids, some of which are essential (i.e., they are not synthesized by the organism but can only be obtained in food). The essential amino acids in fish (as well as higher animals) are lysine, arginine, valine, phenylalanine, leucine, isoleucine, threonine, histidine, methionine, and tryptophan. The presence (in definite proportions) or absence of these amino acids in fish food is an indication of their nutritional value. A significant part of nitrogenous metabolism is transamination, i.e., the interconnected transformation of amino acids by turnover of active amino groups. Two of the important enzymes involved in transamination are aspartate aminotransferase and alanine aminotransferase. These enzymes are often defined by investigators as indicators of condition, characterizing the direction of nitrogenous catabolism. Apart from enzyme activity, intermediate metabolites of protein (nitrogenous) catabolism are important indicators and are components of ATP resynthesis (Hochachka & Somero, 1973, 1984, 2002). In order to identify the direction and intensity of protein anabolism, along with growth and production, the incorporation of marked molecules in proteins is used. This characterizes their biosynthesis. An important indicator of fish condition is the protein composition of blood serum (plasma), which includes proteins such as serum albumin and α-, β- and γ-globulins. Albumin and α- and β-globulins are involved in the transport of nutritive substances to tissues as well as in the transfer of Fe-containing proteins; γ-globulin is involved in immune function. Albumin also plays an important role in colloid-osmotic regulation of the blood. Very significant indicators of condition are the lipoproteins transported by the blood, which comprise both low-density and high-density lipoproteins. The first indicate good and the second bad condition of organism.

In the second half of the last century, due to the development of chromatographic methods, the composition of protein fractions in tissues as well as enzymes began to be widely used in genetic investigations of intraspecific and interspecific differentiation including fish (Tsuyuki et al., 1965; Wilkins, 1967; Altukhov, 1974, 2003; Kirpichnikov, 1987; Lukyanenko et al., 1991; Dobrovolov, 1992).

2.5 Nucleic acid-based indicators

Intensive studies of nucleic acids began in the second half of the twentieth century after the discovery of the double helix of DNA and the deciphering of the genetic code. In ecology the study of DNA and RNA structures resulted in an avalanche of investigations focusing on interspecific and intraspecific differentiation including that in fish (e.g., Jamieson, 1974; Clemmensen, 1994). Studies of nucleic acids not only constitute a major part of molecular biology but are also used in estimating condition and growth of fish. Although the evaluation of these indicators requires complex and costly techniques, their use in specific life stages of fish (basically larvae and juveniles) makes them important in describing the condition of these key life stages that otherwise is difficult to estimate by other indicators.

Figure 2.13 Larvae of a flatfish of the Soleidae family. Photo by Ana Sabatés.

The use of nucleic acid-based indicators has been strongly advocated during the last decades to study the condition and growth rates of ichthyoplankton (Fig 2.13) and juveniles, with special attention to the RNA/DNA ratio, the DNA/dry weight (DNA/DW) index and the DNA/carbon content (DNA/C) index (Bergeron, 1997; Buckley et al., 1999; Chícharo & Chícharo, 2008). Among these indicators, the RNA/DNA ratio has been the most widely used in fish, and was first proposed more than 40 years ago as a biochemical indicator of the condition of aquatic organisms. Since then it has been continuously explored and applied with success in microbial communities, invertebrates, and fish (Chícharo & Chícharo, 2008). This index provides a measure of the synthetic capacity of the cell and usually correlates with nutritional status. The RNA/DNA ratio is based on the assumption that the amount of DNA, the primary carrier of genetic information, is stable under changing environmental situations within the somatic cells of a species, whereas the amount of RNA directly involved in protein synthesis is known to vary with age, life stage, organism size, disease state, and changing environmental conditions. Thus larvae and juveniles in good condition tend to have higher RNA/DNA ratios than do those in poor condition (Chícharo & Chícharo, 2008).

The RNA/DNA ratio of juvenile fish of several species including sole (*Solea solea*) and flounder (*Platichthys flesus*) has also been used to evaluate habitat quality in coastal and estuarine nurseries (see for example Gilliers et al., 2004; Vasconcelos et al., 2009). Other fish species in which nucleic acid-based indicators have been used to evaluate the condition of their larvae are European anchovy *Engraulis encrasicolus* in the Bay of Biscay (Bergeron, 2000), sardine *Sardina pilchardus* on the Iberian coast (Chícharo et al., 1998), cod *Gadus morhua* in the North Atlantic (Caldarone et al., 2003), and sole *Solea solea* on the Portuguese coast (Vinagre et al., 2008).

Other investigations have also proposed the use of multivariate analysis to assess larvae condition, with DNA, RNA, and protein content as input variables, for example in turbot *Scophthalmus maximus* larvae (Cunha et al., 2003). Nevertheless, some authors have raised concerns about the significance of RNA/DNA ratios as a descriptor of larval condition and its ecological significance (see Suthers et al., 1996; Suthers, 2000; Fonseca & Cabral, 2007). For example, Gilliers et al. (2006) found that RNA/DNA ratios of sole appeared to be unreliable during a long-term study because they fluctuate greatly in the short term, while morphometric indicators seemed to be relevant, complementary indicators. Overall, it is widely accepted that RNA/DNA ratio is a short-term and fluctuating biochemical

index that outlines the condition of the last days or weeks. On the other hand, morphometric indicators such as rate of length and weight growth (as well as protein) integrate the entire juvenile life stage and in adults provide information over a longer time frame (Tanner et al., 2009).

Further, the RNA/DNA ratio has also proved to be a reliable index of nutritional condition when determined in the white muscle of adult fish (Chícharo & Chícharo, 2008). For example, this ratio has been used to estimate the nutritional condition of breeding European anchovies inhabiting the French coast of the Atlantic Ocean (Bergeron & Massé, 2012).

2.6 Enzymes and some other indicators of intermediate metabolism

We have already mentioned in the previous sections some key enzymes that can be indicators of the direction and intensity of metabolism. In the following chapter this list will be extended. Approximate indicators of catabolism may include the relationships between the initial and terminal values of these processes, calculated by their content in the body or tissues, for example total pool of free amino acids/total protein; total pool of free fatty acids/total lipids; total pool of glucose/glycogen; and antioxidants. Antioxidants inhibit the negative effects of free radicals and peroxides and include carotenoids, α-tocopherol (vitamin E), glutathione and a number of enzymes such as catalase, superoxide dismutase, peroxidase, glutathione reductase, and glutathione *S*-transferase. All these substances characterize the organism's capacity for resistance to damaging factors.

2.7 Hormones and other biologically active substances

The content and activity of pituitary, thyroid and adrenocortical steroid hormones have been studied very intensively in fish. These hormones include thyroxine, somatotropin, prolactin, epinephrine (adrenaline), catecholamines, gonadotropins, etc. They control energy metabolism, growth, fat accumulation, reproductive function, migrations, and so on. Study of these and other significant hormones will be reviewed later. It is essential to consider these hormones if we are to understand the processes regulating life-history stages, including annual cycles (maturation, feeding and migration) when hormones are important indicators of fish condition. Other substances (vitamins and provitamins, the antioxidants mentioned above, creatine phosphate as energy source during burst muscle effort) also play a significant roles in the evaluation of fish condition.

3 Indicators of functional activity

Summary: Biological evolution and biodiversity are accomplished in fish, as in other animals, by two alternative metabolic strategies. The first strategy involves the intensification of energy metabolism, while the second involves considerable specialization. Fish species and populations which follow the first strategy occupy vast aquatic habitats and have excellent locomotion, abundance and productivity. Those species that follow the second strategy manifest delicate adaptations to narrow ecological niches and have more effective metabolism. All these features are well demonstrated by indicators of oxygen and food consumption, levels of energy stores (lipids, polyenic fatty acids and glycogen), and use of aerobic and anaerobic metabolic substrates.

Key words: biodiversity, locomotion, oxygen, food, lipids, fatty acids, glycogen

3.1 Significance of condition indicators of functional activity in fish

In this chapter we summarize the determination and application of condition indicators of functional activity in species, populations and single organisms, as this characterizes fundamental features of these living systems. The most important element of fish functional activity is natural mobility, which plays a primary role in their behavior, food acquisition, and intraspecific and interspecific competition. This feature is the foundation of biodiversity, which we have considered in detail in earlier publications (Shulman & Love, 1999; Shulman & Tokarev, 2006). According to Severtsev (1934), in animals, including fish, differences in functional activity and natural mobility are based on two alternative strategies (Table 3.1). The first strategy involves the occupation of vast areas and high abundance, biomass and productivity; the second involves the occupation of narrow ecological niches. Naturally, both these strategies are accomplished as a result of essential differences in the level and intensity of metabolism and in differences in structure. Some examples are presented below.

Table 3.1 Scheme of biological evolution and biodiversity

I Expansion
Development of enormous areas
High biomass and productivity
High interspecific and intraspecific differentiation

II Specialization
Occupation of narrow ecological niches
High interspecific and intraspecific differentiation

Source: after Severtsev (1934) and Shulman & Tokarev (2006).

Table 3.2 Rate of oxygen consumption (mL/g per h) by different Black Sea fish

Species	Standard metabolism
Anchovy *Engraulis encrasicolus ponticus*	0.970
Horse-mackerel *Trachurus mediterraneus ponticus*	0.700
Mullet *Mugil cephalus*	0.572
Pickerel *Spicara smaris*	0.572
Red mullet *Mullus barbatus*	0.247
Whiting *Merlangus merlangus euxinus*	0.276
Scorpion fish *Scorpaena porcus*	0.084

Source: after Belokopytin (1993) and Shulman & Love (1999).

Oxygen consumption can be used as a measure of the metabolism of fish. Table 3.2 shows values of the coefficient *a* (eqn. 2.3) for different Black Sea species at "standard" metabolism (Belokopytin, 1993). As can be seen, these values correspond exactly with the degree of natural mobility of the species studied. Similar dependence was observed by Lipskaya (1974) for several Atlantic species in the tropics.

Differences in condition are revealed at the sub-organism level too. Oxygen consumption of the red muscle of Black Sea horse-mackerel *Trachurus mediterraneus ponticus* is higher than that of the same muscles of pickerel *Spicara smaris* and red mullet *Mullus barbatus*, fish that exhibit moderate activity (Stolbov, 1990). Red muscle is responsible for long-term (cruise) swimming. The red muscle of more active fish contains more mitochondria (energy factories of cell) than that of less active fish: 45.5% of total red muscle mass in most active fish (Johnston, 1982; Shindo et al., 1986; Savina, 1992), 25–31% in fish of moderate mobility (Kryvi et al., 1980; Johnston, 1981; Savina, 1992), and only 3–5% in slow-swimming fish (Totland et al., 1981; Savina, 1992). Active fish have a more developed capillary system in red muscle to enhance oxygen transport via hemoglobin to mitochondria and a higher hematocrit (Blaxter, 1988). The muscles of active fish contain more cytochromes and higher cytochrome oxidase activity that facilitates electron transport in the respiratory chain, more intensive respiratory control (oxidative phosphorylation, i.e., P/O coefficient), and more Atkinson's charge (the efficiency of energy utilization, which is accumulated in adenyl nucleotides) (Verzhbinskaya, 1953; Hochachka & Somero, 1977; Johnston, 1981; Demin et al., 1989; Savina, 1992; Soldatov, 2007). The dark color of red muscle is produced by the pigment myoglobin, which like cytochrome takes part in oxygen transport to tissues. Active Atlantic species like herring *Clupea harengus* and mackerel *Scomber scombrus*
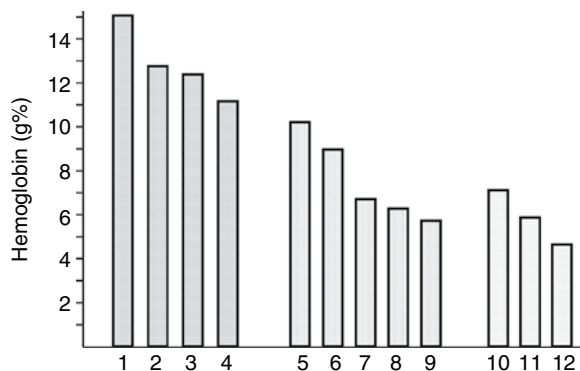
Figure 3.1   Hemoglobin concentration in fish of different mobility. Pelagic active fishes: 1, *Scomber scomber*; 2, *Trachurus mediterraneus*; 3, *Sprattus sprattus*; 4, *Engraulis encrasicolus*. Demersal fish with moderate activity: 5, *Spicara smaris*; 6, *Mullus barbatus*. Bottom-dwelling fish with low activity: 7, *Blennius parvicornis*; 8, *Serranus paracentropsistis*; 9, *Merlangus merlangus*; 10, *Scophthalmus maximus*; 11, *Scorpaea porcus*; 12, *Neogobius melanostomus*. After Tochilina (1990) and Shulman & Tokarev (2006).

contain more myoglobin than relatively less active cod and whiting (Love, 1997). Stocks of Atlantic cod which migrate have more dark-colored red muscle than those stocks which remain in one area throughout their lifespan. Tunas (*Thunnus* sp.), one of the most active fish groups in the world, possess an extra band of red muscle near the backbone in addition to a lateral band under the skin. Salmonidae also possess extra red muscle (Webb, 1971). The percentage content of red muscle in some fish families is as follows: Scombridae, 26.1%; Carangidae, 18.3%; Clupeidae, 18.3%; Sparidae, 17.7%; Mugilidae, 14.5%; Gobiidae, 4.5% (i.e., percentage steadily decreases with decrease in natural mobility). Emerelty (1990a,b) demonstrated a convincing link between succinate dehydrogenase activity in red muscle mitochondria of Black Sea fish and their locomotory activity. This enzyme is one of the most important Krebs cycle enzymes, and controls the intensity of aerobic energy metabolism.

As we have noted, a significant feature of active fish is the extreme development of the circulatory system, manifesting as large blood volume, elevated number of red cells (erythrocytes), and high concentration of the respiratory pigment hemoglobin (Egorova, 1968; Rambhasker & Rao, 1987; Tochilina, 1990) (Fig. 3.1). The oxygen-carrying capacity of hemoglobin is also increased (Table 3.3 and Fig. 3.2) (Klyashtorin, 1982; Soldatov, 2011). Because of this, threshold values of oxygen saturation in water for active fish are higher than those for slow-moving fish (Soldatov, 2011).

Active fish have higher stores of neutral lipids (triacylglycerols), the main energy "fuel" for swimming (Shchepkin, 1972; Shulman, 1972a, 1974; Shulman & Love, 1999) (Table 3.4 and Fig. 3.3). These comprise about 80% of total energy substrates (Belokopytin, 1993). The concentration of non-esterified fatty acids in blood and muscles is higher in active fish than in sluggish (slow-swimming) fish, evidence of more intensive lipid utilization (Shchepkin, 1972). Active fish have more oleic and palmitic fatty acids, which are used to provide energy for swimming, than fish that exhibit low activity (Krueger et al., 1968; Sargent & Henderson, 1980; Ackman, 1989; Yuneva et al., 1991). The same has been demonstrated for the antioxidative enzymes such as superoxide dismutase and catalase (Rudneva-Titova, 1994; Rudneva, 2011). Red muscle, which provides energy for long-term swimming in fish, is characterized by

Table 3.3 Oxygen-carrying capacity in blood of pelagic and bottom-dwelling species of Black sea fish

Species	n	P_{95} (hPa)*	P_{50} (hPa)†
Pelagic fish			
Liza aurata	9	72.7 ± 1.7	26.7 ± 0.8
Mugil saliens	7	74.9 ± 2.1	27.4 ± 1.3
Liza hamatocheila	8	65.8 ± 1.4	22.8 ± 0.7
Trachurus mediterraneus ponticus		72.8 ± 0.8	28.1 ± 0.8
Engraulis encrasicolus ponticus	10	82.6 ± 1.5	31.9 ± 0.8
Bottom-dwelling fish			
Neogobius melanostomus	10	41.9 ± 2.6	17.4 ± 0.6
Mesogobius batrachocephalus	9	56.2 ± 1.6	18.2 ± 0.8
Zosterisessor ophiocephalus	7	63.4 ± 1.5	20.1 ± 0.9
Platichthys flesus	8	62.0 ± 1.3	22.7 ± 0.9
Scorpaena porcus	10	43.0 ± 0.9	14.8 ± 0.5

*P_{95}, partial pressure of oxygen at which hemoglobin is 95% saturated.
†P_{50}, partial pressure of oxygen at which hemoglobin is 50% saturated.
Source: after Soldatov (2011).
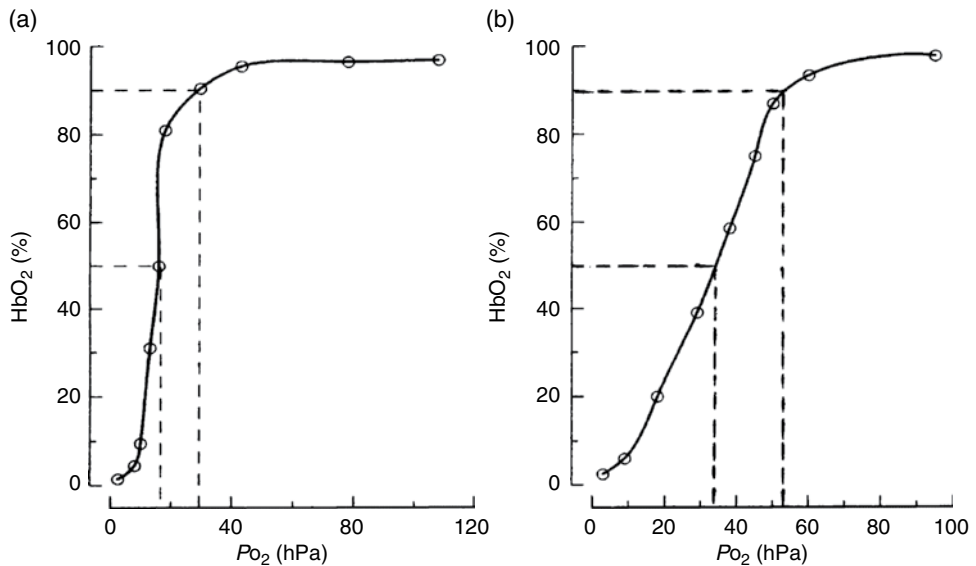
Figure 3.2 Oxygen saturation in (a) scorpion fish *Scorpaena porcus* and (b) golden mullet *Mugil auratus* blood. After Soldatov (2011).

more aerobic metabolism than white muscle, which has a more structural role in fish with high locomotory activity.

In contrast with the first strategy, the second strategy, which is peculiar to sluggish fish that exhibit low activity, is characterized by a low level of oxygen consumption (Table 3.2), low number of erythrocytes, and low content of hemoglobin (Fig. 3.1), as well as a low content of neutral lipids in muscles (Table 3.4). Classic examples of these fish are members of the Gadidae and Pleuronectidae. These fish have small amounts of red muscle (or generally

Table 3.4 Lipid fractions (mg % wet weight of tissue) in muscle and blood serum of horse-mackerel (active) and scorpion fish (sluggish) from the Black Sea

Species	Phospholipids	Cholesterol	Free fatty acids	Triacylglycerols	Cholesterol esters	Total lipids
Horse-mackerel						
Red muscle	2071 ± 145	1379 ± 58	291 ± 32	6170 ± 624	615 ± 67	10,496 ± 649
White muscle	1152 ± 60	1026 ± 53	166 ± 22	2190 ± 155	390 ± 57	4924 ± 146
Blood serum			159 ± 87	1269 ± 194		4615 ± 216
Scorpion fish						
Red muscle	1709 ± 115	1418 ± 109	31 ± 7	1084 ± 91	927 ± 43	5169 ± 274
White muscle	1542 ± 110	605 ± 21	22 ± 6	122 ± 39	530 ± 44	2822 ± 66
Blood serum			80 ± 17	453 ± 60		1597 ± 15

Source: after Shchepkin (1972) and Shulman & Love (1999).
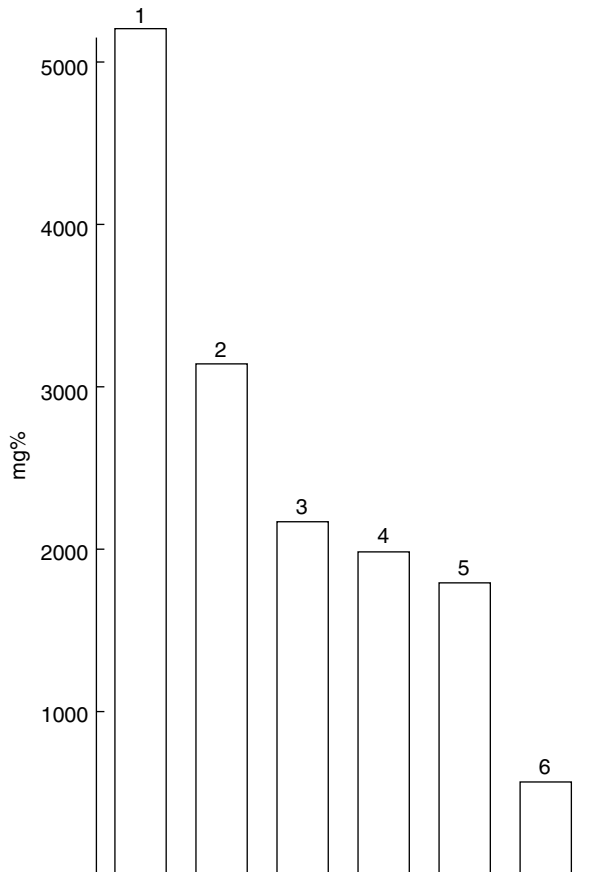
Figure 3.3 Triacylglycerol content in white muscle of Mediterranean fish with different natural mobility: 1, *Scomber scomber*; 2, *Scomberesox saurus*; 3, *Trachurus mediterraneus*; 4, *Diplodus annularis*; 5, *Gadus merlangus*; 6, *Scorpaena scrofa*. After Shulman et al. (1978).

none) and instead there is abundant development of white muscle. Fish that follow the second strategy have undergone metabolic compensation in order to exist in their chosen environment. For example, oxygen affinity to hemoglobin is higher than in active fish (Klyashtorin, 1982; Soldatov, 2002, 2011) (Table 3.3 and Fig. 3.2). Anaerobic metabolism prevails in slow-moving fish, which paradoxically allows short but very explosive (burst) activity (Black, 1958; Drummond, 1967; Prosser, 1991; Sumich, 1999). This is very important for attack on prey by ambush fish (pike, scorpion fish, moray, etc.) or escape from predators (gobies, some coral fish, etc.). Thus these fish may be termed "sprinters" in contrast with the "stayers" (fish using the first strategy). All the enzymes of anaerobic metabolism are very efficient, the main energy substrates of such fish being glycogen and glucose. Glycogen accumulates in the liver, where it can reach large concentrations (Plisetskaya, 1975; Love, 1980). In general, the liver plays a more significant role in the metabolism of fish employing the second strategy (slow-moving fish), providing a quicker transformation of energy for ambush and escape from predators. These fish are able to survive in oxygen-deficient environments by utilizing the catabolism of proteins and nitrogenous substrates,
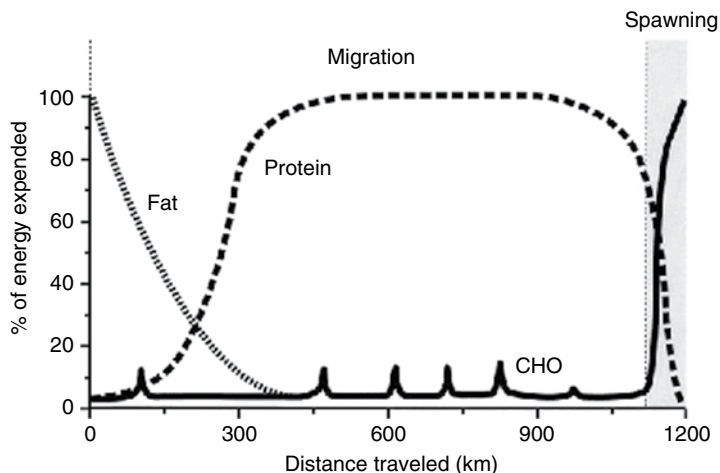
Figure 3.4 Energy substrates during anadromous migration of sockeye salmon *Oncorhynchus nerka*. After Mommsen et al. (1980) and Hochachka & Somero (2002).

including amino acids, to yield energy. It seems paradoxical, but protein accumulation in the liver (demonstrated by incorporation of ^{14}C-labeled protein) is higher not in the more active pickerel but in the slower scorpion fish (Khotkevich, 1974). Liver antioxidative activity is higher in Gobiidae and scorpion fish than in pickerel (Rudneva-Titova, 1994; Rudneva et al., 2011). The content of adenyl nucleotides (ATP, ADP and AMP) is higher in the liver of scorpion fish than in that of horse-mackerel (Savina et al., 1993).

Although we have described two alternative strategies of energy metabolism in fish, there is a third (intermediate) strategy. This strategy is followed by fish with moderate levels of locomotory activity (Sparidae, Myctophidae, Serranidae, Mullidae, etc.). The metabolic characteristics of these fish are transitional between the first and second strategies. Protein and nitrogenous compounds are the main substrates in energy catabolism (Bilinski & Jonas, 1970; Walton & Cowey, 1982; Petrovich et al., 1996; Shulman & Love, 1999). Salmonids have a special interest in this situation as they undertake long-term migrations from the oceans and up the rivers (against the current) for spawning (Fig. 3.4). In the initial stage of this migration they utilize fat stores (Mommsen et al., 1980). In the second stage, when these stores have largely been depleted, the main energy source is protein (and metabolism may be partly anaerobic). During the final stage, spawning, when protein structures have been depleted, glycogen becomes the main energy store. Glycogen is also used intensively for overcoming rapids.

Energy anabolism, as opposed to expenditures, consists of energy accumulation in the form of stores or restoration of those which were mobilized. In fish, substances are stored in the form of neutral lipids and glycogen. Proteins do not form energy stores, but may be used as such during starvation (endogenous feeding). The most suitable indicators of lipid, glycogen and protein anabolism for energy needs are scale, intensity and efficiency of their conversion in individuals and populations. They can be defined by determining their absolute increase and total energy equivalent and the activity of key anabolic enzymes.

The biodiversity of structural components plays a significant role in the energy metabolism of fish. These components include proteins, polar and reserved neutral lipids, glycogen, and intermediate and especially terminal products of their catabolism (free amino acids and

Table 3.5 Mean annual daily intensity of food consumption (ration, % of body weight) and efficiency of its assimilation (K_2) in Black Sea fish

Species	Diurnal ration	K_2
Anchovy	10.17	2.4
Sprat	6.16	4.6
Horse-mackerel	5.43	5.6
Red mullet	1.55	25.1
Pickerel	1.74	13.9
Whiting	0.90	12.8

Source: after Shulman & Urdenko (1989) and Shulman & Tokarev (2006).
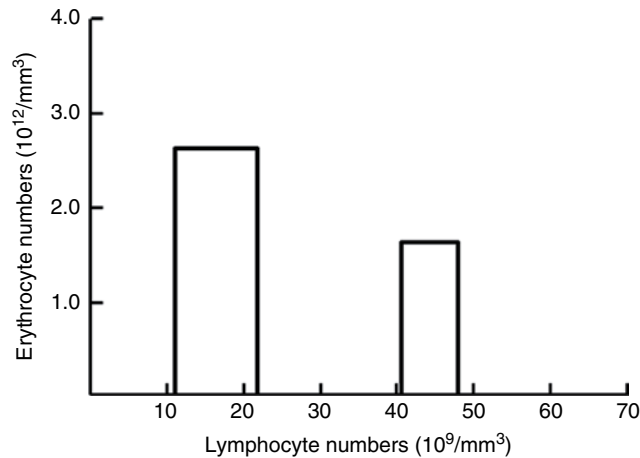
Figure 3.5 Relationship between erythrocyte and lymphocyte numbers in blood of fish: Left column: active swimmers (*Scomber japonicus, Scomberesox saurus, Belone belone, Trachurus mediterraneus mediterraneus, T. m. ponticus, Sprattus sprattus, S.s. phalericus, Alosa kessleri, Engraulis encrasicolus mediterraneus, E.e. ponticus, E.e. maeoticus, Myctophum affinis*). Right column: sluggish swimmers (*Scorpaena scrofa, Trigla lineate, Solea lascarius, Scophthalmus maeoticus, Pleuronectes flesus, Gobius melanostomus*). After Tochilina (Rakitskaya) (1990, 1994) and modified by G.E. Shulman.

fatty acids, glucose, etc.). Food consumption intensity (average annual diurnal ration) of active fishes is considerably larger than that of less active fish (Table 3.5). However, food consumption and efficiency has an inverse relationship, the same as occurs with the intensity and efficiency of energy metabolism in fish employing the first and second strategies. A clear illustration of the inverse relation between intensity of energy metabolism and food consumption is shown in Fig. 3.5, which compares erythrocyte and lymphocyte numbers (concentration) in the blood of different fish species. The first indicator characterizes the level of energy metabolism, the second one the level of food consumption. As Ugolev and Kuzmina (1993) reveal, lymphocytes are involved not only with immune function but also with intracellular digestion. These authors showed that proteolytic enzyme activity in sluggish fish is higher than in active fish. The level of blood serum proteins, transporting assimilated products, is also higher in slow-swimming fishes (Kulikova, 1967). Total polar structural lipids as well as different phospholipids (primarily phosphatidylcholine and
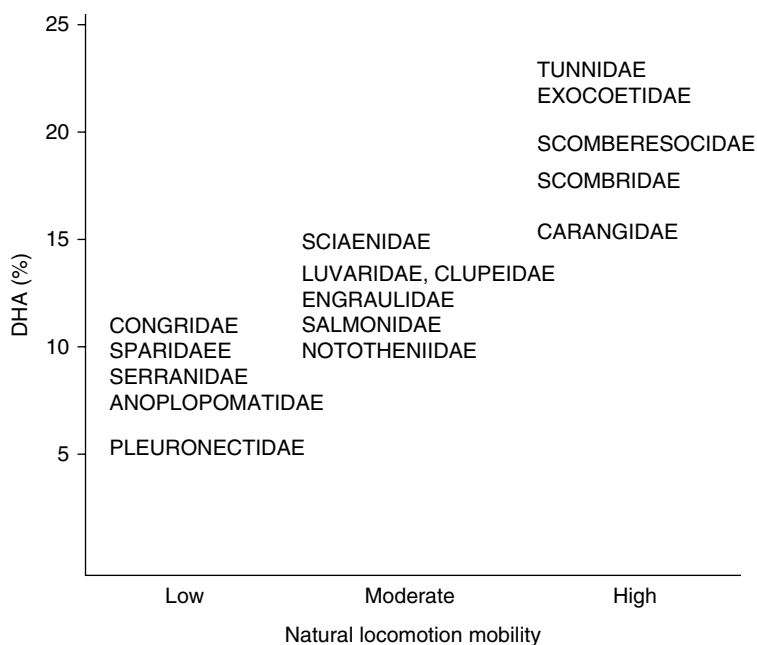
Figure 3.6 DHA content in total lipids of fish taxa of different mobility. From Shulman & Yakovleva (1983) and Shulman & Tokarev (2006).

phosphatidylethanolamine) provide important information about the differences between active and sluggish fish. This relates especially to polyunsaturated fatty acids. In Chapter 5 (section 5.2.1) we discuss the significance of these fatty acids for adaptation to low temperature. Shulman and Yakovleva (1983), Shulman and Yuneva (1990), and Shulman and Love (1999) consider that these structural components of membranes play an important role in the functional activity of fish, especially docosahexaenoic acid (DHA, 22:6 *n*-3) and eicosapentaenoic acid (EPA, 20:5 *n*-3). DHA and EPA have multifunctional roles, being involved in the structure of membranes, membrane ion transport, enzyme activity, and in the formation of biologically active substances such as prostaglandins and leukotrienes (Sargent, 1997; Kolakovska et al., 2003; Kolakowska, 2010). We have revealed a close relationship between the degree of locomotory activity of large taxons of marine fish and the presence of DHA in lipids (Shulman & Yakovleva, 1983) (Fig. 3.6). The same relationship was obtained for interspecific and intraspecific differences (Yuneva et al., 1990, 2011; Tornaritis et al., 1994; Shulman & Love, 1999; Kolakovska et al., 2003; Zlatanos & Laskaridis, 2007; Kolakovska, 2010) as well as for many marine invertebrates (Yuneva et al., 1998). Thus we consider that not only temperature and other abiotic factors (salinity, oxygen deficiency, etc.) but also functional activity (primarily mobility) strongly influence the content of 20- and 22-carbon polyenic fatty acids, which contribute to the condition of cellular and intracellular membranes. Unsurprisingly, omega-3 fatty acid content is higher in red muscle of active fish than in white muscle (Yuneva et al., 1991), but sluggish fishes have a very high content in liver, the most active metabolic organ in the body (Shulman & Yakovleva, 1983; Shulman & Love, 1999; Tanakol et al., 1999). This provides compensation for those fishes with low metabolic activity in comparison with the most active swimmers. So omega-3 acids are exceptional adaptations for fish (and not only for them).

It may seem paradoxical that the activity of some hormones is also higher in sluggish fishes. The scorpion fish has greater activity of adrenocorticoids, produced in the pituitary gland and which regulate catecholamine secretion, than the active horse-mackerel (Plisetskaya, 1975). However, these hormones are involved in carbohydrate mobilization (Plisetskaya, 1975). In addition, the activity of the thyroid gland is also higher in the sluggish scorpion fish than in horse-mackerel (Ivleva, 1989), and this is also evidence of metabolic compensation.

3.2 Applied recommendations

The most useful metabolic indicators of fish functional activity and biodiversity may include the following:

- level of oxygen consumption of whole fish under standard conditions;
- intensity of enzyme activity of oxidative processes;
- efficiency of oxidation (P/O ratio, Atkinson's charge);
- erythrocyte number and hemoglobin concentration in the blood;
- oxygen partial pressure (P_{O_2}) and saturation (S_{O_2}) (provides information about hemoglobin);
- relationship between red and white muscle in body;
- neutral lipid content (triacylglycerols, fats) in red and white muscle and liver;
- the same for glycogen;
- ammonium coefficient (O/N);
- the concentration of terminal metabolic products in blood and muscles (glucose, lactate, free fatty and amino acids);
- content of polyunsaturated omega-3 fatty acids (primarily DHA) in the polar lipids (phospholipids);
- intensity and efficiency of food consumption;
- level of key hormone activity (thyroxine, catecholamines, etc.).

4 Fish condition and life history

Summary: This chapter analyzes the importance of fish condition during the different stages of fish ontogenesis, from larval to juvenile phases, maturation and sexual maturity of fish, senescence and death. The impact of fish condition on life-history traits such as reproductive potential (fecundity, atresia, maturity, recruitment, etc.), growth and natural mortality is described. The consequence of these impacts for future population success is reviewed with examples from marine species worldwide. Annual changes in condition are reviewed, in particular during key periods such as pre-spawning, wintering, and migration. Other cycles in fish condition, such as daily rhythms, are also considered. Finally, the links between fish condition, abundance dynamics, fish behavior, and distribution are explored.

Key words: embryos, larvae, juveniles, maturation, reproduction, spawning, senescence, mortality, growth, wintering, migration

4.1 Fish condition during ontogenesis

The study of fish ontogenesis is concerned with different stages: embryonic; larval (when the post-embryo grows and develops, utilizing endogenous and exogenous supplies); juvenile (when the fry have not yet reached sexual maturity); maturation and sexual maturity of adult fish; and senescence (Severtsev, 1934; Kryzhanovsky, 1949; Shatunovsky, 1980). According to Shulman and Love (1999), ontogenesis is responsible for providing (i) optimal quality of embryos and eggs, making them capable of survival en masse; (ii) optimal development to the beginning of exogenous feeding; (iii) maximum growth rate of fry, which speeds their rescue from predatory stress; (iv) genetically determined growth and development up to maturation; and (v) reproduction (the most prolonged stage in most species). Together with somatic increase, reproduction accounts for the total production of the population.

From a fish ecology perspective, even the final stages of fish (including aging and death) is important for a species, as it eliminates genetically depleted individuals from a population

and supplies the ecosystem with organic substances that arise from their decay. Beginning more than 50 years ago, a large number of studies have dealt with the physiological and biochemical features of the different stages of fish ontogenesis. Some remarkable examples are Needham (1963), Love (1970, 1980), Milman and Yurovitsky (1973), Shulman (1974), Neifach and Timofeeva (1977), Shatunovsky (1980), Cetta and Capuzzo (1982), Ozernyuk (1985, 2000), Novikov (1993, 2000), Sargent et al. (1993, 1999), Ronnestad et al. (2003), Silversand et al. (1996), Sivaloganathan et al. (1998), Shulman and Love (1999), and Kamler (2008).

Generally speaking, fish allocate the assimilated energy following rules determined by its physiological state, and the trade-off between survival, growth and reproduction (Gunderson, 1997). While recruits and post-recruits usually focus on maximizing survival and growth, the adults focus particularly on reproductive activities. All these variables, which constitute important intrinsic factors that are linked to condition during the ontogenesis of fish, are developed in the following sections.

4.1.1   Embryonic (eggs) and post-embryonic (larvae) development

Fertilized and developing eggs contain a full range of substances necessary for their development (the processes of oocyte maturation are discussed in section 4.1.3). In the yolk sac there are proteins, nitrogenous compounds and free amino and fatty acids, phospholipids and polyenic (unsaturated) fatty acids including omega-3 and omega-6 (the latter mostly in freshwater fish), cholesterol, some glycogen and glucose, and certainly nucleic acids, hormones, vitamins, and occasionally carotenoids, among other components.

Omega-3 fatty acids, for instance, promote successful hatching and viability of larvae (Golovachev, 1985; Yuneva et al., 1990). These acids are fundamental components of membranes; they provide osmotic and electrolytic homeostasis, membrane permeability, and ionic transport (Adams, 1999). Docosahexaenoic acid (DHA) has a special significance for the development of larval activity and vision (Brown, 1994; Bell et al., 1995; Sargent, 1995; Bell & Sargent, 1996). DHA accumulates in muscle, retinal rhodopsin, and brain tissue of larvae and provides better orientation during feeding. All polyunsaturated fatty acids, including eicosapentaenoic acids (EPA), are essential for marine fish and are called essential fatty acids (EFAs); they cannot be synthesized and are incorporated into the body only from food (Tocher & Sargent, 1984; Sargent et al., 1993; Sargent, 1995; Geurden et al., 1997). Not only do omega-3 acids constitute a large percentage of polyunsaturated fatty acids, but arachidonic acid 20:4 ω-6 is a precursor for prostaglandins and leukotrienes (Evans et al., 1997).

The fat content of eggs of fish of different species varies widely (0.85 to 22.34% of dry matter) and is almost exclusively composed of neutral lipids, including triglycerides and free saturated and monounsaturated fatty acids as well as cholesterol ethers and wax ethers in some species. These lipids supply energy to the eggs and also contribute to their buoyancy (Kulikova et al., 1996; Bergey et al., 2011). Fat content is sourced mostly (95%) from food (Worthington & Lovell, 1973; Docker et al., 1986).

In embryos and larvae, the processes of protein growth occur by synthesis of free amino acids: firstly they are utilized from yolk sac and then from food, rich in proteins and free amino acids (Conceicao et al., 1997). RNA increases during growth (Clemmensen, 1994). Phospholipids and polyenic fatty acids also play important roles in forming membranes. Neutral lipids (fats) and free fatty acids (14:0, 16:0, 18:0, 16:1, 18:1) have key significance
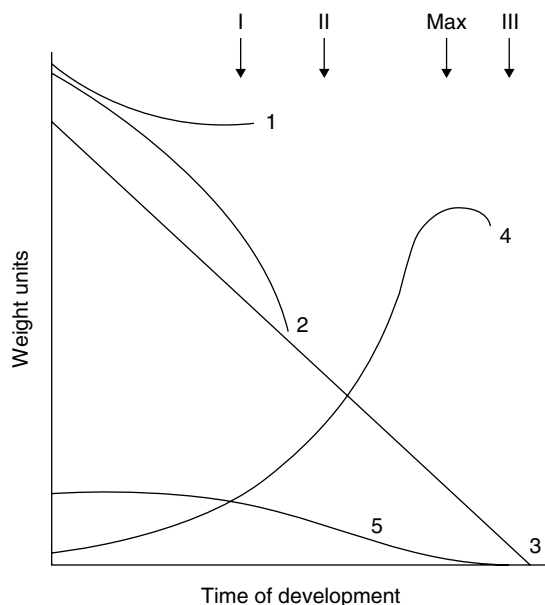
Figure 4.1   Generalized scheme of protein and lipid changes during the development of embryos and larvae (endogenous feeding). Protein in 1, whole egg; 2, egg capsule; 3, yolk; 4, embryo; 5, total lipids. I, hatching from egg; II, transfer to mixed feeding; III, total yolk respiration; Max, maximal body mass. After Shatunovsky (1980), Sidorov (1983), Novikov (1993), and Shulman & Love (1999).

in supplying energy for the structural development of larvae (Blaxter, 1988; Ronnestad et al., 2003).

In the initial phase of embryogenesis, glycogen and glucose are used as energy substrates, whereas in the final phase proteins and nitrogen products are used (Kamler, 2008). All these processes are illustrated in Figures 4.1, 4.2, 4.3 and 4.4. The intensity of oxygen consumption increases in eggs and larvae until larvae attain a definite length and then begins to decrease steadily until the end of adult life (Shamardina, 1954; Ozernyuk, 1985; Ronnestad et al., 2003; Fig. 4.5). During hatching, the endogenous energy stores in the oil globules and proteins and amino acids in the yolk sac are consumed (Kamler, 2008) and the RNA/DNA ratio decreases (Clemmensen, 1994). At this point, larvae have not learnt to obtain exogenous food in the necessary quantity (Folkvord et al., 1996); this is a critical point because it largely determines larval mortality and hence recruitment, causing strong variability in the abundance, biomass, and productivity of fish populations (St John & Land, 1996; Sivaloganathan et al., 1998; Adams, 1999; Lambert & Thorsen, 2003; Kamler, 2008). The ratio of oxygen consumed to nitrogen excreted, the O/N ratio (also named ammonium coefficient, AQ), changes during embryo and larval growth, especially after hatching, to favor the utilization of non-protein substrates (lipid and free fatty acids) compared with proteins and free amino acids (from 4–13% to 36–60%) (Sorvachev & Shatunovsky, 1968; Neifach & Timofeeva, 1977; Houlihan, 1991; Ronnestad et al., 2003; Sivaloganathan et al., 1998).

The role of carbohydrates in larval energy metabolism is significant too (Weatherlay & Gill, 1987; Finn et al., 1995). An important indicator of egg and larval condition, as Gosh (1985) showed, is the degree to which aerobic metabolism (oxygen consumption) dominates
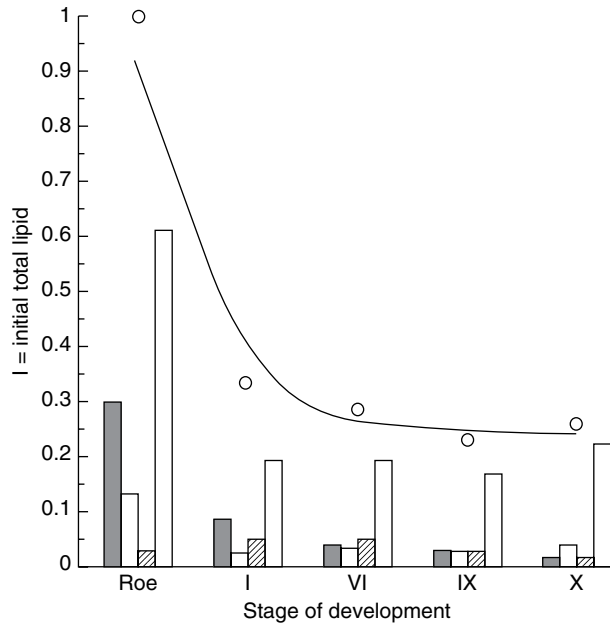
Figure 4.2 Dynamic of lipid fractions in round goby *Gobius melanostomus* from the Sea of Azov during embryonic development. Columns at each stage, left to right: phospholipids, cholesterols, non-esterified fatty acids, triacylglycerols; line curve, total lipids. After Chepurnov & Tkachenko (1983) and Shulman & Love (1999).
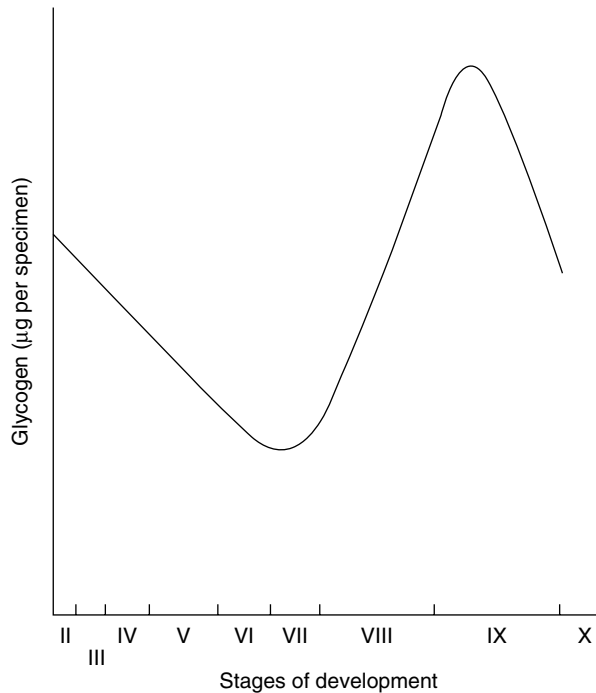
Figure 4.3 Dynamics of glycogen content in eggs of Atlantic salmon *Salmo salar*. II, cell division; III, blastula; IV, gastrula; V, organogenesis; VI, start of mobility, heart pulsation; VII, appearance of blood; VIII, liver–yolk blood circulation; IX, start of continuous moving of burst fins, preparing for hatching; X, hatching. After Novikov (1993, 2000) and Shulman & Love (1999).
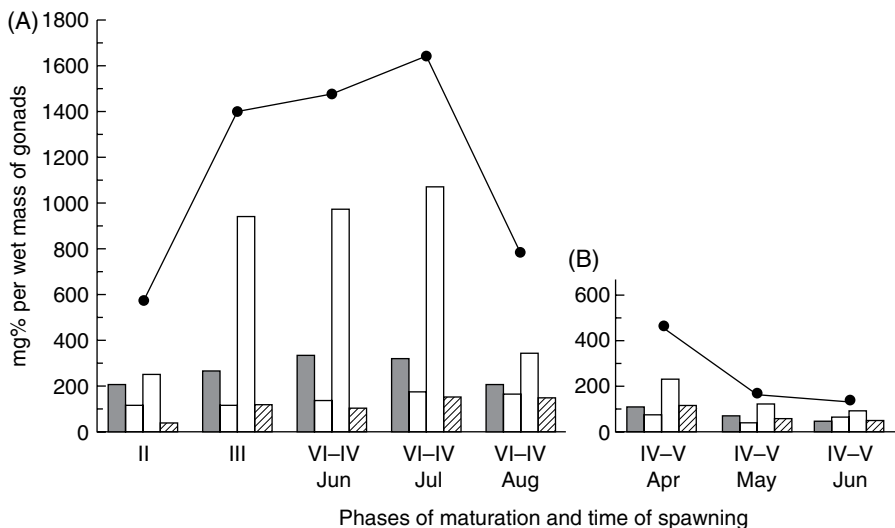
Figure 4.4 Effect of gametogenesis on lipid content in the horse-mackerel (A) and turbot (B) female gonads. Solid columns, phospholipids; light stipple hatching, cholesterol; open columns, triacylglycerols; dark stipple, cholesterol esters; line and closed circles, total lipids. From Chepurnov & Tkachenko (1983) and Shulman & Love (1999).
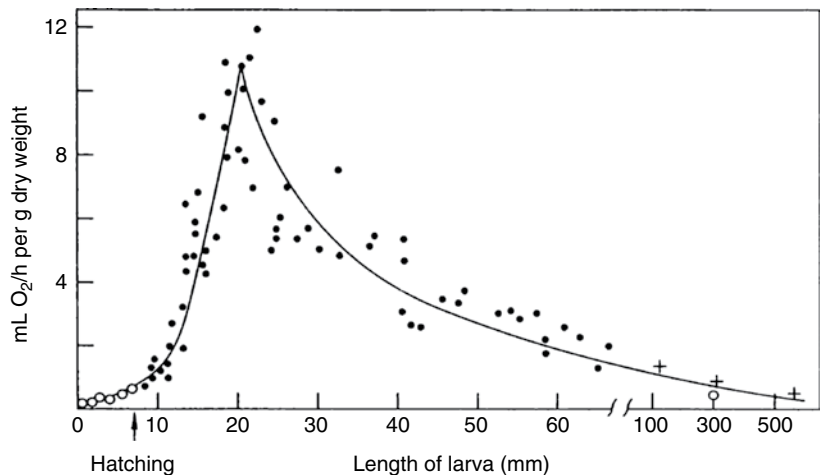
Figure 4.5 Oxygen uptake in pike *Sander lucioperca* during embryonic and larval development: white circles, embryos; black circles, larvae. After Shamardina (1954), Ozernyuk (1985) and Shulman & Love (1999).

anaerobic metabolism (glycolysis). The large quantity of antioxidative carotenoids in salmon eggs is probably related to the large amount of free radicals (Kamler, 2008).

4.1.2 Juveniles

Juveniles and adults show a different energy allocation. In general, juvenile fish usually allocate energy primarily to growth; after maturation the adult fish primarily favors energy

storage for reproductive purposes. This pattern is observed for example in bluefin tuna *Thunnus thynnus* and albacore *Thunnus alalunga* (Goñi & Arrizabalaga, 2010; Chapman et al. 2011).

During the juvenile stage – the stage preceding sexual maturation – intensive protein growth promotes increase in weight and length. Growth has usually been associated with high levels of protein synthesis, following transformation of proteins (from the diet) into amino acids, and high RNA/DNA ratios (Hains, 1973; Shulman, 1974; Shatunovsky, 1980; Kurant et al., 1983; Weatherlay & Gill, 1987). Furthermore, the mineral skeleton develops, juvenile fins and swimming become more stable, and the ratio of red muscle to white muscle increases (Mosse & Hudson, 1977). Condition can change rapidly during early ontogeny as accumulation of energy substrates (neutral lipids in muscles of active fish and glycogen in liver of sluggish ones) occurs. However, this process is slower than protein growth due to the life strategy of the juvenile stage, when it is necessary for fish to reach a maximal size and weight as soon as possible before sexual maturity in order to be able to escape predators, obtain food, and be ready for intensive maturation. Clearly, fecundity is directly proportional to fish size and weight. Hormones such as thyroxine, somatotropin and prolactin regulate the direction and intensity of metabolic processes from early ontogenesis onwards (Thackeray et al., 1989). For juvenile individuals, a few studies have shown that energy reserves usually decline during growth and energy accumulates only when growth is slow. For example, the relative condition index (I_H) of wild juvenile snapper *Pagrus auratus* in the western Pacific Ocean peaks in autumn–winter (when growth is slow) and declines to a minimum in summer, when growth is fast (Fig. 4.6; Francis, 1997). In other studies the negative relationship between condition and growth is very weak (see for example Thackeray et al., 2010). And even some condition indicators that apply to juvenile fish tend to be positively correlated with growth. For example, Fonseca and Cabral (2007) showed that higher RNA/DNA ratios were observed in larvae of species that showed faster growth, thus supporting the idea of higher metabolic investment for protein synthesis. Hence, higher larval and juvenile growth rates and condition indicators (mean RNA/DNA ratios and protein content) were associated with tropical and temperate fish species that occur in complex or variable habitats, respectively coral reefs and estuaries (Fonseca & Cabral, 2007).

4.1.3   Sexual maturation

The mature stage of adult fish is the most prolonged in the life history of the majority of species, excluding freshwater eels and Pacific salmonids of the genus *Oncorhynchus*. During maturation, increases in the levels of lipids and proteins in the gonads are accompanied by increases in the levels of glycogen, vitamins, and other biochemically active substances (Shulman & Love, 1999). Furthermore, in addition to the processes in the reproductive tissues themselves, changes in the quantity and composition of organic substances in the liver are important as a consequence of the increasing metabolism induced by the synthesis of reproductive products. Protein synthesis is switched, to a significant degree, from somatic production only to generative production. In anchovy and sprat from the Black Sea, the annual value of these two types of production is almost equal (Shulman & Urdenko, 1989). In Black Sea fish with a short life cycle, the duration of sexual maturity is threefold to fivefold longer than the juvenile stage, whereas in fish with a long life cycle this relation may be 10- to 15-fold and more. Long-lived fish are able to reproduce up to the end of life. A significant feature of the adult stage is the great increase in fat accumulation in muscles of active fish and the glycogen accumulation in the liver of sluggish fish (Shulman, 1974;
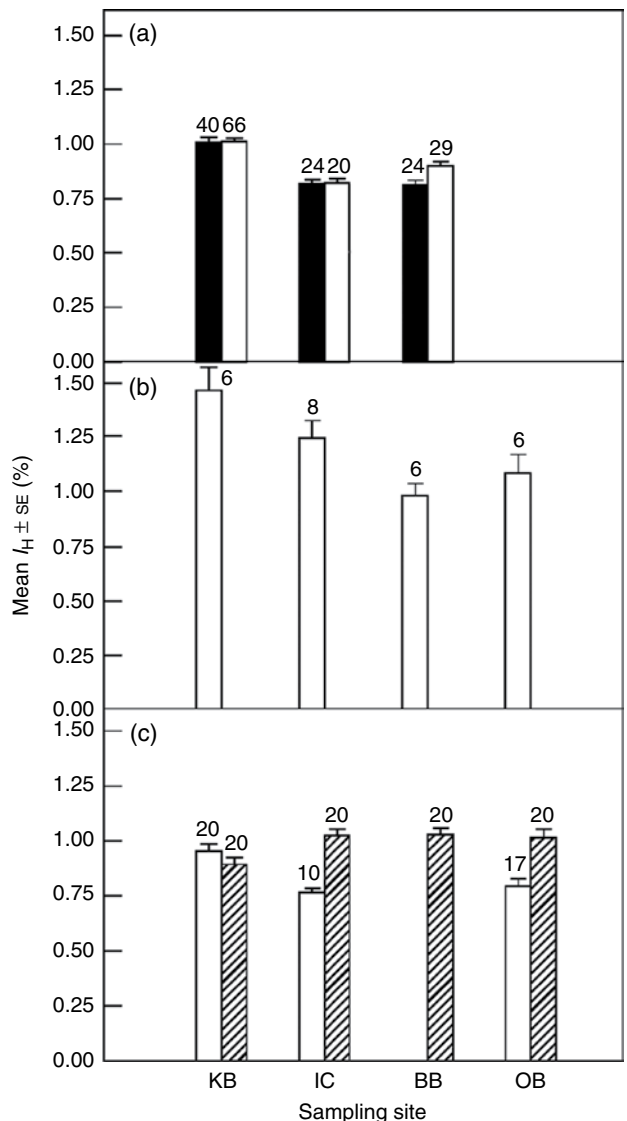
Figure 4.6 Variation in relative condition index (I_H) of juvenile snapper *Pagrus auratus* at four sites in New Zealand waters in (a) February 1988, (b) July 1988, and (c) January 1989. Sample sizes are given above histograms. KB, Kawau Bay; IC, Inner Channel; BB, Bostaquet Bay; OB, Outer Bostaquet. The three different bar motifs represent different year-classes. From Francis (1997).

Love, 1980; Shatunovsky, 1980; Shulman & Love, 1999). These reserves provide energy for somatic and generative growth. If the juvenile stage is characterized by a preponderance of protein synthesis over fat accumulation, the adult stage is characterized by the inverse relation. During growth of Black Sea anchovy and sprat, the relationship between somatic and generative production changes from 2 : 1 somatic : generative to 1 : 2. This is caused by the great increase in significance of reproduction for populations, and thus reproduction becomes more important than somatic growth. All these changes in fish physiology occur
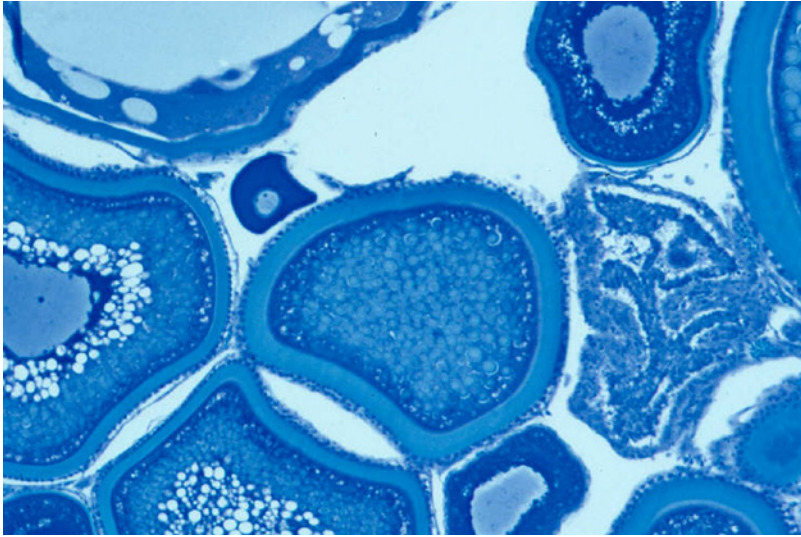
Figure 4.7 Oocytes from *Aspitrigla obscura* in different development stages. Photo by M. Muñoz and S. Vila.

because of a coordinated increase in neurohormonal activity of the hypothalamus, hypophysis (pituitary gland), corticoids, and sex hormones (Fontaine, 1948, 1969; Gerbilsky, 1956; Polenov, 1983; Jensen, 2003).

During maturation, sexual differences in condition appear. Even without dimorphism between females and males, females show higher intensive protein growth than males. This is because of the need to form female gonads. Therefore the range and intensity of reserve fat mobilization for protein biosynthesis is also higher in females. However, energy expenditure in males is higher during spawning due to more active locomotion. This difference in velocity of locomotion causes selectivity of eggs, embryos, and larvae (Oufiero & Garland, 2007). Interesting data were obtained by Bassova (2001) on the sexual differences in biochemical composition and content of the Black Sea turbot *Pleuronectes platessa*. She showed that dry matter, total lipids, triacylglycerols, and cholesterol ethers in female livers are lower, but DNA, RNA, phospholipids and cholesterol higher than in male livers. Female red muscle has more polar lipids and less neutral ones. In female gonads (oocytes, Fig. 4.7) there are more triacylglycerols, 16:1 and 18:1 fatty acids, cholesterol ethers, and RNA/DNA ratio. Glycogen content is higher in spermatocytes. All these differences reflect the functional significance of fertilization and preparation for embryo development. Similar data were obtained earlier (Henderson et al., 1984; Henderson & Almater, 1989). The biochemical composition of female and male gonads and sexual products depends on their destination: in females there are more structural and energy components, which culminate in spawning eggs. The presence of greater quantities of non-esterified fatty acids, glucose, and free amino acids in females indicates that enzyme activity, both anabolic and catabolic, is also higher in females (Liu et al., 1985). Indeed, 22:6 ω-3 content in warm-tolerant Black Sea anchovy is higher in summer, obviously due to more intensive energy and substance metabolism during spawning (Yuneva, 1990). A similar pattern was observed recently in anchovy and sardina *Sardina pilchardus* (Fig. 4.8) in the Mediterranean Sea (Zlatanos & Laskaridis,

Figure 4.8   Sardines (*Sardina pilchardus*) caught in the Mediterranean Sea. Photo by S. Vila.

2007). From our point of view, this is significant with regard to the "temperature paradigm": the relationship between the degree of lipid unsaturation and the temperature environment of fish (see Shulman & Love, 1999). Other than in demersal species, fish eggs contain more fats (triglycerides) in order to provide buoyancy. More phospholipids are present in mature oocytes and spermatocytes than in immature ones (Lizenko et al., 1983; Sidorov, 1983). There are corresponding differences in the liver, which synthesizes and transposes proteins (phosphoproteins, glycoproteins, lipoproteins, and vitellogenins), lipids, glycogen and other bioactive substances to the gonads (Plack et al., 1961; Braekken & Boge, 1962; Jensen, 2003; Hutchinson, 2002; Kamler, 2008). In gonads, during the vitellogenesis and yolk phases of maturity (II and III) the main activity is synthesis of essential amino acids (alanine, leucine, serine, isoleucine, lysine, valine) and formation of structural lipids from free fatty acids. In phase IV (extensive growth) triacylglycerol accumulation prevails. Yolk proteins and lipids are mostly lipoproteins and vitellogenin (De Vlaming et al., 1984; Gershanovich et al., 1991; Hutchinson, 2002). A proportion of the proteinases and other hydrolases of female fish are transferred into the yolk structures of the oocytes as vitellin (Houlihan, 1991), where they are activated as soon as fertilization has taken place (Nemova, 1991). Lysosomal cathepsin D has been studied by Nemova and Sidorov (1990). In spermatocytes aerobic metabolism prevails over glycolysis (Gosh, 1985). Most of the proteins and lipids entering the gonads in phases II and III originate from food, but in phase IV they come mostly from the reserves in the liver and muscle (Wang et al., 1964). A greater quantity of water is accumulated in gonads during phase V, assisting in the oxidation of fatty acids and allowing easier spawning. The main form in which material is transported to the gonads is via lipoalbumins and lipoglobulins (Kulikova, 1967; Ipatov & Lukyanenko, 1979; Kychanov, 1981; MacKay et al., 1985). The accumulation of substances in the gonads is under the control of hormones such as gonadotropins, insulin, corticosteroids, and prolactin (Fontaine, 1969; Plisetskaya et al., 1977; Donaldson et al., 1979; De Vlaming et al., 1984; Sautin, 1985; Jensen, 2003).

In fish that spawn more than once a year, the dynamics of chemical composition differ between groups. The early spawners have an relatively and absolutely richer content of protein and lipid (Shulman & Love, 1999). During the post-spawning period, fatty degeneration of the gonads takes place. The main change in lipid deposition and withdrawal occurs with triacylglycerols, although small changes in phospholipids follow the same pattern, including alteration in the amount of cellular material. In most fish species, the formation of sexual products in the gonads uses up the internal stores of energy and plastic reserves. The situation is even more fraught in species spawning at the end of winter and which are really starving. The classic situation is exemplified by Atlantic cod, in which internal reserves are progressively depleted with each additional year of spawning until the fish is unable to recover from spawning and dies (Love, 1970).

4.1.4 Senescence

During the process of aging, the ratio of protein to lipid in the body shifts toward lipid. The phenomenon is controlled by a change from somatotropin (a growth hormone) to prolactin, which controls lipid accumulation (Donaldson et al., 1979; Sautin, 1985). At the same time, the relative content of triacylglycerols as well as saturated and monounsaturated fatty acids increases. The activity of nucleases declines (Berdyshev, 1968), and the content of serum protein and the ratio of albumin to globulin decreases. On the other hand, the proportion of lipoproteins and non-esterified fatty acids increases (Shatunovsky, 1980). Figure 4.9 convincingly demonstrates the steady decline in intensity and efficiency of food conversion for anabolic processes with the passage of time (Shatunovsky, 1980; Shulman & Urdenko, 1989).

During senescence there is continuous protein growth and fat accumulation (although not so intensive as earlier), which does not occur in many other higher vertebrates. This phenomenon is shown by many fish species, especially "fat" ones (Fig. 4.10) (Shulman,
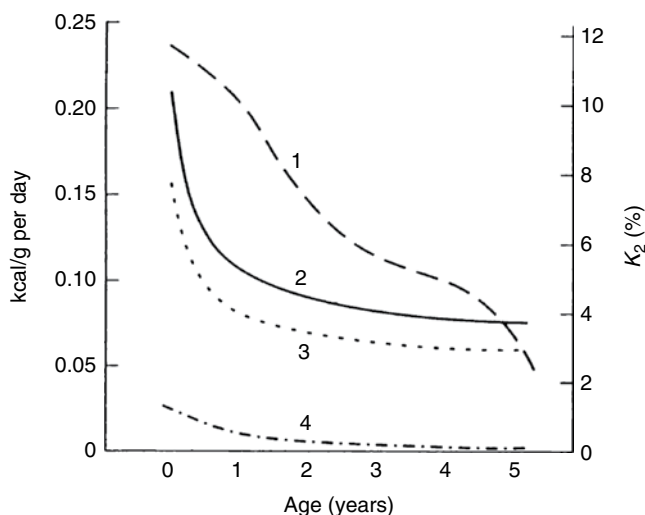
Figure 4.9 Change in metabolic processes during the life cycle of the horse-mackerel *Trachurus mediterraneus*: 1, efficiency of using food for growth; 2, intensity of food consumption; 3, energy metabolism; 4, production. After Shatunovsky (1980), Shulman & Urdenko (1989) and Shulman & Love (1999).
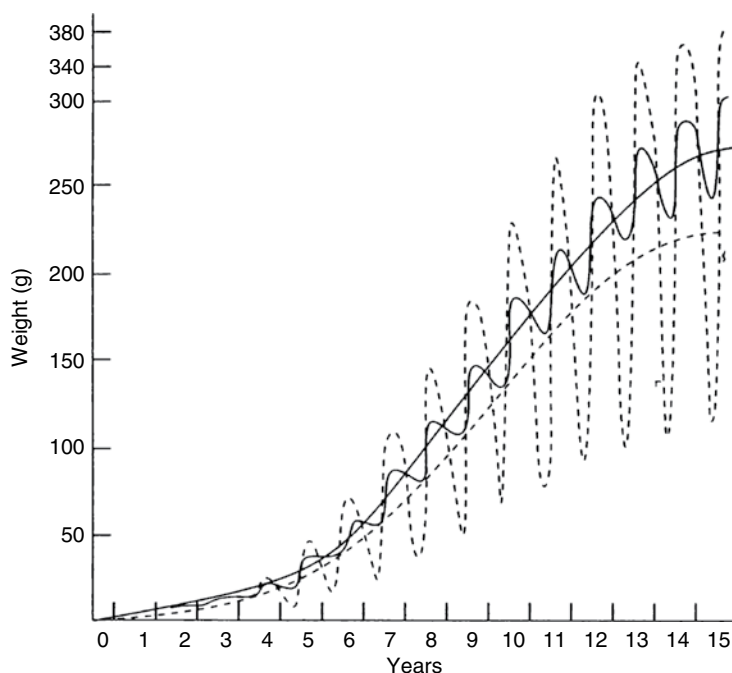
Figure 4.10   Protein growth and fat accumulation in large form of horse-mackerel *Trachurus mediterraneus* during the life cycle. Solid lines, proteins; broken lines, lipids; smooth curves, trends. After Shulman (1972a, 1974) and Shulman & Love (1999).

1972a; Shulman & Love, 1999). The rates of consumption and assimilation of food, metabolic expenditures, and efficiency of conversion decrease considerably. The immune system of old fish becomes very weak (Mikryakov et al., 2001). According to Shatunovsky (1980), the fat content of fish at this stage drops markedly. This indicates a reduced ability to assimilate and synthesize lipids. Under unfavorable conditions, the anchovy and sprat of the Black Sea show a negative relationship between fatness and age (Minyuk et al., 1997). The synthesis of DNA and RNA decreases in old fish (Berdyshev, 1968). The activity of endocrine glands (thyroid, hypophysis, etc.) reduces (Khristophorov, 1975; Woodhead, 1979). This disturbs fish homeostasis.

The lifespan of a given individual fish depends, among other factors, on its condition. Particular species of fish cannot be characterized by lifespan as there is so much variation between individuals (Zotina & Zotin, 1967; Nikolsky, 1974) and populations, and this is because the effect of ecological factors on lifespan is very strong. One factor is the ambient temperature. Atlantic cod in the North Sea mature in 3 years and die at around 8 years of age (Love, 1970), while in the Barents Sea they mature at 11 years and die at around 25 years. The fish *Cynolebias adloffi* lives for about a year at its usual temperature of 22°C, but lives much longer in water at 16°C (Liu & Walford, 1966). Food supply appears to be the most important ecological factor, governing individual variation in lifespan. It differs by several years in diadromous and freshwater forms of the smelt *Osmerus eperlanus* (Ivanova, 1980), normal and dwarf salmon (Krogius, 1978), and white fish (Reshetnikov, 1980). Poor food supply reduces nutritive consumption, and as a consequence reduces metabolic and growth rates, which finally results in premature maturation, aging, and death (Shatunovsky, 1980;

Shulman & Love, 1999). The opposite is observed when food is sufficient. Large forms of Baltic cod, herring and flounder mature later and live longer than their smaller counterparts. Variation in lifespan is obvious in comparison of the Black Sea small horse-mackerel and its hybrid with subspecies from the Mediterranean (Dobrovolov, 2000; Zuev & Melnikova, 2005). Horse-mackerel is a predator and consumes large numbers of small fishes, including sprat juveniles, while the small form feeds on zooplankton. As a result large horse-mackerel live for up to 15 years and reach 50 cm in length and weigh more than 1.2 kg, while the small form lives for only 4–6 years and reaches 20 cm and weighs less than 200 g. Thus favorable environmental conditions allow fish to intensify their metabolism, increasing many functional abilities, including lifespan.

4.1.5   Post-spawning death

In some species, disruption of homeostasis and death occur after spawning, when all or part of the population die. A classic example of unbalanced homeostasis is that of Pacific salmon (genus *Oncorhynchus*), which undergoes post-spawning mass mortality (e.g., Mommsen et al., 1980; Hochachka & Somero, 2002; Kaga & Sato, 2009). Death in Pacific salmon is a result of several factors related to each other: depletion of energy supply, decay of protein–phospholipid structures and, particularly, the enormous quantities of corticosteroids discharged from the adrenal glands into the blood (Idler & Clemens, 1953; Ardashev et al., 1975; Maksimovich, 1988). Kreps et al. (1977) and Tyurin and Gorbunov (1984) consider that this is caused by a stress reaction to the dramatic rise in free radicals in tissues. Dying mature Atlantic cod also have an abnormally high concentration of cortisol in their blood (Idler & Freeman, 1965). The cortisol itself appears to be the lethal agent, rather than a coincidental presence. Robertson et al. (1963) implanted pellets of cortical hormone into immature rainbow trout so that cortisol was continuously released into the bloodstream. The fish lost weight, developed the characteristic infection of the skin, and died within 9 weeks.

Post-spawning mass mortality probably occurs in other fish. The round goby *Neogobius melanostomus* from the Sea of Azov may also be an example of energy depletion. After spawning the male fish guard the spawned egg clutch and do not feed, so that the males turn black, fat stored in the liver is used up, their endocrine system degenerates (Moiseeva, 1969), and death ensues. Nevertheless, it must be considered in all cases that natural death is a genomically programmed phenomenon.

Contrary to Pacific salmon and round goby, in other fish species the adults spawn and live for many years. This is the case for Atlantic salmon *Salmo salar* as well as other anadromic migrants, such as sturgeons (Acipenseridae), and species belonging to Pleuronectidae and Cyprinidae. Furthermore, in many species, post-spawning death does not eliminate the entire population but mainly the senior age groups and the most severely exhausted fish of any age (Shatunovsky, 1980). For example, in the North Sea cod (Love, 1960) and the Barents Sea cod (Borisov & Shatunovsky, 1973), internal reserves are progressively depleted with each additional year of spawning (Love, 1960) until the fish is unable to recover from spawning and dies (Love, 1970). In the case of the round goby in the Sea of Azov not all the fish die after spawning: some restore their energy reserves and spawn the following year (Shulman & Love, 1999). Sex differences in energy depletion can also lead to different mortalities. For example, in the paternal mouthbrooding cardinalfish *Apogon notatus* from southern Japan, the deterioration of somatic condition during breeding was more severe in females, suggesting that selective mortality of females could be due primarily to the depletion of their energy reserves (Okuda, 2001).

4.2 Impact of fish condition on life-history traits

Condition indicators can have major consequences on important fish life-history traits such as reproduction, growth, and natural mortality. These changes in life history may influence population dynamics and affect stock productivity and fishing yield. In particular, the productivity of some fish stocks appears to be especially prone to poor condition. For example, cod stocks in less productive ecosystems have low and highly variable condition values, whereas cod stocks inhabiting more productive ecosystems have high and relatively stable condition values (Dutil et al., 1999, 2003).

4.2.1 Impact on reproductive potential

Maternal condition can have dramatic effects on the reproductive potential of fish, which is a key parameter for stock assessments. Inadequate reserves have been implicated in the reduced reproductive potential of several fish species through reduced fecundity and quality of eggs and larvae or delayed maturation. It has been known for decades that poor condition impairs normal maturation (Newsome & Leduc, 1975; Henderson & Nepszy, 1984; Watanabe, 1985; Heming & Buddington, 1988; Henderson et al., 1996). It seems that fish in poor condition invest in growth rather than gamete development (Yaragina, 2010).

Particularly important are the pre-spawning and spawning periods, when energy (fat) stores decrease up to 1% of body wet mass (Kleiber, 1961; Adams, 1999; Henderson et al., 1988; Rosa et al., 2010). Excessive loss of energy and structure substrates during the pre-spawning period, when these substrates are used for provision of generative maturation (vitellogenesis), worsens reproduction and recruitment (Semlitsch & Gibbons, 1988; Pringe et al., 2000). Therefore, fish condition can provide a valuable additional tool in assessing the reproductive potential of fish. Traditional recruitment models assume that the reproductive potential of a population is proportional to its spawning stock biomass. This implies that the survival rates of offspring are independent of parental age, body size or condition and that total relative fecundity and thus annual egg production per unit mass of adult stock are invariable over time. There is increasing evidence however that direct proportionality between spawning stock biomass and reproductive potential might not exist because the latter may vary substantially among years and stocks depending on environmental and maternal variables. Information on the impact of these variables on the reproductive potential is therefore becoming increasingly important in order to understand the stock–recruitment relationship, a crucial element in population dynamics and stock assessments. Therefore, in any future appraisal of stock–recruitment relationships (as the examples given in this section support) it is important to include information on stock composition and account for size and condition-related features of the spawning stock.

Inadequate energy reserves (particularly during the pre-spawning phase) have been implicated in the reduced reproductive potential of several fish species, such as cod (*Gadus morhua*) in the North Atlantic (Lambert & Dutil, 2000), European hake (*Merluccius merluccius*) in the Mediterranean (Lloret et al., 2008) and the North Atlantic (Murua & Motos, 2006), or bluemouth (*Helicolenus dactylopterus*) in the northwest Mediterranean (Muñoz et al., 2010). In capelin, accumulation of fat is critical for their survival during winter when food supply may be limited and for reproduction in mature fish. In general, capelin needs to reach a fat content (in muscle) of 7.5% by the end of the autumn feeding period to facilitate
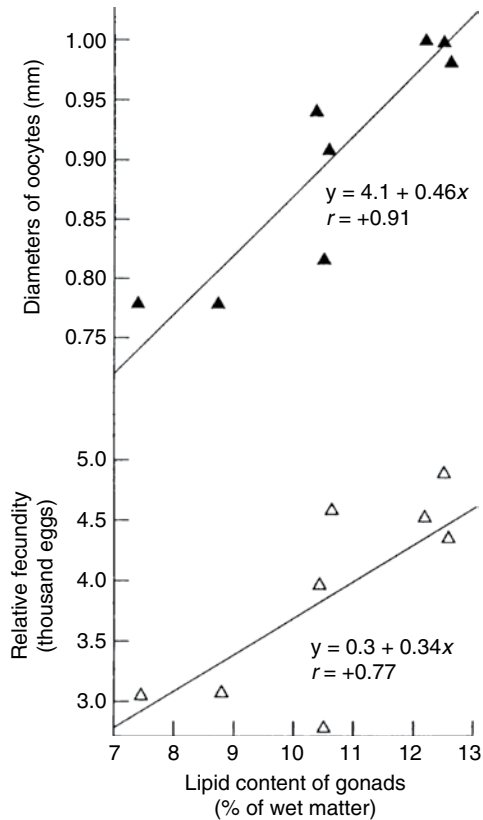
Figure 4.11 Relationship between lipid content of gonads, diameters of oocytes, and relative fecundity in Black Sea horse-mackerel. After Shulman et al. (1970) and Shulman & Love (1999).

the following year's reproduction (Orlova et al., 2002). Poor copepod resources for capelin in certain years led to values below the level required for adequate reproduction the following year (Orlova et al., 2009). In addition, there are examples from decapods showing the link between parental condition and recruitment, for example red shrimp (*Aristeus antennatus*) in the Balearic Islands (Carbonell et al., 2008).

In particular, neutral lipids have a special significance as energy for biosynthesis of generative products (e.g., Shulman, 1972a, 1974; Shatunovsky, 1980; Holdway & Beamish, 1985). Triglyceride deficiency, for example, has a negative effect on gonad development and fecundity (Ackman, 1980; Adams, 1999). On the other hand, in those organisms relying on current energetic incomes ("income breeders") such as octopuses, reproductive potential seems to depend more on the availability of contemporary food resources than on stored reserves (Quetglas et al., 2011). This is the opposite of "capital breeders," where reproduction is fueled by stored reserves. Apart from all these marine species, there are also a large number of studies showing the impact of condition on the reproductive potential of freshwater fish species, such as the three-spined stickleback *Gasterosteus aculeatus* (Chellappa et al., 1989).

There are several ways in fish condition can be related to a population's reproduction, and these are described in the following sections.

4.2.1.1 *Fecundity*

Several studies have shown that the higher the content of structural substances (nucleic acids, proteins, phospholipids, cholesterol) and energy substrates (neutral lipids and triglycerides, sometimes wax ethers, glycogen) in oocytes and spermatozoa, the better the fecundity and development of eggs and larvae (Hester, 1964; Cowey & Sargent, 1972; Shatunovsky, 1980; Adams, 1999; Gosh, 1985; Zhukinsky, 1986; Konovalov, 1989; Rowe et al., 1991; Brown & Taylor, 1992; Henderson & Tocher, 1987; Henderson et al., 1996). For example, there is a positive relationship between lipid content of gonads and relative fecundity in Black Sea horse-mackerel (Fig. 4.11). Numerous studies have also shown that inadequate reserves are implicated in reduced reproductive potential through lower fecundity of several marine species including North Atlantic cod (Kjesbu et al., 1998; Lambert & Dutil, 2000; Marteinsdottir & Begg, 2002), Japanese sardine *Sardinops sagax* (Morimoto, 1996), and Atlantic herring *Clupea harengus* (Kurita et al., 2003). In Atlantic herrring, for example, the relative fecundity showed a significant positive correlation with the muscle dry weight codition factor during the spawning season of this species. Thus, 27% and 16% of the variation in fecundity was explained by muscle dry weight condition factor and somatic condition factor (Kurita et al., 2003).

Laboratory experiments have revealed that fecundity and total egg dry weight are significantly lower in poor-condition female cod (Lambert & Dutil, 2000). Correlation and simulation analyses suggest that, for the northeastern Arctic cod stock, the total lipid energy (TLE) contained in the livers of mature females is proportional to total egg production, making TLE a potential predictor of recruitment of that cod stock (Marshall et al., 2000). In this study, a significant linear relationship between TLE and recruitment to age 3 was observed, which was used to reconstruct and improve the stock–recruit relationship. Similar to this, a study about the eastern Scotian Shelf haddock (*Melanogrammus aeglefinus*) stock during the 1997 and 1999 spawning seasons showed that relative condition factor (K_n) and HSI affected the fecundity of this species, even though consideration of these condition indicators did not result in a dramatic improvement in the relationship between recruitment and stock reproductive potential of haddock (Blanchard et al., 2003).

Furthermore, low maternal condition has been linked to a high incidence of atresia in a number of species including herring (Ma et al., 1998; Oskarsson et al., 2002), sardine (Ganias et al., 2003) and cod (Rideout et al., 2000). Atresia is an involutive process common in the ovaries of fish and other vertebrates in which females may resorb mature follicles at different developmental stages. In general, insufficient nutrient stores before spawning can lead to an increased incidence of egg atresia in cod, and even to spawning failure in the severest cases (Marshall et al., 1998; Rideout et al., 2000). In the case of sardine (*Sardina pilchardus*) in the Mediterranean, early post-spawning females with 100% alpha-atretic oocytes had lower gonadosomatic and hepatosomatic index, compared with reproductively active females (Ganias et al., 2003). In addition, it has been observed in atypical atresic processes leading to cyst formation (swelling of the follicular envelope of oocytes) that cyst prevalence in European hake and cod, which varies inter- and intra-annually, decreases as condition factor increases and increases with length/age of fish (Figs 4.12 and 4.13; Domínguez-Petit et al., 2011). Since cysts would never be released, in practice the impact of cysts on fecundity would be comparable to the effects of atresia and therefore these results support the idea that fish in better condition display higher levels of viable eggs and reproductive potential. However, the relationship between relative intensity of atresia and fish condition has not always been found. For example, no clear relationship between relative intensity of atresia and muscle dry weight condition factor appeared in Atlantic herring *Clupea harengus* (Fig. 4.14; Kurita et al., 2003).
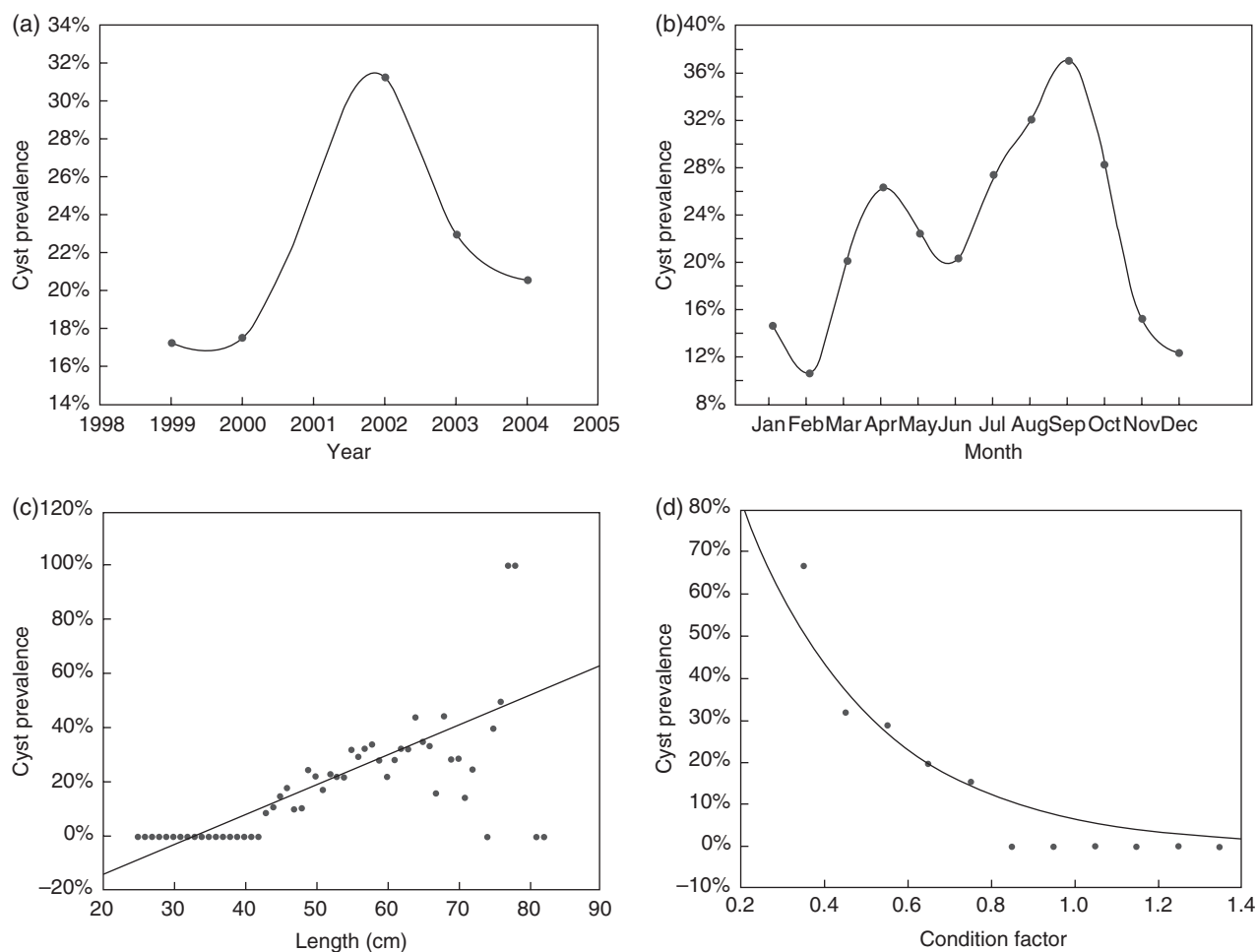
Figure 4.12 Incidence and significance of cystic structures in the ovaries of European hake: (a) annual mean of cyst prevalence; (b) monthly mean of cyst prevalence; (c) linear relationship between cyst prevalence and female length ($y = -0.3557 + 0.0109x$); (d) exponential relationship between cyst prevalence and female condition factor ($y = 1.2251e^{-2.7949x}$). From Domínguez-Petit et al. (2011).
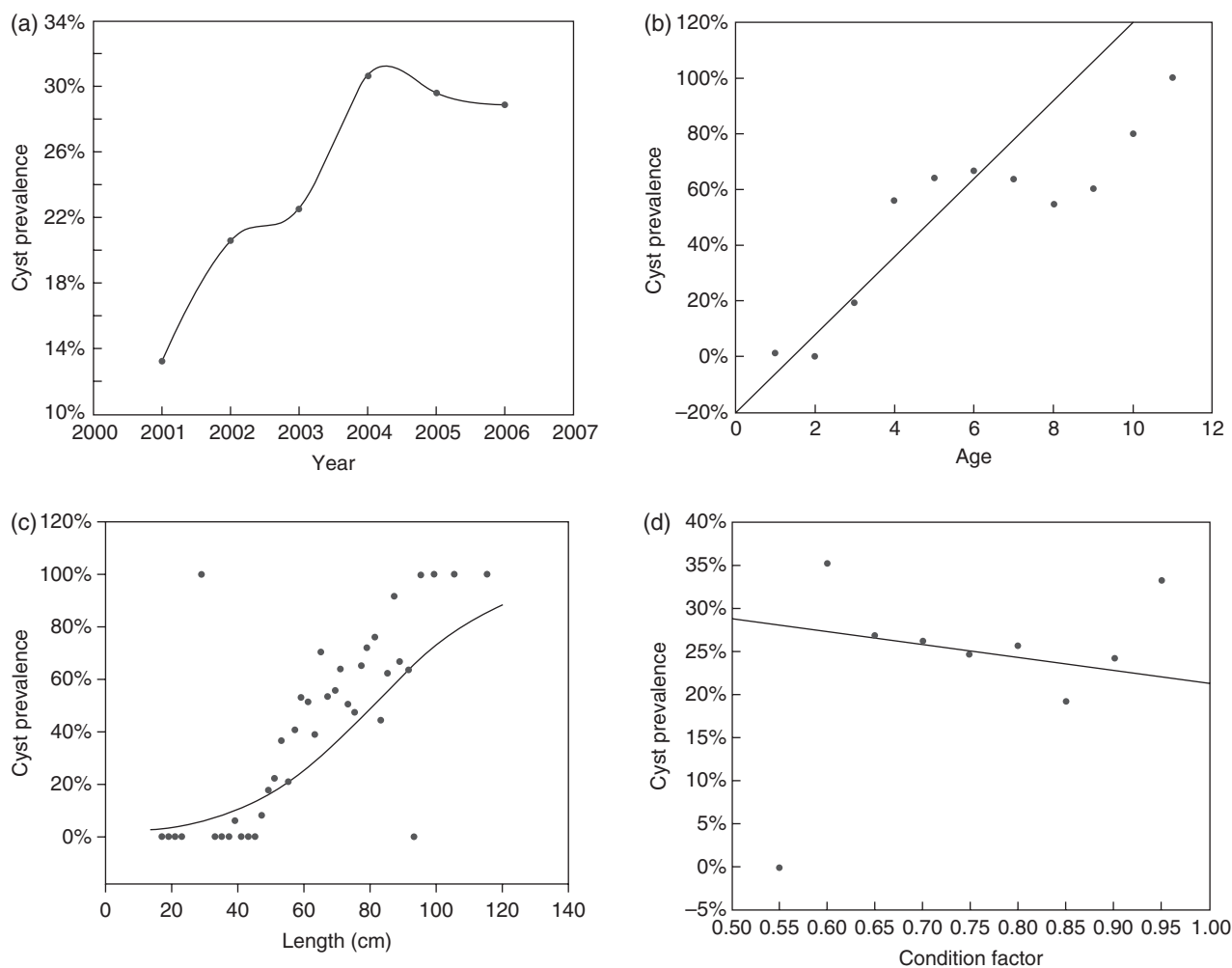
Figure 4.13 Incidence and significance of cystic structures in the ovaries of Atlantic cod: (a) annual mean of cyst prevalence; (b) mean of cyst prevalence by female age ($y=0.1998+0.1403x$); (c) logistic relationship between cyst prevalence and female length $[y=e(-4.247+0.052x)/1+e(-4.247+0.052x)]$; and (d) linear relationship between cyst prevalence and female condition factor ($y=0.3641-0.1509x$). From Domínguez-Petit et al. (2011).
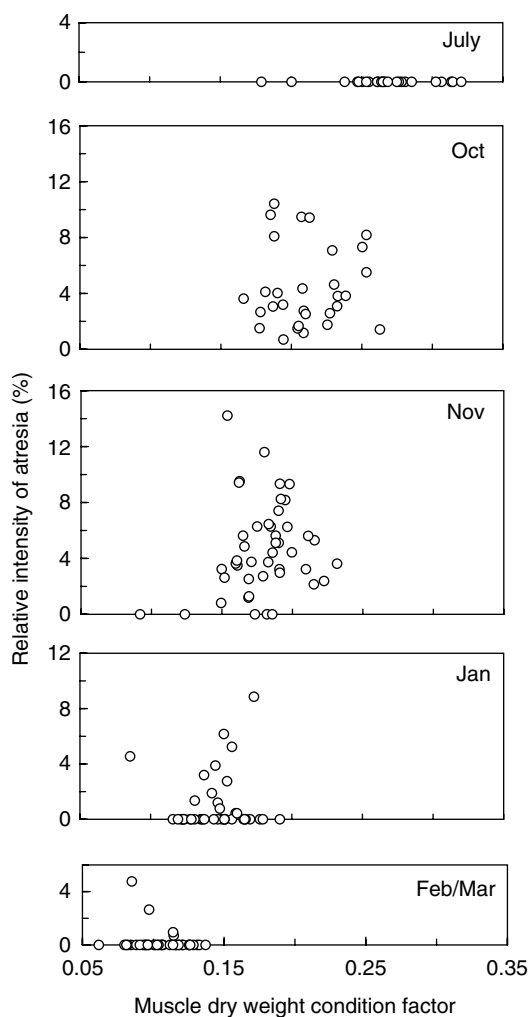
Figure 4.14 Changes in relative intensity of atresia against muscle dry weight condition factor (MDCF; 100 × muscle dry weight/TL³) through the maturation cycle (July 1998 to February/March 1999) for Norwegian spring-spawning herring of 32–37.5 cm TL. From Kurita et al. (2003).

The link between condition and fecundity may not be apparent throughout the spawning season but only at particular times, as the investigation of bluemouth (*Helicolenus dactylopterus*) in the Mediterranean has shown (Muñoz et al., 2010). The relative condition (K_n) of bluemouth had a significant effect on fecundity at the onset of spawning, when potential fecundity is determined. At that particular moment of the spawning season, when females display ovaries with hydrated oocytes but without post-ovulatory follicles, the relationship between the estimated fecundity (F) of females of *H. dactylopterus* and the relative condition factor K_n becomes significant. Therefore, only final potential fecundity fixed prior to spawning is dependent on the condition of the female at that specific moment. In the same sense, Nunes et al. (2011) found a minor effect of body condition on batch fecundity of sardine. The authors argued that when fecundity data were collected during January and February,

sardine condition was commonly at a minimum level and therefore one cannot rule out the possibility that a stronger relationship would exist between batch fecundity and sardine body condition at the beginning of the spawning season when condition is usually maximal (Nunes et al., 2011). This example again shows that the relationship between condition and reproduction can only be found during particular times of the annual life cycle of fish and highlights the complex relationships between condition and reproduction. On the other hand, it has been suggested that the sardine uses not only capitalized (stored) energy to support egg production, but as the reproductive season progresses it could also rely directly on current income (from food) (Ganias, 2009), the relationship between body condition and reproductive investment (fecundity) becoming weaker.

Nevertheless, other studies have failed to detect any effect of condition on fecundity and claimed there is need for greater statistical clarity in analyses of the effects of maternal condition on fecundity in fish (Koops et al., 2004).

4.2.1.2 Skipped spawning

Although the traditional view of iteroparity in fish is one of an annual reproductive cycle that culminates each year in spawning, more recently the potential for mature fish to skip spawning – which refers to a failure to spawn in a given year that does not interfere with the ability to spawn in subsequent years (Rideout et al., 2005) – has been well recognized. Skipped spawning is more common than previously thought (Fig. 4.15; Rideout & Tomkiewicz, 2011). For example, in the years 1999–2004, suppression of reproduction via mass atresia of vitellogenic oocytes occurred in 8.4–55.6% of potential female cod spawners in Newfoundland waters (Rideout & Rose, 2006). Skipped spawning is most commonly attributed to deficient
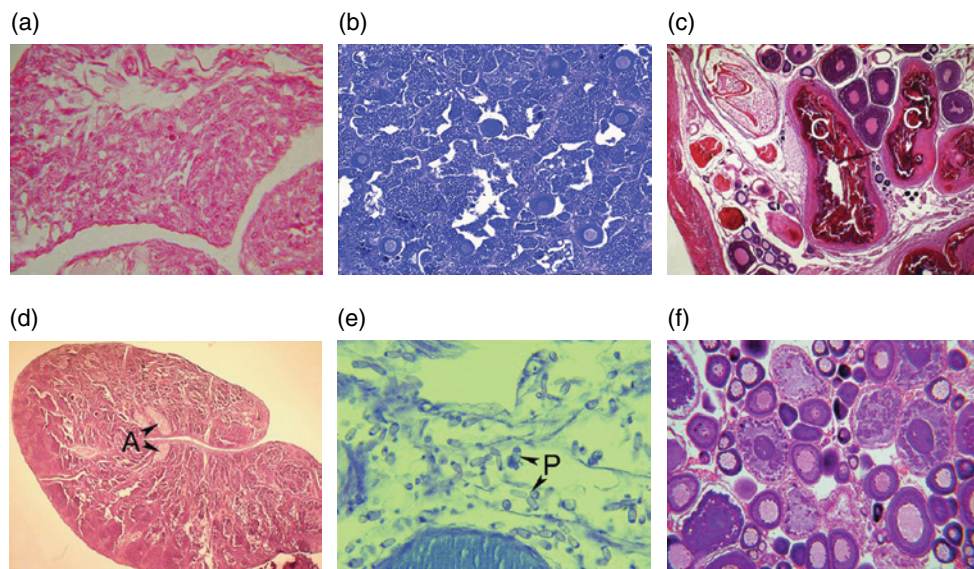
Figure 4.15 Examples of "abnormal" histological observations on fish gonads: (a) sterile Atlantic cod *Gadus morhua* ovary with no oocytes; (b) intersex gonad of eelpout *Zoarces viviparus* with oocytes interspersed throughout spermatogenic cell stages; (c) Atlantic cod ovary with follicular cysts (C); (d) Atlantic herring *Clupea harengus* testis with adipose tissue (A); (e) ovary of a Greenland halibut *Reinhardtius hippoglossoides* with protozoan parasite (P) infection; and (f) ovary of an Atlantic cod that skipped spawning due to mass atresia of oocytes. From Rideout & Tomkiewicz (2011).

diet and poor nutritional condition (reviewed by Rideout & Tomkiewicz, 2011). New research continues to support this generalization. For example, the high incidence of skipped spawning was in particular related to the size and condition of North Atlantic herring (Engelhard & Heino, 2005). The authors found that the mean length, weight, and condition index of first-time herring spawners had significant effects on the fractions of fish skipping reproduction the year after. If first-time spawners had larger body lengths, higher body weights, or higher condition indicators, then skipping of reproduction was less likely to occur in the following year (Engelhard & Heino, 2005). According to the authors, trade-offs between current and future reproduction, growth and survival imply that participation in spawning will only pay off in terms of fitness when herring are sufficiently large and in sufficiently good condition. Thus, it seems that young adult herring, which owing to their small size rely heavily on condition, often need an extra year after first reproduction to regain the energy stores required for reproduction, in contrast to older fish (Engelhard & Heino, 2005).

Recent research on Atlantic cod also indicates that skipped spawning is linked to poor condition. For cod off Newfoundland and Labrador (northwest Atlantic), models of spawning probability based on relative liver condition suggested that female cod in poor condition were more likely to spawn than fish of equal condition (Fig. 4.16; Rideout et al.,
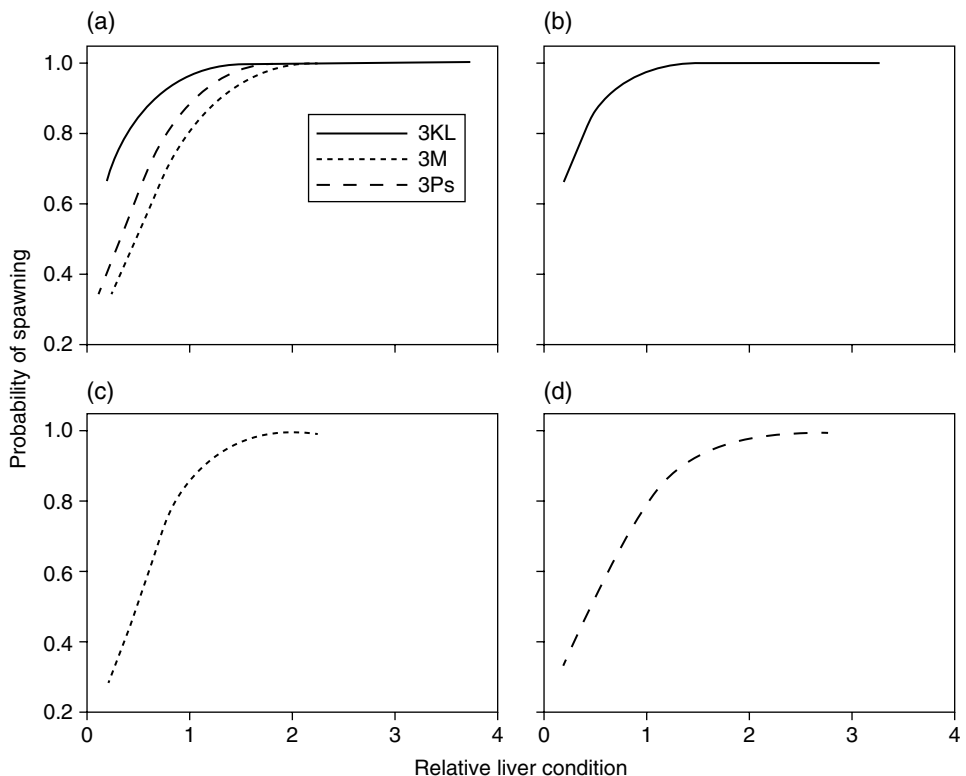
Figure 4.16 The probability of spawning based on relative liver condition for female cod *Gadus morhua* off Newfoundland and Labrador (NAFO areas 3KL, 3 M and 3Ps). (a) Liver condition is expressed relative to the predicted value from a relationship between liver weight and length based on fish from all areas combined and including an area effect in the model. For (b) 3KL, (c) 3 M, and (d) 3Ps, liver condition is expressed relative to fish from the respective areas, and the probability of spawning is modeled separately for each area. From Rideout et al. (2006).

2006). Hence, the relative liver condition had the largest influence on the probability of spawning in Newfoundland and Labrador cod (>17% of the probability of spawning was explained by the relative liver condition, whereas the rest of the factors explained less than 2% of the probability). Furthermore, there was a significant area effect on the relationship between the liver index and the predicted probability of spawning (at very low values of liver index, fish in particular areas were more likely to spawn at low liver index than fish from other areas). Also Yaragina (2010) found that gamete development of northeast Arctic cod could be interrupted by poor liver condition. Additional tools for examining diet and condition are now available for use in exploring aspects of reproductive biology. For example, using white muscle $\delta^{13}C$ signatures Rideout and Rose (2006) demonstrated that medium-sized Atlantic cod feeding on capelin *Mallotus villosus* were less likely to skip spawning than individuals feeding on shrimp.

According to Rideout and Rose (2006), suppressing reproduction in times of poor nutritional condition can increase an individual's likelihood of surviving to spawn in multiple subsequent years. The longer the reproductive lifespan of a species, the more likely it is to suppress reproduction in times of poor condition. In long-lived species such as Atlantic cod, suppressing reproduction when energy reserves are such that spawning may compromise survival can actually increase lifetime reproductive output (Rideout & Rose, 2006).

4.2.1.3 *Egg quality and larval survival*

Low maternal condition has been linked to lower egg quality, whether it is lower total egg dry weight as occurs in cod (Lambert & Dutil 2000; Ouellet et al., 2001) and Atlantic herring (Kurita et al., 2003); a smaller egg diameter as happens in cod (Ouellet et al., 2001), haddock (Trippel & Neil, 2004) and Japanese sardine (Murimoto, 1996); or smaller lipid content in the ovary as occurs in Japanese sardine (Murimoto, 1996) and European hake (Lloret et al., 2008). A smaller lipid content in the ovary has been linked to a smaller egg diameter (e.g. in Black Sea horse-mackerel; Fig. 4.11). For hake, Lloret et al. (2008) showed that there were significant positive relationships between the relative lipid content in the liver and ovary of female pre-spawners in the Mediterranean (Fig. 4.17). Good-condition female pre-spawners, namely those with higher relative lipid reserves in their livers, had higher relative lipid percentage in their ovaries than poor-condition ones, suggesting that better-conditioned females had higher reproductive potential. In fact, a close connection has been revealed between the levels of structural
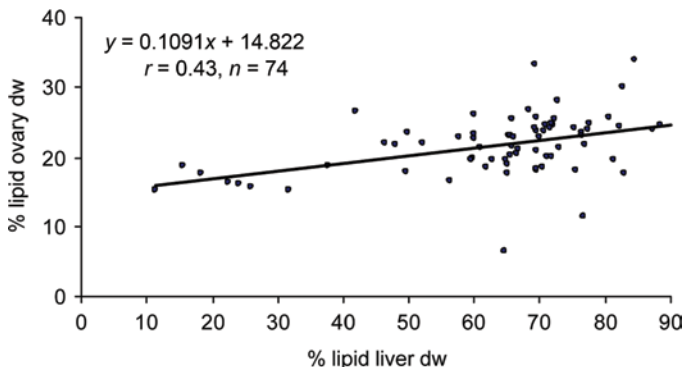
Figure 4.17 Linear relationship between relative lipid content in the ovary and relative lipid content in the liver of European hake *Merluccius merluccius* female pre-spawners in the northwest Mediterranean. From Lloret et al. (2008).

and energy components in the reproductive products of mature fish at spawning, primarily females (Shatunovsky, 1980; Krivobok, 1964; Shulman, 1972a, 1974; Holdway & Beamish, 1985; Shatunovsky & Rychagova, 1996; Rainuzzo et al., 1997; Lambert et al., 2003; Domínguez-Petit & Saborilo-Rey, 2010). The mechanism of is clear: proteins, lipids, glycogen, and other substances from muscles and other tissues of adult fish (e.g., plasma triacylglycerols, cholesterol, total proteins) are transformed in the liver to reproductive products (Shulman & Love, 1999).

The nutritional condition of fish larvae is considered an important factor affecting recruitment fluctuations of the population, either directly by inducing mortality or indirectly by lengthening the duration of larval life and exposure to predators (Ferron & Leggett, 1994; Bergeron, 2000; Ferron, 2000). In particular, Ferron and Leggett (1994) reviewed the use of different condition indices (morphometric, histological and biochemical) in fish larvae taking into account field and laboratory experiments, and provided specific recommendations that need to be considered by ichthyoplanktologists when studying the condition of this particular life stage of fish. Larvae in better condition are presumably less likely to die of starvation or predation on average and thus contribute proportionally more to the numbers at recruitment. Hence, the larvae produced by fish in poor condition may be smaller or have higher specific gravity, and are thus less likely to survive (Marteinsdottir & Steinarsson, 1998; Saborido-Rey et al., 2003). In this sense, Ware (1975a) found that when larvae are deprived of food, big larvae resulting from bigger eggs survive longer than small larvae hatched from smaller eggs. Kamler (1992) revealed that larval survival depends on neutral lipids accumulated in the egg, and Thorpe et al. (1984), Brown and Taylor (1992) and Kamler (1992) demonstrated a positive correlation between the viability of offspring recruits and total concentration of lipids in eggs of several species. Finally, Rainuzzo et al. (1997) and Domínguez-Petit and Saborido-Rey (2010) showed that the level of neutral lipids in females is responsible for fecundity, fertilization, hatching and, to a considerable degree, survival of larvae in the earliest post-embryo stages. All these examples show that ovarian lipids are a primary source of energy at the larval stage before first feeding, and therefore will influence larval condition and survival (Rainuzzo et al., 1997).

There are many examples showing how low maternal energy reserves can lead to a decrease in egg quality and larval survival. Two examples are the sardine *Sardina pilchardus* in northwest Spain (Riveiro et al., 2000) and the Icelandic cod *Gadus morhua*. For Icelandic cod, Marteinsdottir and Steinarsson (1998) revealed that Fulton's *K* condition factor of females was positively correlated with the size of their eggs. Hence, the relative production of large good-quality eggs by females of different sizes and conditions is likely to be an important component in the total production of viable offspring by the Icelandic cod stock. Furthermore, a positive relationship was detected between egg size and some larval viability parameters, including age at first feeding, successful development of a swimbladder, and specific growth rates during the first 15 days after hatching (Marteinsdottir & Steinarsson, 1998). Also in cod, mean larval specific gravity (larval buoyancy) was negatively correlated with female Fulton's *K* condition factor at different larval developmental stages (Fig. 4.18; Saborido-Rey et al., 2003). Hence, larger eggs produced by females with high condition factor produce larvae with a bigger yolk sac and with a lower specific gravity, which can distribute at shallower depths where the chance for more favorable conditions is greater, both in terms of physical environment and food availability for future larvae (Saborido-Rey et al., 2003). Overall, these results reveal that the viability of cod larvae is related to attributes of the spawning females and that this information is important to our understanding of stock–recruitment relationships.
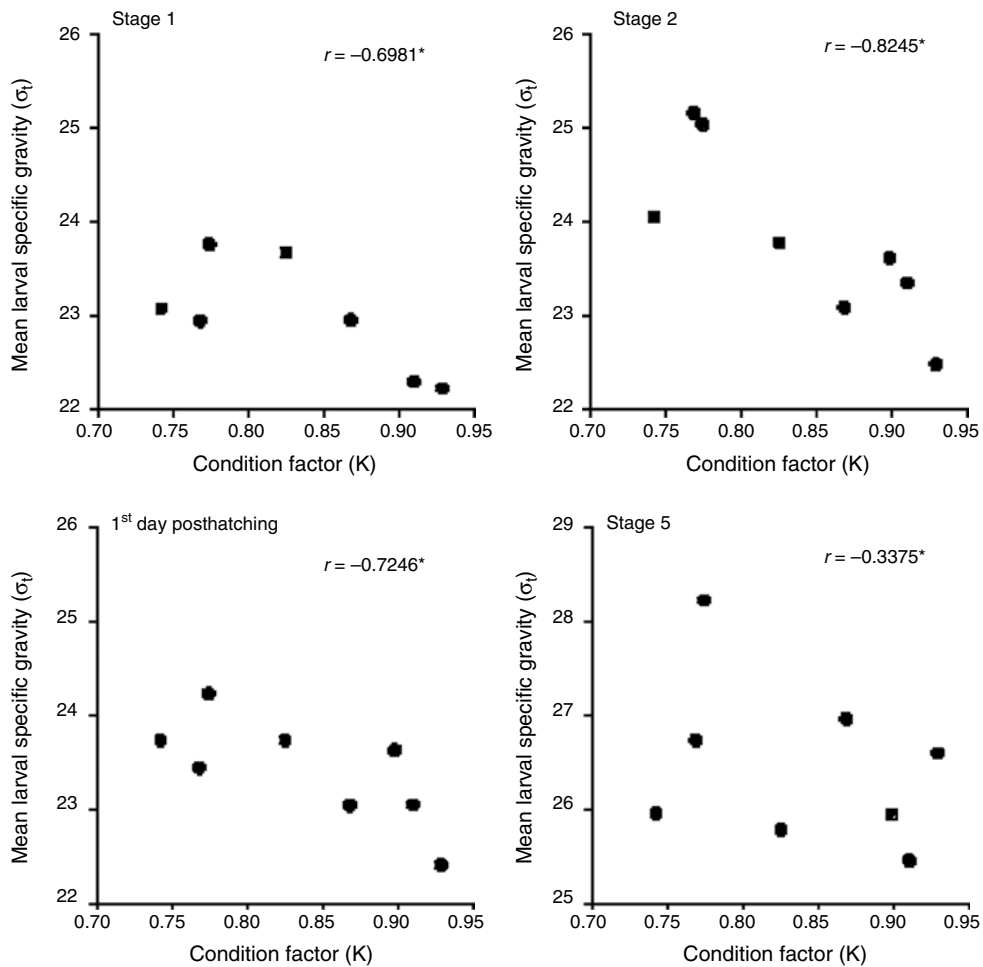
Figure 4.18 Relationships between mean cod larval specific gravity and female mean Fulton's condition (K) at different larval developmental stages. Note that the stage 5 plot has different scaling on the y-axis. From Saborido-Rey et al. (2003).

Better larval condition ameliorates survival and development to juveniles and recruitment (Schmidt-Nielsen, 1984; Thorpe et al., 1984; Post & Evans, 1989; Wagner & Congleton, 2004). In this regard, lipids play a key role, even though carbohydrates and proteins, which also contribute to energy reserves of eggs and larvae, contribute to a minor extent. Higher lipid content in eggs and larvae makes egg and larvae more resistant to unfavorable external conditions (Gall, 1974; Ware, 1975a; Thorpe et al., 1984; Docker et al., Docker et al., 1986; Brown & Taylor, 1992; Kamler, 1992). Unfavorable abiotic factors include inappropriate temperatures, oxygen deficiency, and high levels of water pollution (Wedemeyer et al., 1984). In particular, neutral lipids can provide the energy necessary to resist all these anomalous factors. In this case, sensitivity to these negative factors increases (Shulman & Love, 1999). Unfavorable biotic factors include food deficiency and existence of predators, parasites and diseases. In particular, food deficiency has great significance for larvae during their shift to external feeding (Henderson et al., 1988; Thompson et al.,

1991). Mass mortality occurs during this stage but higher energy stores can reduce this mortality strongly. Further, higher lipid content in eggs fosters the ability of larvae to self-feed earlier and longer than those larvae with fewer energy stores (Brown & Taylor, 1992). Larger larvae can better locate and hunt their prey, as well as escape from predators (Prosser, 1991; Brown & Taylor, 1992).

In particular, polyenic fatty acids play an important role in successful egg and larval development (Sargent, 1978; Sargent & Henderson, 1980; Walton & Cowey, 1982; Yuneva 1990), as higher levels of polar lipids provide higher metabolic activity of cellular and sub-cellular membranes. Higher content of DHA affects the development of sense organs and supports nerve function (Sutharshiny & Sivashanthini, 2011). This, together with higher content of neutral lipids, promotes successful search for food and escape from predators. The investigations by Brown (1994), Sargent (1995), Bell et al. (1995), and Bell and Sargent (1996) showed that 22:6 ω-3 is accumulated in rhodopsin of larval retina and improves vision, contributing greatly to survival of larvae.

However, not all studies support the hypothesis that larvae in better condition are less likely to die of starvation or predation on average and thus contribute proportionally more to the numbers at recruitment. Suthers (2000) stated that the ecological relevance of various condition indicators used for fish larvae still needs further investigation. According to Suthers, future studies should consider in situ small-scale spatial and temporal studies that link mortality and condition of cohorts. We must consider however that on many occasions the quality of eggs and larvae cannot offset the adverse abiotic and biotic conditions. For example, survival of eggs and larvae of pelagic Black Sea fish depends significantly on the temperature regime during the spawning period (Dekhnik, 1979). Finally, the food supply of larvae plays a significant role in their survival (Zaika, 1983) and consequently in recruitment.

Overall, a continuous linkage has been established between quality of producers, quality of reproductive organs, quality of fecundity and fertilization, quality of eggs and hatching, quality of larvae, recruitment value and survival. A sound example of such linkage is the work by Yuneva et al. (1990), obtained during artificial interbreeding of Pacific gorbusha *Oncorhynchus gorbuscha* (Fig. 4.19). The content of DHA in triglycerides
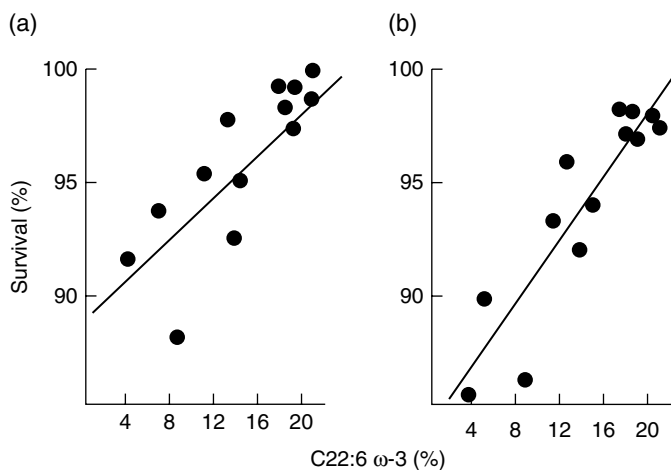
Figure 4.19 Relationship between (a) egg and (b) larval survival and 22:6 ω-3 content in *Oncorhynchus gorbuscha* female. From Yuneva et al. (1990) and Shulman & Love (1999).

of white muscle of females is proportionally related to the survival of eggs and larvae. High levels of neutral lipids are necessary for smoltification and wintering survival of Pacific salmon juveniles (Pavlov et al., 1994). Apart from the direct influence of spawner's quality on egg and larval quality, there is also an indirect effect: the better the condition of the spawner (higher levels of structural and functional constituents and higher protein biosynthesis), the faster it grows. A higher growth rate and increased fish mass leads to an increase in fecundity.

Lipid utilization starts early during maturation. Several studies have shown that poor condition cannot support normal maturation (Newsome & Leduc, 1975; Watanabe, 1985; Heming & Buddington, 1988; Henderson & Nepszy, 1994; Henderson et al., 1996), with decrease in fecundity related to oocyte resorption and follicular atresia (Hester, 1964). Low lipid content negatively affects the immune system of fish (Gurr, 1983) and thus parasites increase in liver and muscles (Graham & Laurs, 1982; Adams, 1999). Male Atlantic salmon stop maturing when mesenterial fat falls to a critical level (Rowe et al., 1991): salmon must have a minimum of 3% fat wet weight for normal reproduction the following fall. In a similar manner, Arctic char *Salvelinus alpinus* must also have a critical level of lipids at maturation (Rowe et al., 1991). These effects are species-specific: for Clupeidae it is carcass lipids, i.e., muscle fat (Love, 1970; Shulman, 1972a, 1974); for flatfishes and Gadidae it is liver fat (Love, 1970; Cowey & Sargent, 1972); for Salmonidae (Rowe et al., 1991) and many Centrarchidae (Adams, 1999), Persidae and Cyprinidae (Henderson et al., 1996) it is mesenterial fat. An exception is plaice (benthic species), for which carcass is the main source of lipids (Adams, 1999). Some representatives of Scorpaenidae, such as scorpion fish and yellowtail rockfish, use liver and mesenterial fat as the source of ovarian development. An example of the effects of inadequate condition is shown by the mass death of maturing female gizzard shad (*Dorosoma* sp.) in late spring during the mid 1980s as a result of severe starvation (Adams, 1999). Shad, which spawn in spring, postponed spawning to the fall: as a result of this postponement, mass death of fish was observed during the fall, which is usually the season where energy accumulation occurs. This postponement did not allow fish enough time to accumulate energy. A significant role in fish condition and mortality is played by stress, mediated by the neuroendocrine system (hypothalamus–pituitary–thyroid–interrenal) (Schrek et al., 2001; Kassahn et al., 2009).

Occasionally other factors rather than fish condition can be more important for reproduction. One is temperature. For example, Domínguez-Petit and Saborido-Rey (2010) showed that spawning success of European hake *Merluccius merluccius* depends more on food supply and temperature than on energy reserves. In striped bass *Morone saxatilis*, maturation worsens when temperature increases (Content, 1987). In this case, especially during oxygen deficiency, sensitivity to diseases and parasites increases (Eure & Esch, 1974). American shad *Alosa sapidissima*, which migrates to rivers, loses fat at a faster rate near the end of migration, due to higher temperatures, than at the beginning of migration and therefore survival of these fish is low (Glebe & Legget, 1981). Another stressor for lipid content in muscles and liver, as shown in sea bass *Dicentrarchus labrax*, is salinity (Roche et al., 1940). In smallmouth bass *Micropterus dolomieui*, hypo-oxidant medium as well as low pH decreases biosynthesis of phosphatidylcholine and phosphatidylethanolamine (Cunnington & Shuter, 1986). In the same species, low pH affects the acidophil system (Kwain et al., 1984).
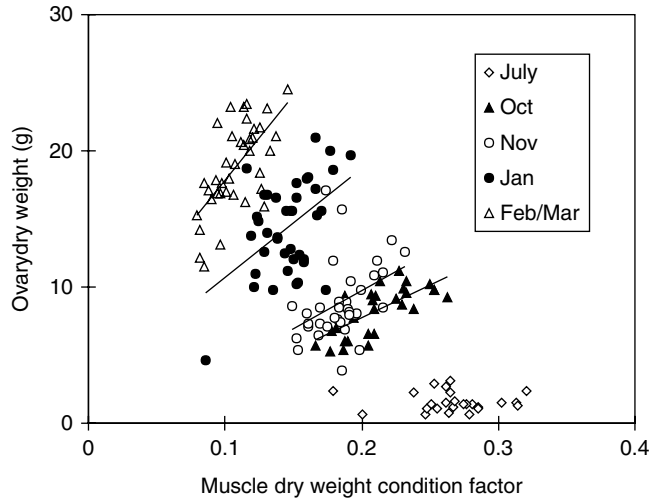
Figure 4.20 Changes in relationships between ovary dry weight standardized to 34 cm TL fish and muscle dry weight condition factor (100 × muscle dry weight/TL³) through the maturation cycle (July 1998 to February/March 1999) for Norwegian spring spawning herring of 32–37.5 cm TL. Each line shows the linear regression line for each month. From Kurita et al. (2003).

4.2.1.4 Ovary weight and gonadosomatic index

Ovary weight and gonadosomatic index (GSI) have been used as indicators of reproductive output or reproductive investment, with both measures varying on an annual basis in many fish species. For example, batch fecundity in European hake is positively related to ovary weight and GSI and therefore both measures could be potentially used as a fecundity index of hake (Murua & Motos 2006). Ovary weights were positively correlated with interannual variations in condition of Atlantic herring (*Clupea harengus*) during the period 1970–1990 in the Newfoundland area (Winters & Wheeler, 1994). Furthermore, there appeared to be a highly significant positive correlation between ovary dry weight and muscle dry weight condition factor in the later maturation cycle of Atlantic herring (Fig. 4.20; Kurita et al., 2003). At a seasonal scale, the inverse relationship between GSI and condition in many fish species indicates that the energy that is stored in the months by fish in resting phase is then utilized to support the production of egg batches during the spawning period (e.g., sardine in the northeast Mediterranean; Ganias et al., 2007).

4.2.1.5 Maturity

Another way in which fish condition can be related to a population's reproductive potential is trough maturity. The probability of a fish being an adult tends to increase with age and size, but it can also be influenced by other factors such as fish condition. The relationship between condition and the probability of being mature (adult) has been well documented for a number of marine species including cod *Gadus morhua* (Marteinsdottir & Begg, 2002; Morgan & Lilly, 2006), American plaice *Hippoglossoides platessoides* (Morgan, 2004), and turbot *Scophthalmus maximus* (Bromley et al., 2000). Thus, for example, Marteinsdottir and
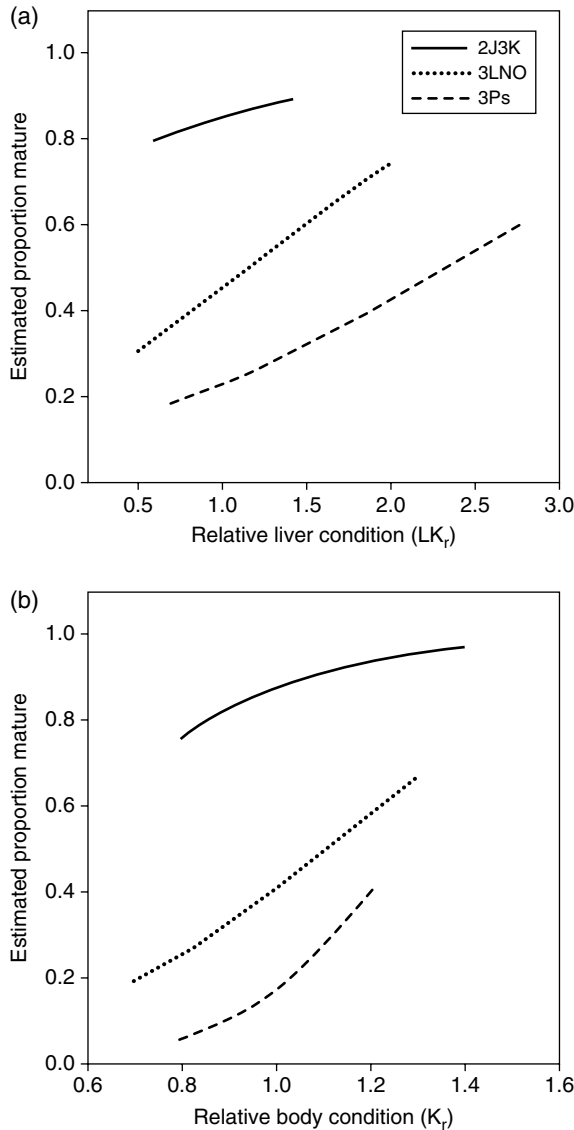
Figure 4.21   American plaice (*Hippoglossoides platessoides*) from the Atlantic coast of Canada. Estimated proportion of females mature at age 8 years and length 33 cm calculated from the fitted model for each population of American plaice. The proportion mature is calculated across the observed range of (a) relative liver condition and (b) relative body condition in each population for a female aged 8 and 33 cm long. From Morgan (2004).

Begg (2002) found an increased proportion of mature Icelandic cod at a given size or age for those fish that were in better condition. Similarly, Morgan and Lilly (2006) found that for Flemish Cap female cod there was a significant effect of the liver condition index on the probability of being an adult, after accounting for the effects of age and length. However, the relative condition index of Flemish Cap cod accounted for only a small portion of the deviance (1%) compared with the deviance accounted for by the combined effects of age and

length (69%). For males there was also no significant effect of relative liver condition on the probability of being an adult (Morgan & Lilly, 2006). Bromley et al. (2000) found that a low food ration in adolescent turbot could lead to failure to mature. Morgan (2004) found a significant positive effect of the relative condition index and the relative condition factor on the probability of being a mature female American plaice *Hippoglossoides platessoides* (Fig. 4.21). However, the additional variation explained by female condition was small when compared with the combined effect of age and length. Furthermore, neither relative liver condition nor relative body condition significantly affected the probability of male American plaice being mature (Morgan, 2004). Furthermore, Yaragina (2010) concluded that it seems likely that the condition factor of northeast Arctic cod in 1984–2006 and the energy content of the liver in particular will affect the chance of becoming mature.

All these examples support the hypothesis that variation in fish condition will have a direct impact on the spawning stock biomass of a population through differences in the maturation schedule of cohorts, particularly the females, with differing condition (Morgan, 2004).

4.2.1.6 Recruitment

Because condition is linked to reproductive variables such as fecundity, maturity or egg quality, condition of spawners can be related to recruitment success. In this sense, positive associations of maternal condition with recruitment have been documented in several species from the North Atlantic, including haddock *Melanogrammus aefleginus* (Marshall & Frank, 1999), cod *Gadus morhua* (Marteinsdottir & Begg, 2002), and anchovy *Engraulis encrasicolus* (Bergeron & Massé, 2012). In this sense, special attention should be given to anomalous low adult condition values since they may precede a recruitment failure, as could have happened with cod in the northern Gulf of St Lawrence during the early 1990s (Lambert & Dutil, 2000). The condition of spent females in this stock indicated that reproductive potential and possibly recruitment may have suffered from that situation and could have contributed to the failure of this stock to recover despite the moratorium on commercial fishing (Lambert & Dutil, 2000). In anchovy from the French coast of the northeast Atlantic Ocean, Bergeron and Massé (2012) demonstrated that the annual recruitment of this pelagic species shows a tight relationship with the mean values for the RNA/DNA ratio determined in the parent fish during the previous breeding season (Fig. 4.22). In the case of red shrimp, there was a significant and positive relationship between the condition of adults during the months prior to spawning and the number of recruits in the following year (Fig. 4.23; Carbonell et al., 2008). This relationship was stronger when only red shrimp male condition was considered, suggesting that males have an important role in the reproductive potential of this species. Results suggest that recruitment of red shrimp in the northwestern Mediterranean is influenced by the condition of adults during the months prior to spawning. Recruitment is higher when pre-spawning individuals are in good condition, i.e., have a higher body weight for a given carapace length. The relationship between the condition and recruitment of red shrimp is stronger when only the condition of males is considered, depicting, in all likelihood, the importance of males during the whole reproductive process (Carbonell et al., 2008). Overall, these results suggested that condition of red shrimp, particularly males, is an important aspect for the reproductive and recruitment success of this species.

Further, in the Portuguese coast, the sardine energy density in a given year explained more than 50% of the variation in the annual catches of the following year, indicating
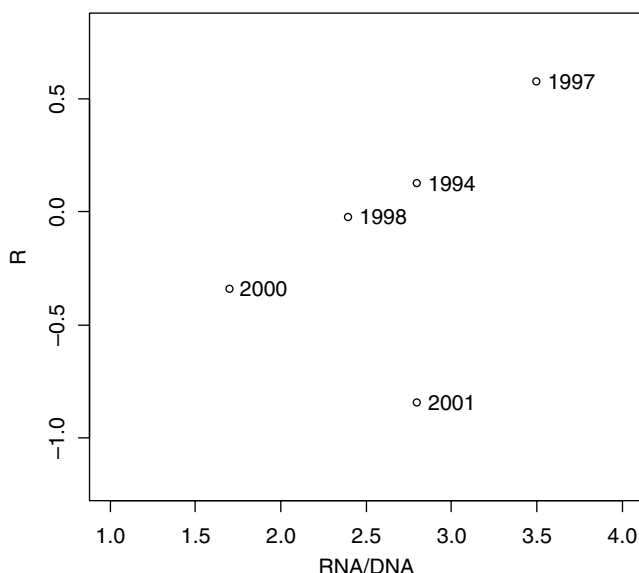
Figure 4.22 Variations in the annual recruitment (R) of the anchovy population in the Bay of Biscay as a function of the mean RNA/DNA ratios determined in the white muscle of individual fish during the breeding season of the preceding year. From Bergeron & Massé (2012).

that adult energetic condition during spawning is partially translated into the fishery through parental effects in recruitment strength (Rosa et al., 2010). In particular, sardine spawners in better physiological condition have higher reproductive outputs (more batches of eggs per season and more viable eggs per batch) than fish in lower condition, which increases the probability of recruitment success and produces a stronger year-class that, apparently, is influential enough to be reflected in the fisheries of the following year (Rosa et al., 2010).

A comparative analysis of Fulton's K condition factor of 10 cod stocks in the North Atlantic in relation to the reproductive potential of cod was carried out by Rätz and Lloret (2003). The results showed that the a coefficients (function's slopes) obtained from standardized Ricker's recruitment–spawning stock biomass (SSB) relationships, which were defined as indicators for the recruitment potential of stocks, were positively correlated with the mean condition factor of the 10 stocks analyzed. Figure 4.24 illustrates the linear model explaining 49% of the observed variation in Ricker's a coefficients. This indicated that stocks consisting of individuals in poor condition appear to be very susceptible to reduced recruitment at low SSB, while the stocks that consist of fish in good condition seem to behave more robustly with a higher probability of good recruitment at low SSB (Rätz & Lloret, 2003). According to the authors, the positive effect of cod condition on their reproductive potential generally implies that the stocks in good condition in the temperate regions of the northeast and west Atlantic can sustain higher exploitation rates than stocks in poor condition in the colder regimes of the northwest Atlantic (Greenland, Labrador and Grand Banks).

Notwithstanding the general positive relationships between condition and recruitment, this relationship has not always been found – indeed, even the inverse has been observed. For example, Engelhard and Heino (2005) unexpectedly found an inverse relationship of the condition index of North Atlantic herring spawners in year y with the fraction surviving to year $y+1$.
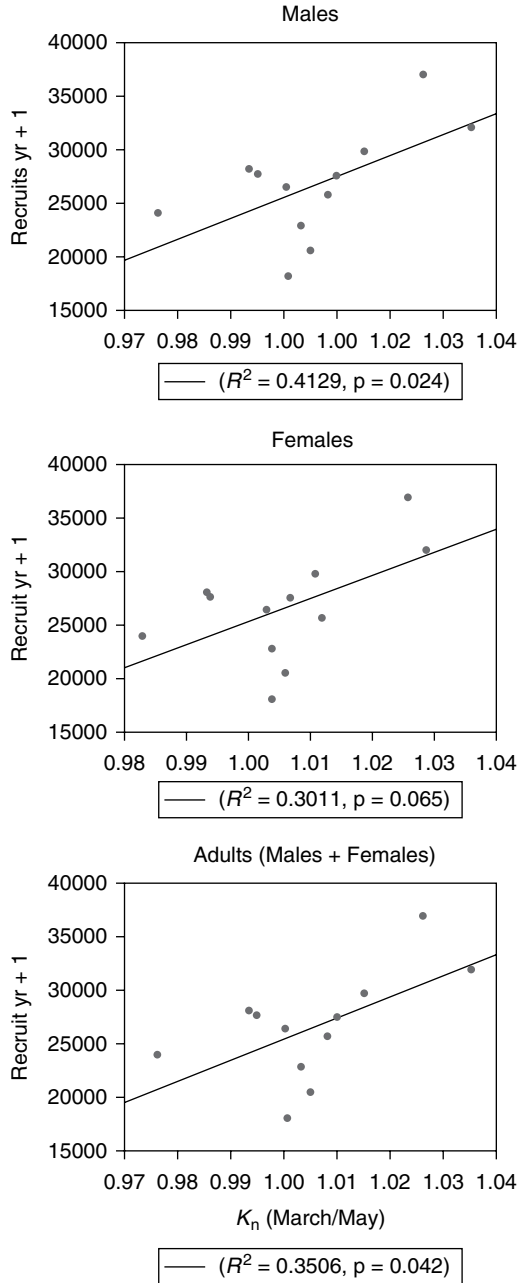
Figure 4.23 Red shrimp (*Aristeus antennatus*) off Balearic Islands (northwest Mediterranean). Linear regression between the relative condition index (K_n) of spawners (average March to May) by sex separately and combined, and recruitment at age-1 in the following year (thousands of individuals) of the red shrimp, 1992–2004. From Carbonell et al. (2008).
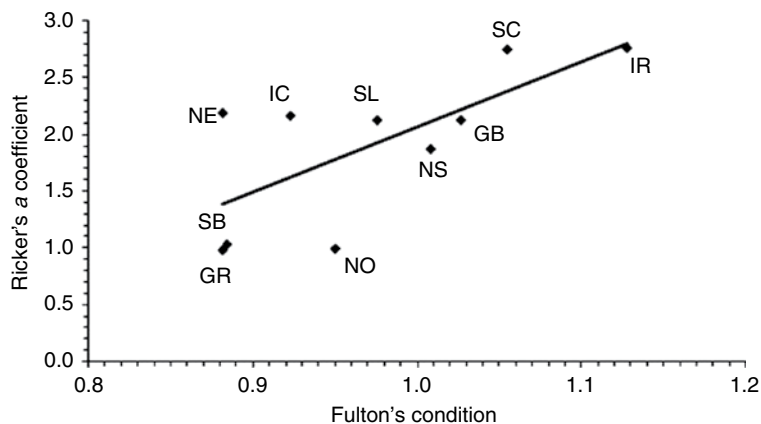
Figure 4.24 Linear regression between Ricker's *a* coefficient (standardized) and Fulton's condition factor for 10 north Atlantic cod stocks. Stock abbreviations: Greenland (GR), Georges Bank (GB), S. Gulf of St Lawrence (SL), Southern Grand Bank (SB), Northern Cod (NO), North-east Arctic (NE), NW Scotland and Ireland (SC), Irish Sea (IR), North Sea (NS), Iceland (IC). From Rätz & Lloret (2003).
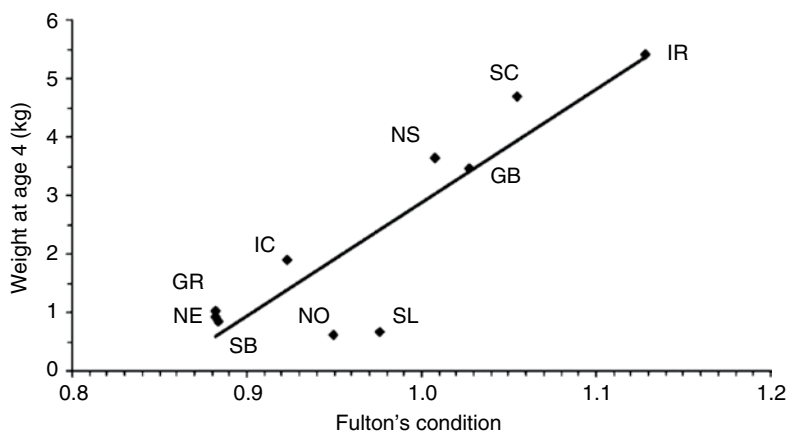
Figure 4.25 Linear regression between weight at age 4 and Fulton's condition factor for 10 north Atlantic cod stocks. Stock abbreviations are given in Fig. 4.24. From Rätz & Lloret (2003).

4.2.2 Impact on growth

There are only a few examples showing that good-condition fish grow faster. A comparative analysis of Fulton's *K* condition factor of 10 cod stocks in the North Atlantic in relation to growth rates revealed that stocks in better condition display significantly higher weights at age 4 than stocks in poor condition (Rätz & Lloret, 2003). Nearly 80% of the observed variation in mean weights at age 4 was explained by the mean condition factor (Fig. 4.25). In general, cod from stocks in better condition are much heavier at age 4 than fish in stocks in poor condition.

In other cases, condition explains a small percentage (sometimes none) of the growth variation. For example, condition factor explained just a small percentage (4%) of the growth

variation of juvenile Atlantic salmon (*Salmo salar*), whereas other factors such as water temperature and seasonal effects explained most of the variability in condition (Bacon et al., 2005). Also Liao et al. (1995) found little evidence for a relationship between the relative condition (W_r) and growth of two freshwater fish (pumpkin seed *Lepomis gibbosus* and golden shiner *Notemigonus crysoleucas*) inhabiting different southern Quebec lakes (Canada). The authors found that there were no significant correlations of size-specific W_r estimates with corresponding size-specific growth estimates among lakes.

4.2.3 Impact on natural mortality

Natural mortality (M) is a very important life-history parameter that is essential for effective management of exploited fish populations. M values have important implications for setting allowable harvest rates because sustainable management of fish populations requires that total mortality – the sum of natural and fishing mortality – remains below a target level. If natural mortality rates are underestimated, allowable harvest rates may be overestimated. Unfortunately, M is difficult to estimate directly because natural deaths are rarely observed. Furthermore, often little is known about factors that affect M. For many fisheries, natural mortality rates are simply assumed to have a constant value, or are inferred from techniques such as catch curve analysis, regression of the instantaneous total mortality rate (Z) on fishing effort, correlation of M with other life-history parameters (e.g., longevity), ecosystem modeling, tagging and visual censuses. While all these techniques are useful for estimating the magnitude of M, they tell us little about the underlying causes of natural mortality. This is where the analysis of fish condition comes into play, because among all factors that can affect M, condition is a very important one.

In general, low energy reserves may lower the chances of survival, leading to an increase in natural mortality. Starvation due to exhaustion of energy reserves, particularly during the non-feeding and reproductive periods, weakens fish and renders them more susceptible to predation and fishery, diseases and to a variety of environmental stressors. In particular, low energy stores decrease fish immunity, which increases their mortality due to diseases (Mikryakov et al., 2001).

There are a few examples in the wild showing that poor-condition fish suffer higher natural mortality rates than good-condition fish because of higher predation (e.g., lower swimming performance) or lower resistance to other factors such as overwinter starvation and breeding costs. In a study about the recruits of the coral reef fish *Pomacentrus moluccensis*, high-condition fish were not only aggressively dominant and preyed more than low-condition fish, but were shown to be at much lower risk of predation (Booth & Beretta, 2004). It was concluded that the condition of new settlers can have an important influence on subsequent juvenile survival. In other studies the importance of fish condition in determining swimming performance of fish with respect to speed and endurance was documented. For example, Martínez et al. (2003) showed that declining condition may decrease metabolic and swimming capacities of cod, and hence their ability to avoid predation. Also low levels of neutral lipids in the muscle and liver characterize a poor swimming performance in horse-mackerel (Yuneva et al., 1991), whereas the swimming performance of juvenile seabream *Diplodus sargus* in the northwest Mediterranean was also affected by body condition (Planes et al., 1997).

Impaired condition due to overwinter starvation can also be an important cause of mortality. For example, the condition factors and hepatosomatic indicators of Gulf of St Lawrence cod in spring were low enough to suggest that winter starvation may cause
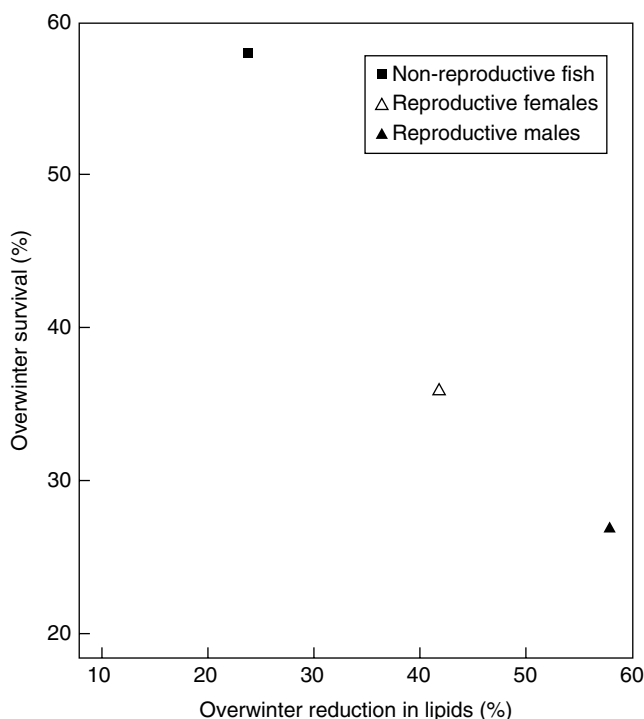
Figure 4.26   Negative association between overwinter (October through April) survival and overwinter reduction in total lipids for reproductive and non-reproductive brook trout (*Salvelinus fontenalis*) in Watern Cove River, Newfoundland. From Hutchings et al. (1999).

mortality in this stock (Schwalme & Chouinard, 1999). There are also many remarkable examples indicating the relationship between fish condition and *M* of fish in freshwater species with respect to overwintering. In general, winter is an energetically stressful period for northern-temperate freshwater fish, as reflected by substantial overwinter declines in lipid content in a number of fish that is linked to the overwintering natural mortality (Hutchings et al., 1999). For example, there is a negative correlation between overwinter survival and overwinter reductions in lipids in brook trout *Salvelinus fontinalis* (Fig. 4.26; Hutchings et al., 1999). A similar link between lipid content and mortality is suggested by the observation that overwinter mortality of Lough Neagh (Northern Ireland) roach (*Rutilus rutilus*) appears to be inversely related to fat content (Griffiths & Kirkwood, 1995).

Moreover, poor condition in some species may increase vulnerability to predators. For example, it was hypothesized that poor-condition cod in Hawke Channel (Canada) due to low capelin abundance may have increased vulnerability to predators, in particular harp seals, which may be abundant in that region during the cod spawning season (Rose & O'Driscoll, 2002). According to the authors, the disappearance of northern cod after first spawning raises the hypothesis that mortality increases as a consequence of poor post-spawning condition coupled with increased predation.

Laboratory studies also provide good indications of the link between *M* and condition of fish. Experiments revealed that energy reserves invested in reproduction by poor-condition cod females increased their risk of mortality (Lambert & Dutil, 1997b) and that natural mortality in cod was inversely related to initial condition factor (Dutil et al., 2006).

Similar, a comparison of several condition indicators from wild cod in the northern Gulf of St Lawrence and cod exposed to a prolonged period of starvation in laboratory experiments revealed that natural mortality from poor condition contributed to lower production, possibly contributing to the decline of that stock (Dutil & Lambert, 2000). Further, laboratory experiments revealed that there was some indication that survival correlated with sardine condition, with better-condition fish being more likely to survive (Marçalo et al., 2010).

However, it is important to notice here that the collapse of several stocks is often due largely to the impact of fishing mortality, not natural mortality. Thus for example, Fu et al. (2001) indicated that it is unlikely that the collapse of the eastern Nova Scotia (Canada) cod stock can be attributed to a sudden increase in M; fishing appears to have been the primary cause for the stock's decline.

Nevertheless, a positive relationship between condition and survival has not always been found and, in some cases, a negative relationship has even been observed. For example, an inverse relationship of the condition index of herring spawners in a given year with the fraction surviving to a year later was found in the Barents Sea by Engelhard and Heino (2005). The authors hypothesized that reduced condition and increased survival may not have cause–effect relationships, but rather be the result of common underlying causes. First, favorable conditions early in life can result in high survival but reduced growth and condition due to reduced per-capita food intake, as a density-dependent effect. Second, strong year-classes, characterized by reduced growth and condition indicators, may suffer less predation and exploitation mortality due to the dilution effect (Engelhard & Heino, 2005).

Overall, it is clear that more detailed and specialized procedures should be applied in order to increase our understanding of the influence that fish health may have on individual and population life-history traits such as natural mortality, growth, and reproductive potential (Lloret et al., 2012). Specialized studies should thus also consider controlled laboratory experiments, altering the health of fish in order to illustrate the direct connection between health, growth, natural mortality, and reproduction of fish. All these studies will require interdisciplinary work among researchers such as parasitologists, fish physiologists and fisheries biologists. Results could be used, for example, to improve natural mortality (M) and growth estimates to be used as input variables in the standard stock assessments. While there are several techniques for estimating M, they tell us little about the underlying causes of natural mortality. Then, given sufficient information on fish health, it may be possible to more exactly evaluate M, growth rates, and spawner quality (and not only quantity) and to forecast temporal changes of these variables (Lloret et al., 2012).

Considerable mortality of some fish species can occur during wintering migration because of reduced condition. Fish, as a rule, do not feed intensively during this period. Azov anchovy that have not accumulated sufficient level of fat stores die in the Sea of Azov when cold weather arrives. Natural losses of this fish are observed during migration to the Black Sea. Also, mass death of migrating birds, as a result of exhaustion, is an analogous example. Death usually occurs in weak fish with low energy content. We assume, according to our experimental data, that the proportion of anchovy dying as a result of this comprises about 15% of all stock (Shulman, 1972a, 1974; Shulman et al., 1978).

4.2.4 Applied recommendations

Overall, the most useful functional and metabolic indicators of fish condition during the different stages of ontogenesis may be the following:

- proximate chemical composition, primarily total lipids, protein, glycogen and water content;
- neutral lipids (fat, triacylglycerol) content;
- wax esters;
- saturated and monounsaturated free fatty acids;
- polyunsaturated fatty acids (DHA 22:6 ω-3, EPA 20:5 ω-3 and also arachidonic 20:4 ω-6);
- polar lipids (phospholipids, primarily phosphatidylethanolamine, phosphatidylcholine, and cholesterol);
- free amino acids (total content);
- RNA, DNA and RNA/DNA ratio;
- hormones (thyroxine, somatotropin, prolactin, corticoids, gonadotropins, etc.).

These indicators are determined in eggs and embryos, larvae, juveniles, and adult fish (total body, red and white muscle, liver and gonads).

4.3   Condition during the annual cycle

Annual cycles are a part of ontogenesis, during which populations undergo processes that are essential for their continued existence (Shulman & Love, 1999). Annual cycles are the manifestation of endogenous rhythms, which developed during evolution and which are under the strong control of exogenous, primarily climatic, factors. These endogenous rhythms are themselves a manifestation of complex functional metabolic processes carried out at the sub-organism (molecular, cell, tissue) and organism levels. Thus annual cycles are endogenous stereotypes that balance populations with the environment. Annual cycles are divided into periods, which for fish include growth, reproduction, feeding, migration, and wintering. Although these periods provide a common basis for understanding the processes involved in annual cycles, there are many exceptions. Thus, for example, migrations are absent in settled species while wintering is absent in those species inhabiting low latitudes, particularly in the tropics.

   In the literature on fish biochemistry, physiology, and ecology, there are thousands of examples of rhythms and their metabolic components (see, for example, Fig. 4.27). Different biochemical patterns do not change equally during the annual cycle (Fig. 4.28). The most
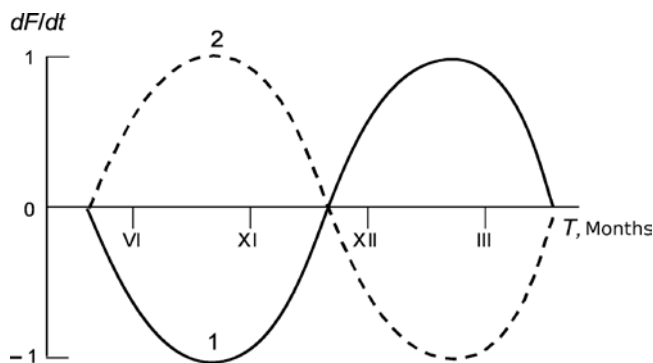
Figure 4.27   Annual dynamics of fat accumulation (dF/dt, mg lipid/g per day) in Black Sea warm-tolerant anchovy (curve 1) and cold-tolerant sprat (curve 2). After Shulman & Love (1999).
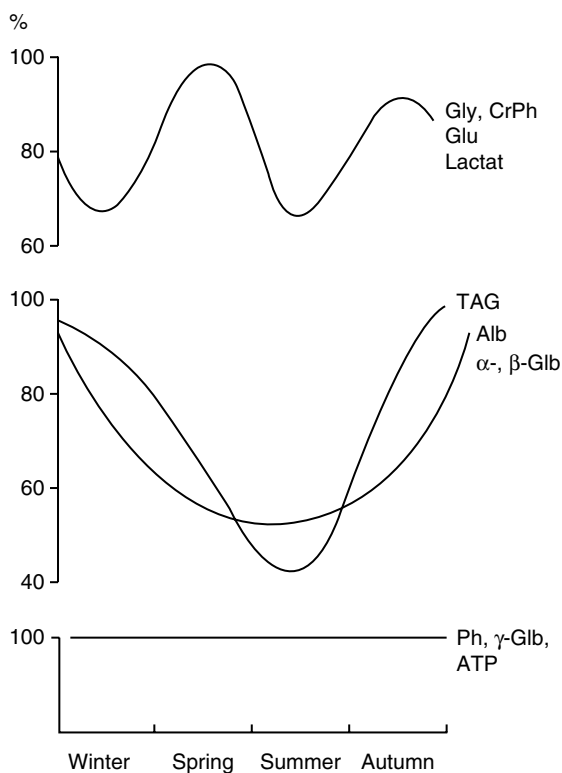
Figure 4.28 Variability of some biochemical patterns during fish annual cycles: Gly, glycogen; CrPh, creatine phosphate; Glu, glucose; TAG, triacylglycerols; Alb, albumin; α-, β-Glb, α- and β-globulins; Ph, phospholipids; γ-Glb, γ-globulins; ATP, adenosine triphosphate. After Shulman (1978).

variability is observed in elements of carbohydrate metabolism (glycogen, glucose, and lactate) as well as creatine phosphate. These substances provide emergency energy to organisms and populations adapting to environmental and functional alterations. Triacylglycerols, serum albumins, and α- and β-globulins are mobilized to provide energy during maturation and feeding. Thus these changes have a monocyclic character. Finally, there is a third group that comprises components with a relatively stable content: ATP, phospholipids, γ-globulins. These constitute a "gold reservoir" where changes are very large despite their low quantity in tissues. Of course, this "gold reservoir" continuously regenerates but remains dynamically stable. Many reviews of this subject are available (Love, 1970, 1980; Shulman, 1972a, 1974; Shatunovsky, 1980; Shulman & Urdenko, 1989; Minyuk et al., 1997; Shulman & Love, 1999). In the last few years, several excellent publications on this subject have appeared (e.g., Adams, 1999; Kolakovska et al., 2003; Kolakovska, 2010).

Often such rhythms are referred to as "seasonal changes." We consider that such terminology is superficial and not exact. Firstly, "seasonal change" is not an accurate term because it does not consider the main cause of these changes, which are not connected with seasons but more with ecological/physiological processes in populations (reproduction, feeding, migration, wintering, etc.). Therefore we prefer to talk about "changes during the annual cycle." Secondly, these changes do not always follow seasons. For instance, in fish from the northern seas, spawning and other cycles (wintering, migration, etc.) can embrace adjacent

(consecutive) seasons, and in different species and populations are dated to different times of the year (Parrish & Saville, 1965; Shatunovsky, 1980; Graham & Laurs, 1982). Thus, for instance, in some species "fall season" means "spawning" but for others "pre-spawning." In warm- and cold-tolerant fish of warm seas, spawning takes place in opposite months of the year. Thus for warm-water species spawning in summer, the "fall period" means "after-spawning feeding" but for cold-water species spawning in winter it means "pre-spawning feeding." Conversely, "spring season" means the opposite. At least in the southern seas (e.g., the Mediterranean), the spawning period in fish is very prolonged and can encompass several seasons (spring, summer and fall). The concept that fish annual cycles are programmed by the genome in close interaction with the environment has been developed in many physiological/biochemical investigations (Fontaine, 1948; Hoar, 1953; Gerbilsky, 1958; Idler & Bitners, 1958; Love, 1970, 1980; Shulman, 1974, 1978; Beamish et al., 1979; Shatunovsly, 1980; Shulman & Love, 1999; Wagner & Congleton, 2004; Kassahn et al., 2009). These and many other works have dealt with the rhythms of oxygen consumption, nitrogen excretion, activity of digestive and tissue enzymes, dynamics of the RNA/DNA ratio, protein growth and fat accumulation, changes in fractional and fatty acid composition in tissue neutral and polar lipids, blood components, endocrine activity, and were summarized in an earlier book (Shulman & Love, 1999). Here our main objective is to reveal functional and metabolic features that can be indicators of the intensity and efficiency of the different periods that integrate fish annual cycles.

4.3.1 Pre-spawning period

We begin with the pre-spawning period (sometimes named pre-spawning feeding). This period can accompany spawning, more specifically pre-spawning migration. It is characterized by intensive processes of protein synthesis, connected with differentiation and growth of generative tissues. At this time, gonad development is in phases III and IV. The liver plays a crucial role in gonad formation, synthesizing and transforming vitellogenins, lipoproteins, glycoproteins, phosphoproteins as well as structural lipids and transporting them attached to transport proteins (albumins, α- and β-globulins) to generative tissues via the blood serum (plasma). Because of this, liver size and weight increases during pre-spawning. During this period, fish mobilize neutral lipids (fats, triglycerides) that were laid down as reserves during food consumption. Parts of these fats are formed by the transformation of carbohydrates and even proteins into fat. Thus, the initial amount of fat accumulated at the onset of maturation has great significance for spawning. If accumulated fat stores are large, more energy can be mobilized to generative synthesis. Thus for a number of fish species (particularly pelagic fish), decrease in fat content can be a good indicator of preparation for spawning. However, other species do not show this pattern. For example, in red mullet *Mullus barbatus* and *Mullus surmuletus* from the Mediterranean Sea, there is no decrease in fat stores because feeding occurs concurrently during this period (Lloret et al., 2007), reflecting prolonged and intensive feeding.

4.3.2 Spawning

The spawning period is characterized by high metabolic activity, which is closely related to spawning of eggs in females and enhanced locomotory activity in males. During the spawning period, and just before laying of the eggs, the gonads of females swell because hydration of eggs occurs (gonad water content greatly increases). Male spermatozoa manifest enhanced content of glycogen and glucose, which it is hypothesized relates to competition between

males to succeed in egg fertilization, as those spermatozoa containing more glycogen and glucose can swim faster (Bassova, 2001).

In most fish species, the formation of sexual products in the gonads consumes the internal stores of energy no matter how great the intake of food. Thus, energy reserves are usually greatly diminished in the muscle, liver and other tissues during breeding in order to supply energy to the ovary for intensive generative synthesis and to supply essential nutrients such as fatty acids and lipid-soluble nutrients, and increase during the post-spawning and regeneration (Chellappa et al., 1989; Wiegand, 1996; Cubillos et al., 2001; Okuda, 2001; Mello & Rose 2005; Ter Hofstede et al., 2007). Thus minimum values in condition are usually observed during or just after spawning. In cod for example, a significant depletion of body reserves during the course of spawning has been well documented (Lambert & Dutil, 1997b). Similar to this, Fulton's *K* condition factor and hepatosomatic index (HSI) of cod off southern Newfoundland were lowest during the spawning season (spring) and increased rapidly during the post-spawning period, reaching maximum values by fall (*K* and HSI increased on average 24% and 82% between spring and fall, respectively, Fig. 4.29; Mello & Rose, 2005). In the cardinalfish *Apogon notatus* from southern Japan, condition factor, hepatosomatic index and fat body–somatic index declined in both sexes during the breeding season (Fig. 4.30; Okuda, 2001). In red drum *Sciaenops ocellatus* from the Gulf of Mexico, the depletion of lipid reserves from liver (a major depot for lipid in this species) and visceral fat in late summer indicates that lipid stored during active spring and summer feeding supplies energy for reproduction (Craig et al., 2000). In English sole *Parophrys vetulus* from the northeast Pacific, an analysis of gross chemical composition revealed a disproportionate transfer of materials from body to the ovaries; 55% of lost body protein, 47% of ash, and 5% of lipids were accounted for by an increase in these constituents in ovarian tissue during sexual maturation (Dygert, 1990). In *Sardinella aurita*, condition is lowest during the breeding season. Thus, mesenteric lipid content of *S. aurita* in the Adriatic showed an inverse relationship, reaching minimum values in spring/summer when the species reproduces (Mustac & Sinovcic, 2012). Similar to this, the mesenteric fat content of *S. aurita* in Mauritanian waters (northwest Africa) reached minimum values just after breeding (Ter Hofstede et al., 2007). Also the condition of megrim *Lepidorhombus whiffiagonis* from the eastern Adriatic Sea is lowest just after spawning (Santic et al., 2012). Again, the seasonality of Fulton's *K* condition factor of several species of barracudas (*Sphyraena* spp.) in Mediterranean Egyptian waters was linked to the reproductive cycle, with lowest values obtained during spring and summer (breeding season) (Allam et al., 2004a). In decapods, there was a significant and negative relationship between GSI and the Le Cren relative condition factor of female red shrimp in the Balearic Islands (northwest Mediterranean), indicating that there is an effective transference of somatic energy to the gonads during reproduction (Carbonell et al., 2008). This is in line with the seasonal fluctuations of condition, with minimum values (i.e., lower energy reserves) occurring during spawning (from May to August) and maximum values (i.e., higher energy reserves) during the mating period from March to April (Eliassen & Vahl, 1982).

Also, in red porgy *Pagrus pagrus* from the Buenos Aires coast (Argentina), despite the fact that females feed throughout the entire spawning period, it was shown that between pre-spawning (September, water temperature 15°C) and mid-spawning (December, water temperature 19°C), they lost 45 and 38% of their muscle and liver lipids, respectively, and 89% of the perivisceral fat associated with the gut and body cavity (Aristizabal, 2007). Further, in several species of the genus *Diplodus* in the Gulf of Tunis (central Mediterranean), the HSI presented an inverse trend to the GSI in relation to breeding activity (Mouine et al., 2012). Also in this study the condition factor showed significant monthly variations affected
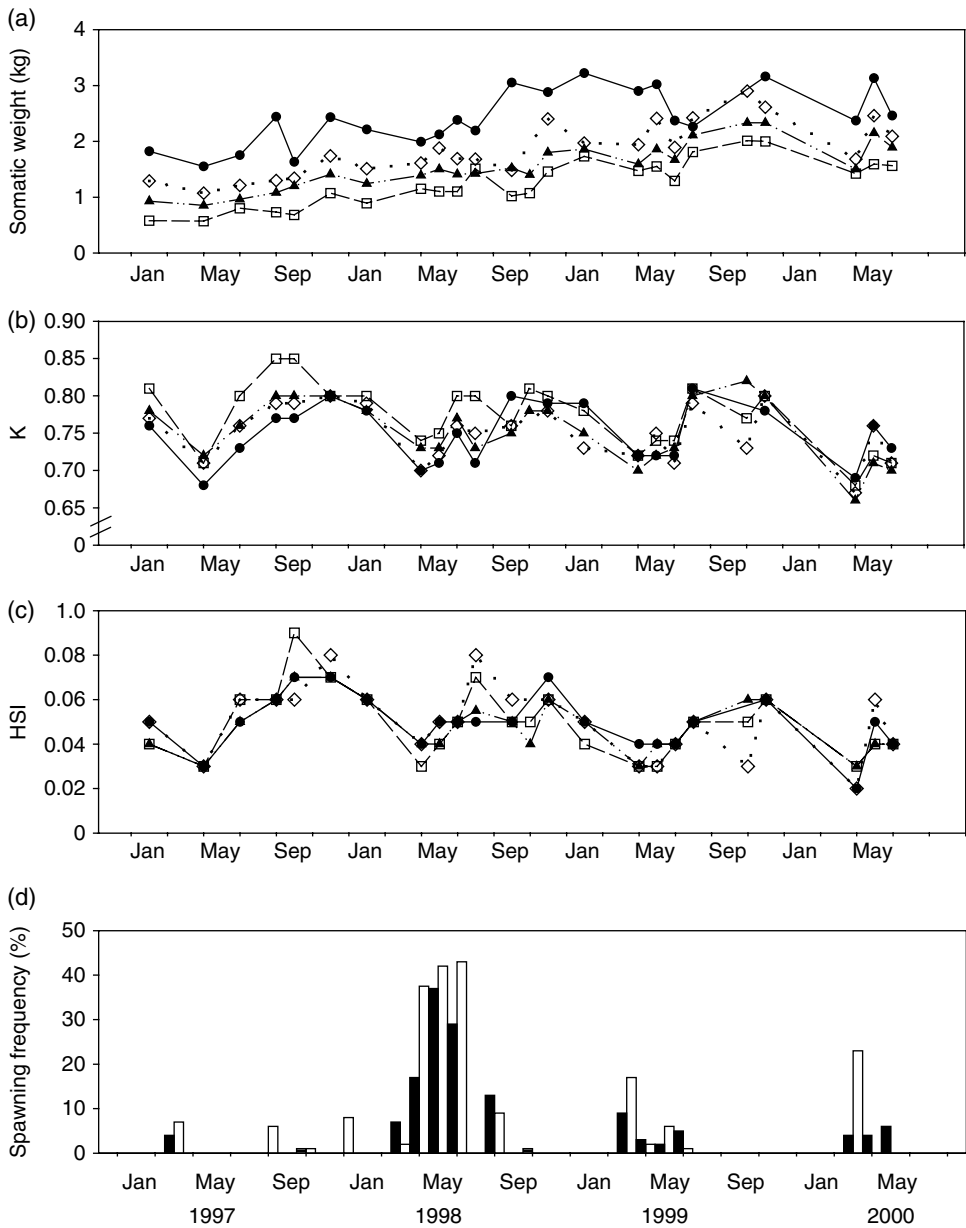
Figure 4.29 Temporal changes in (a) mean somatic weight (kg), (b) Fulton's K condition factor (g/cm³), (c) hepatosomatic index (HSI), and (d) frequency of spawning females (black bar) and males (open bar) from cod sampled during acoustic surveys in Placentia Bay, Newfoundland. Cohorts: black circle, 1990; open diamond, 1991; black triangle, 1992; open square, 1993. From Mello & Rose (2005).

by the sexual cycle of the *Diplodus* species. For example, in white seabream *Diplodus sargus* from the Canary Islands coast, gonadal total lipid values of males and females increased during pre-spawning and spawning (from November to March), particularly in the case of females (Pérez et al., 2007). This explains why in general the ovaries contain, during the
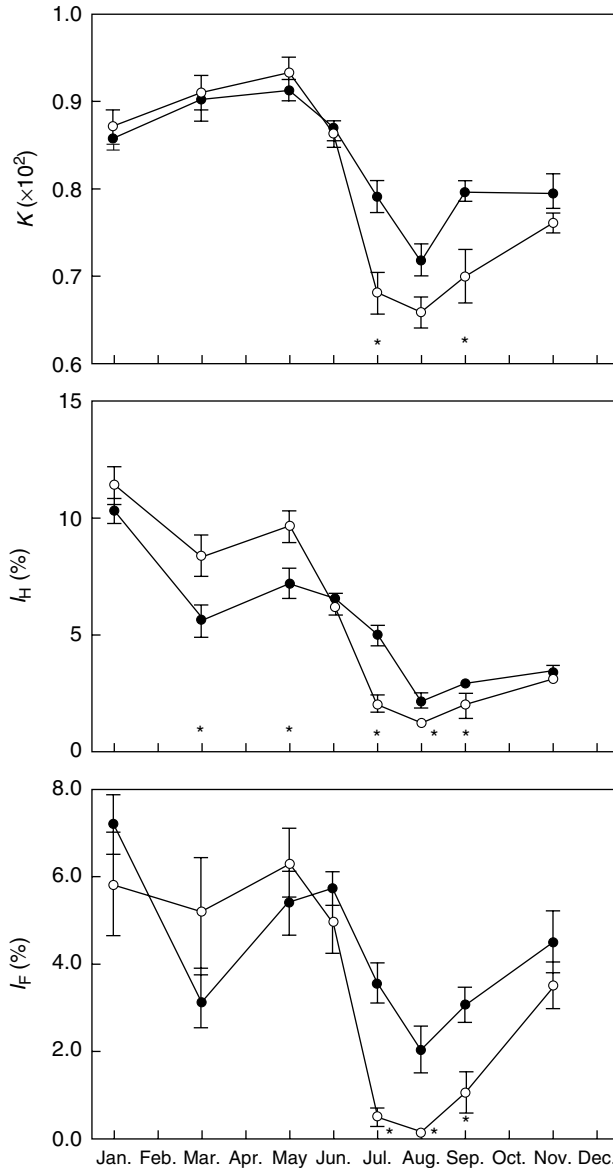
Figure 4.30   Seasonal changes in mean condition factor (K), hepatosomatic index (I_H) and fat body–somatic index (I_F) for males (closed circles) and females (open circles) of cardinalfish *Apogon notatus*, respectively. Vertical lines are standard errors. Asterisks indicate significant sexual difference by Student's *t*-test ($P < 0.05$). From Okuda (2001).

breeding phase, a significant amount of protein and lipid (e.g., 39% and 14% respectively in the case of hake; Rodrigo et al., 1998). The energetic costs of reproduction can also be detected when the condition of reproductive individuals is compared with that of non-reproductive ones. For example, Hutchings et al. (1999) identified an average overwinter
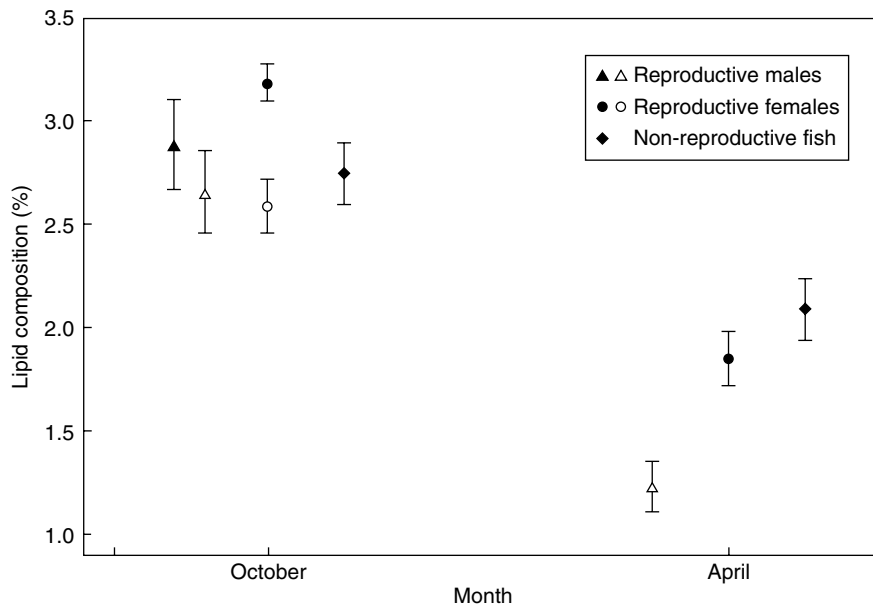
Figure 4.31   Somatic (open symbols) and total (closed symbols) lipid content as percentage of fresh body weight of reproductive male, reproductive female, and non-reproductive brook trout *Salvelinus fontinalis* from Watern Cove River, Newfoundland, in October 1995 and April 1996. Data are means and error bars represent one standard error. From Hutchings et al. (1999).

loss of lipids in reproductive brook trout (males and females combined), as a percentage of total body weight, more than double that of non-reproductive individuals (Fig. 4.31).

However, in some small pelagic fish which used to spawn in areas of substantial biological production to ensure adequate adult feeding and which therefore do not separate feeding and reproduction seasons (Hunter & Leong, 1981; Lisovenko & Andrianov, 1996), the condition increases during the breeding season because feeding not only meets energy requirements of daily spawning but also provides surplus energy. For example, the condition of anchovy in the western Mediterranean and in Cádiz (southwest Spain) increases in late spring and summer (breeding season) due to a greater food availability resulting from the winter and summer primary production peaks (Giráldez & Abad, 1995; Millán, 1999). Similar, the somatic weight of the bay anchovy *Anchoa mitchilli* in Chesapeake Bay (northwest Atlantic) increased by 32 to 33 during the spawning season (Wang & Houde, 1994). Conversely, the muscle lipid content of the Iberian sardine increased when sardines were in the resting stage of reproduction and sharply decreased when they started to reproduce (Garrido et al., 2008).

Species' sex-specific energetic costs of reproduction explain the sex differences that usually exist between condition of males and females. In many fish species, males and females differ in their physiological condition, with females' condition usually being greater than that of males before reproduction to account for the greater energy expenditures of females during breeding (female gonads tend to be larger; Shulman & Love, 1999). For example, in white seabream *Diplodus sargus* in the northwest Mediterranean, mean HSI of female spawners was significantly greater than that of male spawners, indicating that for a given body weight, livers of female spawners were significantly bigger than those of male spawners (Lloret & Planes, 2003). In Icelandic cod, age 5 and 6 year mature females had
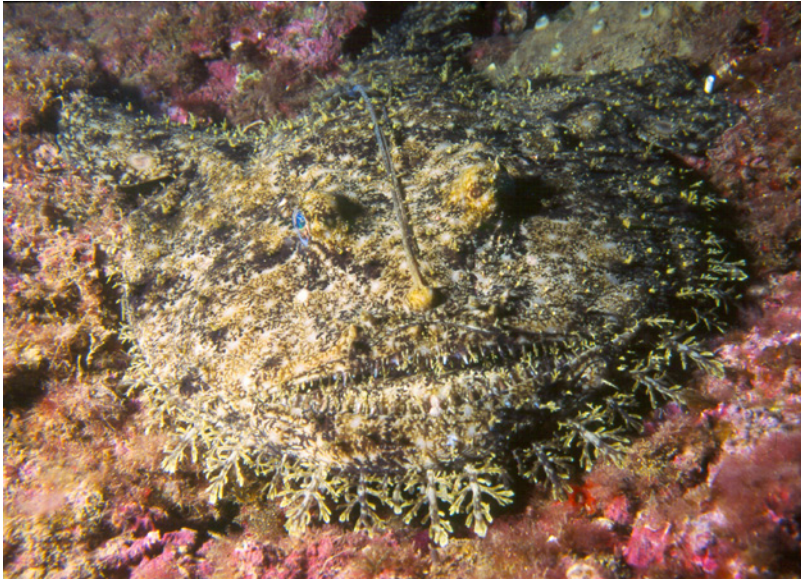
Figure 4.32   *Lophius piscatorius*. Photo by Bernd Mörker.

significantly higher HSI than mature males of the same age (Pardoe et al., 2008). On the northwest Mediterranean shelf, Lloret et al. (2005) found that the HSI of females of *Mullus barbatus* and *Pagellys erythrinus* was significantly better than that of males. Furthermore, Fulton's *K* condition factor of females of *Lophius budegassa*, *Merluccius merluccius*, *Mullus barbatus* and *M. surmuletus* in the northwest Mediterranean was higher than that of males (Lloret et al., 2002). Notwithstanding this general pattern, at the climax of spawning, male fish may incur greater energy losses than females because of greater motor activity due to courtship and competition among males (Shulman & Love, 1999).

Finally, it is also worth noting that immature individuals are usually in worse condition than mature ones. For example, condition of mature female and male cod on the Icelandic shelf is significantly higher than that of immature cod (Pardoe et al., 2008). Similarly, for eight demersal species of the northwest Mediterranean among which were *Lophius piscatorius* (Fig. 4.32) and *Merluccius merluccius*, adults were better conditioned (higher Fulton's *K* condition factor) than juveniles (Fig. 4.33; Lloret et al., 2002). This is because in many fish species, sexual maturity is accompanied by a sharp deceleration in growth; however, body weight and condition continue to increase, usually at a higher rate. It is during this period (i.e., the change from juvenile to adult) when lipid content increases considerably. Furthermore, juvenile fish spend most of the energy for growth and do not accumulate it. However, in other species (e.g., white seabream in the northwest Mediterranean) no difference in lipid content between juveniles and adults has been found (Lloret & Planes, 2003).

4.3.3   Post-spawning feeding

Post-spawning feeding (for many species it is pre-wintering too) is characterized by intensive accumulation of fat stores, which are necessary for normal population survival in those periods of the annual cycle when food consumption ceases completely or
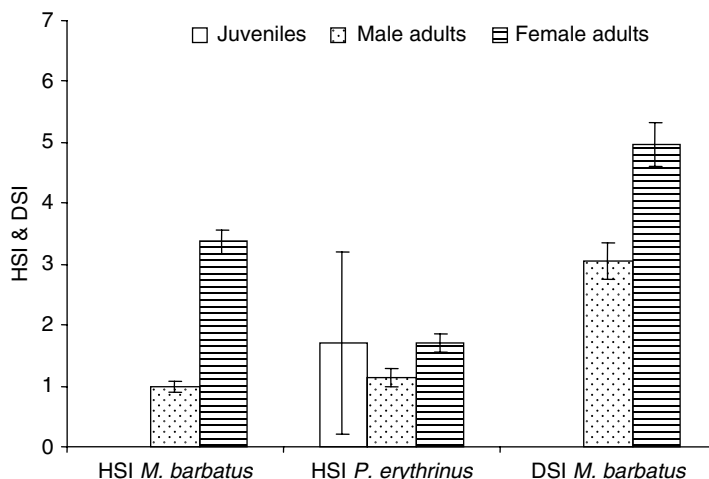
Figure 4.33 Adjusted mean (± SE; 2000–2001) hepatosomatic index (HSI) and digestivosomatic index (DSI) of adult males and females of *M. barbatus* and HSI of juveniles and adult males and females of *P. erythrinus* in the northwest Mediterranean. Adjusted means are the means after adjusting for variation of the covariables (length, depth and latitude). From Lloret et al. (2002).

largely declines, such as during wintering migration and wintering. Together with neutral lipids (triacylglycerols), there are increases in accumulated levels of creatine phosphate in muscle and glycogen levels in muscle, and especially in liver. The concentration of serum proteins (mainly albumin), utilized for gonad maturation, also increases. The heterogeneity of protein fractions in blood serum decreases. Although the processes of protein growth continue, their intensity is considerably less than that of fat accumulation.

4.3.4 Wintering

Wintering (more accurately pre-wintering, but sometimes also called overwintering) is the most critical period for fish (Shuter et al., 1980; Hutchings et al., 1999), especially for juveniles, for which the first winter is the "bottleneck" (Henderson et al., 1988). Water temperature is a major cause of these deep metabolic changes, as cold conditions decrease food digestion and assimilation (Pearse & Achtenberg, 1917).

During wintering, the level of metabolism in populations is strongly reduced. During winter most parameters decrease because fish feeding strongly decreases or completely stops. The RNA/DNA ratio is reduced (e.g., *Dicentrarchus labrax*; Mustafa et al., 1991) and fat reserves are steadily decreased as well as the levels of glycogen and creatine phosphate in tissues. The degree of lipid unsaturation reaches a maximum value in winter, which constitutes an adaptation of warm-water species to cold-water temperatures (Henderson & Tocher, 1987). During wintering protein growth does not occur and the alkaline phosphatase of scales (which controls sclerite formation by calcification of external covers and indirectly corresponds to growth of the bony skeleton) strongly decreases, whereas protein content in blood serum remains stable. Fish are forced to move from exogenous to endogenous feeding (Ackman, 1989): together with fats (particularly triglycerides) and glycogen, a significant fraction of phospholipids and

especially proteins are mobilized to energy metabolism (endogenous feeding), whereas lysosomal activity (lipase, protease, glycogenase, etc.) increases strongly in liver and muscles (Hochachka & Somero, 1984, Hochachka and Somero, 2002; Nemova & Vysotskaya, 2004; Vysotskaya & Nemova, 2008). Lipolysis and proteolysis during wintering are therefore connected to endogenous feeding and starvation. There is also a decrease in enzyme number and a change in the isozyme spectrum (Mommsen et al., 1980; Hochachka & Somero, 1984; Reyiol et al., 2009). During unfavorable wintering conditions, the levels of ATP and ADP strongly decrease in muscle, liver and brain of fish such as carp *Cyprinus carpio* (Zhidenko, 1994). The author considers that mass fish mortality is due to disturbances in ATP resynthesis. Clearly, wintering is the main period when population numbers fall. Many authors have revealed an inverse relation between level of energy stores accumulated by fish at the start of wintering and their survival during this period (Berman, 1956; Kirpichnikov, 1958; Mukhina, 1958; Polyakov, 1975; Higashi et al., 1964; Belyanina, 1966). In particular, triglyceride and phospholipid deficiency have a negative effect on juvenile survival during wintering (Love, 1980; Henderson & Tocher, 1987). The more northerly the region the fish inhabit, the shorter the period of growth and the worse the survival (Miranda & Hubbard, 1994). Juveniles suffer the most (Shuter et al., 1980; Adams, 1999; Hutchings et al., 1999), which is related to metabolic allometry (Post & Evans, 1989; Thompson et al., 1991; Miranda & Hubbard, 1994). Fish juveniles have a more active metabolism and less energy stores, which disturbs maintenance metabolism (Schmidt-Nielsen, 1984; Post & Evans, 1989).

Overall, plasma triglycerides as well as total protein, calcium, and alkaline phosphatase are the best indicators of nutritional status during wintering. Cold adaptation obliges metabolism to take advantage of anaerobic processes and consequently there is increasing utilization of cathepsin B and D. The decrease in phospholipid content disturbs the maintenance of osmotic and electrolyte homeostasis (Lerey et al., 1986; Adams, 1999). Lipids have an important role in nerve impulse conduction (Sutharshiny & Sivashanthini, 2001), and are key for maintenance of osmotic and electrolyte homeostasis (Lerey et al., 1986). Thus, during starvation, membrane ion transport, especially at low temperatures, is disturbed. During starvation, protein biosynthesis and RNA/DNA ratio greatly decrease and begin to recover only at the end of wintering (Mustafa et al., 1991). At the end of winter, in warm-tolerant fish the initial stages of gametogenesis begin (i.e., processes of reproductive tissue differentiation), and these initial stages consume the most energy throughout the whole process of reproductive maturation. Even though these profound metabolic changes play a key role in supporting the necessary level of vigor, sometimes they do not save fish from starvation. At the end of wintering fish become exhausted due to considerable expenditures of neutral lipids (fats) and proteins and this is the main reason for mortality, especially if temperatures during the winter are severe. Furthermore, during wintering (also during maturation), sensitivity to unfavorable environmental factors, including parasites, rises due to exhaustion of fat stores (Ghittino, 1989). The reduction in fat reserves weakens the immune system of fish (Gurr, 1983) and therefore mortality increases. Low energy stores during wintering lead to higher mortality from predators (Kramer & Smith, 1962; Herting & Witt, 1967; Chevalier, 1973; Shelton et al., 1979; Wicker & Jonson, 1987). At the same time food availability decreases (McKinnon, 1973), having broad negative effects, especially for winter-spawning fishes (e.g., Black Sea sprat). Thus, in general, overwintering survival is better when fish have a higher fat content (Wagner & Congleton, 2004).

Among the best examples of these profound metabolic changes during wintering are rainbow trout *Oncorhynchus mykiss* and chinook salmon *Oncorhynchus tshawytscha*. Shulman and Love (1999) showed that metabolism in juveniles of rainbow trout *O. mykiss* during long (147-day) starvation undergoes three phases: (i) glycogen and glucose are critical fuels for the central nervous system, so in order to maintain glucose levels lipids are utilized and proteins preserved; (ii) there is further utilization of lipids and partial utilization of proteins; and (iii) protein depletion occurs which ultimately leads to death. Similar results were also found for chinook salmon *O. tshawytscha* (Navaro & Gutieres, 1995): during starvation, juvenile (smolt) glucose is synthesized using proteins and lipids as substrates (Gutiérrez et al., 1995). In Atlantic cod, Black and Love (1986) showed how endogenous substrates are used during starvation in a similar sequence. Liver lipids and liver and white muscle glycogen begin to be mobilized at the start of starvation; liver lipids are exhausted first until they reach minimum values. Then proteins of white and red muscle and glycogen of red muscle begin to be mobilized in a second step until fish dies.

Different species adapt to wintering condition in different ways. In Atlantic cod, gluconeogenesis decreases during severe starvation. In the liver, the activity of phospho-enolpyruvate carboxylase, fructose diphosphatase and alanine aminotransferase, as Love and Black (1990) showed, decreases considerably after 22 days of starvation. Less active fish species adapted to low glycogen levels stop swimming but more active fish such as rainbow trout continue gluconeogenesis and increase the activity of these enzymes during the 8 weeks of starvation. Furthermore, glycogen content of fish brain and heart is not affected by starvation (Love, 1958, 1970).

4.3.5 Migrations

Migratory fish species such as tuna have a higher and more variable fat content compared with most non-migratory fish species, indicating that lipids are the main source of energy during these long-range migrations (Stansby, 1976). For these species, extensive spawning migrations incur a high energetic cost that is exacerbated by the fact that some do not feed while migrating (Engelhard & Heino, 2005). Some species do not even migrate if energy stores are insufficient. For example, only herring with sufficient energy stores can success-fully migrate and spawn (Slotte & Fisksen, 2000). In others, migration distance has been linked to available fat stores. For example, if anchovy muscle fatness is high, fish migrate to the southeastern part of the Black Sea, whereas if it is low, migration is limited to the central-eastern part (Shulman, 1972a, 1974, 2002). Therefore, the evaluation of muscle lipid content of Sea of Azov and Black Sea anchovy stocks, along with the analysis of sea temperature, have been proposed for forecasting the start of the winter migration of these populations, which in turn has implications for stock availability to the fisheries (Shulman, 1974, 2002; Chashchin & Axelev, 1990; Nikolsky et al., 2009a).

Also there appears to be an excellent link between migration, the reproductive cycle, and condition of *Sardinella aurita* off Mauritania, northwest Africa. From an analysis of seasonality in condition factor, GSI and fat content in the mesenteries of this species, it was concluded that the fish were in good condition when entering the Mauritanian zone, used most of their reserves during the spawning activities, and during the months after spawn-ing, though feeding is maintained, the physical condition of the fish collapses, with fat content rapidly declining (Ter Hofstede et al., 2007). After that they retreat to the south in order to recover.

4.3.6 Applied recommendations

The same indicators apply as given in section 4.2.4.

4.4 Other cycles

4.4.1 Daily rhythms

Although daily (circadian) rhythms may seem to have little relation to indicators of fish condition, this is not true. Many functions and processes of metabolism in fish connect directly or indirectly with time of day, and therefore data obtained at different hours may result in different outcomes. A good example of this is the daily change in energy metabolism associated with differences in locomotion, food consumption and some other forms of functional activity during the day. Different levels of locomotion that account for hunting during light or dark periods (or early in the morning or late in the afternoon) lead to different levels of oxygen consumption during persecution of prey. Belokopytin (1993) deduced the following formula:

$$Q = ab^v \qquad (4.1)$$

where Q represents energy expenditure, v locomotion velocity, and a and b coefficients.

It is curious that daily predators "sleep" many hours during darkness (Radakov & Solovyev, 1959; Belokopytin, 1993), although this "sleep" is completely different from that of higher animals (Karmanova et al., 1976). However, night predators, even sedentary ones such as *Scorpaena porcus*, are very active at night and can swim distances up to 500 m (Belokopytin, 1993; Fig. 4.34). In addition, fish such as sprat undergo daily vertical
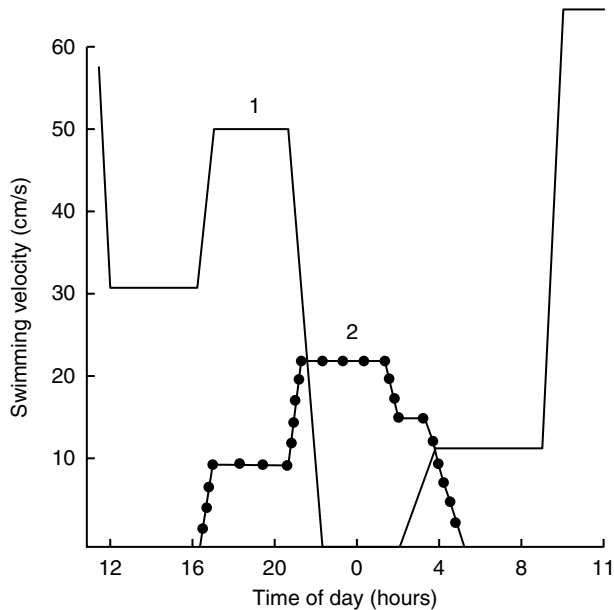
Figure 4.34 Average swimming velocity of fish at different times of the day. Solid line, horse-mackerel; dotted line, scorpionfish. After Belokopytin (1993) and Shulman & Love (1999).

migrations to the surface in order to feed on phytoplankton and zooplankton (these migrations also need large energy expenditures).

It is also known that many fish species feed more intensively in the morning and especially during the evening (twilight) hours. Thus digestive enzyme activity in these fish falls during these periods (Ugolev & Kuzmina, 1993; Kuzmina, 2008). Lipid accumulation (all fractions) also occurs at this time (Lal & Singh, 1999; Khal'ko & Khal'ko, 2001, 2003; Khal'ko et al., 2009). Expenditure of lipids (especially triacylglycerols) mostly occurs during spawning hours (Khal'ko et al., 2009). Processes of protein growth fall during the hours following lipid accumulation (Revina, 1964; Arkhipchuk & Makarova, 1992). It is well known that daily rhythms of fish metabolism are under close neuroendocrine control (hypothalamus–epiphysis–hypophysis), including somatotropin, prolactin, serotonin and norepinephrine (noradrenaline) (Lee & Meier, 1967; Polenov, 1983; Leatherland et al., 1974; Sautin, 1989).

4.4.2   Interannual fluctuations

The literature contains considerable data about interannual fluctuations of fish metabolic characteristics. Most of these fluctuations are caused by climatic change, which affects many aspects of a population's environment. These are considered in Chapter 5. Unfortunately most studies deal with very short time series (2–4 years) and are not long enough for meaningful analyses. Most of these investigations were carried out with fish lipid content because of its large interannual variability and the preservation of samples collected. Examples of such investigations are Lasker's (1962, 1970) work on the Pacific sardine *Sardinops caerulea* from California, which expanded from 1932 until 1956, and also the studies carried out by Smith and Epple (1982) on anchovy *Engraulis ringens* in the same region, as well as those from Rosa et al. (2010) on sardine and anchovy in the Portuguese region and Kolakovska et al. (2003) and Kolakovska (2010) on Baltic herring. There have also been important long-term studies on the lipid fraction content of Californian anchovy *Engraulis mordax* (Håkanson, 1989a,b, 1993) and North Sea sprat (Håkanson et al., 1994).

We have studied the lipid (primarily fat) content of the Black Sea sprat in the summer period of feeding completion, when these stores reach maximal values, during the period from 1960 to 2012 (Shulman, 1974; Shulman et al., 1993, 2009a; Minyuk et al., 1997; Nikolsky & Shulman, 2005; Nikolsky et al., 2009b, 2011). These data will be devoted to monitoring stock and population condition. There is also a short time series (from 1956 to 1973) on lipid (fat) content of Black Sea anchovy in the autumn period of feeding completion (Danilevsky, 1964; Dobrovolov, 1972; Shulman, 1972a, 1974; Danilevsky et al., 1979; Shulman & Dobrovolov, 1979). Data from Chashchin and Axelev (1990), obtained in the 1980s, unfortunately may not allow us to estimate interannual fluctuations. Recently, the investigations about the Black Sea anchovy have been renewed by Nikolsky et al. (2009a, 2011). These comprise data on the interannual changes of fatness of Azov anchovy (Shulman, 1960a,b, 1972a, 1974; Danilevsky, 1964; Taranenko, 1964; Dubrovin et al., 1973; Luts & Rogov, 1978). They cover the time frame from 1953 to 1974. During the first period of these investigations (to 1966), there is good correlation of fatness variability between two different anchovy populations (Sea of Azov and Black Sea) (Shulman, 1972a, 1974). Because both these marine basins inhabit a common climatic zone, fluctuations in hydrological and hydrochemical regimes are similar, which may explain the high degree of coincidence of fatness variability between stocks. After 1966, data on Sea of Azov and Black Sea anchovy

fatness is much more difficult to compare due to discrepancies in collection and elaboration of field samples.

Interannual changes in neutral lipid (fat) content and condition factors in marine fish were observed also by Kaga et al. (2009) and Kaga and Sato (2010) with salmon of the genus *Oncorhynchus* (chum salmon *O. keta*, sockeye salmon *O. nerka* and pink salmon *O. gorbuscha*) in the North Pacific and the Bering Sea; by Lloret et al. (2005, 2007) with several fish species in the Mediterranean; by García-Charton et al. (2004) with Mediterranean rocky reef fish assembles; by Hidalgo et al. (2008) and Domínguez-Petit and Saborido-Rey (2010) with hake *Merluccius merluccius* in the western Mediterranean; and by Marshall et al. (2000, 2004) with North Atlantic cod.

The number of investigations dealing with other metabolic characteristics are few. They relate to some parameters of protein metabolism and blood mineral composition of chinook salmon *O. tshawytscha* (Wagner & Congleton, 2004) and antioxidative enzymes of Black Sea fish (Rudneva, 2006; Rudneva et al., 2011).

4.5 Connection indicators between fish metabolism, abundance dynamics, behavior, and distribution

It is obvious that metabolic rhythms are the foundation of abundance (number) dynamics, behavior and distribution of fish populations and stocks. However, the opposite relation – influence of abundance and complicated forms of behavior on fish metabolism – also has significance. Shulman and Love (1999) have already concentrated on these very important aspects. Now we will consider new results obtained since then as well as some significant ones which were unknown before.

4.5.1 Abundance dynamics

The interaction between metabolism and abundance was first demonstrated for fish by G.V. Nikolsky (1974) using a simple conceptual model. The model connects recruitment, adult stock, total elimination, and food supply of fish. A similar model was also elaborated for other animals such as insects, birds, and mammals (e.g., Elton, 1958). Recruitment of a species depends significantly on abundance of the brood. Abundance, in turn, is influenced in large degree by population fecundity and quality of reproductive products. These products comprise content of nucleic acids, proteins, phospholipids, and cholesterol, and energy substrates (neutral lipids/triacylglycerols, sometimes wax ethers and glycogen). It was shown that the higher the content of these substances in oocytes and spermatozoa, the better the fertility and development of the eggs and larvae (Hester, 1964; Cowey & Sargent, 1972; Shatunovsky, 1980; Gosh, 1985; Zhukinsky, 1986; Konovalov, 1989; Rowe et al., 1991; Brown & Taylor, 1992; Henderson & Tocher, 1987; Henderson et al., 1996; Adams, 1999). Better larval condition improves survival, development to juvenile stage, and then recruitment to the population (Post & Evans, 1980; Schmidt-Nielsen, 1983; Thorpe et al., 1984; Wagner & Congleton, 2004). Food search by larvae and juveniles, as well as escape from predators, is more successful in more developed individuals (Prosser & Brown, 1962; Brown & Taylor, 1992). Higher content of lipids promotes increased resistance of eggs and larvae to unfavorable external conditions (Gall, 1974; Ware, 1975a,b; Thorpe et al., 1984; Docker

et al., 1986; Brown & Taylor, 1992; Kamler, 1992). Abiotic factors include water temperature (which can be higher or lower than the optimal limit), oxygen (especially deficiency), and water pollution among others (Wedemeyer et al., 1984). Adaptation to unfavorable factors requires large energy expenditures, which will be provided by neutral lipids. In this case, sensitivity to these negative factors increases (Shulman & Love, 1999). Biotic factors include food deficiency and presence of predators, parasites and diseases. Food deficiency has great significance for larvae during critical periods of conversion to external feeding (named "bottleneck" by Henderson et al., 1988). It is during this stage that mass mortality occurs. Higher energy stores can strongly reduce this mortality. In particular, polyenic fatty acids play an important role in the successful development of eggs and larvae (Sargent, 1978; Sargent & Henderson, 1980; Walton & Cowey, 1982; Yuneva et al., 1990), because higher levels of polar lipids allow higher metabolic activity of cellular and subcellular membranes. Higher content of DHA also affects the development of sense organs and higher nervous function which, together with higher content of neutral lipids, promotes successful predation and escape from predators. In this regard, the outstanding investigations of Sargent (1995), Bell et al. (1995), and Bell and Sargent (1996) showed that 22:6 ω-3 is accumulated in rhodopsin of the larval retina, promoting improvement in vision and helping survival. Higher lipid content in eggs increases the ability of larvae to begin self-feeding earlier and to continue it longer than larvae with lower energy stores (Brown & Taylor, 1992). Larger larvae can better find prey, as well as to escape from predators. They have more ability to grow and survive. Lipids play a significant role in nerve impulse conduction of larvae and juveniles (Sutharshiny & Sivashanthini, 2011). Carbohydrates and proteins also contribute to the energy potential of eggs and larvae, although this contribution is not so large as that of lipids (Pepin & Myers, 1991).

There is a close relationship between levels of plastic and energy components in generative products and bodies of spawners, particularly in females (Shatunovsky, 1980; Krivobok, 1964; Shulman, 1972, 1974; Holdway & Beamish, 1985; Shatunovsky & Rychagova, 1996; Rainuzzo et al., 1997; Lambert et al., 2003; Dominguez-Petit & Saborido-Rey, 2010). The mechanism behind such connection is rather clear: proteins, lipids, glycogen and other substances from muscles and other tissues of adult fish (for example, plasma TAG, cholesterols and total proteins) that undergo transformation process in liver "shift" to generative products (Shulman & Love, 1999).

Poor fish condition impairs normal maturation (Newsome & Leduc; 1975; Henderson & Nepszy, 1984; Watanabe, 1985; Heming & Buddington, 1988; Henderson et al., 1996). In particular, neutral lipid (triacylglycerols, fat) content has particular importance because the energy from these lipids is used to power biosynthesis of reproductive products (Shulman, 1972a, 1974; Shatunovsky, 1980; Holdway & Beamish, 1985; Hochachka & Somero, 1984; see, for example, Fig. 4.11). Therefore, a continuous linkage is established between quality of spawners, quality of gonads, fecundity and fertilization, quantity and quality of eggs and hatching, quantity and quality of larvae, and recruitment and survival.

A vivid example of such linkage is given by Yuneva et al. (1990) obtained by artificial interbreeding of Pacific gorbusha *O. gorbuscha* (see Fig. 4.19). The content of DHA in female white muscle is proportionally related to survival of eggs and larvae. Also, high levels of neutral lipids are necessary for smoltification and wintering survival of Pacific salmon juveniles (Pavlov et al., 1994). There is also an indirect influence of spawner quality (condition) on recruitment via growth rates. Good-condition spawners (higher content of the most important structural and functional constituents, higher protein biosynthesis) usually grow faster, and this relates to an increase in fecundity and recruitment.

Later in the book (Chapter 5) we will show that the level of energy stores accumulated by fish and their growth rate (defined by protein biosynthesis) are closely related to food supply. Consequently, the higher this supply, the higher the quality of spawners, and this, as noted above, increases recruitment. Therefore, when the fodder base (quantity of food) remains more or less stable, a worsening of the population's food supply is expected as the number of consumers increases due to higher recruitment. A decreasing food supply "untwists" the process: when feeding conditions worsen, the level of accumulated energy stores and growth rate decrease, the quality of reproductive products and fecundity decline, recruitment decreases and consequently also population abundance. This reduction in population numbers leads again to an improvement in food supply and the cycle renews. In this way, according to Nikolsky (1974), there is self-regulation of population number. A sound example of such self-regulation is provided by the interannual change in fat content of sprat populations in the Black Sea (Shulman et al., 2005): levels of fat increase up to a threshold, after which it begins to fall steadily (see Chapter 6, section 6.3). This is, of course, a simplified scheme because it is difficult to foresee that food quantity is more stable than number of consumers. In fact, abiotic and biotic conditions may affect egg and larval survival to a greater degree than spawner quality, and therefore quality cannot effectively protect against loss of individuals. For example, survival of eggs and larvae of pelagic Black Sea fish depend greatly on temperature regime during the spawning period. In this case, the generative products of producers not only benefit from the shift (to the gonads) of the initial substrates stored in organs (e.g., liver) and tissues, but also from intensive food consumption during the pre-spawning period. Finally, the food supply of larvae plays a significant role in their survival (Zaika, 1983) and consequently in recruitment to the adult stock. For example, Domínguez-Petit and Saborido-Rey (2010) showed that spawning success of European hake *Merluccius merluccius* depends more on food supply and temperature than on energy reserves stored in adult fish. Nevertheless, as a first working hypothesis, the principle of population number self-regulation may be accepted.

It is especially important to consider in detail the negative factors that cause fish to die during the annual cycle. The most important are deficiency of food supply and inappropriate (usually lower at high latitudes) water temperature, which affects particularly the early stages of fish ontogenesis (eggs, larvae, juveniles). The elimination of these early life stages usually accounts for more than 90% of the natural mortality observed in fish populations (Nikolsky, 1974). Also the rest of the population suffers these factors, to which we could add other environmental factors such as water pollution and oxygen deficiency.

Undoubtedly, self-regulation of population abundance also occurs in adult fish during the spawning period. A classic example is the post-spawning death of Pacific salmon (genus *Oncorhynchus*). This is caused by two main factors: (i) total exhaustion of fish due to the huge expenditures of energy used during maturation and spawning periods in association with anadromous migrations upstream; and (ii) strong hydrocortisone–catecholamine hormonal stress (Black, 1958; Idler & Truscoft, 1972; Ardashev et al., 1975; Ando, 1986; Maksimovich, 1988). Perhaps fish of many other species also experience loss during pre-spawning and spawning periods for similar reasons. In general, expenditure of energy stores in adult fish is almost complete during these two periods. A number of authors (Henderson et al., 1988; Adams, 1999; Kolakovska et al., 2003; Kolakovska, 2010; Rosa et al., 2010) showed that a reproducer's death occurs at a given threshold, for example when the decline

in fat content reaches 1.0–1.4% of wet mass (we do not consider here inactive sedentary fish). At this time, fish lose up to 50% of body mass (Kleiber, 1961). Frequently death occurs in older age groups, as old fish suffer from more energy loss than younger fish under unfavorable environmental conditions (Shatunovsky, 1980). In our studies, males of Sea of Azov round goby *Gobius melanostomus* lose fat dramatically (almost to zero) after spawning in order to protect eggs (at this time they remain inactive and do not eat for long periods; Shulman, 1967). As a consequence, most of these gobies die after spawning and only a few of the exhausted males survive and later reestablish their energy stores in order to be involved in spawning the following year (Rashcheperin, 1967). The same could happen in other types of animals (e.g., octopus). Low energy stores decrease fish immunity, which increases their susceptibility to diseases and therefore their mortality (Mikryakov et al., 2001). It is necessary to remember that energy and structural substrates are allocated to the gonad during maturation (i.e., they are shifted from muscles and some mesenterial tissues in liver). Thus, liver mass may increase and this can be used as a condition indicator (HSI), together with fat content in this organ, of pre-spawning fish (a proxy for successful preparation for spawning; Shatunovsky, 1980).

However, the most critical period (bottleneck) in the life history of fish is undoubtedly the wintering period, especially for juveniles (Shuter et al., 1980). As explained in detail in section 4.2, during this period feeding greatly decreases or even stops, and fish are forced to shift from exogenous to endogenous feeding using reserves of neutral lipids and even polar lipids as well as proteins (Ackman, 1989). The low temperatures experienced during wintering also contributes to this bottleneck, as lower temperatures induce a decrease in food digestion and assimilation (Pearse & Achtenberg, 1917). The termination or decrease in fish feeding leads to a profound transformation of fish metabolism (see section 4.2).

Apart from wintering, other critical periods in the life of fish (albeit less threatening than overwintering) are the pre-spawning and spawning periods (see sections 4.3.1 and 4.3.2 for a detailed discussion), when energy (fat) stores can be exhausted until they reach just 1% of body wet mass because fat stores are used for gonad development (Kleiber, 1961; Henderson et al., 1988; Adams, 1999; Rosa et al., 2010). This level of energy is insufficient for normal existence.

4.5.2 Behavior

Metabolic processes and behavior of fish populations and individuals interact during every period of the annual cycle. Population behavior is specific and precisely determined in every period, from pre-spawning migration and feeding to spawning, post-spawning, wintering and wintering migration. The transition of a population from one period of the annual cycle to another is possible only after the completion of complex metabolic processes that contribute to the aims of the periods. The fraction of the population not able to carry out these processes dies. We have shown in section 4.2.3 several examples of such mortality during periods of wintering migration and wintering. Post-spawning death is a different story, as fish have had time before death occurs to fulfill their aim (i.e., take part in reproduction). Atlantic cod can die shortly after spawning due to exhaustion or food deficiency (Love, 1970). Failure of gametogenesis in the pre-spawning period may not lead to death in poor-condition producers: disturbance of vitellogenesis and trophoplastic growth or resorption of reproductive growth are often observed during sharp downturns in condition. This endangers populations, as it decreases or undermines recruitment. Finally, resorption
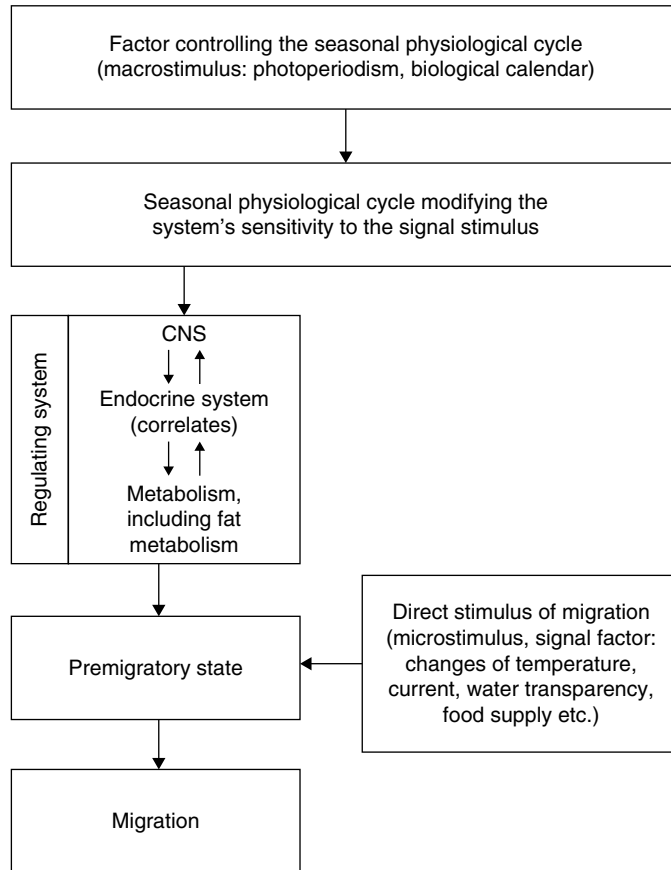
Figure 4.35 Role of light in bird and fish migrations. From Dolnik (1965), Shulman (1972a) and Shulman (1974).

of reproductive products in fish may be the result of considerable water pollution (Lukyanenko, 1989; Evgenieva et al., 1989; Nemova & Vysotskaya, 2003; Vysotskaya & Nemova, 2008).

In section 4.4.1 we dealt with the "biological clock." Now it is necessary to consider the "biological calendar" (i.e., consider the whole year). The duration of daylight is the cardinal trigger of rhythmic processes in fish whatever the latitude. Daylight allows fish, as well as birds and other animals, to compare their biological calendar. Daylight plays an essential role in the orientation of fish, preceding other factors such as climate (including temperature) in importance. Climatic factors can fluctuate considerably from year to year and from season to season. Furthermore, they may influence the population in a negative way. For instance, a strong decrease in water temperature may lead to death of fish or considerably reduce their metabolic activity. In fact, the effect of daylight on bird and fish migrations has been studied rather well (Fig. 4.35; Shulman, 1972a). In fish, a number of studies have been published on the role of daylight on fish behavior (Fontaine, 1948; Hoar, 1953; Gerbilsky, 1956; Barannikova, 1975; Maksimovich, 1988, 1989). These studies mostly considered anadromous migrating fishes (Acipenseridae and Salmonidae). In the Salmonidae, it is sufficient to refer to the studies on smoltification

of Atlantic and Pacific salmon (Barannikova, 1975; Thorpe et al., 1982; Klyashtorin & Smirnov, 1990). A similar phenomenon also occurs in marine fish. The most important links between daylight and fish behavior involve the neuroendocrine and endocrine systems (Gerbilsky, 1958): hypothalamus–hypophysis (pituitary)–thyroid gland–adrenal cortex–gonads. All these systems transform and communicate the "light impulse" to all aspects of metabolism and through this they affect population behavior. It is known that an increase in duration of daylight stimulates development of reproductive products in birds (Dolnik, 1965) and warm-tolerant fish species (Gerbilsky, 1958). Conversely, a decrease in the duration of daylight impedes spawning and prompts fish to start pre-wintering feeding. We have already explained how the direction and intensity of metabolism change during that time. Here we will discuss the significance of external (exogenous) and internal (endogenous) factors in the development of annual cycle periods. Obviously, these factors must be observed in permanent connection with each other. Duration of daylight is an initial exogenous factor. Endogenous factors promote the completion of metabolic processes, without which the annual cycle cannot take place. These endogenous factors must be specific for every period of the annual cycle. For instance, during the spawning period the endogenous factor is protein biosynthesis of reproductive tissue. During the pre-wintering phase, it is formation of fat stores, which provides for maintenance of the population during wintering. During wintering, it is structural and energy metabolism at the necessary (maintenance) level. In this regard, neuroendocrine hormones of the hypothalamus (catecholamines, glucocorticoids) and hypophysis (somatotropin and prolactin) play the most significant role in regulating the direction and intensity of protein and lipid metabolism (Lee & Meier, 1967; Sautin & Romanenko, 1982; Sautin, 1989; Trenkler & Samenkova, 1990; Kassahn et al., 2009).

These endogenous factors strictly control population behavior. During the pre-spawning period, intensive feeding stimulates migration to spawning regions. During the spawning period, dispersion to these regions occurs, and for many species a decrease in feeding intensity takes place too. During post-spawning or pre-wintering periods, intensive feeding occurs and, as fat store accumulation decreases, food-obtaining reactions diminish and there is increased schooling. During migration, this causes formation of compact schools. In anchovy, migration is necessary (as noted above) to accumulate sufficient fat for use during wintering. Only after a sufficient level of fat stores is achieved do anchovy perceive temperature reduction as an impulse to start migration. In previous work (Shulman, 1960a,b, 1972a, 1974, 2002) we revealed that the average fat content in the adult anchovy population necessary to start migration should be higher than 14% of wet fish mass. Anchovy populations that do not attain this level of fat content (i.e., do not finish feeding) do not begin migration under any circumstances, even if temperature decreases strongly, remaining in Sea of Azov until December and dying because of low temperatures. Occurrences of such mass anchovy mortality have occurred in several years. For example in 1953, when fodder base of anchovy in Sea of Azov weakened greatly due to a sharp fall in river runoff (which brings nutrients necessary for plankton productivity). As a consequence, in November and December 1953, the northern coast of the Kerch Peninsula (Ukraine) was full of dead anchovies. At the end of the 1980s and the start of the 1990s, another mass death of anchovy was observed, due to low fodder base caused by invasion of the alien ctenophore *Mnemiopsis leidyi* (Studenikina et al., 1991). Fat content of anchovy which remain in the Sea of Azov and do not migrate is always lower than 14%. In this situation anchovy generally do not form migrating schools.

Schematically, the interaction between external and internal factors leading to anchovy migration or not is illustrated in Fig. 4.35. This scheme is similar to the one Dolnik (1965) elaborated for migrating birds. The only difference is that, for fish, the temperature signal has more significance because it indicates the imminent approach of worsening weather in areas where schools are gathering for migration. In contrast to birds, fish usually migrate within the limits of the same climatic zone, and thus the warning signal has great significance. Overall, it is clear that the transition to the migrating condition, as happens in other animals, occurs as a result of close interaction between exogenous and endogenous factors. Therefore, the external signal may not only involve temperature, but some other factors. For example, external factors related to cod migration in the southern Gulf of St Lawrence include photoperiodism, oxygen concentration, temperature, and food supply (Comeu et al., 2002). There is also increasing evidence of endogenous seasonal variation in appetite. Cod migration is related to food obtained from consumption of herring and capelin. When food decreases, this causes an increase in appetite and motivates movement to feeding regions (Jobling, 1983). For example, food deficiency primes cod to start summer migration. In chinook salmon *O. tshawytscha*, Wagner and Congleton (2004) found that condition indicators of smoltification and nutrition of juveniles included lipid concentration in plasma (triacylglycerols and other fractions), glucose, total protein and cortisol, activity of lactate dehydrogenase, keratin kinase, and alkaline phosphatase. In rainbow trout *O. mykiss*, smoltification indicator is ATP activity (Gale et al., 2009), while in Arctic char *Salvelinus alpinus* (Glemet et al., 1998) and bluefin tuna *Thunnus thunnus* in the Bay of Biscay (Goñi & Arrizabalaga, 2010), significant factors for migration were lipid store levels.

From this it is clear that the debate between scientists that took place during the 1930s and 1940s regarding the superiority of exogenous over endogenous impulses for migration has no sense. In this debate, Marti (1932) considered that only temperature has significance as an impulse for migration for Sea of Azov anchovy, whereas Lebedev (1940) tried to prove that the impulse to migrate is apparent only when anchovy reach a certain degree of fatness (expressed as "condition factor") and that temperature did not trigger migration at all. Vorobiev (1945) was the first researcher who remarked on the unsubstantiated nature of these "extreme" points of view. He considered that interaction between both factors is significant for the anchovy's transition to the migrating condition, even though this was a purely speculative statement because at that time he had no data about fat store levels in anchovy populations. It was not until the 1950s when we revealed the level of fat content at which anchovy can perceive the temperature impulse (Shulman, 1960a,b).

However, the interaction between internal and external factors is not simple. In Sea of Azov anchovy we revealed that there is a delicate "resetting" of both factors to each other (Shulman, 1960a,b, 1972, 1974). It may be named the principle of "dosage." It was found that fish sensitivity (degree of external impulse, signal perception and its threshold value) is closely related with the same level of fat stores (Fig. 4.36).

Anchovy populations that have accumulated maximal fat stores (>22%) during the feeding process perceive the impulse to migrate when the water temperature in the Sea of Azov falls in the range 16–19°C; in this event, the temperature drop may be very small. Populations that have accumulated 17–20% fat perceive the impulse to migrate when there is a temperature drop in the range 13–15°C. Population which have accumulated 14–16% fat perceive the impulse to migrate only when the temperature drops in the range 9–12°C. Finally, populations that have accumulated less than 14% fat generally do not perceive a temperature drop as an impulse to migrate. Thus the migration of anchovy from the Sea of
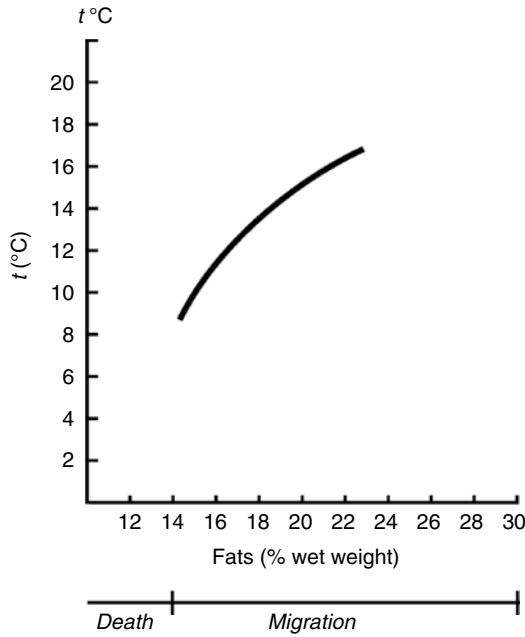
Figure 4.36 Relationship between anchovy fatness and temperature migration factor. From Shulman & Love (1999) and Shulman (2002).
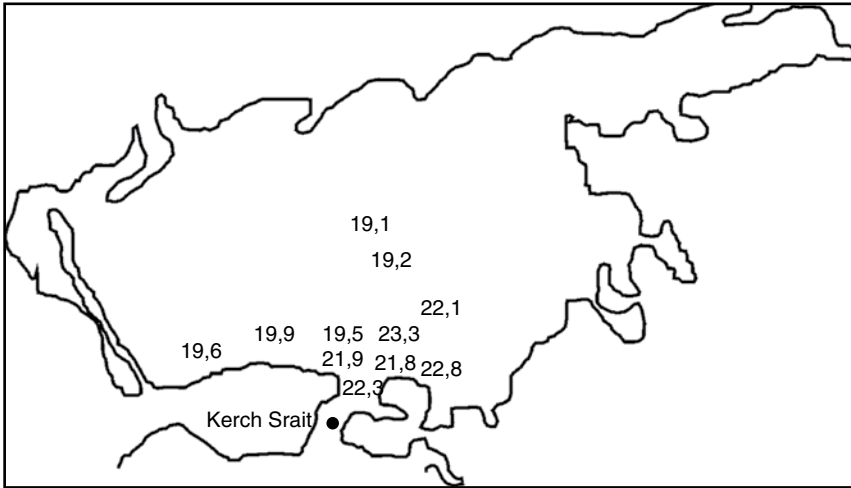
Figure 4.37 Fatness of anchovy populations in the Sea of Azov in October 1995. Numbers refer to fat content (% wet weight). After Shchepkin et al. (2002) and Shulman (2002).

Azov to the Black Sea through the Kerch Strait depends on fat stores accumulated by the populations before migration. Anchovies with higher fat stores can move out from the Sea of Azov by the end of September, whereas fish with average fat stores usually migrate during October and the poorest conditioned anchovies (lowest fat stores) in November. In addition, the data obtained during the expedition to the Sea of Azov at the beginning of
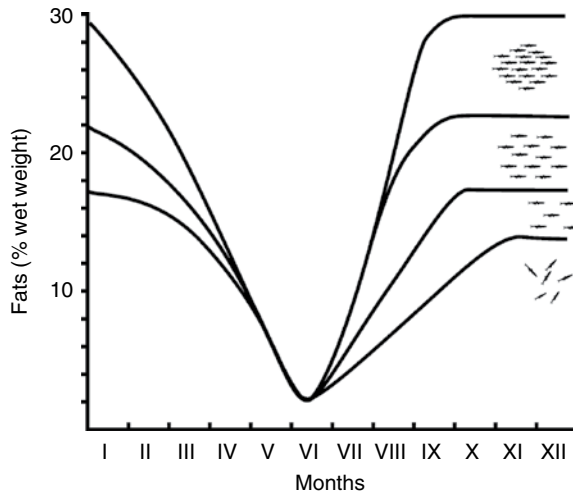
Figure 4.38 Intensity of wintering migration of Sea of Azov anchovy through the Kerch Strait. From Shulman (2002).

October 1995 showed that anchovy populations with the highest fat stores were near the Kerch Strait, i.e., they were more ready to start migrating (Fig. 4.37; Shchepkin et al., 2002; Shulman, 2002). Returning to the relationship between internal factors and fish behavior (in this case, fat store levels and density of migrating schools), fish with the highest level of fat stores migrate through the strait in extremely dense schools as they swim rigorously and in unison (Shulman, 1972a, 1974, 2002; Fig. 4.38). Conversely, the poorest-condition fish swim slowly in small schools, and their movement through the strait is sluggish. Knowledge of the regularity of the wintering migrations of Azov anchovy is important for predicting migration intensity and the periods when fish move through the Kerch Strait (Shulman, 1972a, 1974, 2002). Fishery institutions in Kerch and Rostov carry out a survey every fall on anchovy condition in the Sea of Azov and, with the help of a hydrometeorological forecast, advise on the rational development of the fishery (Shulman, 2002).

4.5.3 Distribution

All that has been discussed in relation to behavior is also related to distribution. Localization in wintering areas, displacement of migrating schools, dispersal during spawning period, feeding concentrations, and migrating schools are all important features of distribution closely connected with population metabolism. Lets take as an example the Black Sea sprat. In contrast to anchovy, this fish does not undertake long-term migrations. Sprat populations localize in certain regions and undergo rather small movements: in fall, they move offshore for spawning that will take place in winter; in spring and summer, they move onshore for feeding. Despite weak migration behavior, sprat are characterized by the same pattern explained before for anchovy, as movements of sprat are also closely related to fat reserves. In years when nutritional conditions are good (and provide high level of fat stores), sprat form dense schools that move to the coastal zone. This can be observed in all shelf regions of the Black Sea, especially the northwestern part. Conversely, in years when food supply is poor (leading to low fat stores), schools are strongly dispersed, localized far from the coast,
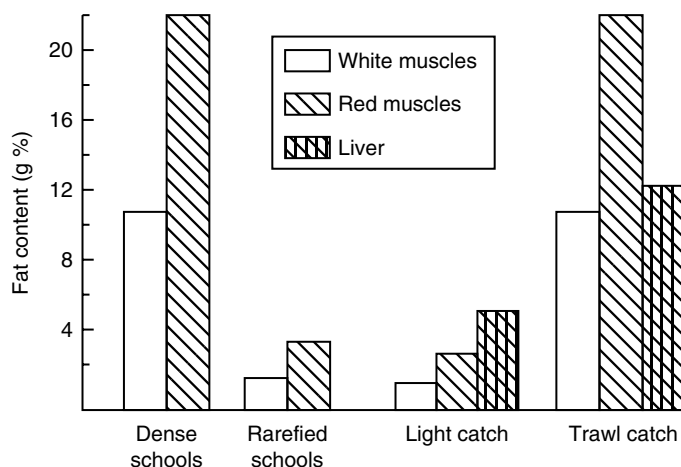
Figure 4.39   Fat content of sprat at different densities. After Gusar et al. (1987).
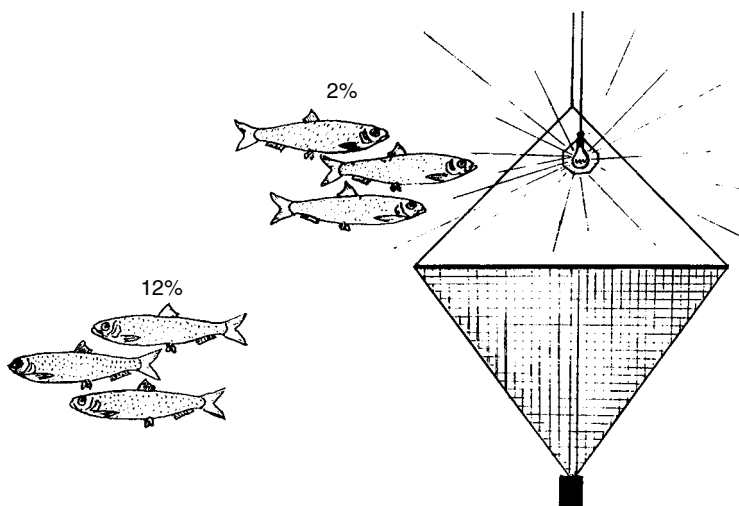
Figure 4.40   Difference in fat content of sprat caught by trawl and artificial light. After Gusar et al. (1987).

and do not concentrate on the coastal zone. There is a close connection between sprat fatness during the summer period (feeding period) and value of the catch by fishery effort (Gusar et al., 1987). This shows the significance of fat stores in forming schools not only in migrating but also in non-migrating fish species. However, many studies completely ignore the significance of physiological condition in forming fish schools, these reports mostly concentrating on oceanographic and meteorological conditions (Shulman, 2002). Interesting results have been obtained regarding the connection between density of sprat schools and fat content (Fig. 4.39). Sprat fatness is lower in less dense groups, which have a strong attraction to artificial light, than in those groups captured with trawls (Gusar & Getmantsev, 1985; Gusar et al., 1987). The explanation is that fish in the less dense groups are hungry and the orientation reflex (Pavlov, 1932) develops strongly, while those fish captured in trawls have accumulated large fat stores, are in dense schools and their feeding reflex is reduced (Fig. 4.40).
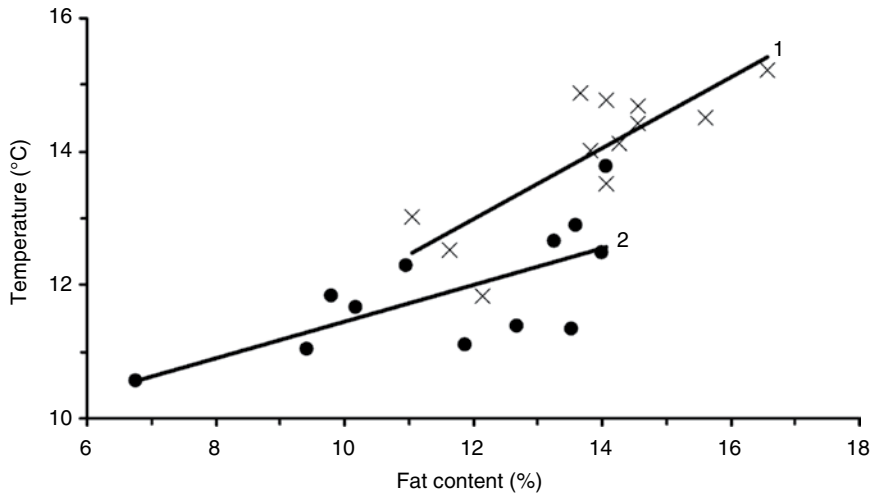
Figure 4.41 Relationship between neutral lipid content of Black Sea anchovy and temperature during approach to Caucasus Coast. 1, Adults; 2, juveniles. After Chashchin & Axelev (1990) and Shulman (2002).

An exact relationship between distribution and degree of feeding (fatness) has been revealed for different schools of Black Sea anchovy (Danilevsky, 1964; Chashchin & Axelev, 1990) (Fig. 4.41), Caspian kilka *Clupeonella engrauliformes* (Rychagova, 1989), Japanese sardine *Sardinops melanostictus* (Shwidky, 1988), Pacific saira *Cololabis saira* (Filatov & Shwidky, 1988), yellowfish *Pleurogrammus monopterygius* (Vdovin & Shwidky, 1993), and walleye pollack *Theragra chalcogramma* (Shwidky & Vdovin, 1991). Recently Nikolsky et al. (2011) established a similar connection between lipid content of anchovy in the north-western Black Sea at the end of fall feeding and the lipid content of anchovy near the Turkish coast at the same time. Lipid content in anchovy near the Turkish coast is generally higher. As anchovy migrate for wintering from northern regions to southern ones, these data show that schools arriving on the Turkish coast have more fat stores.

To finalize this section, it is important to consider a very significant phenomenon which we have not yet discussed. It is necessary to remember that populations are not only complex genetic and morphophysiological systems but energetic ones too. Thus not all individuals achieve the threshold required to perform the behavior stereotype. Swimming experiments on fish in a hydrodynamic tube can determine the exact distribution of individuals with regard to capacity for long-term resistance to stream velocity. During such experiments on Black Sea horse-mackerel, "leaders" are revealed that can swim for long periods in cruise mode (many hours, sometimes more than 24 hours) (Shulman et al., 1978). Conversely, a rather large fraction of the experimental group fail to maintain their position during the first hour (sometimes only after a few minutes) and these have been termed "recessive" individuals or "outsiders." Among horse-mackerel we found 16% of such recessives, and the same proportion was found in red mullet. It is possible that this consistency across species is not coincidental and reflects a more or less constant proportion of weak individuals in a population. In horse-mackerel, it is just these individuals that have the lowest value of energy-containing lipid fractions: triacylglycerols constituted 55 mg% in red muscle and 32 mg% in white muscle compared with levels of 102 and 87 mg% in normally swimming

fish (Shulman et al., 1978). In addition, weak fish have much lower levels of other significant lipid components, primarily DHA in phosphatidylethanolamine of red and white muscle. Conversely, increase in lipid unsaturation relates positively with metabolic activity (Shulman & Yuneva, 1990). In analogous experiments in hydrodynamic tubes, divergence in swimming capacity of coho salmon *Oncorhynchus kisutch* juveniles was observed (Zaporozhets, 1991). Juveniles of the cichlid *Aequidens pulcher* with higher catecholamines (L-dopa and dopamine) in the brain have a marked "searching" reaction (Nechaev et al., 1991). It is these juveniles that dominate in subpopulations of young fish and show more high activity for settling apart. We discussed the functional and biochemical heterogeneity of *O. gorbuscha* spawning group in section 4.5.1. During swimming in hydrodynamic tubes, blood parameters of weak fishes are lower than normal ones (Belokopytin & Rakitskaya, 1981). The detailed "social–hierarchic" population structure of *O. nerka* was studied by Semenchenko (1988) and Chebanov (1994). These authors' data show that the stress condition of subdominant individuals negatively affects the number and quality of spawned generative products. Specific differences in growth rate in juvenile yellowfish cause the appearance of leaders and outsiders (Vdovin & Shwidky, 1993). In this case there is higher elimination of outsiders. The problem of the interrelationships between hierarchies in fish has been presented by Thorpe et al. (1982).

The effect of physiological and biochemical parameters on behavior and distribution of Atlantic cod has been revealed by Love (1970) and Love et al. (1977). In this species greater locomotory activity is provided by higher glycogen and lipid content in liver, development of the vascular system (vascularization) and gemine pigment content, which is necessary for the supply of energy to contractile tissues with maximal efficiency. A good visible indicator of fish activity is pigmentation of red muscle, the degree of which changes in cod schools in different regions (Love, 1980). The most migratory form of cod exhibits a deep red color of these muscles (especially the lateral band). Such cod have been observed in regions to the south of Spitsbergen. The school undertakes long-term migration in the direction of North Cape and as far as Lofoten, where the fish spawn. Other schools, for example near the Faroe Bank, are located in specific regions and have a pale brown color of the lateral band in their red muscle. Cod populations in every region exhibit a Gaussian distribution of red muscle color, showing differences in fish condition as "leaders," "outsiders" and intermediate forms. Azov anchovy too exhibit a Gaussian distribution of fatness during wintering migration through the Kerch Strait (Fig. 4.42; Shulman 1972a).
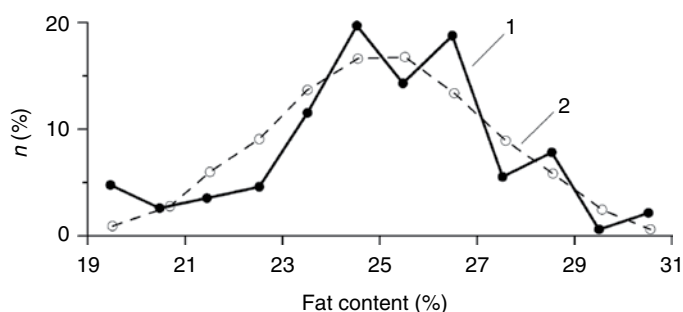
Figure 4.42 Fat content distribution in anchovy population during migration through Kerch Strait. 1, Real data; 2, normal Gaussian curve. After Shulman (1972a, 1974).

5 Influence of environmental and anthropogenic factors on fish condition

Summary: Apart from the intrinsic factors that affect fish condition, which are observable during ontogenesis, condition is affected by external factors including food availability, environmental variables, and anthropogenic factors. This chapter focuses on all these external factors. Among biotic factors, food consumption (food quantity and diet), density effects, and parasitism are major factors impinging on fish condition. Among abiotic factors, temperature, climate change, and oxygen deficiency are the most important. Anthropogenic factors such as pollution, fishing and aquaculture activities are also considered in this chapter.

Key words: food quantity, food quality, diet, parasites, sea warming, oxygen, trawling, aquaculture, contamination, climatic indices, winds, oxygen

5.1 Biotic factors

5.1.1 Food consumption

Along with reproduction, the quantity and quality of prey consumed are key for fish condition. The dependence of condition on food consumption has been thoroughly demonstrated experimentally and indirectly in the field, where there is usually a close relationship between food consumption and levels of energy reserves, whether in tropical fish such as *Pomacentrus amboinensis* in Australian waters (Kerrigan, 1994), cold-water fish such as cod in the northeastern Arctic (Jorgensen, 1992), or temperate fish species such as sprat in the Black Sea (Shulman et al., 2005). For example, in the tropical fish *P. amboinensis*, partially fed and fully fed juveniles attained not only large size but also weighed more and exhibited higher concentrations of total lipid in their tissues than starved and field fish (Kerrigan, 1994). These results support the close relationship between feeding history, somatic growth, and levels of energy reserves, principally total lipid concentration. In this regard, lipid class evaluation can reveal different feeding strategies. For example, Lança et al.

(2013) distinguished two feeding strategies in the sea lamprey *Petromyzon marinus* from the Portuguese west coast based on the analysis of lipid profile.

In previous sections we have considered that food (together with oxygen) constitutes one of the two basal channels connecting organisms and populations with the environment. Food provides organisms and populations with structural components from which they form and develop their bodies, and supplies them with energy substrates for numerous vital functions. Naturally, food is profoundly transformed once ingested. Therefore revealing the features of food supply involves not only information about quantity of consumed fodder organisms but also their quality or composition (content of proteins, lipids, carbohydrates, amino acids, fatty acids, vitamins, macroelements, and microelements), scope, intensity and efficiency of food consumption and transformation, and significance of food for survival and reproduction. Also we need to study food availability and intraspecific and interspecific competition for food.

Sometimes the combination of quantity and quality of food as well as the number of consumers (both the species under examination and competitors) and specific feeding conditions such as temperature (on which digestion rate depends) and transparency of the water (which influences the rate of hunting) is called "food supply" (Shulman & Love, 1999). With regard to the relationship between fish condition and food consumption and the difficulties in evaluating fish consumption, the evaluation of lipid accumulated by the population at the end of the feeding period was proposed by Shulman and Love (1999) as an indirect surrogate approach to estimating food consumption. It should be borne in mind that in this case lipids cannot characterize the food supply over the whole annual cycle, but only in that period when lipids are accumulated rather than consumed.

The most informative results obtained on marine fish concern the relationship between number of available nutritive organisms and defined physiological and biochemical characteristics of fish populations and individuals. It was shown many years ago in the Sea of Azov that there is an association between neutral lipid (fat) content (all data are percent per wet mass) in Azov Sea anchovy *Engraulis encrasicolus maeoticus* during the post-spawning feeding period and the zooplankton concentration (Shulman, 1972a, 1974) (Fig. 5.1). Similarly, the
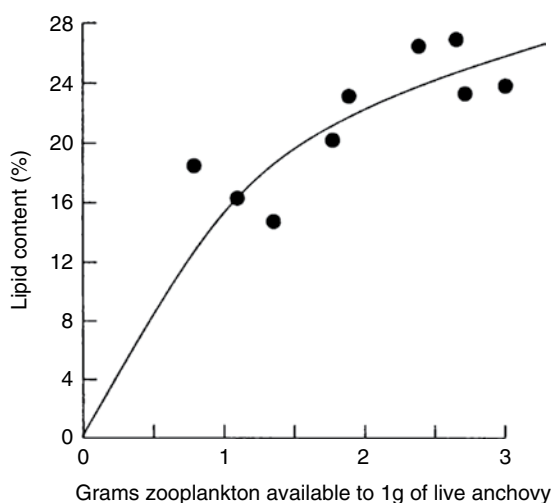
Figure 5.1 Dependence between fat content in the Azov anchovy and zooplankton concentration in the Sea of Azov. After Shulman (1972a, 1974) and Shulman & Love (1999).
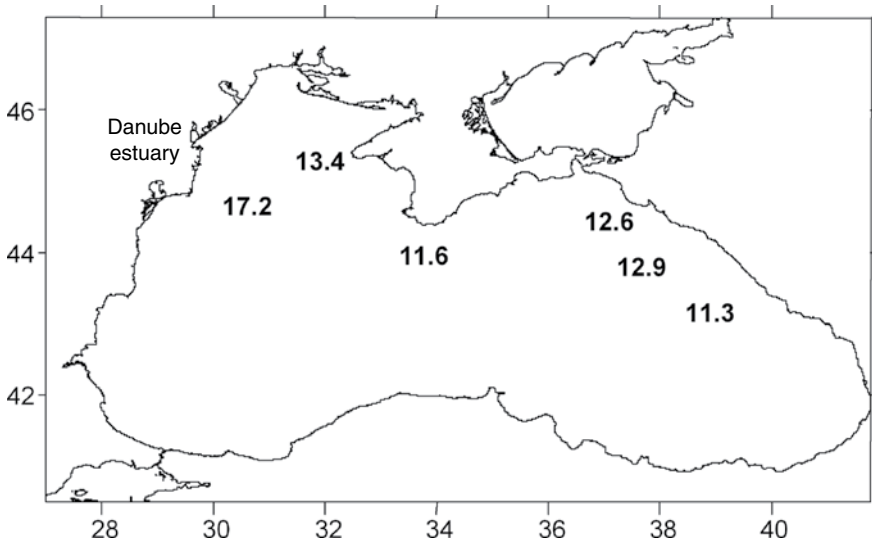
Figure 5.2 Fat content of sprat in different regions of the Black Sea. From Minyuk et al. (1997).
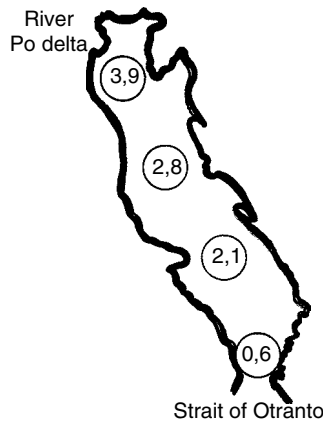
Figure 5.3 Fat content (%) of red mullet *Mullus surmuletus* juveniles in the Adriatic Sea. From Shulman (1972b).

highest fat content in Black Sea sprat *Sprattus sprattus phalericus* (Fig. 5.2) is found in the population that feeds in the region adjacent to the Danube estuary (Minyuk et al., 1997). This region has the largest abundance of nutrients, brought to the sea by Danube runoff, and from which phytoplankton and zooplankton develop. Also supporting this association are data on fat content in juvenile red mullet *Mullus surmuletus* in the Adriatic Sea along a gradient from the River Po delta (Fig. 5.3). The food supply gradient, which decreases from the River Po delta towards the Strait of Otranto, causes the fat content of juveniles to decrease from 3.9% in the delta region to 0.6% in the Strait of Otranto (Shulman, 1974). Food concentration also influences the lipid content of California sardine *Sardinops caerulea*, as was demonstrated for larvae by Hakanson (1989a,b) and for adults by Lasker (1970) and Smith and Epple (1982). The same was found for other pelagic species from the same region by Bailey and Robison (1986).

5.1.1.1 Food quantity

The relationship between food quantity (i.e., the amount of food in a unit area of water) and fish condition has been established in several studies. Interannual variability in food quantity has been shown to affect the condition of fish. For example, interannual fluctuations in the liver condition index (LCI) of five length classes of northeast Arctic cod were non-linearly related to capelin stock biomass such that the LCI decreased rapidly when capelin stock biomass was below 1 million tonnes (Fig. 5.4; Yaragina & Marshall, 2000). Yaragina and
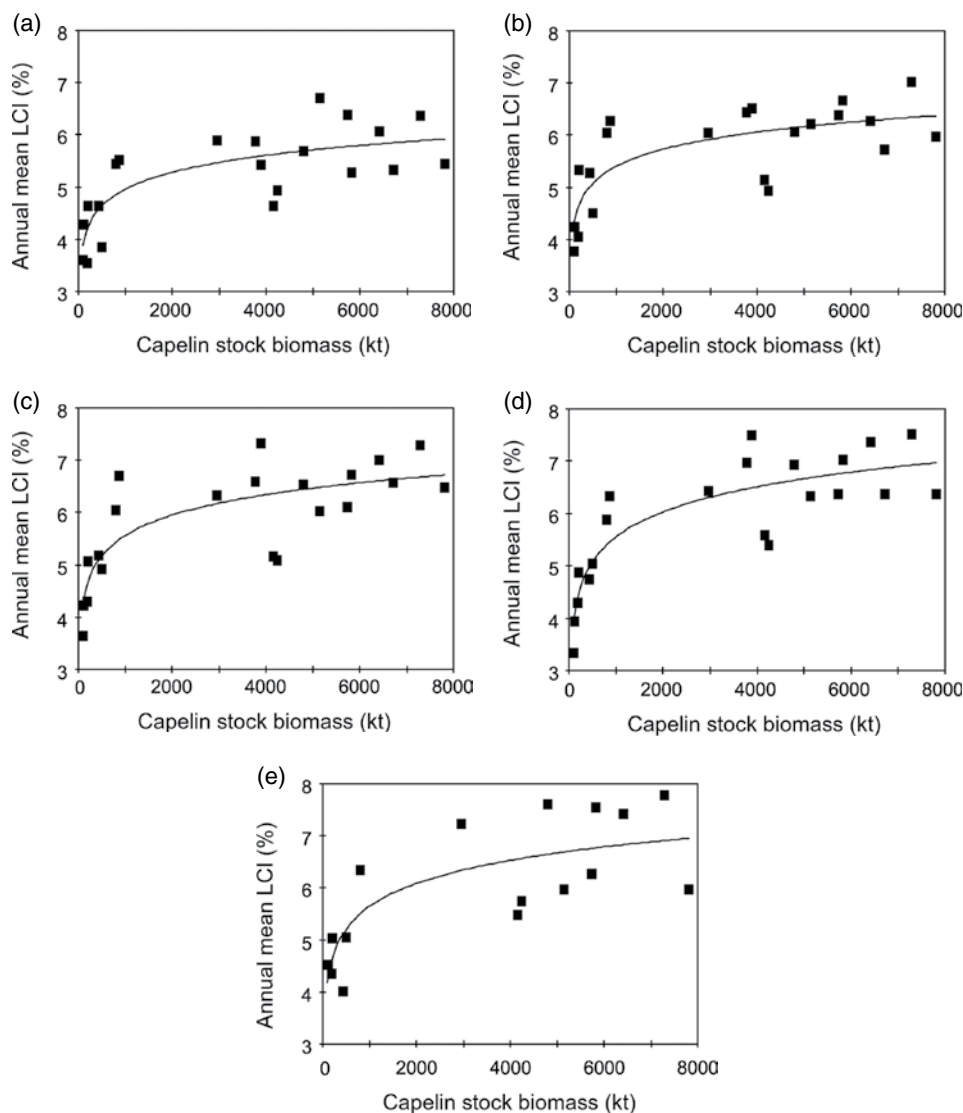
Figure 5.4 Relationship between annual mean liver condition index (LCI, %) of northeast Arctic cod (*Gadus morhua*) and capelin (*Mallotus villosus*) stock biomass (1973–1996) in the Barents Sea. Solid lines indicate the regression relationships between the annual mean LCI and ln-transformed capelin stock biomass: (a) 41–50 cm cod, (b) 51–60 cm cod, (c) 61–70 cm cod, (d) 71–80 cm cod, and (e) 81–90 cm cod. From Yaragina & Marshall (2000).

Figure 5.5 *Pelagia noctiluca*, a jellyfish species in the Mediterranean Sea. Photo by Toni Font.

Marshall (2000) also showed that the LCI of north Arctic cod were positively correlated with the frequency of occurrence of capelin in cod stomachs. These results could explain the marked decline in condition factor in the last half of the 1980s, which coincided with a dramatic reduction in relative capelin abundance (Jorgensen, 1992).

Competition for food between populations and between individual fish often explains the differences in fish condition. In several cases, declines in condition of small pelagic plank-tivorous fish have been associated with decreased food supply as a consequence of an increase in jellyfish species (Fig. 5.5), which are strong competitors of these fish for food resources. For example, in the 1980s a decrease in lipid content of sprat from 13% to 10% by the end of the feeding period was recorded in the Black Sea (Shulman et al., 2005). This drop was attributed to a reduction in the food supply for each fish as a consequence of the increase in the jellyfish species *Aurelia aurita*. Similarly, the introduction of another competitor, the ctenophore *Mnemiopsis leidyi*, into the Black Sea in the late 1980s also damaged the food supply of sprat and provoked another decrease in their fat content (Shulman & Love, 1999). *Mnemiopsis* made an even greater impact on the nutritive base of anchovy.

For many temperate and cold-water species, lipid reserves may be vital for survival over the winter months as prey resources decline and this is why lipid storage typically increases just prior to the onset of winter. For example, cod has to cope with periodic food shortages in winter (Schwalme & Chouinard, 1999; Dutil et al., 2003). When deprivation or shortages occur, cod must draw energy from body reserves accumulated during periods when food was available (Black & Love, 1986; Lambert & Dutil, 1997a). As a result, cod exhibits marked seasonal variations in energy reserves, with peak somatic condition factors being reached in the fall (Lambert & Dutil, 1997b; Schwalme & Chouinard, 1999). Thereafter, declines in carcass and liver weights occur steadily throughout winter (December to April) when very little feeding occurs, the animals losing 25–27% of carcass weight and 76–84% of liver dry weight during this time (Schwalme & Chouinard, 1999). About 90% of the loss in carcass and liver dry weight of overwintering Newfoundland cod is being used to

meet metabolic energy requirements. For these authors, the condition factors and hepatosomatic indicators of cod in spring were low enough to suggest that winter starvation may cause mortality in this stock. In fact, in starved cod the lipid content of the liver can be as low as 2% (Love, 1958) when lipids normally constitute more than 50% of the liver weight of cod (Lambert & Dutil, 1997b). This pattern has also been shown in juvenile fish. For example, O-group sand smelt *Atherina boyeri* lays down fat prior to the onset of winter because during the winter the fish do not feed for some 100 days and rely on this fat for energy (Henderson et al., 1988). Similar results have been reported in freshwater fish inhabiting temperate and cold rivers. For example, a study on juvenile bluegill sunfish *Lepomis macrochirus* in northern United States rivers (Booth & Keast, 1986) showed that over the winter period (October–April) condition dropped and lipids were depleted to supply the energy requirements of inactive wintering fish. Condition was restored to summer levels within the following month and fat reserves were built up from 7 to 14% of body weight over the entire summer.

Not only food availability can determine food consumption but also behavior due to reproduction. For instance, Atlantic cod cease feeding for as much as 70 days during pre-spawning and much of the spawning period (Fordham & Trippel, 1999). The relationship between condition and food availability exists not only for adults but has also been well documented in fish larvae. For example, RNA/DNA ratios of anchovy larvae from the Catalan Sea showed a positive correlation with food availability (fluorescence and microzooplankton organic matter). Nevertheless, this relationship was not observed with anchovy larvae from the Gulf of Lions (García et al., 1998).

For small pelagic species, the seasonality in condition often matches primary productivity. For example, the hepatosomatic index of sardine in the northeast Mediterranean matched the spring burst of primary productivity and was followed by fluctuations in somatic condition and mesenteric fat by a 2-month lag (Ganias et al., 2007).

Notwithstanding the seasonal pattern of condition of many shallow-water species, seasonal variation in feeding and condition appear small or nonexistent in deep-sea top predators such as macrourids (Drazen, 2002). The lack of seasonality has been attributed to many factors including the ability of deep-sea predators to switch to whatever food is available or the absence of seasonality in reproduction and growth of these fish (Drazen, 2002). Also in many studies food is not observed as a limiting condition in juveniles in the highly productive estuaries, indicating that prey resources are often likely adequate for newly settled fish in these highly productive habitats. For example, the RNA/DNA ratio of southern flounder (*Paralichthys lethostigma*) from the Galveston Bay Estuary (Texas) was well above a minimum ratio for fed larvae of winter flounder and starved Japanese flounder, suggesting food was not a limiting factor in any of the bays or habitats sampled (Fig. 5.6; Glass et al., 2008). Moreover, this study showed that the RNA/DNA ratio of the individuals indicated that over 90% of the southern flounder were in the same nutritional condition as well-fed fish from laboratory studies (fed versus starved). Also Rooker and Holt (1997) reported the RNA/DNA ratio of wild red drum from Texas estuaries and found that the nutritional condition of wild-caught individuals was well above the minimum or starved baseline estimate.

It is also possible to consider a wider geographical scale. For example, zooplankton concentration in the Sea of Azov (200–500 mg/m^3) is higher than that in the Black Sea (100–200 mg/m^3), and this is higher than that in the Mediterranean (average 50 mg/m^3) (Zenkevich, 1963). Fatness of three subspecies of anchovy (*Engraulis encrasicolus maeoticus*, *E. e. ponticus* and *E. e. mediterraneus*) at the end of feeding periods were found to be
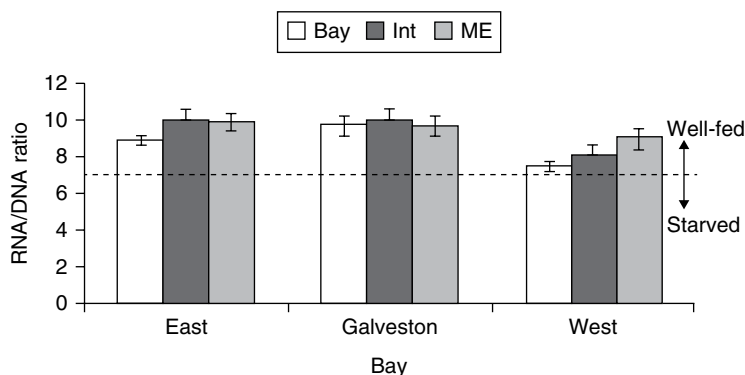
Figure 5.6 Mean RNA/DNA ratio for newly settled southern flounder *Paralichthys lethostigma* from the Galveston Bay Estuary in 2005. Ratios are given by bay and habitat. Error bars represent 1 SE. Habitat codes: Bay, bay zone ; Int, intermediate zone ; ME, marsh edge. RNA/DNA ratios above dashed line are in a "well-fed" range versus below the line in a "starved" range as found in laboratory studies. From Glass et al. (2008).

20–25%, 10–15% and less than 7% in the Sea of Azov, Black Sea and Mediterranean, respectively (Shulman, 1974). Compare this with the zooplankton concentration in the Baltic and Caspian seas (200–300 mg/m^3) (Zenkevich, 1963) and the fatness of Baltic sprat *Sprattus sprattus* (Biryukov, 1980; Kolakovska et al., 2003) and Caspian kilka *Clupeonella engrauliformis* (Rychagova, 1989), which both have 15–20% fat content, as well as Sea of Azov kilka *Clupeonella cultiventris*, which has fatness close to that of Azov anchovy. Recent research by Lloret et al. (2005) showed that levels of accumulated lipids in *Pagellus acarne* and *P. erythrinus*, as well as *Diplodus sargus*, are higher in the Gulf of Lions than in the Catalan Sea. It is known that Gulf of Lions is more productive than the Catalan Sea as it is enriched by nutrients from the strong wind mixing and from the Rhone river flow. It is possible to conclude that each sea basin (more exactly its pelagic zone) has specific trophic capacity which may supply pelagic fish populations with specific fat stores.

Although many of the examples cited above refer to the so-called "fatty" fish (those with large lipid stores that accumulate principally in the muscle), "lean" fish (low muscle lipid stores) such as Atlantic cod *Gadus morhua*, which accumulates its energy stores in the form of lipids and glycogen in liver, also show a relationship between fat accumulation and food quantity. For example, Love (1974) and Black and Love (1986), who studied cod in a wide area of the northern Atlantic, showed that population inhabiting the Faroe Bank region has maximal energy stores. This region is strongly affected by upwelling which transports large quantities of food. In this sense, data on hepatosomatic index of Atlantic cod showed that accumulated energy stores are related to stock numbers of capelin *Mallotus villosus* (the basal food of cod) in the Norwegian and Barents seas (Marshall et al., 1999, 2000) Aditionally, several studies have also shown that interannual fluctuations of fat content are closely related with the environment (primarily with nutrition factor).

5.1.1.2 *Food quality (diet)*

The second important part of investigations dealing with fish food is the quality (composition) of the food, particularly the characterization of the essential components and their physiological effects. This comprises the relation between biochemical characteristics of

Table 5.1 Seasonal variation in the principal fatty acids (%) in the muscle of capelin *Mallotus villosus*

Fatty acid	January	August	January	August
14:0	4.8	5.9	3.8	7.6
16:0	22.1	25.1	19.3	23.0
16:1	8.5	8.3	6.9	7.8
18:0	1.8	1.2	1.4	1.1
18:1	26.0	28.0	20.8	22.4
18:2	1.4	1.8	1.4	1.7
18:4	1.4	4.6	1.5	4.2
20:1	2.3	1.3	4.1	4.4
20:5	13.8	10.8	16.8	10.7
22:1	2.0	1.0	3.8	4.4
22:6	11.3	6.7	15.4	8.3

*Source*: after Henderson et al. (1984) and Shulman & Love (1999).

fodder organisms consumed by fish and the biochemical characteristics of the corresponding fish populations. The most relevant investigations deal with the influence of food on the composition of fish lipids. This problem is the subject of classic work by Lovern (1964), Ackman (1967, 1989, 2005), Sargent (1976) and Sargent et al. (1987, 1995). Yet in the first half of the last century it was found that fatty acid composition of fish lipids has the same composition as that of food (Lovern, 1964). This relates especially to neutral lipids (triglycerides, fats) stored by fish during the feeding period. Their composition includes saturated, monoenic, and dienic acids (main ones listed in Table 5.1, which have high calorific value and are used for energy requirements of organisms). Polyenic (polyunsaturated) fatty acids are mainly accumulated in the form of polar lipids (phospholipids): phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, and others. Their initial forms are linolenic (*n*-3) and linoleic (*n*-6) fatty acids. It was shown that marine fish contain mainly linolenic acids and freshwater fish mainly linoleic acids (Lovern, 1964). This is a reflection of lipid composition in algae; in marine waters they synthesize linolenic acids (18:3 *n*-3), while in fresh water they synthesize linoleic acids (18:2 *n*-6). These acids are then transmitted through trophic chains to higher trophic levels (fish and even birds and mammals). During the processes of elongation and desaturation, the linolenic and linoleic acids are transformed into polyenic or polyunsaturated fatty acids (PUFAs), especially highly unsaturated fatty acids (HUFAs). In freshwater fish these comprise arachidonic acid 20:4 *n*-6 and others. However, as Sargent (1976, 1995), Fraser et al. (1989), Sargent et al. (1995), and Bell and Sargent (1996) found, marine fish have lost their capacity to transform linolenic and linoleic acids to polyenic acids, so they obtain PUFAs and HUFAs only from food and therefore such acids are essential for marine fish. The most significant are eicosapentaenoic acid (20:5 *n*-3) and docosahexaenoic acid (22:6 *n*-3). They are important constituents of the bilayers comprising cellular and subcellular membranes, and are essential for ion transport and for regulating the viscosity of membranes (and consequently metabolic and functional activity of the whole body). Important indicators of this activity include not only their content in phospholipids of tissues, cells, membranes and organelles but their ratio to each other [coefficients $\Sigma(n\text{-}3)/\Sigma(n\text{-}6)$ and $22:6n\text{-}3/20:5n\text{-}3$]. For marine fish, comparison of their polyenic acid composition with that of food shows preliminary consumption of defined fodder organisms (Sargent et al.,

1995; Iverson et al., 2002). With regard to neutral lipids, it must be remembered that wax ethers play an important role in feeding in some marine fish (Sargent, 1976; Henderson et al., 1984. Fish consume wax ethers when they eat copepods and retain them in this form for long periods until they are transformed into triglycerides (when triglycerides stores are consumed).

The influence of other food components on the biochemical composition of marine fish has been less well studied. Proteins of fodder organisms significantly affect the protein and nitrogenous metabolism of fish. The importance of essential amino acids as a stimulant of fish growth was shown by Cowey et al. (1962). The presence of essential amino acids in a fish characterizes its nutrition. The processes of transamination and desamination have great significance in protein and nitrogenous anabolism and catabolism, which are closely connected with lipogenesis and gluconeogenesis, i.e., accumulation of fats and glycogens (Wagner & Congleton, 2004).

Overall, it is clear that differences in fish condition are explained not only by the quantity of food consumed but also by variations in diet because different prey have different quality/energetic content. Although in most studies the nutritional quality of prey is assumed to be constant over space and time, species can exhibit considerable variation in their value as prey items depending on length/age, season or year. Thus, for example, a significant positive relationship was found between length and total lipid content of Atlantic herring (*Clupea harengus*) from the Bay of Fundy, Canada (Lane et al., 2011). Atlantic herring from different years and seasons also had significantly different fatty acid signatures, with summer fish being significantly fatter than winter fish.

The diet of cod in Newfoundland and Labrador waters affects not only growth and reproduction but also fish condition (Rose & O'Driscoll, 2002; Sherwood et al., 2007). According to Sherwood et al. (2007) the benefits of a more pelagic diet in medium-sized (30–69 cm) cod included higher somatic condition, higher liver index (lipid stores), and greater spawning potential (decreased incidence of atresia). In some cases, the abundance of a particular prey determines the condition of a fish, as occurs with cod and capelin. Several examples show that optimum abundance of capelin is not a luxury but a necessity for cod, and that without abundance of capelin the growth, condition, and reproductive potential of northern cod will decline (Rose & O'Driscoll, 2002). Hence, seasonally adjusted cod liver condition in Newfoundland waters was strongly associated with capelin availability, and the liver index was highly correlated with gonad size in mature females (Rose & O'Driscoll, 2002). Cod of the northern stock in the Hawke Channel in the 1990s were in poor condition compared with those from the same stock further south because of lower availability of capelin in the north. The main food item of cod in the Hawke Channel was pandalid shrimp, which are not such a good source of lipid for cod as capelin or herring *Clupea harengus* (Rose & O'Driscoll, 2002). According to these authors, shrimp represent a good source of protein but not lipid, and are therefore a relatively low-energy food source for cod. Furthermore, whole shrimp contain much indigestible chitin, which may lower their overall digestibility and nutritional value to cod. Accordingly, the authors suggested that the inability of Hawke Channel cod to regain condition quickly after spawning relates directly to the lack of capelin in adjacent waters. In conclusion, the evidence suggests that northern cod were in poor condition in Newfoundland waters in the 1990s and that some populations suffered poor recruitment and high levels of adult mortality where capelin were not abundant. In view of the links between capelin availability and cod diet, growth, condition, and reproductive potential, the authors suggested that northern cod stock rebuilding requires capelin.
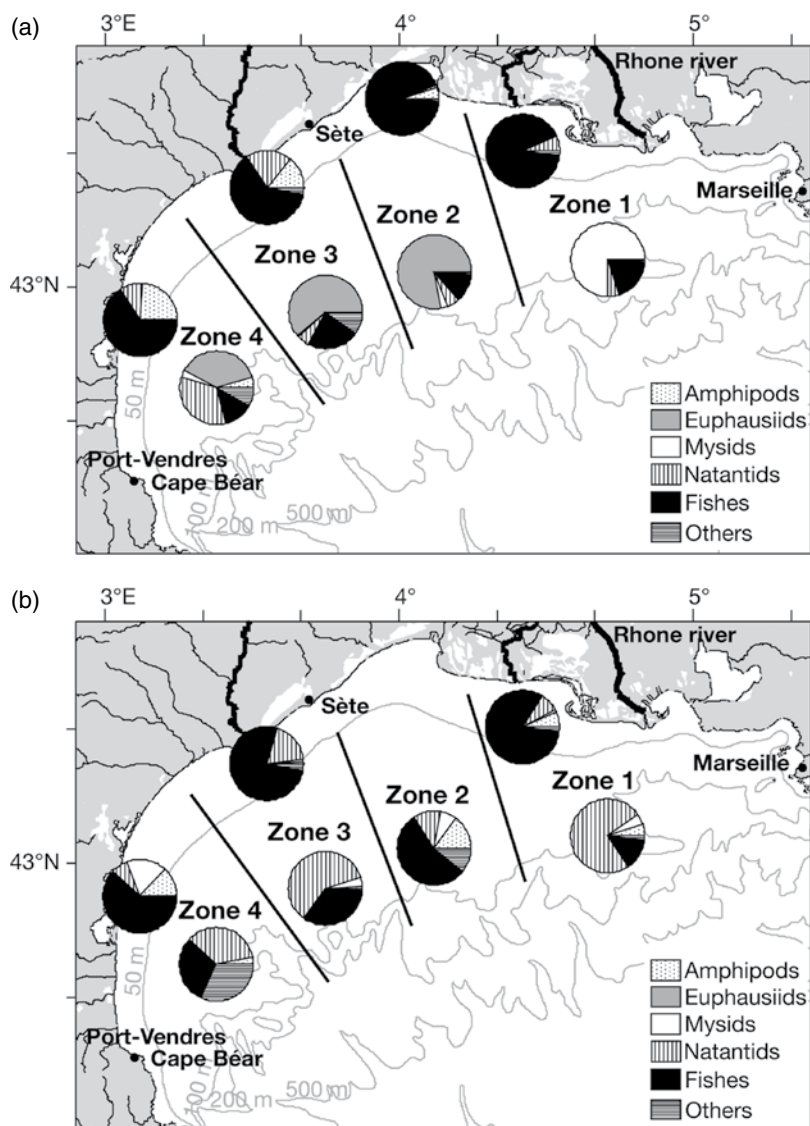
Figure 5.7 Hake (*Merluccius merluccius*) in the Gulf of Lions (northwest Mediterranean). Spatial and annual variability in diet composition (%IRI) of juveniles of 10–14 cm total length sampled in (a) 2002 and (b) 2003. (One area not shown in 2003 because of small sample size, *n* = 3.) From Ferraton et al. (2007).

In the Gulf of Lions (northwest Mediterranean), diet was also shown to affect condition and abundance of juvenile European hake (Ferraton et al., 2007). This study revealed that spatial and annual variability in diet composition of juveniles (Fig. 5.7) significantly influenced the condition of hake. Hence, the relative condition of juveniles feeding on mysids and euphausiids in a given year was higher than that of juveniles that preyed on natantid shrimps a year after. Thus for example, in 2003, juvenile hake located in deep waters had a lower condition factor than those living in shallow waters (Ferraton et al., 2007). According
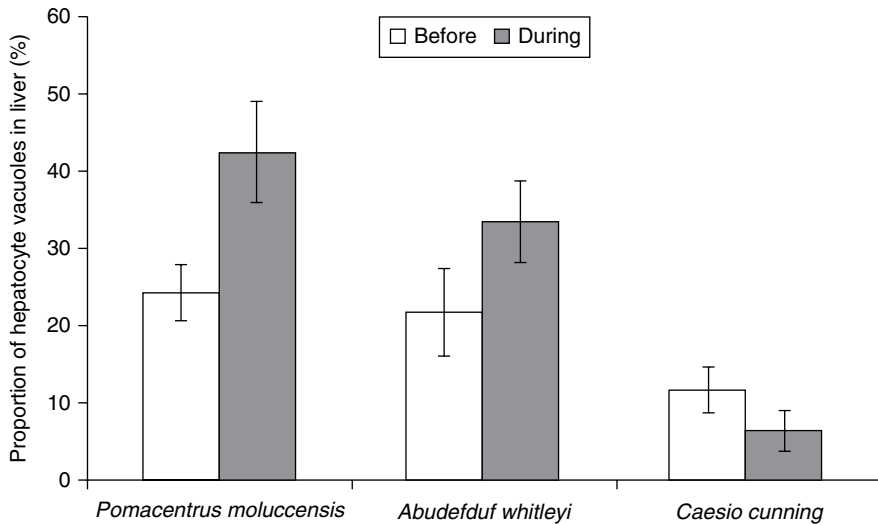
Figure 5.8 Mean proportion (±SE) of lipid vacuoles found in sections of liver of three planktivorous fish collected before and during mass spawning of corals from the northern Great Barrier Reef. Data are pooled across replicate sites ($n = 20$). From Pratchett et al. (2001).

to the authors, the main difference in the feeding of juveniles in deep waters was a diet composed mainly of shrimps in 2003, which provided a less energetic source of food than fish, the main prey in shallow waters, and small crustaceans (mysids and euphausiids), the main prey in deep waters in 2002, when no bathymetric differences in condition of juvenile hake were found (Ferraton et al., 2007). Considering these results, Ferraton et al. (2007) hypothesized that enhanced survival after benthic settlement of hake could be partly related to the availability of an adequate food supply in a given year. Similar to this, Paul et al. (1990) revealed for pollock (*Theragra chalcogramma*) in the North Pacific that the poorest growth in weight is shown by pollock fed amphipods, the prey with the lowest energy content, while the best growth is obtained by pollock fed herring, which have the highest energy content.

In the case of the Iberian sardine (*Sardina pilchardus*) it was shown that dietary composition varied seasonally and geographically and depended to some extent on the prevailing food conditions, and these differences were strong enough to be reflected in the lipid content of fish, which can have a strong impact on reproduction success for this species (Garrido et al., 2008). Also, two coral reef fish (*Pomacentrus moluccensis* and *Abudefduf whitleyi*) from the Great Coral Reef feeding extensively on coral eggs, which have a very high lipid content (50–70%), amassed considerable lipid stores during the period of coral spawning (Fig. 5.8; Pratchett et al., 2001). Thus, the pulse of lipid-rich prey provided by mass spawning of corals to particular coral reef fish contributes to the increase in energy reserves of these fish, which according to the authors could greatly improve the quality and survivorship of their progeny.

Diet is also an important factor influencing condition of capelin. The spatial and interannual changes in the diet of capelin, which are linked to sea temperature (Fig. 5.9), influences the condition of this species in the North Atlantic (Orlova et al., 2010). Fat content was highest in northeastern regions and strongly influenced by capelin diet. Capelin appears to select for the Arctic copepod *Calanus glacialis*, which is considerably larger and contains
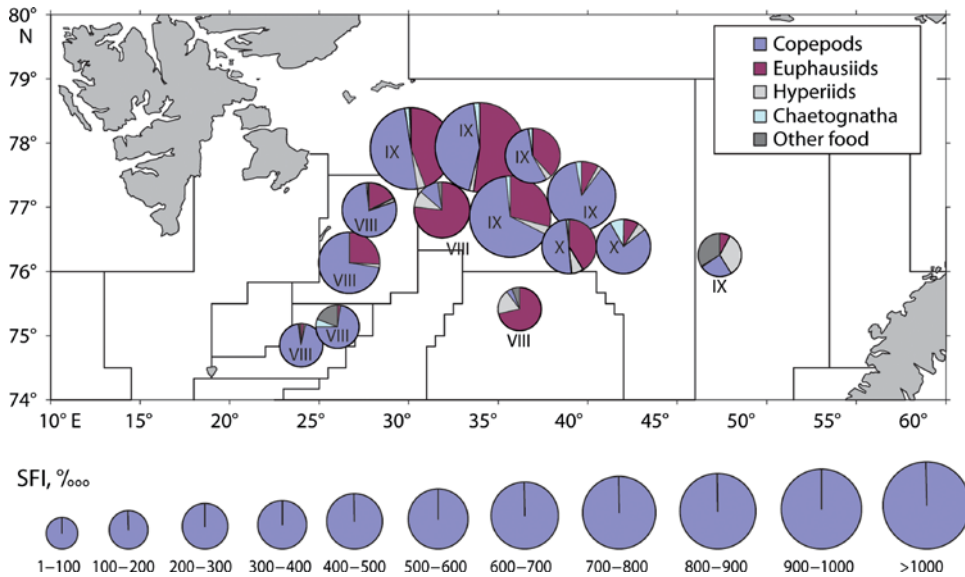
Figure 5.9 Capelin (*Mallotus villosus*) in the Barents Sea. Capelin feeding intensity and dietary composition in a very cold year (1978). Size of circle indicates stomach fullness index (SFI) and shading of each circular chart indicates mean percentage (by mass) contribution of each prey group. All size classes are combined into one circular chart per station. Roman numeral in each circle indicates sampling month. From Orlova et al. (2010).

more lipid than the Atlantic-boreal *C. finmarchicus* (Scott et al., 2002), and for larger (and more fat-rich) life stages (Orlova et al., 2010).

Of all the constituents of the diet of fish, it seems that the amount of essential fatty acids (EFAs) plays an important role in determining condition. EFAs are produced only by plants and must be obtained by fish through their diet because they are required as components of hormones and cell membranes and thus deficiency can limit the reproduction of marine invertebrates and fish. For example, natural variability in EFA content of common wolffish *Anarhichas lupus* eggs correlates with hatching and survival rates (Halfyard et al., 2001). Furthermore, field-collected Atlantic herring *Clupea harengus* larvae show order of magnitude variability in EFA and lipid content that correlates with dietary EFA restriction and poor nutritional condition (Fraser et al., 1987). Larvae of other fish species show similar variability in lipid content that correlates with diet quality (Lochmann et al., 1995). The potential for EFA limitation is likely highest for larval and juvenile fish because rapid growth produces high EFA requirements (Tocher, 2003). Fat content, which is an important indicator of capelin reproductive potential and their food value for predators, was strongly influenced by capelin diet (Orlova et al., 2010).

It also seems that on some occasions condition is related to prey diversity. Thus, De Raedemaecker et al. (2011) found a significant correlation between a morphological condition index and prey diversity in the gut, suggesting that fish which specialize on a limited number of prey items (perhaps due to a greater abundance of certain prey) may do better than fish which feed on a wide range of prey types.

Sometimes, low quantity and quality of prey may coincide, leading to a critical situation for the condition of their predators. For example, declines in the condition of the coral-feeding butterflyfish *Chaetodon lunatus* in Australian waters occurred in

conjunction with a period of coral bleaching that provoked a reduction in the quantity and quality of coral prey (Pratchett et al., 2004). Conversely, when high quantity and quality of prey coincide, condition increases greatly. Furthermore, increased condition of wild fish associated with farms has been observed in Mediterranean horse-mackerel (*Trachurus mediterraneus*) by Fernández-Jover et al. (2007) and in saithe (*Pollachius virens*) by Skog et al. (2003) because of direct feeding of fish on pellets lost from farms. In fact, net-cage fish farms attract a great number of wild fish, altering their behavior and possibly their physiology.

5.1.2   Density effects on condition

Occasionally, density effects due to competition for food are behind the inverse relation between condition and abundance that has been observed in a number of stocks. Evidence of density-dependent effects on the condition factor of North Sea sole (*Solea solea*) has been reported by Rijnsdorp et al. (1991), whereas in the Pacific Ocean the decreases in fatness of Japanese sardines were strongly related to the cumulative sum of population abundance that each year-class experienced (Kawabata et al., 2011). It is thought that insufficient food owing to the density-dependent effect of an abundant population at feeding grounds resulted in a decrease in the growth rate for small-bodied sardines that invest their energy intake in body growth, and a decrease in fatness for large-bodied adults that accumulate fat for the next reproduction. Similar to this, Vasconcelos et al. (2009) indicated that the negative relationships between the RNA/DNA ratio and juvenile densities of sole (*Solea solea*) and flounder (*Platichthys flesus*) in several estuaries along the Portuguese coast were due to density-dependent processes that may exist in estuarine areas with higher juvenile densities. In laboratory experiments, Lambert and Dutil (2000) also found a negative effect of increased stocking density of cod on the condition index and postulated decreased food intake as the cause. On some occasions, the density effects depend on other factors such as depth. Furthermore, adult energetic condition at the end of the main feeding period significantly correlates with variation in catches of sardine (*Sardina pilchardus*) on Portuguese coasts one year later (Rosa et al., 2010).

Another example is that of sprat in the Black Sea. The outburst of sprat numbers in the Black Sea at the beginning of the 1980s resulted in strong decreases in its fat stores. The competition between different species also has great importance. The outbreaks of medusa *Aurelia aurita* at the beginning of the 1980s and the mass invasion at the end of that decade by the ctenophore *Mnemiopsis leidyi* undermined the fodder base of planktivorous fish and decreased sprat fatness at the end of the feeding period from 15.5% to 9% (Shulman et al., 2009b; Minyuk et al., 1997). An even higher impact was observed with *Mnemiopsis* on anchovy in the Sea of Azov: anchovy fatness decreased from 20–30% to 10–15% (Studenikina et al., 1991), resulting in collapse of the fishery.

However, other studies did not find any density-dependent effect on condition, possible because the abundance of prey may fluctuate as much as the abundance of the stock, thereby obscuring a possible density-dependent condition regulatory mechanism (Jorgensen, 1992). For example, no evidence of density-dependent effects was observed in the condition of North Sea cod (Rijnsdorp et al., 1991). And some studies even indicated a positive relationship between stock density and fish condition, supporting the idea that individuals aggregate selectively at areas that provide optimal feeding conditions, and that optimal habitats enable greater accumulation of body lipid reserves than do suboptimal habitats. In the North Sea, for example, the hepatosomatic index of haddock was better at stations where this species
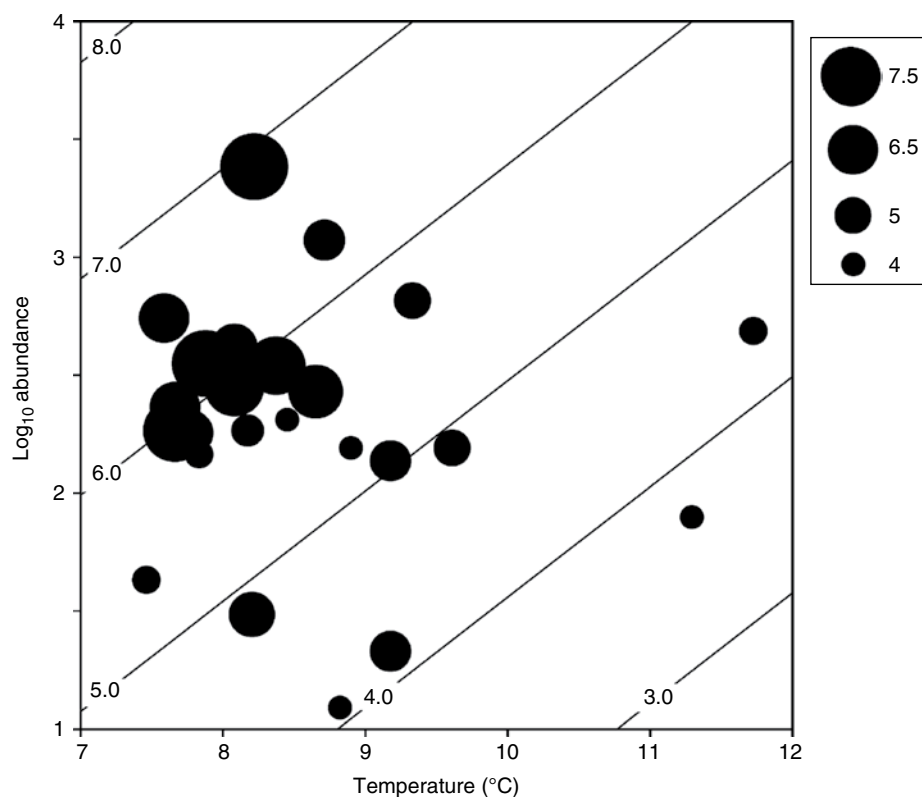
Figure 5.10 Haddock hepatosomatic index (I_H, filled circles) in relation to temperature and abundance. Circle size indicates magnitude of I_H. The fitted regression of I_H is plotted as contour lines. From Hiddink et al. (2005).

were most abundant and where temperature was low (Fig. 5.10; Hiddink et al., 2005). The authors hypothesized that energy intake was higher at the high-abundance locations because more food was available per fish at these locations. According to them, despite the fact that no data describing food availability for haddock were collected, since the better condition could not be explained by less energy expenditure in relation to temperature, energy intake must have been higher at the high-abundance locations. Therefore, it is likely that more food was available per fish at the high-abundance locations. Similar to this, Francis (1997) found that a site supporting the highest density of juvenile snapper *Pagrus auratus* in New Zealand waters had the highest hepatosomatic index, suggesting that juvenile snapper aggregate at selected sites that provide the best feeding conditions. Further, Jørgensen et al. (1993) found that the condition of Arctic char (*Salvelinus alpinus*) reared at high stocking densities improved with time, while the condition of the same fish stocked at low densities for the same time did not. Further, Shulman et al. (2005) found that the dynamics of fat content in Black Sea sprat was, to a large degree, similar to the long-term changes in stock biomass of this fish. The positive relationship between fat content and sprat biomass was attributed to the food supply: during periods of high food supply, the condition increased with the consequent and immediate benefits for the productivity of this short-lived species (Shulman et al., 2005).

Figure 5.11   Parasitic copepod attached to the gill of European hake *Merluccius merluccius*. Photo by Dolors Ferrer.

5.1.3 Parasites

Parasitic infections in fish are a specific form of interspecific cooperation. Parasitism and its potential negative influence on fish may be considered a natural factor, developed during phylogenesis, and is a significant component of the original ecosystem of parasite and host. Parasites (Fig. 5.11) can disturb the intensity and efficiency of food utilization in fish, worsen their physiological condition, and decrease abundance, biomass and production of populations. On the other hand, they constitute a catalyst for natural selection, regulating the proportion of each fish species and population in the marine and freshwater ecosystems.

Low energy reserves decrease the immunity of fish, increasing mortality through disease (Mikryakov, 1978, and other examples). Several studies have established an inverse relationship between condition factor and disease prevalence. In laboratory experiments, for example, no mortality or sign of disease was observed among cod with the highest condition factors, whereas all cod with the lowest condition factors died within a short period of time when refed following a period of food deprivation (Dutil et al., 2006). Among diseases,

parasites have been quite extensively studied in relation to fish condition. Fish serve as hosts to a range of parasites that are taxonomically diverse and that exhibit a wide variety of life-cycle strategies (Barber et al., 2000). Several studies have shown that parasites can reduce growth, fecundity and survival, and change behavior and sexual characteristics of the infected host, and these changes could have significant consequences not only at the individual level but also at population, community and ecosystem levels (Barber et al., 2000; Marcogliese, 2002; Iwanowicz, 2011). Host–parasite relationships are often unbalanced and parasites can severely compromise the condition of their hosts and even kill them (Jones et al., 2005). Most of the studies showing the effects of parasites on condition of wild fish populations have been carried out in freshwater ecosystems (see, for example, Neff & Cargnelli, 2004; Moreira et al., 2010; Heins & Baker, 2011; Ferguson et al., 2012). However, parasites are often overlooked as a factor affecting fish condition and stock productivity in marine ecosystems (Lloret et al., 2012). In marine ecosystems, most of the severe fish parasitic infections have been reported in aquaculture, normally related to the artificial culture conditions, where fish densities are abnormally high (Rohde & Littlewood, 2005). The effects of parasites on wild marine fish populations are often underestimated and the relationships between parasitism, condition, reproduction, and natural mortality of marine exploited species remains largely unknown.

Basic measurements of parasite infection include the prevalence (percentage of individuals of a particular host species infected by a particular parasite species), intensity (number of individuals of a particular parasite species in a single infected host), and abundance (total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of individuals of the host species examined) (Bush et al., 1997).

Several studies have revealed negative relationships between high parasite loads and condition of marine fish (Adlard & Lester, 1994; Faliex & Morand, 1994; Williams & Jones, 1994; Durieux et al., 2007; Fogelman et al., 2009). Pelagic fish constitute well-known examples where parasites affect the energy reserves of the individuals and populations. In the case of anchovy in the Black Sea, the presence of parasites affects anchovy's lipid content (Shchepkina, 1985), besides the effect of food supply (Shulman, 1974, 2002). In particular, infection by helminths is an important factor influencing the lipid content of the Black Sea anchovy population. Individuals with high levels of parasitism exhibit lower levels of lipid content, particularly triglycerides, which reduces the state of readiness of the anchovy for migration and wintering and may therefore influence the reproduction of this species (Shchepkina, 1985). Shchepkina (1980a,b) showed that infestation of Black Sea anchovy larvae by the nematode *Controcaecum aduncum* considerably decreases triglyceride content in fish liver and red and white muscle (Fig. 5.12). The trematode *Cryptocotyle concavum* causes a similar effect on round goby *Neogobius melanostomus* (Fig. 5.13). Decrease in triglyceride content varies in both cases from 25 to 71% in relation to tissue and studied species. Calculations show that production of total lipids is less than 20–25% in infested fish in comparison with ones that are free of parasites.

In addition, parasitism affects the condition of herring in the North Atlantic. On the individual macroscopic level, infection by a fungus-like protozoan in Norwegian spring-spawning herring significantly reduced not only body condition but also reproductive capacity (Kramer-Schadt et al., 2010). There is a strong seasonality in infection prevalence within years, with peaks in summer and winter. Summer peaks consisted of infected fish unable to follow the migration routes to the feeding grounds (Kramer-Schadt et al., 2010).
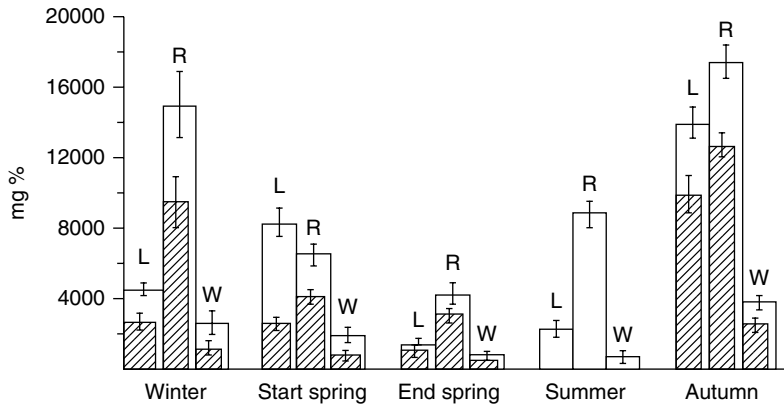
Figure 5.12 Influence of nematode larvae on triacylglycerol content (mg % wet mass) in anchovy *Engraulis encrasicolus ponticus*. L, liver; R, red muscle; W, white muscle; unshaded columns, weakly infested; shaded columns, heavily infested.
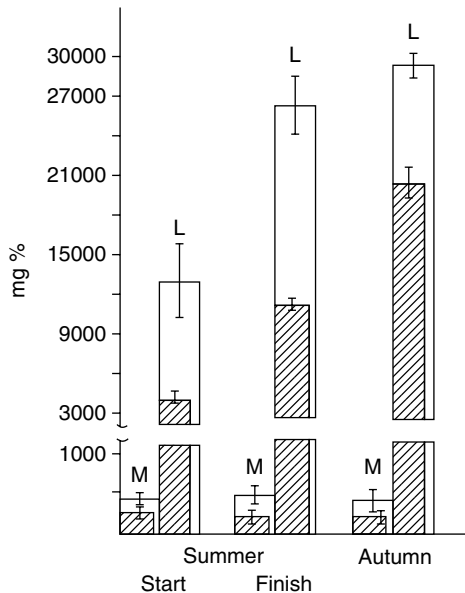
Figure 5.13 Influence of trematodes on triacylglycerol content (mg % wet mass) in round goby *Neogobius melanostomus*. L, liver; M, muscles; unshaded columns, weakly infested; shaded columns, heavily infested. After Shchepkina (1980).

Guryanova (1980) and Sidorov (1983) have shown that infestation with helminths such as plerocercoids of the broad tapeworm *Diphyllobothrium vogeli* leads to changes in phospholipid content and composition of tissues of stickleback *Pungitius pungitius* and turbot *Scophthalmus maximus*. Most pronounced is the drop in phosphatidylcholine and, occasionally, phosphatidylethanolamine concentrations. Such a disturbed phospholipid status of biomembranes usually affects permeability. It has also been shown that parasites selectively absorb essential amino acids of the host (Sidorov & Guryanova, 1981). In the

Figure 5.14 Sunfish *Mola mola*, a highly parasitized fish. Photo by Bernd Mörker.

developing eggs of Atlantic salmon *Salmo salar* infected with saprolegniasis, the store of glycogen was found to be depleted, with consequent loss of viable eggs (Timeyko, 1992). Carp *Cyprinus carpio* infested with ectoparasites exhibits reduces oxygen consumption whereas the content of hemoglobin in the blood and the activity of oxidative enzymes in other tissues decline (Kititsina & Kurovskaya, 1991). On the other hand, good feeding conditions increase the resistance of fish to diseases.

In the Indian Ocean, the condition of sardines (*Sardinella* spp.) is affected by parasites. The condition factor calculated for sardines infected by the copepod *Peroderma sardinellae* was 0.3, whereas for non-parasitized sardines it was 0.9. Furthermore, infected sardines had retarded maturity conditions when compared with non-parasitized fishes in similar length groups (Ranjitsingh & Padmalatha, 1997).

Another example of parasite impact on fish condition is that of Arctic cod. Arctic cod infected by a hematophagous parasite had significantly lower condition factor, hepatic and gonadal somatic indicators, and hematocrit than parasitized fish (Khan et al., 1997). Females harbored the largest numbers of the parasite and showed the most dramatic changes. This result parallels that for other gadoids parasitized with hematophagous parasites, which impair growth and reproduction (Khan et al., 1997).

Other species provide only indirect examples of the effects of parasitism on fish condition. This is the case with ocean sunfish *Mola mola* (Fig. 5.14), the world's heaviest bony fish (Macías et al., 2004). The ocean sunfish is often infected by a high number of parasites, including larvae of the cestode *Molicola horridus* (Gibson et al., 2011). Some studies indicate that the heavy parasite loads observed in ocean sunfish apparently hamper its liver function and condition, although the exact degree of damage is difficult to estimate (reviewed by Lloret et al., 2012).

Notwithstanding all these studies supporting the negative effects of parasites on the energy reserves of fish, other studies did not find any relationship between parasitism and fish condition. Thus, for example, studies on European eel, European hake, and lesser-spotted

Figure 5.15 Catshark *Scyliorhinus canicula*, a highly parasitized elasmobranch. Photo by Natàlia Martínez.

catshark in the Mediterranean Sea indicate that parasites found in these species do not affect their energy reserves, even in the case of the highly parasitized lesser-spotted catshark *Scyliorhinus canicula* (Fig. 5.15), or in eels affected by an introduced parasite (Lloret et al., 2012). In the case of the small-spotted catshark, one of the most abundant sharks in the by-catch of trawl fishery, it has been shown that the condition of this species in the Mediterranean is unaffected by its high parasite load. In the case of the eel, the introduced hematophagous parasite *Anguillicoloides crassus* is known to damage the swimbladder of the eel and affect its physiology, which would have implications for eel reproduction. Migration of infected eels will be impaired because spawners will have difficulties crossing the Atlantic Ocean and reaching the breeding sites (Kirk, 2003; Kennedy, 2007). Despite this, it remains difficult to demonstrate a negative effect on the condition of the infected eel (Barus & Prokes, 1996; Koops & Hartmann, 1989), their hepatosomatic index (Möller et al., 1991), or cell count or hematocrit (see Boon et al., 1989; Palikova & Navratil, 2001 or Kangur et al., 2010). This suggests that naturally infected eels are able to compensate the costs induced by the parasites. In the case of amberjacks (*Seriola* spp.), high intensities of skin or gill parasites have been reported to cause outbreaks of monogeneans in cultured individuals (see Whittington & Chisholm, 2008 for revision). Although experiments revealed that up to 100% of the fish living in affected tanks died, fish condition factors were normal and only a decrease in hematocrit was found as these monogeneans are blood-feeders (Montero et al., 2004). Another example that failed to provide a link between condition and parasitism is that of European hake in the Mediterranean. The high parasite loads and diversity of parasites affecting this species do not affect the Le Cren condition factor, neither the hepatosomatic index nor the lipid content in the liver of this species (reviewed by Lloret et al., 2012). Nevertheless, high parasite loads in European hake negatively affects the gonadosomatic index of this species, particularly during spawning (Fig. 5.16), thus indicating a negative impact of the parasites on the reproductive potential of hake.
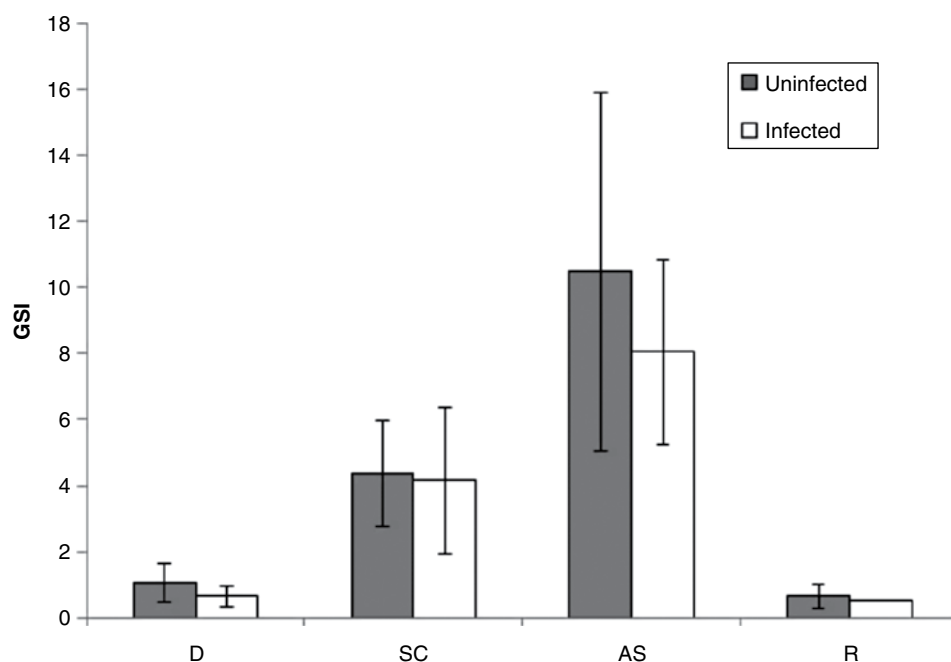
Figure 5.16 Average gonadosomatic index (GSI) of female European hake (*Merluccius merluccius*) in different states of maturity, infected and uninfected by copepods (NW Mediterranean). D: developing, SC: spawning capable; AS: actively spawning: R: regressing. After Lloret et al. (2012).

In some cases the opposite could be expected: fish able to feed more intensively are prone to incorporate more parasites during the feeding process and improve their condition, and in this case one could imagine a positive relationship between fish parasitism and condition.

Finally, it is important to note that there is considerable variation in parasite virulence and host tolerance which may have a genetic and/or environmental basis. In a recent study on the effects of a striking mouth-dwelling, blood-feeding isopod parasite on the life history and physiological condition of two Mediterranean populations of the coastal fish *Lithognathus mormyrus*, it was found that the growth and hepatosomatic index of fish in a heavily human-exploited population were severely impacted by this parasite (Sala-Bozano et al., 2012). In the exploited population, the parasite load explained 34.4% of the variation in hepatosomatic index. Conversely, the parasite showed negligible virulence in fish close to a marine protected area: the parasite load only explained 0.3% of the variation in hepatosomatic index (Sala-Bozano et al., 2012).

5.2 Abiotic factors

Several studies have shown the variability in the condition of pelagic and demersal fishes associated with environmental variables. Among the environmental variables studied, water temperature (including climate change/sea warming) and oxygen deficiency have been largely considered as the main factors impinging on condition of fish.

5.2.1 Temperature

Temperature is a basic characteristic of the environment that impinges on the biology and physiology of fish and other ectotherms (affects rates and direction of metabolic processes, behavior and distribution, etc.). From Fry (1957) and Brett (1979), temperature is the main factor controlling the vitality of aquatic poikilotherms. According to the van 't Hoff–Arrhenius law, all processes in aquatic poikilotherms at the borders of the biokinetic zone have a direct dependence on temperature (Prosser, 1967, 1991; Schmidt-Nielsen, 1983; Ivleva, 1983). It is worth remembering that the Q_{10} for aquatic animals during a temperature change of 10°C is on average 2.2 (fluctuations usually are in the limits 2.0–2.5) (Winberg, 1956, 1960; Brett, 1973, 1979; Belokopytin & Shulman, 1987).

Environmental temperature has a great effect on rhythmic processes, and this is related to the completion of annual cycles (Shulman & Love, 1999), tissue enzyme activity (Hochachka & Somero, 1973, 1984, 2002), structural and functional features of lipids (e.g., degree of unsaturation of their fatty acid ethers; Ackman, 1967; Kreps, 1981), and energy provision for locomotion (Hochachka & Somero, 1973, 1984, 2002), among others. It must be remembered that during study of the ecology of all aquatic ectotherms including fish, each physiological and biochemical indicator needs to be defined at a certain temperature that is appropriate to the habitat and period of the annual cycle, i.e., the temperature the fish is actually experiencing (Hochachka & Somero, 1973, 1984, 2002; Emeretly, 1990; Ugolev & Kuzmina, 1993; Shulman & Love, 1999). Thus the activity of fish tissue enzymes must not be defined at a predetermined temperature of 25°C (the favorite temperature of classical biochemists studying poikilotherms), but at the temperature of the habitat at the real moment of time (Fig. 5.17).

The influence of temperature on fish occurs via a direct pathway that involves sensory perception of temperature and which ultimately leads to the release of the thyroid hormones thyroxine and triiodothyronine and these increase oxygen consumption (Ruland, 1969; Leatherland, 1994). Therefore global warming, which began at the end of the last century, has had an impact on the fat reserves of pelagic fish in the Black Sea: in warm-water anchovy the fat reserves increased, while those in cold-water sprat decreased (Shulman et al., 2009b; Nikolsky et al., 2011). With increasing water temperature, many species (including thermophilous fish) undergo a change in the character of energy catabolism, for example common carp and silver carp *Hypophthalmichthys molitrix* shift metabolism, to a considerable degree, from aerobic (lipids) to anaerobic (carbohydrate) pathways of energy utilization (Eckberg, 1962; Freed, 1965; Ramaswami & Sushella, 1974; Wells, 1978; Arsan, 1986; Bilyk, 1989; Romanenko et al, 1991). It is also known that glycolysis is less dependent on temperature than aerobic metabolism. In such a situation, the content of glucose and other products of glycolysis (oxaloacetate, pyruvate, lactate) increase (Prosser, 1967; Hochachka & Somero, 1973). The pentose phosphate shunt also increases (Hochachka & Hayes, 1962; Yamaguchi et al., 1976; Malinovskaya, 1988; Kudryavtseva, 1990). Mullet *Liza* sp. adapted to low temperature increases its content of neutral lipids in muscles by inhibition of aerobic catabolism (Soldatov, 1993). Protein synthesis (Ray & Medda, 1975; Berezhnaya et al., 1981; Saez et al., 1982) and somatic growth rate (Ryzhkov, 1976) also proceed more intensively at high temperatures. Relationships between protein, lipid and carbohydrate catabolism and anabolism change as temperature changes (Romanenko et al., 1991). During temperature adaptation, hormones such as somatotropin, cortisol and prolactin play an active role in regulating energy metabolism (resynthesis of ATP and macroenergy utilization)
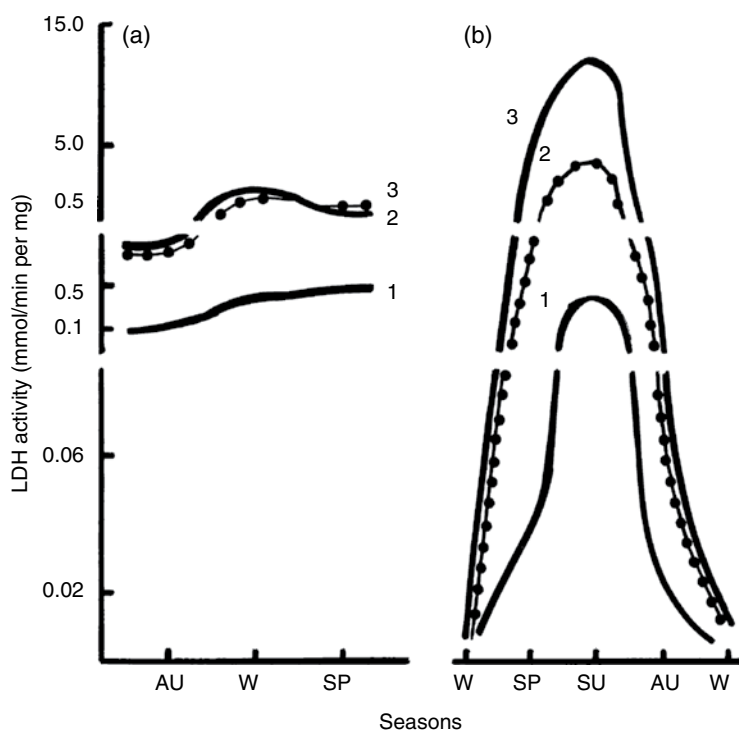
Figure 5.17   Lactate dehydrogenase (LDH) activity of fish at natural temperature of habitation: (a) cold-tolerant whiting *Odontogadus merlangus euxinus*; (b) warm-tolerant horse-mackerel *Trachurus mediterraneus ponticus*. 1 – white muscles, 2 – red muscles, 3 – liver. After Emeretly (1990a,b) and Shulman & Love (1999).

(Sautin, 1985; Trenkler & Samenkova, 1990; King et al., 2006). At very high temperatures (39°C or more) the level of metabolism in many species decreases and respiration and phosphorylation become disconnected (Johnston et al., 1983; Romanenko et al., 1991). As a result, heat dissipation in fish increases (Fomovsky, 1981). Conversely, Antarctic fishes (e.g., Notothenidae) live at temperatures of about –1.8°C in water with a very high oxygen concentration. Large quantities of food and high oxygen concentrations stimulate aerobic metabolism and inhibit enzymes of carbohydrate catabolism (Hemmingsen & Douglas, 1970; Johnston, 1985). The same occurs with the ice fish *Chaenocephalus aceratus*, which has no hemoglobin but exhibits high concentrations of oxygen in blood plasma.

Over many decades the following question has arisen: Is there any compensation of the rate of aerobic catabolism in fish (determined by oxygen consumption) at low temperatures? The answer is that it occurs at a biochemical (sub-organism) level, and this confirms numerous results already discussed. However, the suggestion of such compensation at a physiological (organism) level has been widely criticized by other researchers (Holeton, 1974; Ivleva, 1983; Karamushko, 2007). We consider that there are no serious reasons for disagreement around this question. It is worth remembering that integration of metabolic processes occurs at individual and population levels, and also at lower levels (tissue, cellular, subcellular) and that, as a rule, this integration leads to a decrease in their intensity. Primarily this relates to oxygen consumption. Taking this into account, the metabolic level of natural populations

should be based on active metabolism (Ivlev, 1959; Belokopytin, 1993; Shulman & Love, 1999; Karamushko, 2007), whose definition, as shown in Chapter 2, is extremely complicated. Therefore, from our point of view, there is no reason for the disagreement. These indicators of fish adaptations to temperature may be used to characterize populations and individuals and at subcellular levels. It is only necessary to consider exactly what structural–functional levels these characteristics indicate.

Accepting the close relation between temperature and level of metabolic activity in fish, temperature also affects the composition and conformation of macromolecules, triggering the expression of specific isoenzymes involved in muscle contraction (Johnston, 1993) and changing the protein ultrastructure of muscles (Crockford & Johnston, 1993). The temperature of the fish habitat induces thermoresistance of enzymes (succinate dehydrogenase, aldolase, adenylate cyclase, alkaline phosphatase, acetylcholinesterase, and proteinase) and structural proteins (actomyosin, collagen, hemoglobin, serum albumin, and globulin) (Ushakov, 1963; Tsukuda & Ohsawa, 1971; Alexandrov, 1975; Johnston et al., 1975). Differences in thermoresistance related to temperature of the environment has been shown for ATPase of myofibrils in 19 species of fish mainly from the North Sea and the Mediterranean (Johnston et al., 1973). Differences have been observed between collagens of subcutaneous tissues in warm-tolerant and cold-tolerant species (Gustavson, 1953; Takahashi & Yokoyama, 1954; Gowri & Joseph, 1968). Similarly, in cod *Gadus morhua* and whiting *Merlangius merlangus* the stability of collagen of subcutaneous tissue depends on the temperature of the habitats occupied by populations in the different regions (Andreeva, 1971).

The temperature of the environment that fish occupy is responsible for the degree of unsaturated lipids in their bodies (Kizevetter, 1942; Shkorbatov, 1961; Ackman, 1967; Hilditch & Williams, 1964; Lunde, 1973). With decrease in temperature the level of unsaturation rises, and this prevents transformation of lipid from a liquid to a liquid-crystal state. The degree of lipid unsaturation depends on the climatic–geographic zone of habitation, seasonal temperature changes, and adaptations to different temperature regimes in experimental conditions (Hoar & Cottle, 1952; Lewis, 1962; Farkas & Herodek, 1964; Privolnev & Brizinova, 1964). The degree of unsaturation is regulated by the content of polyenic fatty acids, which are consumed as part of the diet (Bell et al., 1986; Sargent & Henderson, 1980). An inverse relationship between the temperature of the environment that fish occupy and the percentage content of unsaturated fatty acids in their bodies has been shown by Johnston and Roots (1964), Ugolev and Kuzmina (1993), and many others. This desaturation process is mediated by Δ^5-, Δ^6- and Δ^9-desaturases in freshwater fish (Christiansen, 1984; Hagar & Hazel, 1985). In marine fish, because of the inactivity of the necessary enzymes, desaturation is achieved by consumption of exogenous sources of unsaturated lipids as food (Sargent, 1987). Seasonal changes of polyenic acid content are inversely related to temperature of the environment. This has been shown in horse-mackerel *Trachurus trachurus* from the eastern and northern Atlantic (Dobrusin, 1978), little tunny *Euthynnus alletteratus* (El Saed, 1984), mackerel *Scomber scombrus* and anchovy *Engraulis encrasicolus* from the Atlantic sector of Argentina (Pozo et al., 1992), sprat *Sprattus sprattus* from the Adriatic (Viviani et al., 1973), Atlantic herring *Clupea harengus* (Henderson & Almater, 1989), cod *Gadus morhua* from the northern Atlantic (Jangaard et al., 1967), sardine *Sardina pilchardus* (Zlatanos & Laskaridis, 2007) from the Mediterranean, skates *Rhinobatos cemiculus* and *Rhinoptera marginata* from the tropical Atlantic (El Kebir et al., 2003), blenny *Enedrias nebulosus* (Pekkarinen, 1980), and grey mullet *Mugil cephalus* (Deng et al., 1976). These changes are well shown by capelin *Mallotus villosus* (Henderson et al., 1984; Table 5.1). Kreps et al. (1977) and Kreps (1981) demonstrated higher levels of C20 and C22 fatty acids
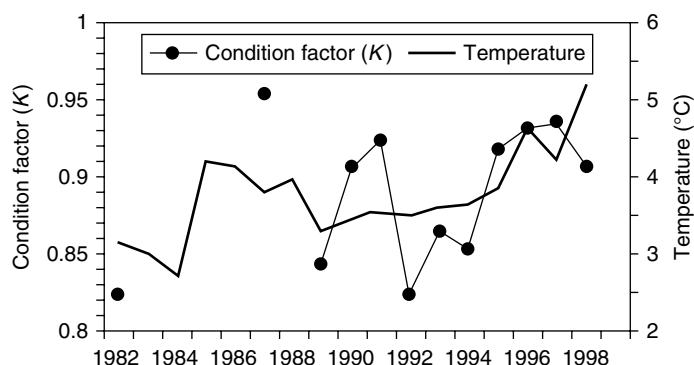
Figure 5.18   Interannual variations in temperature and condition factor (calculated with total weights) of cod *Gadus morhua* captured during autumn (September–November) groundfish surveys between 1982 and 1998 off Greenland. Data for 1983–1986 and 1988 on condition factor were not available. From Lloret & Rätz (2000).

in phospholipids of brain tissue in deep-sea tropical fish compared with fish that inhabit surface layers at higher temperatures. Excluding polyenic acids, fish which live at low temperatures have increased content of phospholipid fractions (phosphatidylethanolamine, phosphatidylcholine, etc.) in triglycerides (Hazel & Schuster, 1976; Shatunovsly, 1980; Lapin & Shatunovsky, 1981; Van den Thillart & Bruin, 1981; Sidorov, 1983; Hazel & Landrey, 1988; Hazel & Williams, 1990). Cholesterol, another structural lipid that provides "durability" of membranes, also plays an active role in temperature adaptations (Kreps, 1981; Chebotareva, 1983; Shatunovsky, 1980; Sautin, 1989). An important role in adaptation to over-low temperatures belongs to glycoproteins, which protect blood from freezing (De Vries, 1970, 1971; Bielar et al., 1982; Hochachka & Somero, 1973).

The effect of temperature on condition of fish has been extensively investigated, both in the field and in the laboratory. In gadoid species (usually considered cold-water species), good condition has usually been associated with warm waters. For example, in Greenlandic waters Lloret and Rätz (2000) showed that annual mean condition factor values of cod were positively correlated with annual weighted mean temperatures (Fig. 5.18). In the North Atlantic, Rätz and Lloret (2003) reported a positive relationship between the condition factor of 10 cod stocks and the near-bottom temperature of their habitats. The authors concluded that cod stocks living in temperate waters on average are in better condition than stocks living in cold waters. For juvenile and adult Atlantic cod (*Gadus morhua*) inhabiting the southern Grand Bank of Newfoundland (Canada), Morgan et al. (2010) found that gutted body condition was highest at warm temperatures, although liver condition was highest at low temperatures. In addition, adult pouting (*Trisopterus luscus*) were found to be in better condition in the warmer waters of southern Portugal than in the colder waters of the north (Tanner et al., 2009). Total protein content increased from north to south along the coast, according to an increase in sea surface temperature (SST) in the same direction. According to the authors, the higher temperature towards the optimal might enable higher protein turnover and allow individuals from warmer waters to produce and accumulate more energy reserves in the form of proteins in white muscle, enhancing growth in mass. In the southern California region, the condition factors of mackerel (*Scomber japonicus*) and jack mackerel (*Trachurus symmetricus*) were correlated with water temperature (Parrish & Mallicoate, 1995).
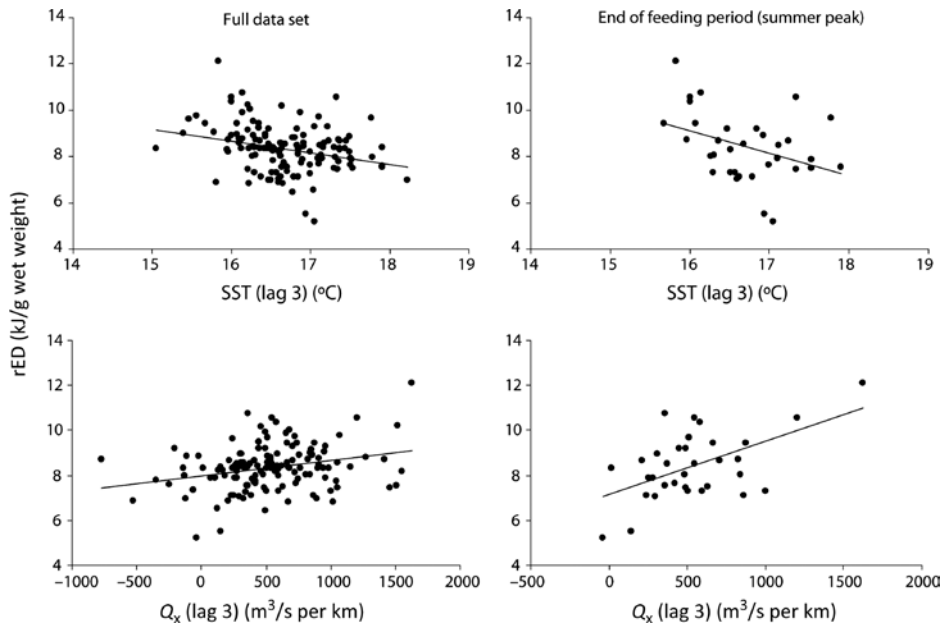
Figure 5.19   Sardina (*Sardina pilchardus*) off the Iberian Atlantic coast. Relationships between seasonally adjusted sardine energy (rED, kJ/g wet weight) and earlier oceanographic conditions, namely seasonally adjusted sea surface temperature (SST) and Ekman transport (Q_x) with a 3 month lag, during the entire studied period (left panels) or restricted to the end of the main feeding period (summer peak; right panels). From Rosa et al. (2010).

In other cases, a negative relationship between water temperature and fish condition has been found. For example, in several Portuguese estuaries a negative relationship has been observed between the condition of sole and temperature (Vasconcelos et al., 2009), which may be explained by increased physiological stress of sole in warm waters (Lefrançois & Claireaux, 2003). Furthermore, Rosa et al. (2010) found a negative effect of temperature on condition of sardine on the Atlantic coast of the Iberian Peninsula. When comparing the entire time series of adult sardine energy density (whole body) with SST and offshore Ekman transport, the authors found significant lagged associations with SST and Ekman transport (lags of 2 and 3 months). Lagged SST was negatively associated with sardine energy, and lagged Ekman transport was positively associated with sardine condition (Fig. 5.19). In this case, water temperature and Ekman transport probably affected sardine condition through changes in prey availability. Hence, the combination of environmental conditions that triggered positive anomalies in sardine energy density – colder ocean temperatures associated with positive Ekman transport values – corresponded to upwelling conditions favorable for the spring/early summer phytoplankton and zooplankton blooms. According to Rosa et al. (2010), these environmental conditions are critical for adult fish feeding in order to maximize their late summer energy peak before starting into the prolonged winter spawning season.

Not only is adult and juvenile condition affected by sea temperature but also by larval condition. The RNA/DNA ratio of anchovy larvae from the Catalan Sea (west Mediterranean) correlated negatively with sea temperature, while in the Gulf of Lions there was a positive correlation with temperature (García et al., 1998). Environmental factors are also behind the higher larval and juvenile growth rates and condition indicators (*K*, mean RNA/DNA ratios
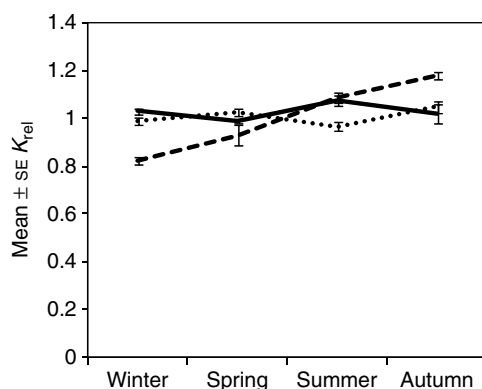
Figure 5.20 Mean ± standard error of relative condition factor (K_{rel}) of each predator (continuous line, *Sphyraena viridensis*; dotted line, *Sphyraena sphyraena*; dashed line, *Sphyraena chrysotaenia*) by season. From Kalogirou et al. (2012).

and protein content) of fish inhabiting tropical and temperate seas that occur in complex or variable habitats (respectively coral reefs and estuaries; Fonseca & Cabral, 2007). It seems that these species invest highly in rapid growth in order to increase survival probability to counter high mortality rates during early stages or unstable habitat conditions.

Nevertheless, other studies (e.g., cod in Iceland, Pardoe et al., 2008) have suggested that temperature does not determine condition directly but instead acts as a proxy for other more important factors such as food supply. Furthermore, the effect of temperature on fish condition has been hypothesized as a limiting factor of the expansion of invasive species in certain areas. For example, low-temperature conditions in winter in the Mediterranean may limit the further expansion of barracuda *Sphyraena chrysotaenia*, a species of Indo-Pacific Ocean origin, from the southeastern Mediterranean towards the north and west (Kalogirou et al., 2012). During winter, the condition factor of the non-indigenous *S. chrysotaenia* was significantly lower than that of the indigenous *S. sphyraena* and *S. viridiensis* (Fig. 5.20). According to the authors, the non-indigenous *S. chrysotaenia*'s lower condition factor in winter indicates that temperature minima may be a limiting factor for growth through reduced feeding, as is generally accepted for many fish species (Fonds et al., 1992). Furthermore, as the two indigenous species were stable throughout the year, except for *S. sphyraena*, which exhibited a slightly lower condition factor in summer, the authors suggested that the indigenous sphyraenid species were in good condition and that the condition had not been affected to any large extent by the establishment of a non-indigenous barracuda. On the contrary, the non-indigenous *S. chrysotaenia* in Egyptian waters of the Mediterranean had a higher Fulton's condition factor than the indigenous species *S. sphyraena*, *S. flavicauda*, and *S. viridensis* (Allam et al., 2004a), and also reproduces at a smaller size than the indigenous species, indicating abilities which could favor its successful establishment (Allam et al., 2004b).

5.2.2 Climate change

Global climate and ocean changes resulting from anthropogenic greenhouse-gas emissions are currently affecting and are expected to continue to affect marine organisms (reviewed by Cheung et al., 2009). These impacts are fundamentally linked to the close relationship

between ocean conditions and the ecophysiology of marine organisms. Climate variability affects the abundance and biogeography of fish (McFarlane et al., 2000; Reid et al., 2001; Genner et al., 2004) through species-specific physiological thresholds of temperature tolerance. Despite many studies focusing on the implication of thermal tolerance for the distribution range of fish, few studies have assessed the integrated responses of changes in ecophysiology. Thus, only a small number of studies have assessed the impact of climate change on fish condition, particularly regarding the impact of sea warming, which is a well-known feature observed in nearly all seas and oceans, from shallow to deep waters.

Although the link between water temperature and fish condition has been studied, other environmental variables that have also been linked to condition of fish include salinity, bottom characteristics, and winds. In the southern California region, the condition factors of mackerel (*Scomber japonicus*) and jack mackerel (*Trachurus symmetricus*) were correlated with proxy indicators of alongshore advection (sea level), offshore advection (Ekman transport), and salinity (Parrish & Mallicoate, 1995). In the Bay of Biscay (northeast Atlantic), strong winds affect the larval condition of anchovy (Bergeron, 2000).

Climate variability can also influence the condition of fish by changing the duration of suitable feeding conditions. For example, by influencing the length of zooplankton reproduction and development seasons, climate variability can also influence the duration of suitable feeding conditions for capelin in the Barents Sea (Orlova et al., 2010). In this study, capelin biomass and the NAO climatic index explained more than 28% of the interannual variability in maximum fat content, suggesting that climatic conditions partly determine feeding conditions for capelin, and may influence population biomass accumulation and fat content simultaneously. The authors hypothesized that climate change may result in increased primary productivity and significantly alter the Barents Sea zooplankton community by reducing *Calanus glacialis* abundance by 50%, while only modestly increasing the *C. finmarchicus* population (Ellingsen et al., 2008). This scenario could have strong negative implications for capelin feeding and condition because capelin appears to select for *C. glacialis* (contains more lipid than the Atlantic-boreal *C. finmarchicus*) and for larger (and more fat-rich) life stages. Thus, climate can affect both biomass and condition of a given species, as well as the biomass of its main prey, but in a complex manner. On the other hand, Engelhard and Heino (2006) indicated that a year of poor environmental conditions, by affecting growth and condition of herring *Clupea harengus*, could have a threefold negative effect on reproductive potential by (i) increasing mortality, (ii) reducing mean fecundity per individual spawner, and (iii) increasing the fraction of adults skipping reproduction.

In addition, climate shifts could also change EFA production (a key component of fish diet that affects condition) either by changing phytoplankton species composition or by changing EFA production within phytoplankton taxa. One of the best-studied examples of changing EFA production in response to environmental change comes from the Baltic Sea, where eutrophication has shifted phytoplankton dominance from diatoms to flagellates, apparently resulting in changes in EFA ratios throughout the food web, and possibly leading to a chronic reproductive disease in salmon *Salmo salar* (Ahlgren et al., 2005). Also, Litzow et al. (2006) provided strong indications that changes in lipid content of different fish communities were the result of climate-mediated changes in the availability of essential fatty acids. The authors found that following the 1970s Pacific Decadal Oscillation regime shift, walleye pollock *Theragra chalcogramma* and other demersal lipid-poor species (mostly demersal) increased in abundance in four boreal zones (Bering Sea, Gulf of Alaska, North Sea and Scotian shelf), while small pelagic lipid-rich species such as capelin *Mallotus villosus* declined (Fig. 5.21). Therefore, the four examples of community reorganization that Litzwow et al. (2006) utilized
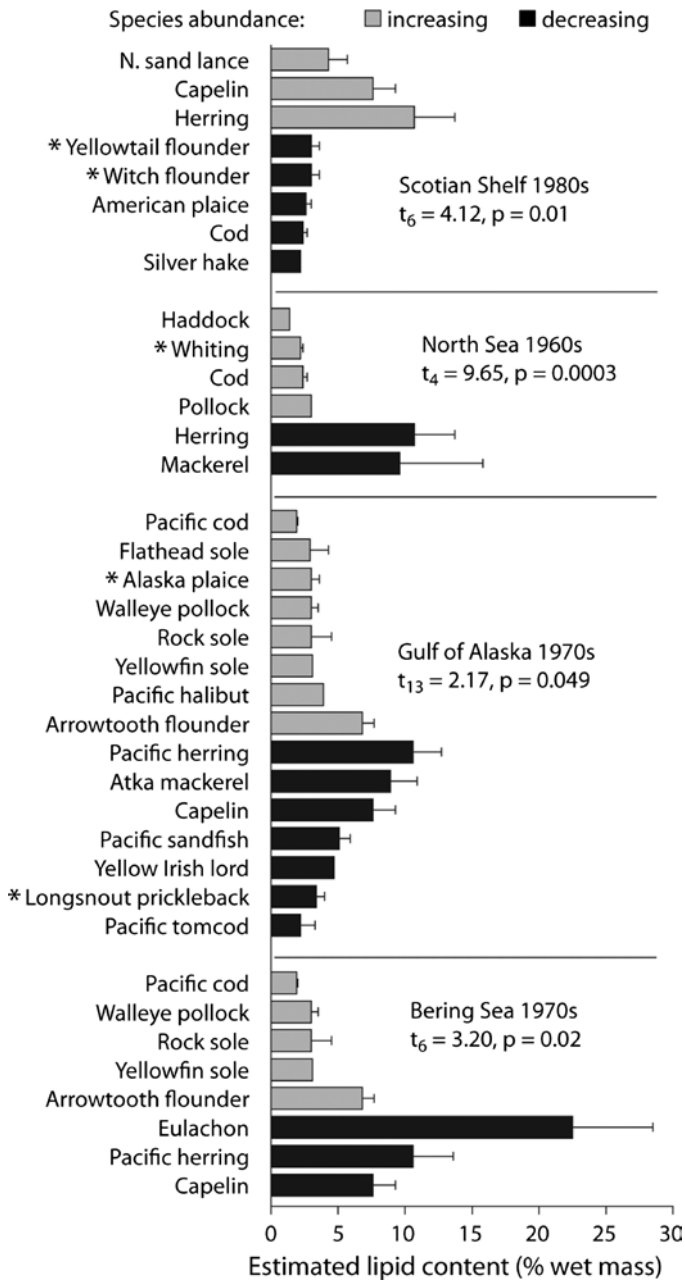
Figure 5.21 Opposite population trajectories in lipid-rich and lipid-poor species following climate regime shifts: analysis of previously published data on fish population trajectories and lipid content. In each instance lipid content was significantly different between groups that increased and decreased in abundance. Asterisks indicate lipid data estimated from family mean; error bars + SE. From Litzow et al. (2006).

in their study all involved shifts between pelagic and demersal communities, and they hypothesize that these changes in fish community lipid content were the result of climate-mediated changes in the availability of EFAs. Shifts between pelagic and demersal species have implications for community lipid content and lipid availability for higher predators, as pelagic fish are typically richer in lipids than demersal species (Anthony et al., 2000; Iverson et al., 2002). This situation is also recognized in the Gulf of Alaska, where a climate regime shift in the 1970s resulted in population decreases in lipid-rich pelagic species (capelin *Mallotus villosus* and Pacific herring *Clupea pallasii*) and steady population increases in lipid-poor gadoids and pleuronectids (Anderson & Piatt, 1999; Mueter & Norcross, 2000). The resulting low-lipid state has persisted for approximately 20 years and is hypothesized to have had negative effects on piscivores in the region (Anderson & Piatt, 1999).

There are also some recent indications showing detrimental effects of ocean warming on condition of some species. For example, condition of Atlantic salmon *Salmo salar* has fallen as sea surface temperature (SST) anomaly has risen, and for each year-class the midwinter (January) SST anomalies they experienced at sea correlated negatively with their final condition on migratory return during the subsequent summer months (Todd et al., 2008). According to the authors, the probability of the poorest condition salmon successfully migrating upriver and surviving to spawn in November is likely to be extremely low. While a direct physiological effect of ocean warming on fish like salmon is possible, other evidence suggests it is more likely that the negative correlations between condition and sea temperature are manifest in bottom-up control (i.e., reduced prey availability) (Todd et al., 2008).

In freshwater ecosystems there is also good evidence that climate change can affect body condition of fish. In Ontario, for example, changes in body condition of two species of coregonid fish (lake herring *Coregonus artedi* and lake whitefish *C. clupeaformis*) were related to regional differences in the degree of climate change during the time period investigated (Rennie et al., 2010). Climate data from northwestern, northeastern, and southern Ontario showed a general warming trend in all regions over the period of study. However, greater temporal changes in climate were observed in the northwest where warming was more intense and precipitation declined over the study period compared with relatively little change in southern or northeastern Ontario (Rennie et al., 2010). Correspondingly, northwestern Ontario coregonid populations demonstrated significantly greater declines in body condition relative to those from northeastern or southern Ontario. Because fish condition affects both reproductive success and overwinter survival, the authors suggested that observed condition declines of the magnitude reported here could have profound implications for the structure of freshwater aquatic ecosystems in a warming climate.

Also the condition of larvae is affected by environmental variables. For example, low nutritional condition of anchovy (*E. encrasicolus*) larvae in the Bay of Biscay (northeast Atlantic) was linked to strong winds with speeds of 20–30 knots, and supported the notion that some environmental stability is required for good feeding conditions (Bergeron, 2000).

A recent study has shown that both theory and empirical observations support the hypothesis that sea warming and reduced oxygen will reduce body size of marine fish (Cheung et al., 2013). Specifically, the maximum body weight of marine fish and invertebrates is fundamentally limited by the balance between energy demand and supply. Results suggest that oxygen-limited growth in aquatic water-breathing animals and species' range shift will translate, given their physiological responses to warming and changes in oxygen level, into a reduction in individual- and assemblage-level body size (Cheung et al., 2012). Further examples on how climate change and sea warming affect the condition of fish through changes in the fodder base are given in section 6.3. The relationship between temperature and fodder base for many fish is illustrated in general by the scheme shown in Fig. 6.23.

5.2.3 Oxygen deficiency

We previously noted the significant role of oxygen for fish life (see Fig. 2.1). Naturally high water oxygen saturation guarantees normal population existence. In the oceans and in many seas, the oxygen concentration is 7–8 mg/L, which is optimal for the majority fish species (i.e., this is the "normal" environment). However, we cannot ignore the fact that a number of fish species may (and some do) live in lower oxygen concentrations (0.1 mg/L and even lower). These are the so-called "extremophiles." There is nothing surprising in this. Many freshwater species of the Cyprinidae family (especially those in closed, weakly flowing or inflowing basins) suffer a decline in oxygen concentration to very low values and survive strong hypoxic conditions. What is more, marine species inhabiting coastal areas such as the northwestern Black Sea (Zaitsev, 1992) and the Sea of Azov (Kostyuchenko, 1961), where the depth does not exceed 16 m, as well as in the gulfs of Venice and Trieste in the Adriatic (Orel et al., 1986), are permanently exposed to hypoxic stress. This stress is caused by strong eutrophication, which leads to higher concentrations of phytoplankton and even plankton blooms (including red tides). Although phytoplankton liberate oxygen into the water, the oxygen required for oxidation of dead plants exceeds the amount liberated by the plankton. Other factors that contribute to eutrophication are high water temperatures during the summer (which thoroughly warms all water layers to the bottom) and pollution, which has increased strongly during the last decades due to the input of organic toxicants and heavy metals of terrestrial origin (via river discharges). In summer months, especially during long periods of calm weather, oxygen is depleted and this causes the death of immense numbers of fish by asphyxia. Nevertheless, some fish are adapted to these difficult environmental conditions, including numerous species of the Gobiidae family. Some of them, to avoid these difficult conditions, penetrate into fresh waters where they swim against the current, while others move to further sea basins. Recently, the round goby *Neogobius melanostomus* spread from the Sea of Azov and the Black Sea towards the Baltic Sea and has been introduced as an invasive species in the Great Lakes of North America. It is known that in oceans and open seas there are numerous regions where low oxygen concentrations prevail (0.1 mg/L) (Fig. 5.22).

The reduction in oxygen concentration extends to considerable depths (between 200 and 1000 m; Fig. 5.23) and is caused by the massive development and death of phytoplankton (after death the organic substances are oxidized and sink to deeper waters as "detritus," but the products of excretion of living phytoplankton such as soluble organic substances are also responsible). This paradox is seen in the Arabian Sea (Kukharev et al., 1988) and in the Pacific coastal zone of North America (Vinogradov et al., 1992): in areas of low oxygen concentration not only is there higher abundance of zooplankton and squids, but also schools of large fish of the families Myctophidae and Gonostomatidae, and also Scombriidae and Carangidae. Fish are also found in the hydrothermal regions on the borders of zones with very low oxygen concentrations (Gal'chenko et al., 1988; Tunnicliffe, 1991; Gebruk et al., 1997). These examples force us to think about the metabolic mechanisms leading to fish adaptation to "extreme" environmental conditions, from the point of view of "standard" considerations. Here, protein substrates and products of protein and nitrogenous catabolism play an important role in marine fish adaptation to hypoxia. Experiments have been conducted on several species of Black Sea fish in which it was shown that short-term hypoxic stress (reduction in oxygen concentration from 8.6 to 1.7 mg/L over 1–3 hours) of scorpionfish *Scorpaena porcus*, annular bream *Diplodus annularis*, and horse-mackerel *Trachurus mediterraneus ponticus* caused a marked fall in oxygen consumption and significant rise in ammonia nitrogen excretion
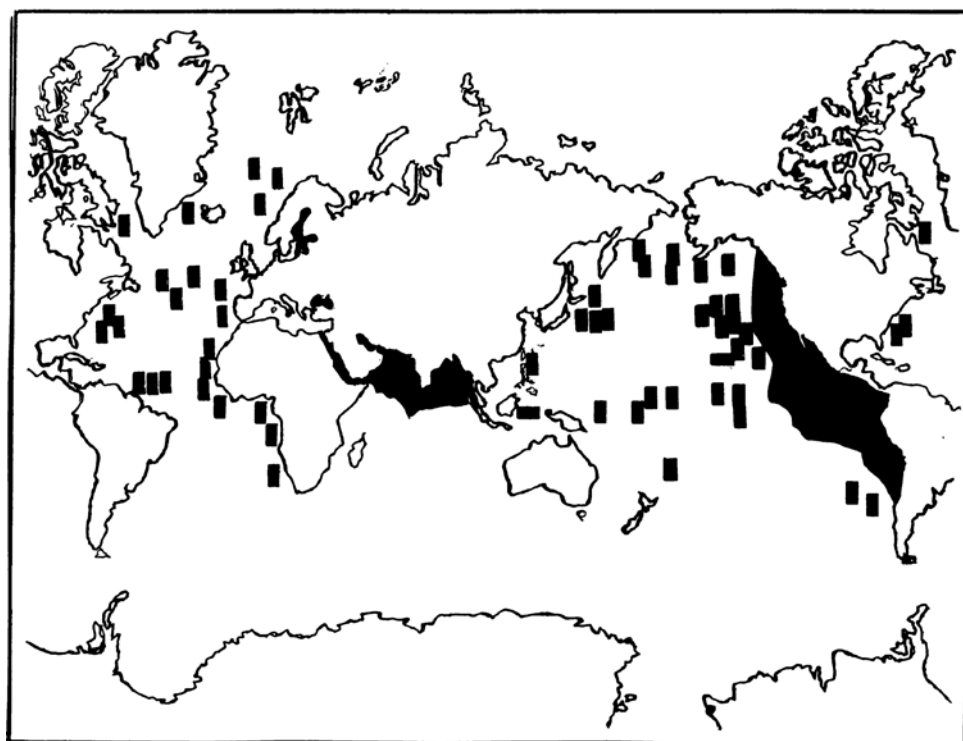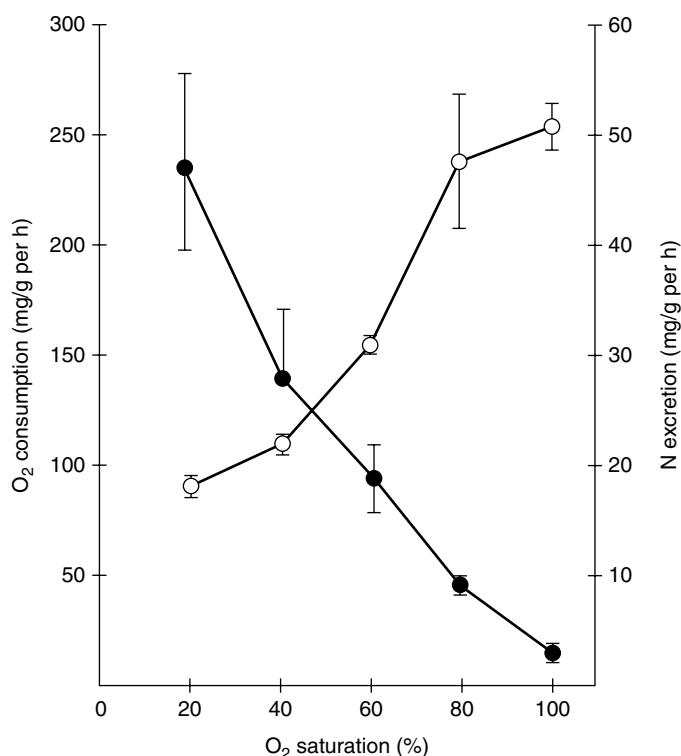
Figure 5.22 Distribution of hypoxic zones in the world's oceans. After Kamykowski & Zentara (1990).
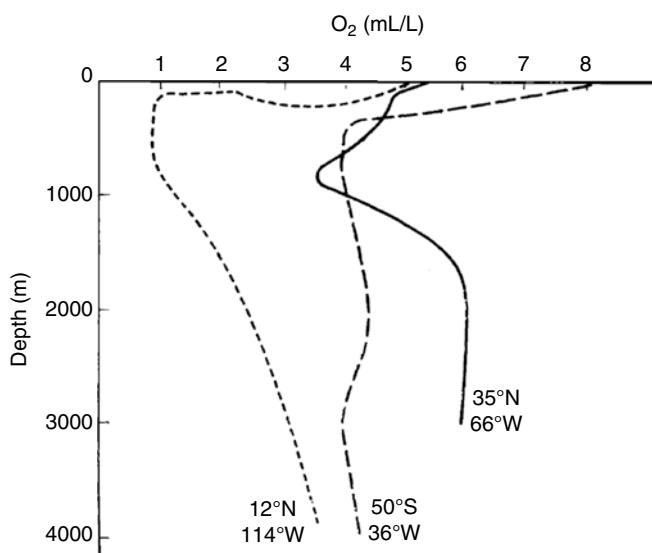

Figure 5.23 Dependence of oxygen deficiency on ocean depth. After Raymont (1963).

Figure 5.24 Changes in oxygen consumption and nitrogen excretion by horse-mackerel *Trachurus mediterraneus ponticus* during short-term hypoxia. Open circles, oxygen; filled circles, nitrogen. After Stolbov et al. (1995) and Shulman & Love (1999).

(Fig. 5.24). It is known that ammonia nitrogen comprises 80–90% of total nitrogen excreted by fish (ultimately in the form of urea) (Stroganov, 1962; Mann, 1965; Waarde, 1983). Concurrently, the ratio of ammonia excreted to oxygen consumed (ammonia quotient or AQ) increases as does the ratio of oxygen consumed to nitrogen excreted (O/N) (Stroganov, 1956; Prosser & Brown, 1962; Mathur, 1967; Kutty, 1968, 1972). Energy consumption at AQ >30 is mainly the result of lipid and carbohydrate metabolism; at AQ = 20–30, protein and non-protein substrates are used in metabolism; and at AQ <20, energy catabolism switches to proteins and products of their metabolism. At AQ = 8.67, all the oxygen is utilized by the protein–nitrogen complex; at AQ <8.67, a fraction of proteins and nitrogen products are catabolized in anaerobic processes. Under controlled experiments, when AQ fell to very low values (Stolbov et al., 1995; Fig. 5.25), at 60% oxygen saturation of water, protein–nitrogen catabolism begins to dominate; at 20–40% oxygen saturation, some protein and nitrogen products are used in anaerobic pathways (Fig. 5.26). However, can the results of these experiments be translated to the natural environment? The experiments of Stolbov et al. (1997), carried out under long-term hypoxic conditions with scorpionfish maintained over 13 hours in water with an oxygen saturation of 12–16% (1.0–1.4 mg/L) (Fig. 5.27), confirmed the short-term consequences of hypoxia for this coastal species. Further, a 29-day experiment with scorpionfish in water at 30% oxygen saturation revealed an AQ of 15.8 (Figs 5.28 and 5.29) (Shulman et al., 2003). In the same experiment, golden mullet *Mugil auratus* in water at 54% oxygen saturation exhibited an AQ of 4.9. Blood condition indicators
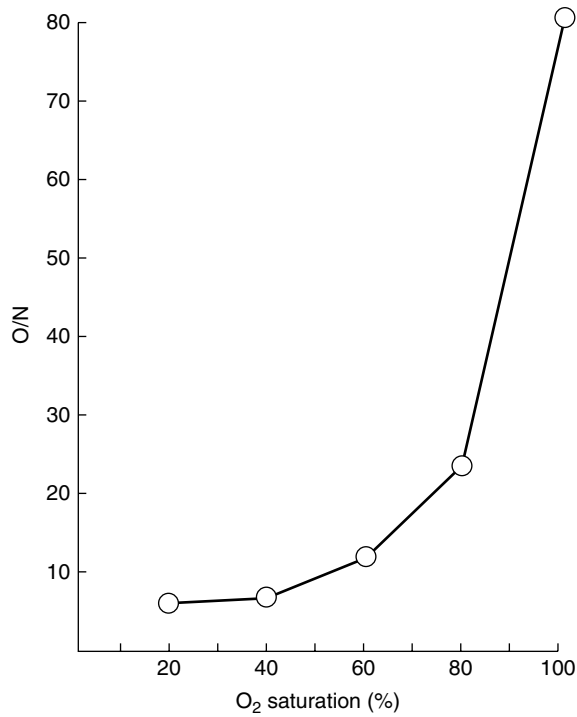
Figure 5.25 Change in ammonium coefficient (AQ) in horse-mackerel during short-term hypoxia. After Stolbov et al. (1995) and Shulman & Love (1999).
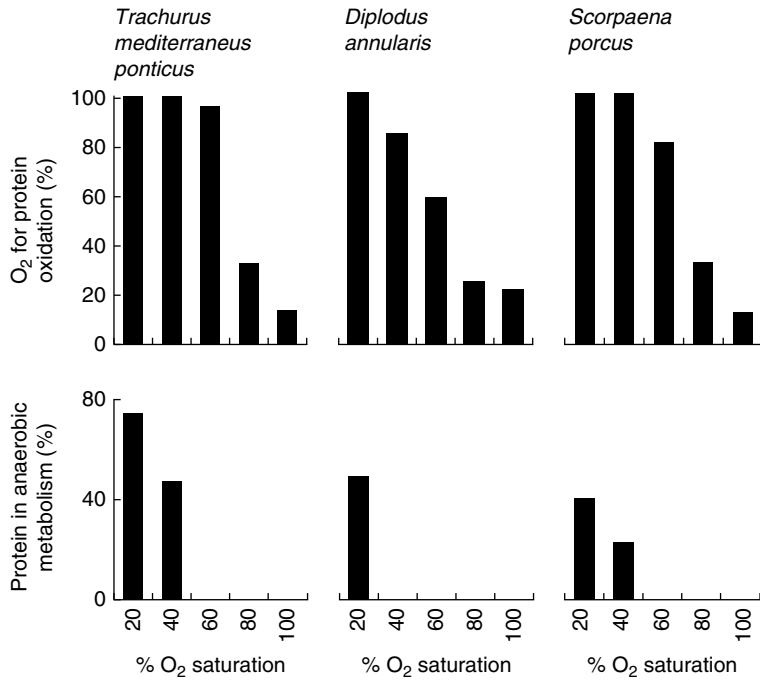
Figure 5.26 Protein and nitrogen products used for anaerobic metabolism during short-term hypoxia: upper graphs show percentage of oxygen used for oxidation of protein; lower graphs show quantity of protein involved in anaerobic metabolism. From Stolbov et al. (1995) and Shulman & Love (1999).
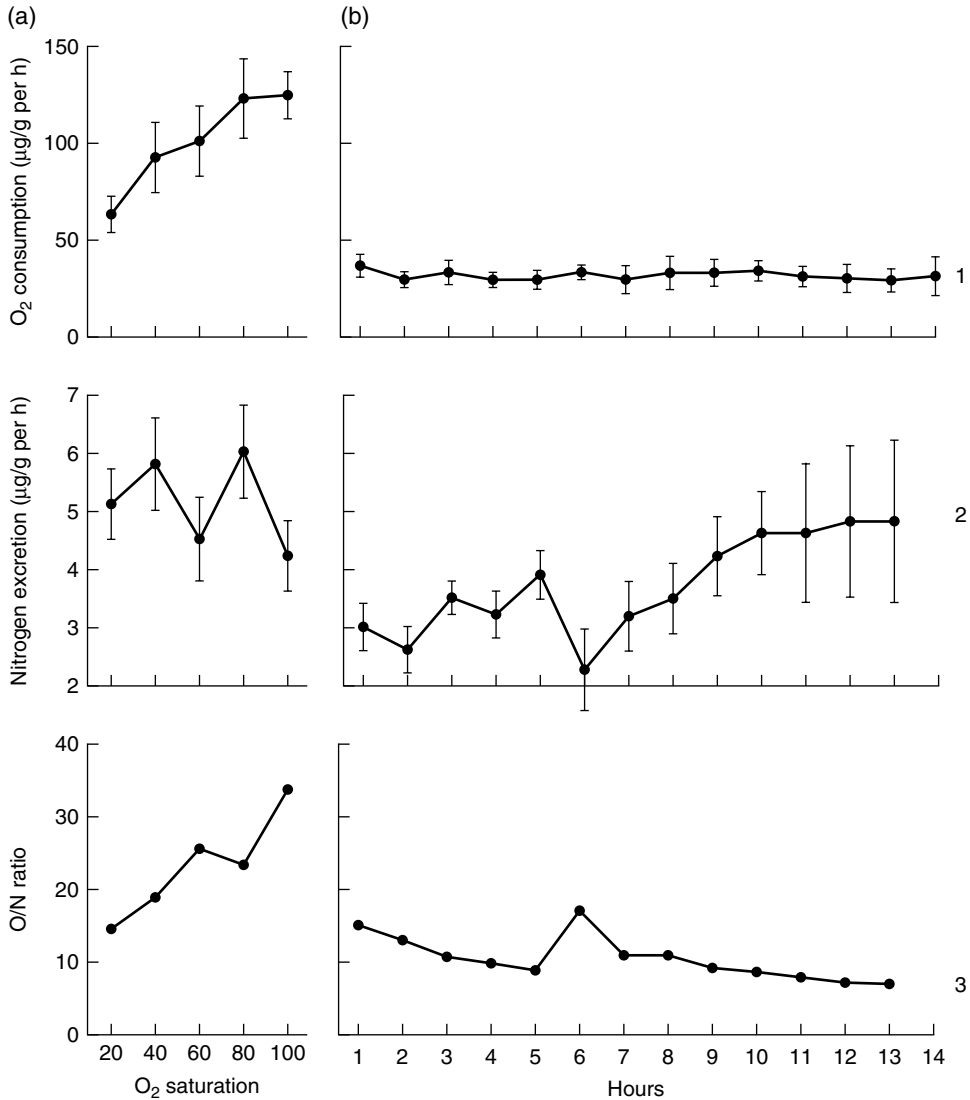
Figure 5.27 Dynamics of oxygen consumption and nitrogen excretion in *Scorpaena porcus* during 13 hours of hypoxia. (a) Short-term phase (some hours); (b) long-term phase (13 hours). After Stolbov et al. (1997).

(hemoglobin concentration, erythrocyte number, and hematocrit) were normal. These results show that selected fish adapted rather well to hypoxic conditions, which often occur in the Black Sea. Scorpionfish is a sedentary species whereas golden mullet exhibits moderate locomotory activity. We do not know how highly mobile species such as pelagics behave under long-term oxygen deficiency. However, Artemov (personal communication) observed that sprat schools in the Black Sea often penetrate to zones adjacent to areas containing hydrogen sulfide where oxygen deficiency is very marked (0.1–0.4 mg/L), and these schools rest here for long periods. This confirms data obtained earlier by Zuev and Melnikova (2000). By the way, all these examples from the marine environment agree with results from
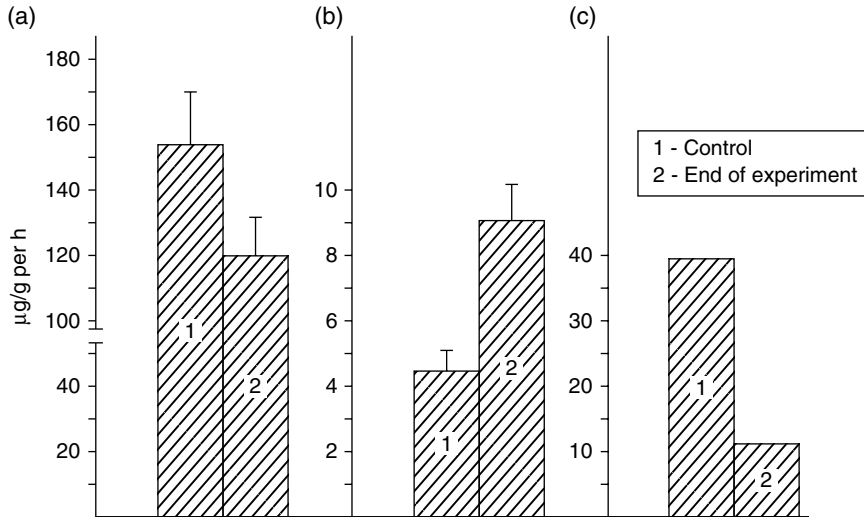
Figure 5.28 Oxygen consumption (a), nitrogen excretion (b) and O/N ratio (c) in *Scorpaena porcus* during long-term hypoxia. After Shulman et al. (2003).

Figure 5.29 Scorpionfish *Scorpaena porcus*. Photo by Josep Lloret.

studies on freshwater fish, where the respiratory quotient (RQ, ratio of excreted carbon dioxide to consumed oxygen) is 0.7–0.9 (Stroganov, 1956; Mathur, 1967; Kutty, 1968; Alikin, 1975; Kutty & Mohamed, 1975; Solomatina et al., 1989). Unfortunately the use of RQ in marine fish is impossible due to the carbonate buffer characteristics of sea water. Anaerobic utilization of protein is also observed in fish during so-called functional hypoxia, when long-term intensive swimming causes deficiency of oxygen used in oxidation of lipid and carbohydrate substrates (Lukyanenko, 1987), as well as during "overfeeding" when the quantity of protein consumed by the fish exceeds the ability of aerobic oxidation to metabolize it (Sukumaran & Kutty, 1977; Shulman & Love, 1999).

The ability of fish to utilize proteins and products of the anaerobic catabolism of nitrogen is spread widely among marine animals belonging to numerous taxa (Shulman et al., 1993; Shulman & Love, 1999; Soldatov, 2011), for example annelids, crustaceans, mollusks, and echinoderms. During oxygen deficiency, utilization of protein occurs before non-protein substrates. Catabolism of lipids requires more oxygen, while glycogen content in fish and many marine invertebrates is too low to maintain anaerobic metabolism for long periods. Glycogen is used by fish as a short-term adaptation to oxygen deficiency (Brand, 1946) and for short-term energy-demanding "explosive" work. The significance of carbohydrates in adaptation to hypoxia has been observed by Hochachka and Somero (1973, 1984, 2002), Murat (1976), Mohamed and Kutty (1983a,b). Proteins, peptides and amino acids are used in anaerobic metabolism not only directly but also by gluconeogenesis (Hochachka & Mustafa, 1972; Hochachka et al., 1973; Driedzic & Hochachka, 1975; Savina & Plisetskaya, 1976). Anaerobic utilization of proteins and carbohydrates in mitochondria of fish muscles has been studied by Owen and Hochachka (1974) and Savina (1992), who showed that eventually, under conditions of hypoxia, the pool of free amino acids becomes an important supply of energy. A number of enzyme and metabolic indicators characterize features of the transformation of nitrogenous products and carbohydrates during energy utilization, including lactate dehydrogenase, malate dehydrogenase, glucose phosphorylase, glycogen phosphorylase, aspartate aminotransferase, and alanine aminotransferase. All may be used for estimating fish condition during oxygen deficiency.

An outstanding example of protein utilization (during oxygen deficiency) is the dramatic degeneration of muscle tissues in Pacific salmon of the genus *Oncorhynchus* during anadromous spawning migrations (Idler & Bitners, 1958; Mommsen et al., 1980; Ando, 1986). In this situation migratory fish undoubtedly survive functional hypoxia caused by swimming against the river current and jumping rapids. Thus proteins, as well as being utilized for maturation, are exploited as an energy source via anaerobic catabolism. By the way, oxygen deficiency increases the level of catecholamines and these affect carbohydrate metabolism. Oxygen deficiency also influences fish lipids. Closely related species of the Gobiidae family from the Sea of Azov exhibit different degrees of lipid unsaturation (Table 5.2) that exactly corresponds with the variable oxygen regime in their biotope in summer months (Shulman, 1974; Shulman & Love, 1999). The lowest oxygen concentrations are found in silts on muddy bottoms, are higher in sandy bottoms, with the highest values found on rocky bottoms of the coast. The lowest level of energy metabolism is found in *Neogobius syrman* which inhabits silts (Shulman et al., 1957; Skazkina, 1972); energy metabolism is 1.5–2.0 times higher in *N. melanostomus* which inhabits sand, and is also higher in *N. batrachocephalus* which inhabits rocky bottoms (Skazkina, 1972). The degree of lipid unsaturation changes in tandem with these changes in energy metabolism. Thus fish

Table 5.2 Iodine value (a measure of lipid unsaturation) in the liver lipids of different species of goby from the Sea of Azov

Species	Males	Females
Neogobius syrman	81.7	80.7
Neogobius melanostomus	128.8	113.7
Neogobius fluviatilis	153.7	145.0
Neogobius rattan	160.0	—
Mesogobius batrachocephalus	160.0	169.0

Source: after Shulman & Love (1999).

lipids depend not only on food and temperature, but also on oxygen supply. In particular, toxic compounds have a specific effect on muscle structure, blocking the transfer of oxygen to tissues. Oxygen deficiency promotes the mobilization of structural proteins in muscles, causing disintegration of the sarcoplasmic reticulum and myofibrils and degeneration of collagenous structures. This was shown first for the Volga-Caspian sturgeon *Acipenser guldenstadti* but occurs in many other species in fresh and marine waters worldwide (Evgenieva et al., 1989; Lukyanenko et al., 1991; Nemova, 1991).

We have previously observed the influence of oxygen deficiency on the biochemical features of fish. However, it is important to understand the influence of hypoxia on their physiological (functional) features. Such investigations have been mainly carried out on freshwater fish (Itazawa & Takeda, 1978; Klyashtorin, 1982). However, marine fish of the Sea of Azov and Black Sea basins have been studied by Soldatov and colleagues (Soldatov, 1996, 1998, 2011; Soldatov et al., 2004, 2010). He revealed a decrease in P_{O_2} in blood and muscle tissues of fish under experimental hypoxic conditions. In this situation the velocity of oxygen transport via hemoglobin in erythrocytes and oxygen diffusion to tissues falls. As a result P_{O_2} in tissues also decreases. However, during long-term experimental hypoxia, the decrease in P_{O_2} in blood and tissues is compensated by a rise in efficiency of oxygen diffusion. Very large decreases in P_{O_2} promote the transition to anaerobic metabolism. The strong warming of the Black Sea and the Sea of Azov has led to considerable expansion of the hypoxic zones and this has significant effects on the number and composition of fish species tolerant of low and high levels of oxygen.

We have not discussed in this book the effect of carbon dioxide on marine fish, even though this has significance for fish in freshwater basins and aquaculture (Romanenko et al., 1980; Shulman & Love, 1999; Esmark & Belfyord, 2005). We also do not consider significant factors such as salinity and water pressure as these have been discussed in detail in the book by Shulman and Love (1999).

5.3 Anthropogenic factors

5.3.1 Pollution

Because coastal and estuarine areas are under threat of chronic and accidental release of a wide range of anthropogenic pollutants, coastal fish are often exposed to pollution. In particular, juveniles of numerous commercial marine flatfish species, which use coastal and estuarine habitats as nurseries, are likely to be exposed to a number of anthropogenic pollutants.

Although alterations in fish condition are sometimes indicative of toxicant effects (Mayer et al., 1992), the impact of pollution on condition can be very different depending on the species and the area. Several studies have related lower sublethal physiological condition, particularly in juveniles, with pollution. In particular, a number of studies have demonstrated that nurseries located in or near harbors or polluted estuaries do not provide high-quality habitats for juvenile fish, as reflected by their lower growth and condition. The general hypothesis behind this relationship is that metabolic costs may be increased during exposure to pollutants, thus decreasing production processes such as growth and lipid storage (Amara et al, 2007; Martínez-Gómez et al., 2012).

Gilliers et al. (2012) carried out mesocosm experiments to determine whether high exposure to petroleum hydrocarbons, similar to what happens after an oil spill on coastal areas, affected survival and biological (growth, body condition and lipid reserve) performance

of juvenile common sole, which live on nearshore and estuarine nursery grounds. Results demonstrated that this type of exposure significantly reduces not only body condition and energy storage but also survival and growth of the juvenile fish, especially over the medium term (3 months after the exposure). The most dramatic difference was observed for lipid content, exposed fish presenting highly depleted lipid reserves, with triacylglycerol to free sterol ratios close to zero. These medium-term consequences affect future recruitment of this long-lived species. Furthermore, Azmat et al. (2007) showed a statistically significant relation between fluoride concentration and condition of the coastal fish *Johnius belangerii* in the Arabian Sea. Further, in laboratory experiments, physiological deterioration was apparent in fish exposed simultaneously to an oil refinery effluent and nitrite, with unremittingly decreasing liver somatic index even after the withdrawal of the contaminants (Shailaja et al., 2006). Similarly, Amara et al. (2009) found that condition (RNA/DNA ratio and morphometric Fulton's *K*) and growth of 0-group European flounder *Platichthys flesus* was lower in the Seine estuary, heavily impacted by artificial modifications and one of the most contaminated in Europe, than in two adjacent cleaner estuaries (low domestic, agricultural and industrial effluents). The authors concluded that metal contaminants were the most likely cause of condition differences among estuaries.

Morphometric (Fulton's *K* condition index) and lipid (triacylglycerols/sterols ratio) condition indicators measured on 0-group juveniles of common sole along the French coast of the Southern Bight of the North Sea and the eastern English Channel correlated well with anthropogenic disturbance (Amara et al., 2007). Sites with highest levels of chemical contaminants in sediment had the lowest habitat quality and, through growth and lipid-storage limitation, could dramatically lower overwinter survival of the juveniles living in these nursery grounds. Furthermore, 0-group sole grew slowest in nursery sites characterized by intense industrial activity and urbanization.

Similarly, Martínez-Gómez et al. (2012) found that red mullet *Mullus barbatus* from a highly metal-polluted coastal area in the southwestern Mediterranean Sea (the Portmán area) had significantly lower condition factor, muscle lipid content, and gonadosomatic index, as well as the lowest levels of DNA integrity and the highest ethoxyresorufin-*O*-deethylase (EROD) activity in liver, compared with less-polluted areas nearby (Santa Pola, Ebre Delta and Valencia; Table 5.3). Further, in the polluted area red mullets accumulated the highest concentrations of mercury, lead and arsenic and also of some polycyclic aromatic hydrocarbons and polychlorinated biphenyls congeners (Table 5.3). According to the authors, the low nutritional and reproductive status in fish from the highly polluted site could be partially explained not only by variation in quality and lower prey abundance due to the effect of contaminants, but also by cumulative environmental and chemical stressors that impose a higher energy demand on the fish deriving from their natural detoxification processes.

In freshwater ecosystems, condition indicators have been frequently used for assessing the effects of pollutant stress on fish. The use of bioindicators such as condition indicators and indicators related to lipid biochemistry and histopathology have been shown to reflect impaired lipid metabolism, immune and reproductive system dysfunction, and reduced growth potential of fish (Adams et al., 1989). A number of investigations report lower Fulton's condition factor and other metrics of morphometric condition in yellow perch (*Perca flavescens*) from metal-contaminated North American lakes relative to fish from reference lakes (Eastwood & Couture, 2002; Rajotte & Couture, 2002). Similarly, significant correlations between metal concentrations (Cd, Hg, and Pb) in organs and the condition factor of bream *Abramis brama* of the Lake of Balaton (Hungary) were found, especially for

Table 5.3 General physiological indicators, hepatic biomarker responses, and contaminant concentrations in muscle tissue of red mullet *Mullus barbatus*

	Santa Pola	Portmán	Valencia	Delta Ebro
Biological measurements				
Eviscerated weight (g)	28.2 ± 2.5 (29)	23.5 ± 1.1 (47)	29.8 ± 1.9 (39)	34.4 ± 1.8 (38)
Total length (cm)	13.8 ± 0.3 (29)	13.5 ± 0.2 (47)	14.1 ± 0.2 (39)	14.5 ± 0.2 (38)
Condition factor in female	1.06 ± 0.01 (8)	0.95 ± 0.010* (21)	1.09 ± 0.05 (6)	1.12 ± 0.02 (26)
Condition factor in male	0.97 ± 0.01 (21)	0.92 ± 0.01 (23)	1.02 ± 0.02 (32)	1.09 ± 0.05 (12)
GSI in female stage II	1.99 ± 0.22 (8)	1.56 ± 0.10 (22)	2.48 ± 0.32** (6)	2.79 ± 0.19 (26)
GSI in male stage II	1.90 ± 0.54 (20)	1.97 ± 0.14 (24)	2.93 ± 0.17 (33)	2.67 ± 0.23 (12)
Lipid content in female (g/kg)	41.56 ± 13.20 (5)	16.62 ± 2.45 (10)	17.40 (1)	56.55 ± 7.20 (12)
Lipid content in male (g/kg)	16.21 ± 2.56 (14)	11.65 ± 1.34 (9)	27.84 ± 3.18** (18)	26.47 ± 4.07 (8)
HSI in female	2.25 ± 0.21 (7)	1.98 ± 0.10 (21)	2.04 ± 0.42 (6)	2.45 ± 0.09 (26)
HSI in male	1.40 ± 0.07 (21)	1.56 ± 0.07 (23)	1.46 ± 0.10 (32)	1.58 ± 0.09 (12)
EROD activity (pmol/min per mg microsomal protein)	81.24 ± 11.91 (21)	152.79 ± 18.12** (35)	93.86 ± 8.87 (31)	100.77 ± 7.77** (34)
DNA damage (elution constant K/mL×10³)	102.8 ± 8.2 (24)	152.4 ± 10.4** (36)	126.2 ± 7.3 (33)	151.8 ± 8.9 (35)
Metallothionein in female	462.60 ± 61.15 (8)	281.16 ± 22.96* (19)	423.14 ± 76.41 (5)	601.47 ± 61.17 (25)
Metallothionein in male (µg/g hepatic wet tissue)	266.89 ± 20.54 (14)	291.65 ± 23.97 (18)	290.05 ± 20.86 (26)	283.94 ± 20.80 (10)
Chemical measurements				
As (mg/kg)	15.8 ± 3.5 (9)	19.8 ± 1.6 (10)	17.7 ± 2.5 (10)	6.9 ± 0.9 (8)
Cd (µg/kg)	1.10 ± 0.12 (9)	1.06 ± 0.06 (10)	1.24 ± 0.11 (10)	1.07 ± 0.04 (7)
Cu (mg/kg)	0.444 ± 0.036 (9)	0.347 ± 0.018** (10)	0.430 ± 0.020 (10)	0.487 ± 0.021 (8)
Hg (mg/kg)	0.078 ± 0.009 (8)	0.121 ± 0.012* (10)	0.093 ± 0.018 (10)	0.074 ± 0.004 (8)
Pb (mg/kg)	< DL (10)	0.050 ± 0.004** (10)	< DL (10)	< DL (10)
Zn (mg/kg)	3.36 ± 0.19 (9)	3.65 ± 0.07 (10)	3.38 ± 0.08 (9)	4.06 ± 0.17** (8)
Σ4PAHs (µg/kg)	3.05 ± 0.37 (10)	4.00 ± 0.45* (10)	2.06 ± 0.15 (10)	2.68 ± 0.21 (9)
Σ9CBs (µg/kg)	442.7 ± 76.0 (8)	942.2 ± 205.7 (9)	741.2 ± 74.4 (9)	847.4 ± 119.6 (10)
ΣDDTs (µg/kg)	201.6 ± 38.6 (9)	293.8 ± 44.9 (9)	255.7 ± 25.5 (9)	615.9 ± 173.7 (10)
HCB (µg/kg)	< DL (10)	< DL (10)	3.27 ± 0.42 (9)	5.27 ± 0.47 (9)
Trans-nonachlor (µg/kg)†	< DL (9)	< DL (9)	1.95 ± 0.38 (9)	1.58 ± 0.37 (10)
Lindane (µg/kg)†	3.35 ± 0.91 (9)	< DL (10)	< DL (9)	4.97 ± 0.77 (10)
Dieldrin (µg/kg)†	8.50 ± 2.56 (9)	7.65 ± 2.53 (10)	21.16 ± 3.38** (9)	16.54 ± 1.70 (10)

Fish were sampled in April 2006 in four areas of the Spanish Mediterranean coast (mean ± SE). Number of samples analyzed in parentheses. All contaminant concentrations are expressed as wet weight. Outlier values excluded.
*, Significant differences in comparison with Santa Pola (control area), $P < 0.05$. **, Significant differences in comparison with Santa Pola (control area), $P < 0.01$.
< DL, below detection limit.
† Concentrations are referred on a lipid basis.
Source: Martínez-Gómez et al. (2012).

the gill and liver (Farkas et al., 2001). Further, the changes in condition of roach *Rutilus rutilus* and bleak *Alburnus alburnus* clearly coincided with the variation in water quality in a eutrophic reservoir in the Iberian Peninsula (Benejam et al., 2008). Also Norris et al. (2000) found significant site differences in hepatosomatic index of brown trout *Salmo trutta* exposed to heavy metals (mainly Cd and Zn), with hepatosomatic index greater in fish living at the uncontaminated site. In fact, a wide range of physiological effects has been associated with poor water quality, including circulatory changes, altered heart rate, reduced blood oxygen saturation, change in respiratory quotient, and change in spleen, liver and kidney weights (Seager et al., 2000). In addition, Burke et al. (1993) found declining growth and condition in Atlantic croaker *Micropogonias undulates* between different estuarine stations along a pollution gradient.

In particular, biochemical parameters such as lipid, glucose, glycogen, and lactate are very sensitive to sublethal concentrations of stress agents such as pesticides. For example, liver glycogen and lipid content in European eel *Anguilla angilla* have been shown to decrease significantly after exposure to fenitrothion, a pesticide widely used in agriculture for crop protection (Sancho et al., 1998). Further, liver and blood lactate values increased during pesticide exposure, while proteins were decreased in comparison to unexposed controls. The observed effects of the pesticide on eel biochemical parameters were attributed to a stress syndrome (Sancho et al., 1998).

Notwithstanding these results, other studies have suggested that condition factor values of fish exposed to pollutants are not significantly different from those of controls (e.g., Van der Oost et al., 1998; Gadagbui & Goksøyr, 1996), while others have pointed out that an increase in condition factor is sometimes indicative of toxicant effects. For example, condition factor and hepatosomatic index of mullet *Liza saliens* from a polluted lagoon on the Portuguese coast were higher compared with mullet from sea (Fernandes et al., 2008). Similarly, the hepatosomatic index of sculpin *Myoxocephalus scorpius* caught in polluted areas of the North Atlantic was higher than that of individuals caught in unpolluted areas (Stephensen et al., 2000). Results from studies with fish exposed to different pollutants, such as polychlorinated biphenyls, organochlorine pesticides and polychlorinated dibenzodioxins, also showed an increase in hepatosomatic index (e.g., Arnold et al., 1995; Gadagbui & Goksøyr, 1996). In these cases, the authors consider that the higher condition of individuals found in polluted areas can be indicative of increased activity of detoxifying enzymes

Furthermore, Gilliers et al. (2006) found that the growth and condition indicators of juvenile sole *Solea solea* from nursery grounds exposed to a major oil spill that occurred in December 1999 along the Atlantic French coast were relatively high. These results led the authors to suggest that there was no obvious impact of this event on the health of juvenile sole and on the quality of the exposed nursery grounds a few months after the event.

5.3.2 Fishing

Fishing may provoke a decrease in the condition of certain marine species via the removal of available prey items for these species, the induction of stress, or the removal of individuals in the population. In particular, bottom trawling negatively affects biomass and production of benthic communities (Jennings et al., 2001) that are the main food source for a number of demersal species (Hoines & Bergstad, 1999). As a result, bottom trawling has the potential to have the biggest effect on the condition of demersal species, as some studies support. In northwestern Mediterranean waters, Lloret et al. (2007) analyzed the lipid
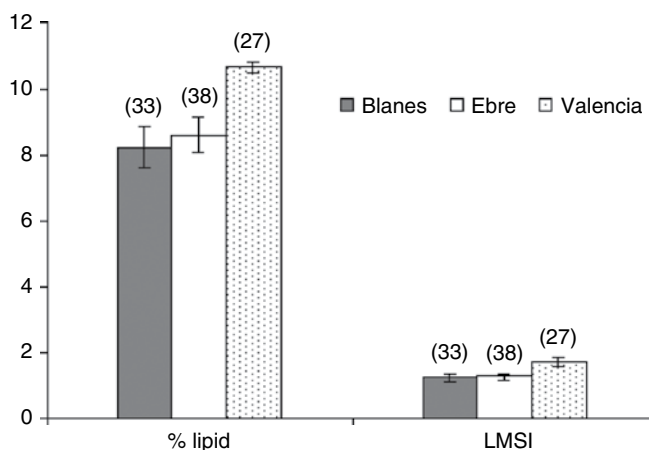
Figure 5.30 Differences in muscle lipid content (% dry weight, mean ± SE) and lipid musculosomatic index (LMSI, mean ± SE) of red mullet *Mullus barbatus* female pre-spawners between the study areas (Blanes, Ebre and Valencia). Number of samples are given in parentheses. From Lloret et al. (2007).
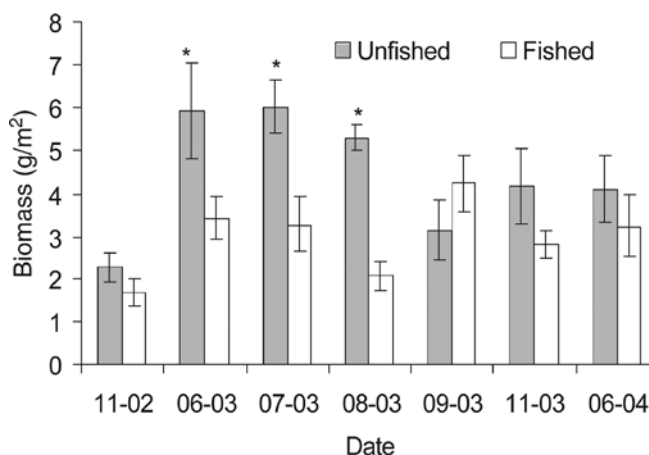
Figure 5.31 Differences in biomass (g/m², mean ± SE) of polychaetes between a fished and an unfished zone within the Ebre area in the seven benthic surveys: November 2002, June, July, August, September and November 2003, and June 2004. The asterisks denote that the difference is significant ($P<0.05$).

content of pre-spawners of red mullet *Mullus barbatus* in three different areas in relation to trawling activities, and showed that fish with the highest muscle lipid levels inhabited the area where fishing impact (in terms of number of trawlers, fishing days, total capacity, and total machine power) was lowest, namely the Valencia area (Fig. 5.30). Furthermore, the abundance and biomass of polychaetes, which represent the main food source for the red mullet, was lower in trawled zones than in unfished ones (Fig. 5.31). Thus the authors suggested that red mullet were fatter in the Valencia area because the lower fishing impact there allows greater abundance and biomass of polychaetes, which allows a higher lipid content in red mullet.

Similarly, in the North Sea, Hiddink et al. (2005) concluded that bottom trawling had the potential to negatively affect the condition and distribution of haddock. Furthermore, Chícharo et al. (2002) studied the chronic effects of dredging on condition of the clam *Spisula solida* in Portuguese waters. Laboratory simulations revealed that dredging-induced stress provoked a decrease in the RNA/DNA ratio and lipid composition of bivalves. Although responses were sublethal, decreases in condition suggested that bivalves are more susceptible to predation when they have been left in the seabed after the dredging activity, particularly during spawning (Chícharo et al., 2003). Furthermore, the noise from fishing operations (e.g., from towed nets or from motors) may induce stress on the fish and reduce its condition. For example, Anderson et al. (2011) examined stress responses to chronic noise exposure in the lined seahorse *Hippocampus erectus* and found that seahorses exposed
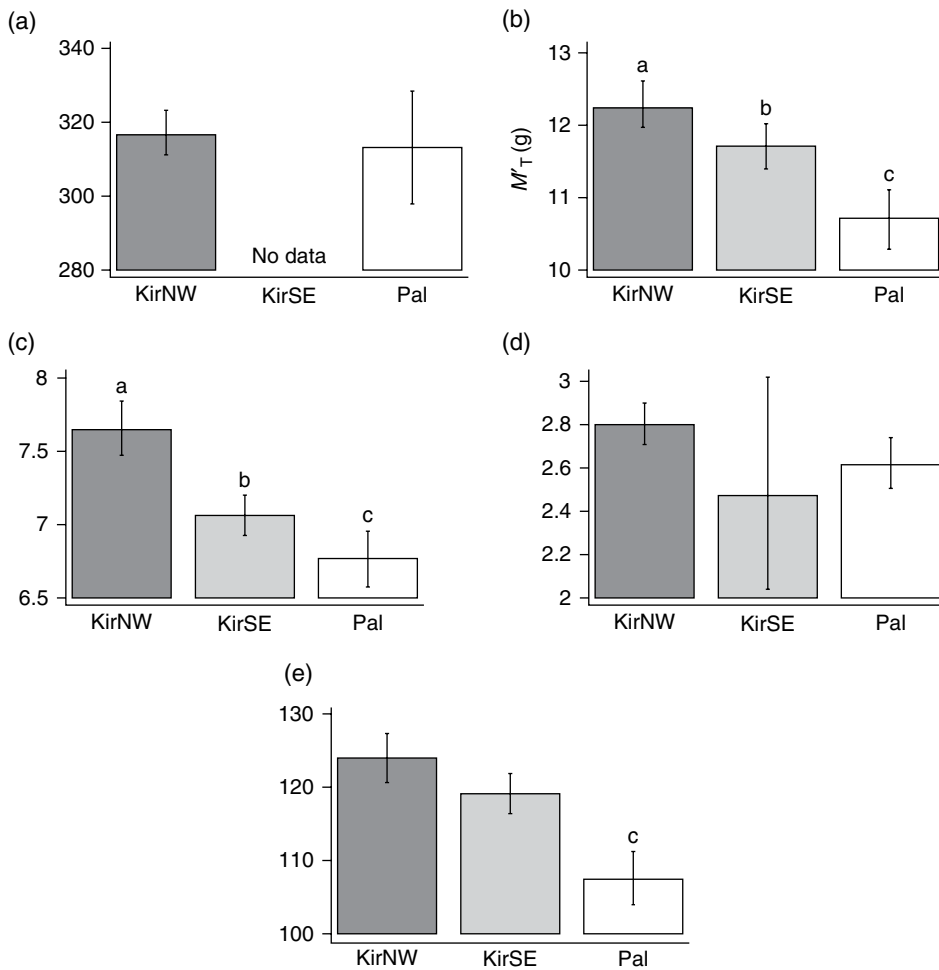
Figure 5.32 Adjusted mean total body mass (M_T) and 95% CI by regions [northwestern Kiritimati (KirNW), southeastern Kiritimati (KirSE) and Palmyra (Pal)] for (a) *Lutjanus bohar*, (b) *Paracirrhites arcatus*, (c) *Plectroglyphidodon dickii*, (d) *Chromis margaritifer* and (e) *Acanthurus nigricans*. The M_T that were determined to be significantly different by *t*-test with Holm's sequential Bonferroni correction are indicated by lower-case letters. From Walsh et al. (2012).

to loud ambient noise in aquaria exhibited stress responses that reduced their Fulton's condition factor and weight. In the laboratory experiment, seahorses in loud tanks declined in weight and condition factor. Therefore, long-term exposure to loud ambient noise can act as a chronic stressor to fish, resulting in responses among condition indicators (Anderson et al., 2011).

However, fishing may provoke density-dependent effects on condition. For example, *M. barbatus* showed a higher condition factor in trawled areas/years in northern Sicily (Giacalone et al., 2010), a fact that the authors attributed to density-dependent effects linked to the increased biomass in untrawled areas.

Furthermore, a growing number of studies have also provided indications that fishing top predators may not only have direct effects on prey abundance (e.g., Terborgh & Estes, 2010) but also can affect condition of fish. For example, Walsh et al. (2012) showed that fishing top predators may indirectly increase prey-fish body condition and energy reserves by reducing predator abundance and associated predation rates and predation risk. Their study examined the indirect effect of fishing predators on prey energy allocation by comparing proxies of condition and reproductive potential in a diverse suite of species from reefs with different levels of fishing in the northern Line Islands (central Pacific Ocean). Results showed that three prey fish from multiple trophic levels (*Paracirrhites arcatus*, *Plectroglyphidodon dickii* and *Acanthurus nigricans*) at reefs with high fishing intensity (northwestern Kiritimati atoll) are not just heavier at a given length, but have heavier livers (for a certain total body mass) than those found at reefs with low (southeastern Kiritimati Atoll) or null (Palmira atoll) fishing intensity (Figs 5.32 and 5.33; Walsh et al., 2012). According to the authors, when predators are scarce, as they are in the highly fished north-western Kiritimati atoll due to high fishing pressure, prey do not need to allocate as much energy to functions that increase survivorship and instead can direct this energy to growth, storage and reproduction. Thus, under low abundance of predators, prey can spend less time avoiding predators and can forage more often and over larger areas. Because time that prey spend avoiding predators is time that cannot be spent foraging, under low abundance predators prey may be able to consume more food or seek out higher-quality food. In addition, prey exposed to lower predation risk has lower mass-specific metabolic rates, meaning that less energy is required for maintenance (Walsh et al., 2012). Overall, it is clear that behavioral or physiological changes in prey under low predator conditions may result in higher net energy intake, which may translate into greater mass gain or storage of energy in fat reserves (Garvey et al., 2004; Pérez-Tris et al., 2004).

It is also important to note that in studies evaluating the condition and general health status of fish stocks, the method used for catching the fish is seldom considered a factor of importance (Fig. 5.34). However, there can be differences in condition indices of fish caught in different gear types. Ovegård et al. (2012) demonstrated that fishing method is related to fish condition; when condition indices were compared between cod caught in pots, gillnets, and on hooks in the same geographical area, the results showed that cod *Gadus morhua* caught on baited gear types (pots and hooks) generally displayed a lower condition and an older age compared with cod caught in gillnets. The results showed that there is indeed substance in the claims made by local fishers that, on a majority of the sampling occasions, fish caught in baited gear types such as hooks and pots exhibit significantly lower condition compared with fish caught in gillnets (Overgard et al., 2012). According to the authors, there are several possible explanations for the lower condition of fish in the baited gear types, with the most obvious one being that fish in poor condition (i.e., in greater need of energy than fish in good
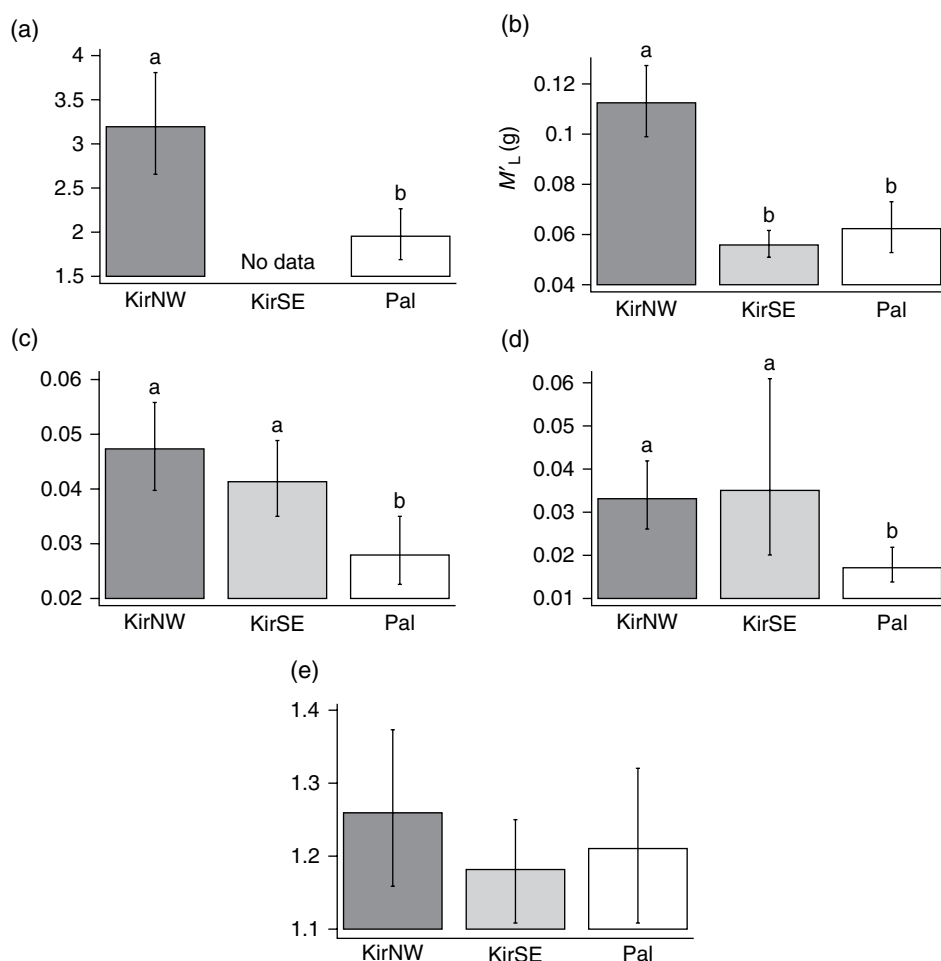
Figure 5.33   Adjusted mean liver mass (M_L) and 95% CI by regions [northwestern Kiritimati (KirNW), southeastern Kiritimati (KirSE) and Palmyra (Pal)] for (a) *Lutjanus bohar*, (b) *Paracirrhites arcatus*, (c) *Plectroglyphidodon dickii*, (d) *Chromis margaritifer* and (e) *Acanthurus nigricans*. The M_L that were determined to be significantly different by *t*-test with Holm's sequential Bonferroni correction are indicated by lower-case letters. From Walsh et al. (2012).

condition) are more likely to be actively searching for food and are therefore more prone to be attracted by the stimuli of the bait.

Finally, overfishing is threatening many fish stocks despite the importance of seafood for a healthy human diet. The depletion of these fish stocks, particularly pelagic oily fish populations, is reducing the potential supply of long-chain omega-3 fats. The current recommendations of government health agencies to people in developed countries to increase their intakes of fatty fish by at least two to three times are incongruent with the collapse of global fish stocks (Jenkins et al., 2009). This raises the necessity to better manage fisheries in the Mediterranean in order to avoid overexploitation and allow stock recovery and, at the same time, to seek alternative sources of omega-3 fatty acids such as marine algae, microorganisms, and plants (Surette, 2008).

Figure 5.34 Longline coastal fishery in the Mediterranean. Photo by Josep Lloret.

5.3.3 Aquaculture

Because of the particular feeding of farmed fish (food pellets), the proximate composition of these fish, including total lipid content and fatty acid profile, is different from that of wild fish. Usually, the fat percentage is higher in reared than wild fish because the former always feed abundantly. Furthermore, Ackman and Takeuchi (1986) reported that the percentage of omega-3 PUFAs in farmed marine fish is usually lower than that in their wild relatives, presumably because of the lack of lipids originating from algae and marine phytoplankton (Jobling, 1985, 1993; Sargent, 1995). For example, fat percentage in the muscle of reared red porgy *Pagrus pagrus* (3.0%) is higher than that in wild fish (0.65%), whereas wild red porgy shows higher levels of omega-3 PUFAs than reared, reflecting dietary lipids (Rueda et al., 1997). Similarly, Skog et al. (2003) found that wild saithe associated with a single fjord-based farm in Norway had higher condition than control fish taken from within the same fjord.

Another example is found in salmon and some other aquaculture species, which were fed with *Artemia salina* nauplies containing high level of omega-3 fatty acids. In this way the level of omega-3 acids in these fries also became higher (Sargent et al., 1999).

Furthermore, there are recent indications that rearing of fish in coastal sea-cages using food pellets containing terrestrial plant products impacts wild fish that aggregate around farms by altering their natural diet in a way that leads to a change in body condition and fatty acid composition. The production of fish such as seabream *Sparus aurata* and seabass *Dicentrarchus labrax* in coastal fish farms is carried out using food pellets composed partly of vegetable oils. The corn or soya used in food production gives a high concentration of oleic acid, linoleic acid, and linolenic acid (Fernández-Jover et al., 2007). The introduction

Table 5.4 Fulton's condition index (CI) and liver somatic index (LSI) for wild Mediterranean horse-mackerel *Trachurus mediterraneus*

Location	Length (mm)	Weight (g)	CI	LSI
Vilajoiosa control	291.7 ± 27.9	222.9 ± 87.7	0.86 ± 0.06	1.32 ± 0.50
Alacant control	250.3 ± 15.2	134.4 ± 20.3	0.85 ± 0.04	1.60 ± 0.61
Campello Farm	378.7 ± 20.6	570.9 ± 107.5	1.04 ± 0.11	1.02 ± 0.34
Guardamar Farm	302.9 ± 30.9	275.71 ± 63.0	1.02 ± 0.24	1.11 ± 0.47

Fish were sampled from populations aggregated around two Mediterranean fish farms and from two natural control populations ($n = 10$). Values represent means ± standard deviation.
Source: Fernández-Jover et al. (2007).

Table 5.5 Fatty acid composition of *Trachurus mediterraneus* associated and not associated with fish farms

Name	Vilajoiosa Control	Alacant Control	Campello Farm	Guardamar Farm
C12:0	0.25 ± 0.51	0.16 ± 0.24	0.05 ± 0.08	0.14 ± 0.15
C13:0	1.02 ± 3.23	0.00 ± 0	0.00 ± 0	0.00 ± 0
C14:0	2.03 ± 0.42	2.53 ± 1.09	3.58 ± 0.55	3.32 ± 0.69
C14:1ω5	0.00 ± 0	0.00 ± 0	0.00 ± 0	0.04 ± 0.12
C15:0	0.46 ± 0.35	0.57 ± 0.36	0.35 ± 0.19	0.49 ± 0.22
C15:1ω5	0.00 ± 0	0.00 ± 0	0.00 ± 0	0.02 ± 0.06
C16:0	21.84 ± 1.94	23.39 ± 3.11	19.73 ± 1.79	18.81 ± 2.74
C16:1ω7	1.97 ± 0.4	2.66 ± 1.28	4.49 ± 0.62	4.63 ± 1.83
C17:0	0.66 ± 0.24	0.55 ± 0.3	0.48 ± 0.21	0.67 ± 0.29
C17:1	0.24 ± 0.27	0.27 ± 0.22	0.21 ± 0.16	0.31 ± 0.28
C18:0	8.63 ± 2.67	7.85 ± 1.2	7.28 ± 2.88	7.66 ± 3.69
C18:1ω9	9.30 ± 1.63	11.34 ± 2.45	18.81 ± 2.68	20.60 ± 9.09
C18:1ω7	1.65 ± 0.2	1.73 ± 0.99	2.69 ± 0.23	2.70 ± 0.69
C18:2ω6	2.69 ± 1.22	2.77 ± 2.88	14.26 ± 2.11	12.02 ± 5.59
C20:0	0.15 ± 0.2	0.20 ± 0.17	0.23 ± 0.18	0.29 ± 0.14
C18:3ω6	0.59 ± 1.08	0.40 ± 0.62	0.66 ± 1.11	1.81 ± 3.22
C20:1ω9	0.45 ± 0.33	0.98 ± 1.31	1.90 ± 0.82	1.93 ± 1.16
C18:3ω3	0.24 ± 0.27	0.51 ± 0.71	1.60 ± 0.24	1.29 ± 0.61
C18:4ω3	0.07 ± 0.12	0.19 ± 0.25	0.50 ± 0.31	0.57 ± 0.43
C20:2ω6	0.21 ± 0.27	0.44 ± 0.33	0.56 ± 0.4	0.64 ± 0.26
C22:0	0.35 ± 0.31	0.33 ± 0.22	0.18 ± 0.17	0.19 ± 0.21
C20:3ω6	0.18 ± 0.31	0.13 ± 0.31	0.23 ± 0.39	0.78 ± 1.32
C22:1ω9	0.02 ± 0.06	0.12 ± 0.28	0.28 ± 0.25	0.20 ± 0.28
C20:3ω3	0.00 ± 0	0.06 ± 0.17	0.05 ± 0.09	0.04 ± 0.09
C20:4ω6	1.45 ± 0.25	1.42 ± 0.36	0.68 ± 0.41	0.68 ± 0.21
C23:0	0.00 ± 0	0.00 ± 0	0.00 ± 0	0.15 ± 0.48
C24:0	0.72 ± 0.3	0.46 ± 0.31	0.14 ± 0.17	0.18 ± 0.24
C20:5ω3	5.83 ± 1.09	6.18 ± 1.61	6.04 ± 1.67	4.53 ± 1.74
C24:1ω9	0.98 ± 0.37	0.90 ± 0.37	0.40 ± 0.23	0.51 ± 0.2
C22:4ω6	0.05 ± 0.1	0.02 ± 0.07	0.05 ± 0.08	0.02 ± 0.05
C22:5ω3	1.36 ± 0.53	1.29 ± 0.56	1.39 ± 0.24	1.11 ± 0.43
C22:6ω3	36.61 ± 4.01	32.56 ± 6.55	13.19 ± 2.63	13.64 ± 3.72
ω3	44.11 ± 5.2	40.79 ± 6.99	22.76 ± 4.31	21.19 ± 4.63
ω6	5.17 ± 1.82	5.19 ± 2.82	16.43 ± 2.93	15.95 ± 5.27
ω5	0.00 ± 0	0.00 ± 0	0.00 ± 0	0.06 ± 0.13
ω7	3.62 ± 0.57	4.38 ± 2.11	7.18 ± 0.82	7.33 ± 2.47
ω9	10.75 ± 1.51	13.34 ± 3.75	21.40 ± 3.15	23.25 ± 9.01
Saturated	36.10 ± 4.77	36.04 ± 3.16	32.02 ± 4.18	31.91 ± 6.81
Monounsaturated	14.62 ± 1.88	17.99 ± 5.45	28.79 ± 3.16	30.95 ± 10.86
Polyunsaturated	49.28 ± 5.22	45.98 ± 6.44	39.19 ± 4.17	37.14 ± 7.81

Fish were sampled from populations aggregated around two Mediterranean fish farms and from two natural control populations ($n = 10$, percent of total fatty acids).
Source: Fernández-Jover et al. (2007).

of this source of food to the marine environment could modify the fatty acid composition of wild fish that feed on lost pellets as well as their total fat content due to the high availability of food. This has been observed for wild Mediterranean horse-mackerel *Trachurus mediterraneus* sampled from populations aggregated around two Mediterranean fish farms and from two natural control populations (Fernández-Jover et al., 2007). Wild fish aggregated at farms mainly ate food pellets while control fish fed principally on juvenile fish and cephalopods. Farm-associated fish had a higher Condition Index than found in control fish (Table 5.4; Fernandez-Jover et al., 2007). In contrast, the Liver Somatic Index was higher in control fish than in farm-associated fish (Table 5.4). Wild fish that fed around the cages had a significantly higher body fat content than the control fish (7.3% and 2.4%, respectively) and a different fatty acid composition (Table 5.5; Fernández-Jover et al., 2007). Despite these discrepancies, the authors hypothesized that the increased condition of wild fish associated with farms could increase the spawning ability of coastal fish populations, if wild fish are protected from fishing while they are present at farms, and that the fatty acid composition could also serve as biomarkers to infer the influence of a fish farm on the local fish community, helping to better describe the environmental impact of fish farming. According to Fernández-Jover (2007), such a method would be a key tool to infer the scale and magnitude of the influence of a net-cage fish farm on the local fauna, not only on fish that directly feed on lost food pellets but on the whole food web.

6 Fish condition as a measure of environmental status and habitat quality

Summary: Temporal and spatial changes in fish condition can reveal changes in environmental conditions and can therefore be used as indicators of environmental status. In this chapter we provide evidence that the use of condition indicators is fundamental for predicting species' responses to acute environmental or human constraints. This chapter shows how condition indicators can be used as indicators of the quality of habitats that fish inhabit, thus contributing to the identification of essential fish habitats, evaluation of the effect of habitat protection (marine reserves), or determination of the value of particular depths and areas for the productivity of fish stocks. In particular, the condition of small pelagic species can be a valuable indicator of anomalous environmental conditions. Furthermore, the consequences of anomalous fish condition on top predators (e.g., seabirds and marine mammals) are also considered. Finally, the chapter supports with examples the integration of both genetic and ecophysiological approaches for interspecific and intraspecific differentiation.

Key words: habitat quality, spatial differences, bathymetric differences, marine protected areas, essential fish habitats, top predators, interspecific and intraspecific differentiation

Because fish condition, if appropriately interpreted, may characterize components of the environment in which fish exist (e.g., habitat, prey availability, competition), temporal and spatial changes in fish condition can reveal changes in environmental conditions and can therefore be used as indicators of environmental status. The use of condition indicators is fundamental for predicting species' responses to acute environmental or human constraints, especially in a global climate change scenario. In particular, the health of small pelagic species can be a valuable indicator of anomalous environmental conditions (Lloret et al., 2012). Moreover, changes affecting marine food webs are ongoing and may accelerate in the future given threats such as climate change, the introduction of exotic species, and overexploitation. Long-term monitoring of stock health as well as the health of their prey can contribute to knowledge of how exploited fish stocks are responding to these changes. Fish health could be used as an additional ecosystem indicator for fisheries

management other than those proposed recently by different authors (see for example Cury & Christiensen, 2005) and policy documents (e.g., Marine Strategy Framework Directive from the European Union). Fish health could serve as associated indicators that need to be identified so as to guide progress towards achieving good environmental status in the marine environment. Because the physiological condition information could also aid in understanding productivity and habitat relationships and steer management actions (e.g., establishment of marine reserves in those habitats where fish are better conditioned), fish condition could also be used as an indicator of habitat quality in the Mediterranean in order to determine and protect the habitats that are more valuable for fishery production, namely to identify essential fish habitats (EFHs), apart from the measurement of standard parameters such as abundance and biomass and the vulnerability of each habitat (Lloret et al., 2012).

6.1   Fish condition as a measure of habitat quality

We have seen that spatial changes in environmental factors (abiotic or biotic) contribute to the observed variability in fish condition between areas, seas/oceans, depths, and habitats. Therefore condition indicators can be used as indicators of the quality of habitats that fish inhabit, thus contributing to the identification of EFHs, evaluation of the effect of habitat protection (marine reserves), or determination of the value of particular depths and areas for the productivity of fish stocks. Hence, using condition indicators to compare habitats assumes that the indicators reflect the habitat's value in which the fish live. Most studies have dealt with differences in abundance and abundance and/or mean length and age between habitats as a way to evaluate habitat quality, but seldom have used the condition approach. Taking into account these differences in condition related to habitat and the importance of individual condition for the overall success of the population, condition indicators could be used as markers of habitat quality and can therefore constitute a useful tool for identifying high-quality habitats or EFHs for exploited species, as well for identifying the effects of the establishment of marine protected areas (MPAs) as a tool for fisheries conservation.

In fact, in freshwater environments and estuaries, fish condition assessment is playing an increasing role in both fishery management and environmental monitoring policy because useful health indicators are capable of detecting responses to different habitat characteristics that can affect the fitness of an organism with population-level consequences (Leamon et al., 2000). In several rivers and lakes around the world, a close relationship has usually been found between water quality and fish condition (e.g., Leamon et al., 2000; Benejam et al., 2008).

### 6.1.1   Large-scale differences in fish condition

Large-scale differences in condition occur in both demersal and pelagic fish species and express differences in habitat quality among regions of the oceans and seas. With regard to demersal fish, a comparative analysis of Fulton's K condition factor of 10 cod stocks in the North Atlantic showed that they display different levels of mean condition, partly due to the different temperature regimes of their habitats (Rätz & Lloret, 2003). Cod living in colder waters, such as Greenland, Labrador and Grand Bank stocks, were found to be in poorer condition than cod living in warmer waters, for example North Sea and Irish Sea stocks (Fig. 6.1). Thus, different areas in the North Atlantic display differences in temperature
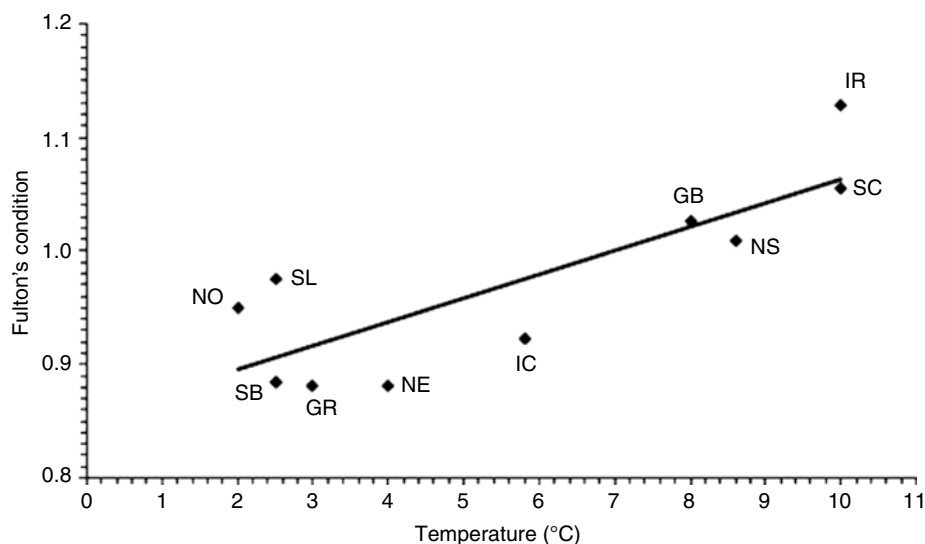
Figure 6.1 Linear regression between condition factor and average bottom temperature (°C) for 10 North Atlantic cod stocks. Stock abbreviations: Greenland (GR), Georges Bank (GB), S. Gulf of St Lawrence (SL), Southern Grand Bank (SB), Northern Cod (NO), North-east Arctic (NE), NW Scotland and Ireland (SC), Irish Sea (IR), North Sea (NS), Iceland (IC). From Rätz & Lloret (2003).

regimes that seem to determine in part the condition of resident cod. Yet Love et al. (1974) had already indicated in the early 1970s the large-scale differences in the condition of cod, showing that the Faroe Bank cod are uniquely corpulent compared with any others inhabiting other fishing grounds, and that their livers were unusually large and creamy and their muscle had greater amounts of total protein and glycogen than cod from the Faroe Plateau, Fillyas Bank, Minch or Fiskernaes grounds. According to the authors, the better-conditioned Faroe Bank cod benefit from an unusually large food supply because of the particular oceanographic features of that area. Similar, Lee and Khan (2000) found that cod taken at southern localities off coastal Labrador (northwest Atlantic) were predominantly in better condition than those from northern areas. Furthermore, Pardoe et al. (2008) showed strong interannual and spatial variation of mean hepatosomatic index (HSI) (Fig. 6.2) and relative condition factor of immature and mature cod in shallow and deep waters.

Additionally, in the northwest Mediterranean, geographic and bathymetric differences in the condition of several groundfish species have been observed. For example, the lipid content in the muscle of *Pagellus acarne* and *Pagellus erythrinus* was higher in the Gulf of Lions than in the Catalan Sea (Fig. 6.3; Lloret et al., 2005). Similar results were reported by Shulman (1972a), who found that small pelagics such as sprats and sardines off the Gulf of Lions had considerably higher fat content than that of sprat *Sprattus sprattus* and sardine *Sardina pilchardus* in other areas of the Mediterranean Sea. The differences in lipid content between individuals from the Gulf of Lions and the Catalan Sea were probably due to differences in productivity between both areas. The Gulf of Lions, located at the western and northernmost part of the Mediterranean Sea, is one of the most productive areas of the Mediterranean Sea due to a number of hydrographic features, including a wide shelf, important river runoff (Rhône River), strong vertical mixing in winter, and occasional coastal upwelling. The waters from the Catalan Sea are less productive due to a narrow shelf
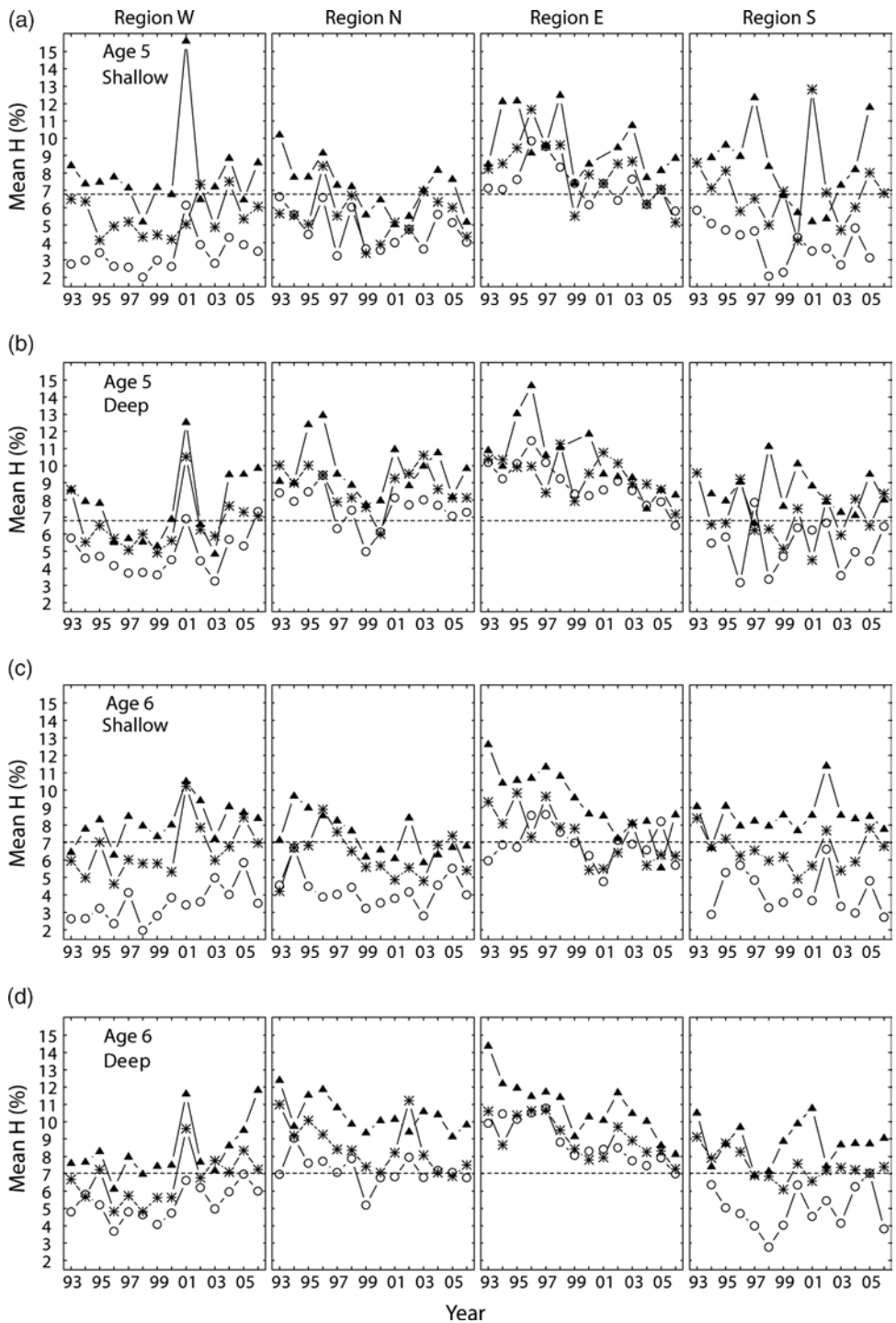
Figure 6.2 Cod (*Gadus morhua*) off Iceland. Interannual (1993–2006) and spatial variation of mean hepatosomatic index (H, %) of age 5 year immature and mature cod in (a) shallow (<200 m) and (b) deep (>200 m) water, and age 6 year immature cod in (c) shallow and (d) deep water. The horizontal dashed line shows the overall mean for the age present in each plot. Open circles, both sexes combined; triangles, mature females; asterisks, mature males. From Pardoe et al. (2008).
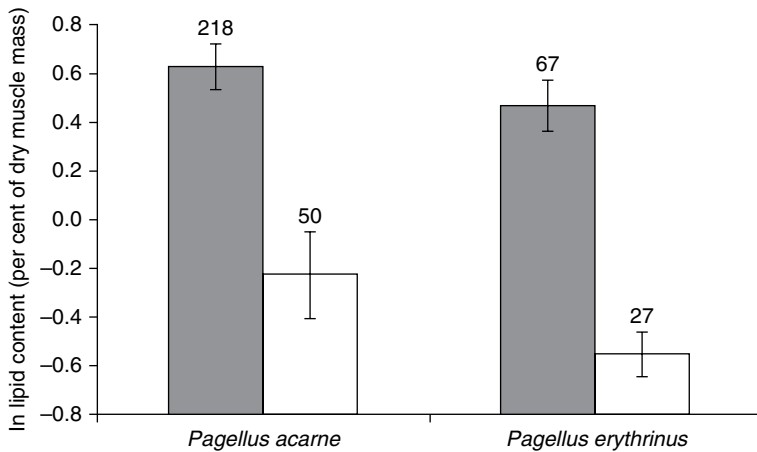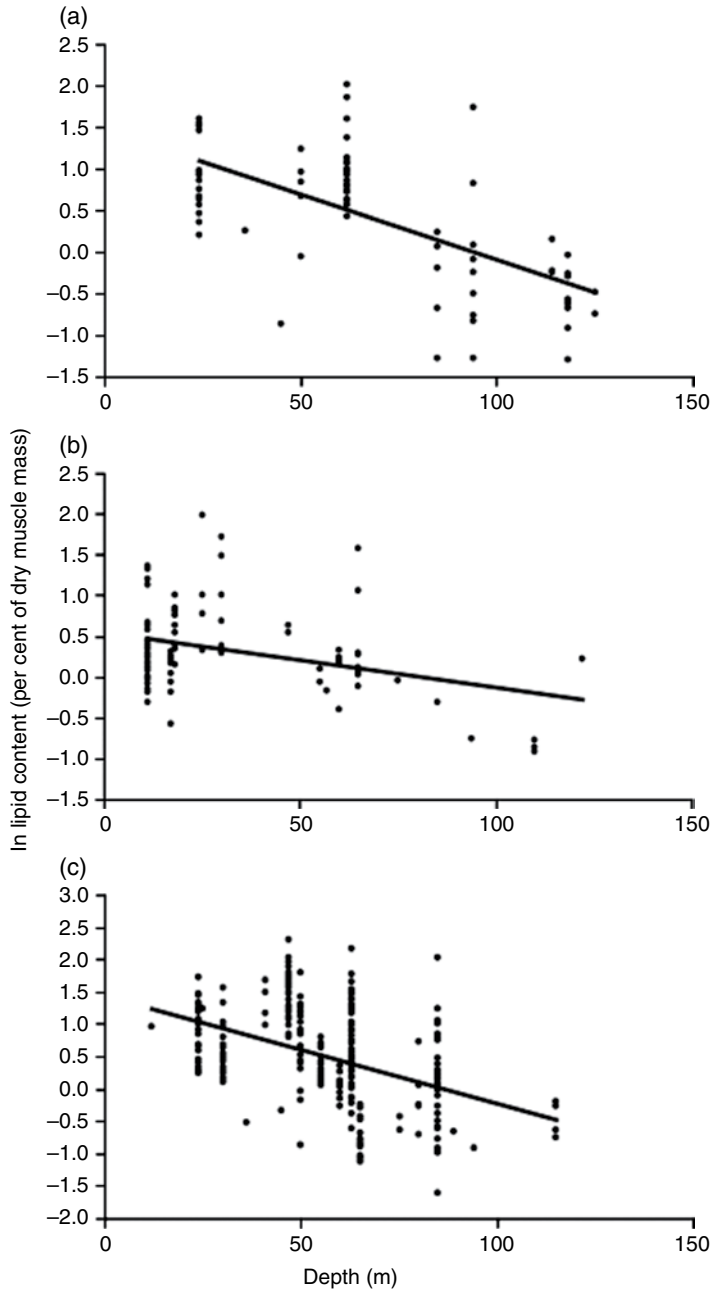
Figure 6.3 Muscle lipid content (ln transformed data, mean ± SE) of *Pagellus acarne* and *Pagellus erythrinus* between the Gulf of Lions (shaded columns) and the Catalan Sea (unshaded columns). For *P. acarne*, these are the adjusted means (means after adjusting for the variation of the covariate depth). Number of samples are given above the bars. From Lloret et al. (2005).

and less important river runoff and winds. Thus, the Gulf of Lions seems to provide good food supply for some fish species.

Furthermore, there was a significant effect of depth on lipid content of *Mullus barbatus*, *M. surmuletus* and *P. acarne*, fish with the highest lipid levels inhabiting the shallowest parts of the Gulf of Lions (all three species) and the Catalan Sea (last two species; Fig. 6.4; Lloret et al., 2005). The bathymetric trends of lipid content suggest that deeper areas of distribution of some species represent a marginal habitat in terms of food resources. In contrast, shallow areas of distribution, which fish with higher lipid levels inhabit, may constitute EFHs for some demersal species. In coastal areas there is often more food available than in the open sea, so fish tend to be better nourished.

This spatial and bathymetric condition of fish in the northwest Mediterranean has been particularly studied for hake (Lloret et al., 2002; Ferraton et al., 2007; Hidalgo et al., 2008). Condition of juvenile hake in the Gulf of Lions was found to be lower in deep waters and in areas where the food supply is low (Lloret et al., 2002; Ferraton et al., 2007). Thus for example, in 2003, juvenile hake located in deep waters had a lower condition factor than those living in shallow waters (Ferraton et al., 2007). According to the authors, the main difference in the feeding of juveniles in deep waters was a diet composed mainly of shrimps in 2003, which provided a less energetic source of food than fish (the main prey in shallow waters), and small crustaceans (mysids and euphausiids), the main prey in deep waters in 2002, when no bathymetric differences in condition of juvenile hake were found (Ferraton et al., 2007). In the Balearic Islands the HSI of recruits, post-recruits and young adults was higher in the northern part (Sóller area) than in the southern part (Cabrera area) for all sizes analyzed, a fact that was attributed to the different oceanographic conditions existing in the north and the south (Hidalgo et al., 2008). According to the authors, the observed differences in the condition of recruits between areas could be a consequence of the fact that the northern coast of the Balearic Islands is more subject to periodic productivity events and the earlier arrival of waters formed in more productive areas. This was consistent with fullness index values, which were higher at the northern coast than at the southern coast, suggesting

Figure 6.4 Linear relationships between depth and muscle lipid content (ln transformed data) of (a) *Mullus barbatus* in the Gulf of Lions ($y=1.490-0.016x$; $n=76$, $r^2=0.423$, $P<0.001$), (b) *Mullus surmuletus* in the Gulf of Lions and Catalan Sea ($y=0.552-0.007x$; $n=90$, $r^2=0.125$, $P<0.001$) and (c) *Pagellus acarne* in the Gulf of Lions and Catalan Sea ($y=1.434-0.017x$; $n=268$, $r^2=0.174$, $P<0.001$). From Lloret et al. (2005).

higher food consumption by hake (more food supply) at the northern coast, which seems to enhance the condition of hake in that area (Hidalgo et al., 2008). Also in the Adriatic Sea, the fatness of juvenile red mullet decreases from the Delta of the Po River towards the Strait of Otranto, paralleling a decrease in the food supply (see section 5.1.1. and Figure 5.3).

Large-scale spatial variability in fish condition is also observed in pelagic species. For example, spatial differences in condition have been found in anchovy in the Mediterranean Sea, Sea of Azov and Black Sea. The most important indicator of condition and food supply for anchovies from these seas, namely the energy levels stored in the form of neutral lipids that individuals accumulate in their muscles at the end of the autumn feeding period (Shulman, 1974; Shulman & Love, 1999; Nikolsky et al., 2009a,b), displays strong spatial and interannual variability. Compared with Azov and Black Sea anchovy, whose muscle lipid content ranges between 15 and 20% of wet mass respectively at the end of the feeding season, Mediterranean anchovy accumulate less than 10% lipid in their muscles (Shulman, 1974; Zlatanos & Laskaridis, 2007). In general, fish from the Mediterranean Sea (mackerel, horse-mackerel, red mullet and pilchard) are less fatty than those from the Black Sea (Shulman & Love, 1999). These spatial differences in muscle lipid levels of several species from the Black Sea and the Mediterranean probably reflect the marked differences in productivity between the areas and hence the differences in habitat quality: the highest food supply (i.e., higher zooplankton biomass productivity) is seen in the Sea of Azov and the Black Sea compared with the Mediterranean Sea, and is likely to be linked with the strong riverine nutrient inputs from the large rivers flowing into the Sea of Azov and the Black Sea (Danube and Dnieper rivers in the Black Sea and Don and Kuban rivers in the Sea of Azov; Ludwig et al., 2009). In the Sea of Azov, the lipid content of anchovy also varies spatially, with good agreement between the distribution of zooplankton biomass in late summer and the fatness of this species (Shulman & Love, 1999). And in the Black Sea, highest fat content in sprat *Sprattus sprattus phalericus* is found in the population that feeds in the region adjacent to the Danube estuary (Fig. 5.2; Minyuk et al., 1997).

In addition, capelin fat content was highest in northeastern regions of the Barents Sea (Orlova et al., 2010). Thus, capelin from the northeastern region had significantly higher fat levels (10.1%) than those from either the northwestern (8.8%) or central (8.4%) regions in 2006. Finally, on the Portuguese Atlantic coast, spatial variation in sardine *Sardina pilchardus* dietary fatty acids was detected in their muscle composition, specifically for *n*-3 polyunsaturated fatty acids, namely eicosapentaenoic acid (EPA) and the ratio of EPA to docosahexaenoic acid (DHA) and the *n*-3/*n*-6 ratio, which were higher in sardines from the west coast, where upwelling is stronger and prey (phytoplankton) availability is higher than is the case of the south coast (Garrido et al., 2008).

Spatial differences in fish condition are more apparent in freshwater ecosystems, where confined water masses facilitate the existence of populations with different biological characteristics. For example, lipid values for several freshwater fish species inhabiting the Great Lakes significantly differed among lakes, with Lake Erie values being significantly higher than those for Lakes Huron and Superior for example (Neff et al., 2012).

## 6.1.2 Small-scale differences in fish condition

Small-scale variation in fish condition has been widely studied for several species, particularly in coastal areas and estuaries, in order to characterize suitable habitat for settlement of juveniles or suitable habitat for feeding. Characterization of suitable habitat for settlement of juvenile fish is important for the management of nursery areas. In this sense, several studies have confirmed the validity of physiological condition performance of juvenile fish

as habitat quality indicators in highly variable environments such as estuarine nurseries. Thus for example, in Galveston Bay (United States), the differences in RNA/DNA ratios between recruits of southern flounder *Paralichthys lethostigma* inhabiting different areas suggested that not all parts of the bay function equally as nurseries (Glass et al., 2008). Southern flounder from West Bay had significantly lower RNA/DNA ratios than those from East Bay and Galveston Bay, with no significant difference in condition between East Bay and Galveston Bay (Glass et al., 2008). In contrast, at a smaller scale (i.e., habitat-specific), differences in density, condition, and growth were not evident between habitats in close proximity, suggesting a variety of habitats maintained conditions favorable for condition of newly settled southern flounder.

In the Seine estuary (France), the RNA/DNA ratio and morphometric (Fulton's *K*) condition indicators of 0-group European flounder *Platichthys flesus* were used as indicators of estuarine habitat quality (Amara et al., 2009). The authors compared the condition in the Seine estuary to two smaller estuaries considered as clean (low domestic, agricultural and industrial effluents) and found that RNA/DNA ratio and *K* values were significantly lower in individuals from the Seine compared with the cleaner estuaries (Fig. 6.5). On the basis of the results, the authors suggested that habitat-specific fish growth and condition are useful proxies for habitat quality since they integrate the effect of multiple environmental factors. De Raedemaecker et al. (2011) found significant differences in condition of juvenile plaice *Pleuronectes platessa* between hauls and between beaches from the southwest coast of Ireland. The results highlighted the importance of considering small-scale variation when attempting to link habitat quality to feeding, growth, and condition of juvenile flatfish. In the Bay of Biscay, indicators of growth and condition were used to compare the habitat quality of nurseries of juvenile sole *Solea solea* (Gilliers et al., 2006). Condition indicators did not show any latitudinal pattern but highlighted low condition values in semi-enclosed sheltered marsh areas little affected by rivers, compared with the Gironde estuarine areas (Gilliers et al., 2006). In addition, morphometric (Fulton's *K* condition index) and lipid (triacylglycerols/sterols ratio) condition indicators measured on 0-group juveniles of common sole were used to compare the habitat quality of different nursery grounds along the French coast of the Southern Bight of the North Sea and the eastern English Channel with differing levels of anthropogenic pressure (Amara et al., 2007). Results indicated consistent differences in the condition indicators of 0-group sole among different sites. Sole had the lowest condition indicators at two sites located in intensively developed and industrialized harbor areas such as Dunkerque and Calais, and the highest condition indicators at three other sites located near small estuaries less subject to human pressure such as Canche, Authie, and Somme (Amara et al., 2007). The indicators measured in this study correlated well with anthropogenic disturbance and may provide a useful tool for assessing habitat quality. Sites with the highest levels of chemical contaminants in sediment had the lowest habitat quality and, through growth and lipid-storage limitation, could dramatically lower overwinter survival of the juveniles living in these nursery grounds.

Furthermore, RNA/DNA ratio and Fulton's *K* condition factor were determined in juveniles of *Solea solea*, *Solea senegalensis*, *Platichthys flesus*, *Diplodus vulgaris* and *Dicentrarchus labrax* collected in several putative nursery areas of nine estuaries along the Portuguese coast (Vasconcelos et al., 2009). Results highlighted species-specific trends in the variability of condition amongst estuaries as some estuaries had higher juvenile condition for more than one species (Table 6.1). Furthermore, intra-estuarine variation in condition of the studied species was also observed (Table 6.2). Within the analyzed estuaries, higher condition values were generally found in sheltered areas with intertidal flats, and in the case of *S. solea* and *P. flesus* in sites where juvenile densities were not the highest,
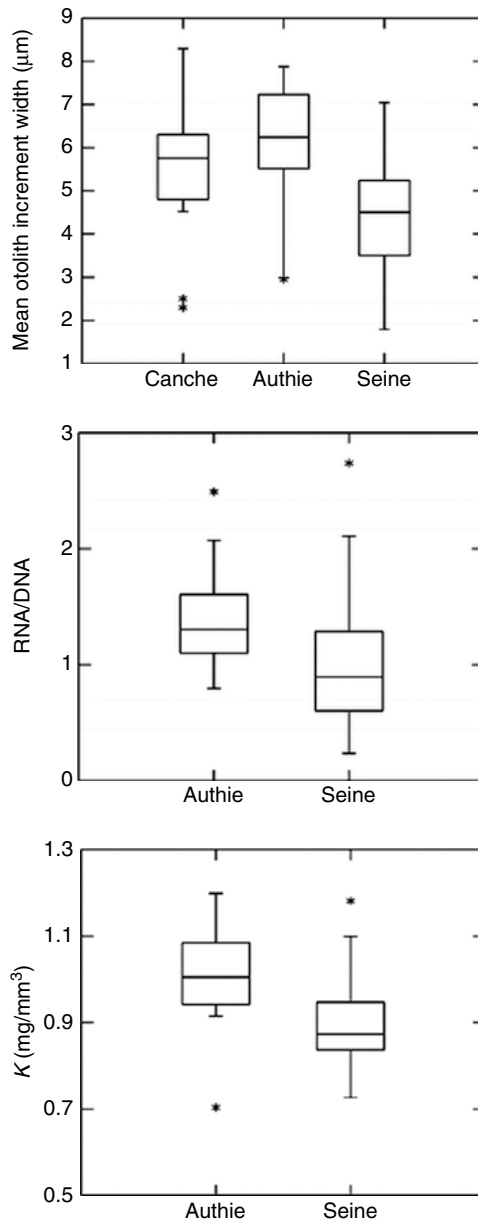
Figure 6.5 Differences in recent growth (mean otolith increment widths for the previous 10 days), RNA/DNA ratio, and Fulton's condition index (K) for 0-group flounder *Platichthys flesus* juveniles caught in the Canche, Authie, and Seine estuaries (French Atlantic Ocean). The whiskers extend to the highest and lowest values, excluding outliers. The line across the box indicates the median. From Amara et al. (2009).

as well as in areas with seagrass beds (Vasconcelos et al., 2009). According to the authors, intra-estuarine variability of species' RNA/DNA ratios provides key insight on differential habitat quality within estuaries and may indicate sites that provide enhanced nutritional status and growth and which may be in higher agreement with the nursery role hypothesis than others.

Table 6.1 Inter-estuarine variation in condition indices

Species	Year	n	df	RNA/DNA'		Fulton's K'	
				F	P	F	P
S. solea	2005 & 2006	211	5	24.86	<0.001	4.67	<0.001
	2005	85	3	5.23	<0.01	5.29	<0.01
	2006	126	5	22.91	<0.001	2.42	<0.05
S. senegalensis	2005 & 2006	91	2	17.17	<0.001	5.27	<0.01
	2005	59	1	16.33	<0.001	1.96	NS
	2006	32	1	0.04	NS	10.66	<0.01
P. flesus	2005 & 2006	136	3	28.74	<0.001	2.49	NS
	2005	77	2	18.31	<0.001	1.71	NS
	2006	59	3	18.94	<0.001	2.80	<0.05
D. vulgaris	2005 & 2006	356	6	22.28	<0.001	4.82	<0.001
	2005	162	5	9.99	<0.001	2.11	NS
	2006	194	5	20.29	<0.001	3.48	<0.01
D. labrax	2005 & 2006	203	3	5.12	<0.01	3.78	<0.05
	2005	138	3	3.54	<0.05	3.95	<0.01
	2006	65	3	2.48	NS	2.25	NS

Results of ANOVA comparisons of RNA/DNA' and Fulton's K' (both indices corrected for fish length) in juvenile *Solea solea*, *Solea senegalensis*, *Platichthys flesus*, *Diplodus vulgaris* and *Dicentrarchus labrax* amongst estuaries along the Portuguese coast (in July 2005 and 2006).
*Source*: Vasconcelos et al. (2009).

Table 6.2 Intra-estuarine variation in condition indices

Species	Estuary	Sites	Year	df	RNA/DNA'		Fulton's K'	
					t	P	t	P
S. solea	Mondego	a vs. b	2005 & 2006	68	−2.67	<0.01	−0.58	NS
			2005	13	−0.89	NS	−0.46	NS
			2006	53	−2.58	<0.05	−0.51	NS
	Mira	a vs. b	2006	41	−6.19	<0.001	2.02	NS
P. flesus	Minho	a vs. b	2006	18	−1.60	NS	0.62	NS
	Douro	a vs. b	2005	25	−0.32	NS	1.26	NS
D. vulgaris	Mondego	b vs. c	2006	36	0.28	NS	−0.61	NS
	Sado	b vs. c	2005	28	−0.05	NS	0.42	NS
	Mira	b vs. c	2005 & 2006	51	−1.13	NS	−0.80	NS
			2005	22	−2.26	<0.05	0.51	NS
			2006	27	0.77	NS	−1.06	NS
	R. Formosa	a vs. b	2006	54	−5.80	<0.001	−1.29	NS
D. labrax	Tejo	a vs. b	2005	39	2.30	<0.05	−2.14	<0.05

t-test results of RNA/DNA' and Fulton's K' (both indices corrected for fish length) in juvenile *Solea solea*, *Platichthys flesus*, *Diplodus vulgaris* and *Dicentrarchus labrax* between sites within a Portuguese estuary (in July 2005 and 2006).
*Source*: Vasconcelos et al. (2009).

Further, Tanner et al. (2009) assessed condition of pouting *Trisopterus luscus* in nursery areas and other coastal areas along the Portuguese coast using different indicators. Fulton's K condition factor, RNA/DNA ratio, and total protein content were determined for 0-group pouting collected in three shallow nursery grounds (Fig. 6.6). Adult pouting were sampled
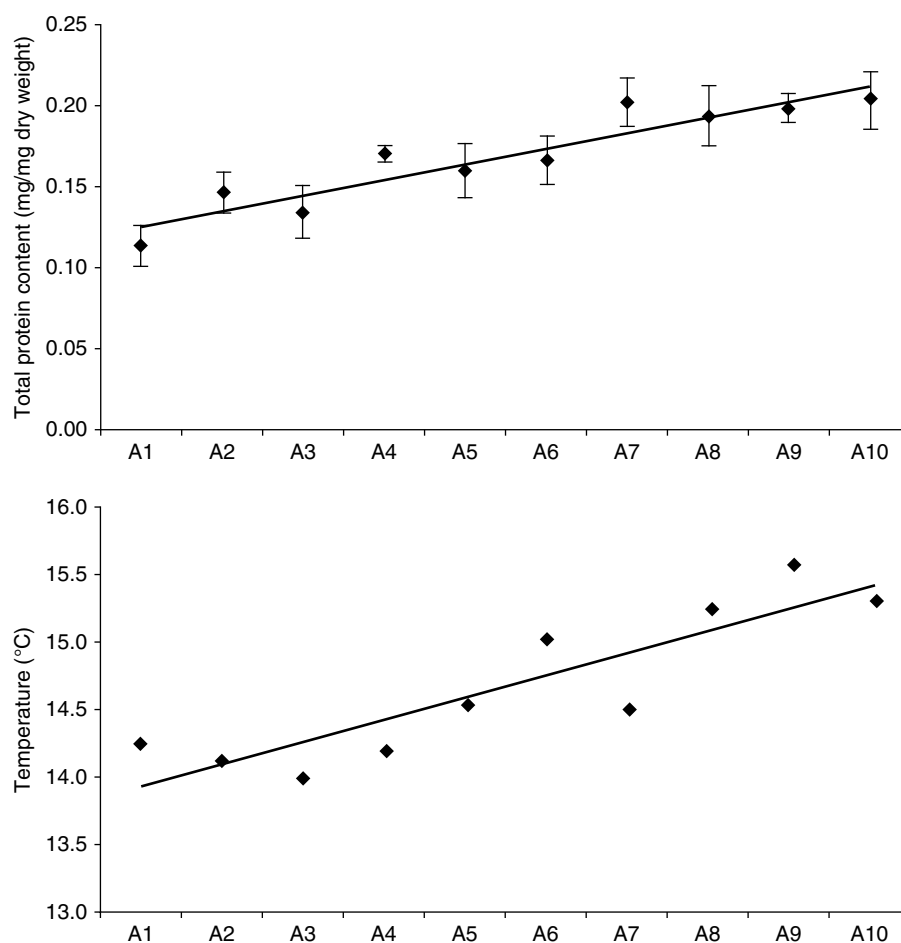
Figure 6.6   Total protein content variations in white muscle tissue (mean ± standard deviation) of *Trisopterus luscus* adults, and sea surface temperature (SST, monthly mean values) along sampling locations off the Portuguese coast. Sampling locations follow a latitudinal gradient, from A1 northernmost position to A10 southernmost position. From Tanner et al. (2009).

in 10 sites along the coast and Fulton's *K*, HSI, total protein content, and water content were determined as indicators of condition. For 0-group individuals, only total protein content showed significant differences among the sites, whereas in adults all the indicators evaluated differed significantly among the sites.

Small-scale spatial differences in condition of white seabream *Diplodus sargus* in the northwest Mediterranean were clearly attributed to differences in habitat quality too (Lloret & Planes, 2003; Lloret et al., 2005). These studies revealed differences in the condition of white seabream between adjacent habitats: during the juvenile phase, fish condition was generally higher on the rocky coast than on the sandy coast (Fig. 6.7). On the basis of these results, the authors indicated that rocky areas may potentially contribute more to production of white seabream than the sandy coasts and are therefore potential EFHs for this species; and that MPAs might offer increased production for white seabream, providing long-term
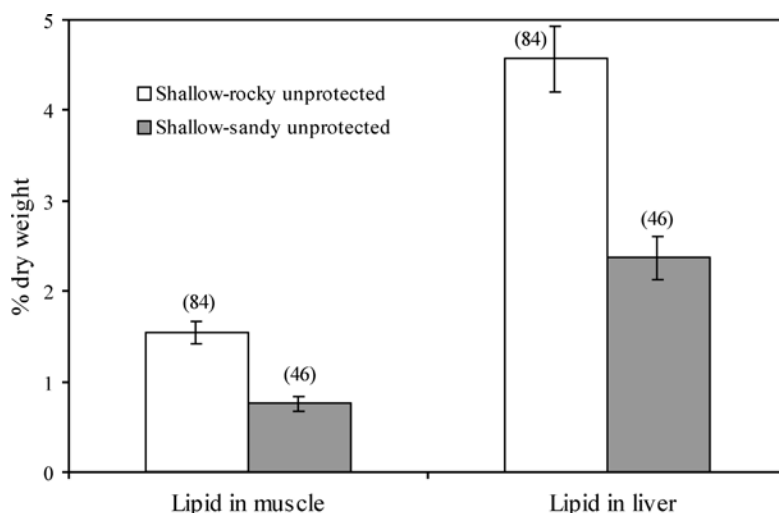
Figure 6.7   Differences in lipid content of muscle and liver of juvenile white seabream *Diplodus sargus* between the unprotected shallow zones of rocky and sandy habitats in the northwest Mediterranean. Values are mean ± SE. Number of samples given in parentheses. From Lloret & Planes (2003).

benefits to the local fishery of this species. The rocky habitat might provide a better environment in terms of food, predation risk, or energy expenditure.

Other studies indicate that wide variations in condition indicators exist between geographically proximate estuaries, which are caused by variable microhabitats or differences in resource partitioning. Thus, for example, large variation in liver glycogen content, liver–somatic index, condition factor, and RNA/DNA ratio exists among four health indicators in natural populations of the estuarine fish *Fundulus heteroclitus* from five geographically proximate estuaries in southeastern Connecticut (Leamon et al., 2000). In this study the authors discuss the significance of this variability and its impact on the use of the indicators as bioindicators of environmental perturbation in estuaries.

At sea, age-0 pollock *Theragra chalcogramma* collected offshore of a hydrographic front in the Bering Sea were found to have a significantly higher Fulton's K condition factor that those found inshore of the front (Brodeur et al., 2000). The significantly higher condition factors in the offshore habitat imply that conversion efficiency may be better there than at the front of inshore. Since the authors were unable to detect any direct trophic benefits for young pollock remaining in these frontal regions, as opposed to residing in offshore waters, they indicated that other considerations (i.e., refuge from predation) make occupying this habitat more beneficial (Brodeur et al., 2000). Similarly, the nutritional condition of anchovy larvae in the Bay of Biscay (northeast Atlantic) showed a clear spatial pattern, with better-conditioned larvae found in stations influenced by freshwater discharge (Bergeron, 2000). This makes these stations particularly valuable for the anchovy larvae.

In the Balearic Islands, for example, the condition of red scorpionfish *Scorpaena notata* was affected by the algal biomass (mostly red algae) (Ordines et al., 2009). Hence results from this study showed that the somatic condition of *S. notata* were positively correlated with algal biomass (Fig. 6.8). The algal biomass present in the bottoms positively affected not only the condition of *S. notata* but also its feeding potential and abundance. In addition, individuals inhabiting bottoms with the highest algal biomass showed faster
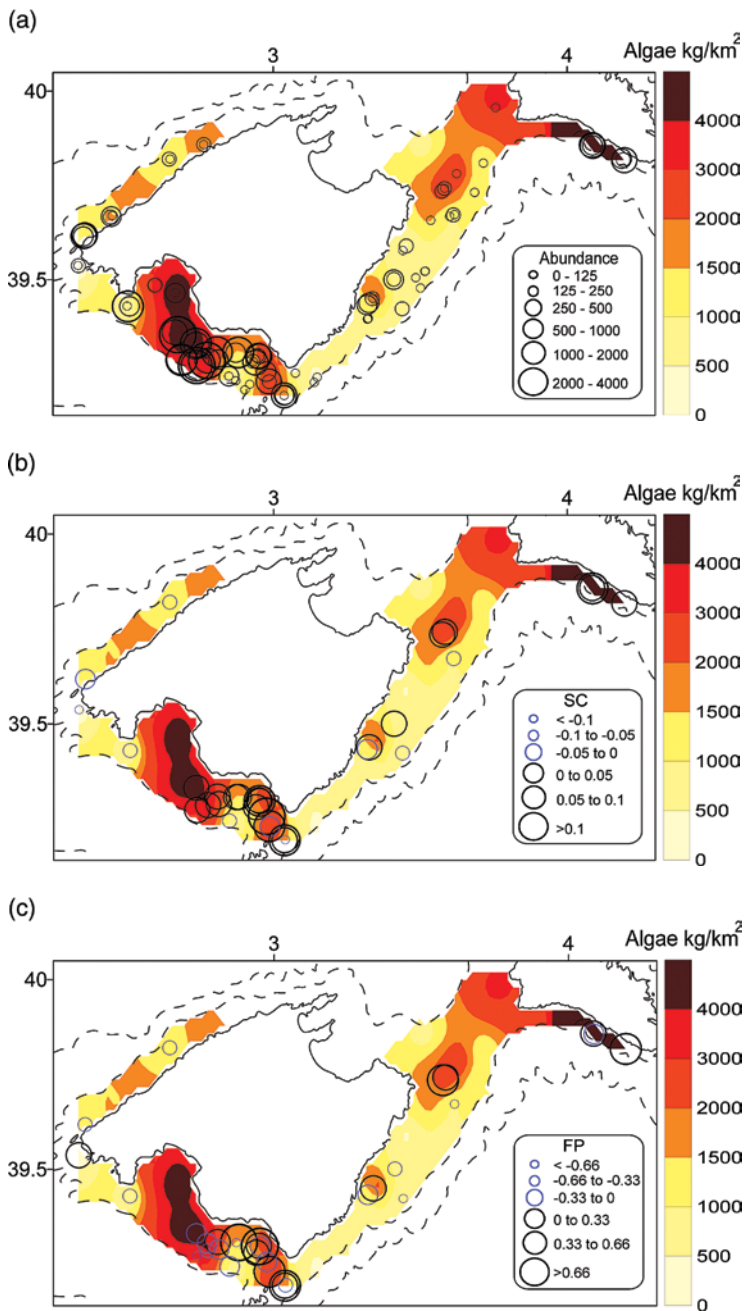
Figure 6.8 Bubble maps representing (a) abundance (individuals/km²), (b) somatic condition (SC), and (c) feeding potential (FP) of red scorpionfish *Scorpaena notata* in the Mediterranean, plotted over the contour map showing algal biomass. From Ordines et al. (2009).

growth than the entire population analyzed together. According to the authors, both the structural complexity and the availability of prey in the facies with red algae are revealed as advantageous traits for the life history of fish. These results highlight, using condition indicators, the importance of the facies with red algae as an oasis of high

productivity where benthic fish can circumvent the general oligotrophic conditions of the Mediterranean, and the necessity of urgent management measures in order to protect them from human impacts (Ordines et al., 2009).

Also in the Mediterranean the relative body and liver masses and stomach contents of red mullet *Mullus barbatus* caught on maerl beds of the Aegean Sea were found to be higher than those caught on adjacent muddy bottoms (Somarakis et al., 2004). Similar indications were provided by Lloret et al. (2007), who found better-conditioned *M. barbatus* in the eastern part of Valencia, where the presence of maerl beds is important. In Newfoundland waters, small to medium-sized cod in Smith Sound were generally in poorer nutritional condition than those in nearby Placentia Bay (Rideout & Rose, 2006). This spatial difference was explained by the fact that good-conditioned Placentia Bay cod fed more heavily on capelin than those in Smith Sound and that they fed year-round, whereas poor-conditioned Smith Sound cod ceased feeding during winter (Rideout & Rose, 2006).

In some cases the positive correlation between abundance and condition of fish has been hypothesized to be indicative of habitat selection. For example, an examination of the condition of haddock *Melanogrammus aeglefinus* at 24 sites in the North Sea in 2004 revealed that at a given temperature, HSI was better at stations where haddock were most abundant, indicating that there was some habitat selection by haddock (Hiddink et al., 2005).

Not only total fat but also lipid composition can vary among different habitats. Thus, for example, the fatty acid profile of the galaxiid fish *Galaxias maculatus* collected from an estuarine habitat (a river mouth), a freshwater creek, and two land-locked freshwater lakes in Victoria (Australia) were investigated by De Silva et al. (1998) with a view to evaluating habitat influences on the fatty acid profile. The study concluded that in addition to the diet, other habitat-related factors could influence the fatty acid profile in *G. maculatus*. Also the lipid composition and content of the small common wrasse *Symphodus ocellatus* was different according to the quality of the biotope. Young fish from a *Caulerpa taxifolia* meadow in France contained less lipids (−21%) and a different lipid class distribution than those from a *Posidona oceanica* bed (Levi et al., 2005; Fig. 6.9). The authors attributed these differences to the fact that the invasive alga *C. taxifolia* diminishes the availability of benthic prey for fish (particularly mollusks, amphipods, and copepods).

Figure 6.9 *Posidonia oceanica* meadows in the Mediterranean Sea. Photo by Toni Font.
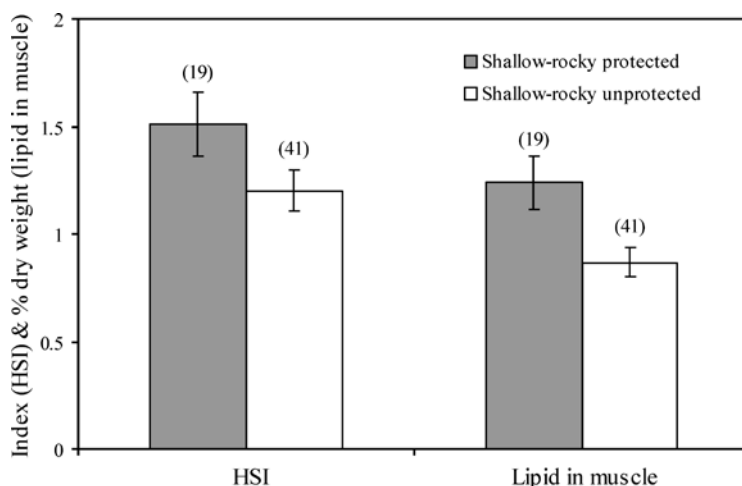
Figure 6.10   Differences in hepatosomatic index (HSI) and muscle lipid content of post-spawners of white seabream between the shallow zones of rocky protected and rocky unprotected areas. Means are indicated with the SE. No. of samples given in brackets. From Lloret & Planes (2003).

6.1.3   Condition as an indicator of habitat protection

Many studies have shown that marine protected areas (MPAs) enhance species richness, abundance, biomass, and stock size structure (see Roberts & Polunin, 1991 for a review) and thus have the potential to enhance fisheries in adjacent areas through export of biomass. Furthermore, a few studies have shown that habitat protection may also affect fish condition positively. In the northwest Mediterranean, Lloret and Planes (2003) and Lloret et al. (2005) found that adult white seabream *D. sargus* were better conditioned within the rocky areas of the MPAs of Banyuls-Cerbere (France) and Cap de Creus (Spain) than in adjacent unprotected rocky areas of the northwest Mediterranean coast. In the MPA of Banyuls-Cerbere (Lloret & Planes, 2003), higher muscle lipid levels and HSI were detected in post-spawners in the shallow rocky protected area compared with the shallow rocky unprotected area (Fig. 6.10). In the MPA of Cape Creus, spawners caught in the protected rocky area of the marine reserve had higher muscle lipid content than those caught in the adjacent unprotected rocky areas (Lloret et al., 2005). The findings of these investigations support the importance of rocky coastal protected habitats for the energy reserves of *D. sargus* in the Mediterranean Sea. Thus, it seems that marine reserves may enhance the condition of fish through an increase in food availability, better habitat quality or less stress from fishing activities.

6.1.4   Bathymetric quality

In some cases fish condition has been related to depth, with deep waters being considered as marginal habitat for demersal species in terms of food resources as the poorest conditioned individuals were found there. In the southern Gulf of St Lawrence (Canada), for example, condition of cod was low in deep waters (Fig. 6.11; Chouinard & Swain, 2002). Also, there was a significant effect of depth on lipid content of *Mullus barbatus*, *Mullus surmuletus* and
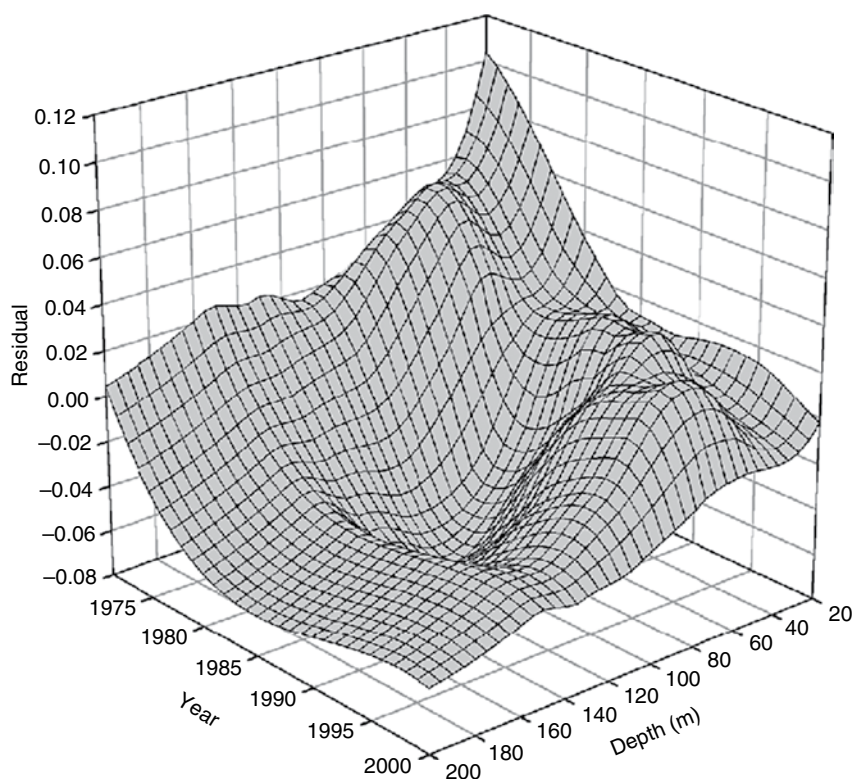
Figure 6.11 Loess response surface of condition for southern Gulf of St Lawrence (Canada) cod *Gadus morhua* by depth from 1971 to 2000. From Chouinard & Swain (2002).

Pagellus acarne in the Mediterranean, fish with the highest lipid levels inhabiting the shallowest parts of the Gulf of Lions (all three species) and the Catalan Sea in the northwest Mediterranean (last two species; Lloret et al., 2005). The poorer overall feeding conditions of deep water for the accumulation of energy reserves in several fish species in the northwest Mediterranean was also observed by Lloret et al. (2002). In this case, lower K and HSI values were found in deep waters for several demersal fish species. Overall, results from these studies support the relative importance of shallow areas of the continental shelf for the productivity of different exploited fishes.

6.2 Condition of exploited fish and their predators

A reduction in fish energy reserves does not only affect the specific population success but may also impact on the reproductive output of their predators. Thus for example, low energy values of sandeel *Ammodytes marinus* and sprat *Sprattus sprattus* appeared to be the proximate cause of breeding failure in 2004 of the common guillemot *Uria aalge*, the most abundant seabird species in the North Sea (Wanless et al., 2005). Many seabirds in the North Sea feed on lesser sandeels during the breeding season. The reasons for the poor fish condition in this part of the North Sea are unknown, but the results provide further evidence of major changes in the North Sea food web. Similar to this, Litzow et al. (2002) found that
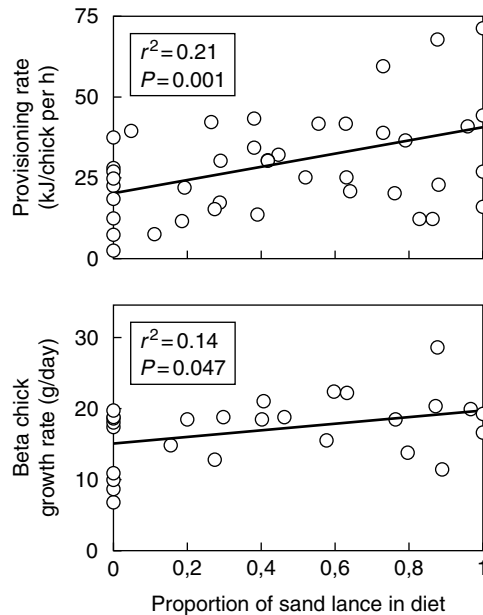
Figure 6.12 The effect of high-lipid prey on nestling provisioning and growth rates of the pigeon guillemot *Cepphus columba* in the Gulf of Alaska and Bering Sea. Beta chick growth rates are for chicks aged 5–20 days. Fourteen data points lie on the *y*-axis in the top panel, 12 in the bottom panel. From Litzow et al. (2002).

recovery of pigeon guillemot *Cepphus columba* populations in the Gulf of Alaska and Bering Sea from the effects of the *Exxon Valdez* oil spill was limited by availability of lipid-rich fish prey. Thus, scarcity of lipid-rich schooling fish such as sand lance *Ammodytes hexapterus* negatively affected pigeon guillemot populations in the Gulf of Alaska. This is because the presence of sand lance in the diet was associated with higher rates of energy delivery to the nest, higher chick growth rates and shortened duration of chick rearing (Fig. 6.12), improved chick survival, decreased brood reduction, and increased reproductive success (Litzow et al., 2002). It is important to thoroughly characterize the lipid content and fatty acid composition of fish such as sand lances, sprats, and sardines that serve as food for many predators because these variables may be different from species to species (even in the same genus). For example, Kaneniwa et al. (1997) found that one species of sand lance, *A. personatus*, contains higher levels of total lipid in muscle and viscera than another sand lance species, *A. hexapterus*, on the coast of northern Hokkaido (Japan). Similar to this, Payne et al. (1999) evaluated the proximate composition of some northeastern Pacific forage fish species, and found differences ranging from 6.7 to 19.4% for protein, from 1.2 to 21.4% for lipid, from 64.6 to 91% for moisture, and from 0.6 to 4% for ash.

The response of other piscivores to the variable abundance of high-lipid and low-lipid prey has also been observed in the Gulf of Alaska. A shift to a warm-water regime in 1977 resulted in a more than 90% decline in populations of capelin *Mallotus villosus*, a lipid-rich schooling fish, and a greater than 250% increase in populations of lipid-poor demersal fish (mostly Gadidae and Pleuronectidae) (Anderson & Piatt, 1999). This dramatic ecosystem change apparently cascaded through higher trophic levels, as the proportion of capelin and other lipid-rich fish in diets of piscivorous birds and mammals declined, and populations of

Figure 6.13 Dolphins and seabirds feeding on fish along the Gulf of Lions coast in the northwest Mediterranean. Photo by Gemma González, projecte NINAM, www.projecteninam.org.

these high trophic level consumers declined by as much as 95% (Merrick et al., 1997; Agler et al., 1999).

In addition, fatness of capelin is directly linked to their value for higher predators, such as Atlantic cod, seabirds, and marine mammals. Small variations in fat content of prey can be critical for reproduction and survival of Arctic top predators (e.g., Karnovsky et al., 2003), and this may act in combination with variations in stock size, distribution, and age structure of capelin to determine dynamics of their predators. Also, Ball et al. (2007) determined proximate composition and energetic value of a suite of potential forage fish collected from an estuary on the Yukon–Kuskokwim Delta, Alaska and based on observed energetic differences concluded that variation in fish size, quantity, and species composition of the prey community could have important consequences for piscivorous predators.

Overall, these results support the importance of evaluating the condition of forage fish (mostly small pelagic fish such as sand lances, capelin, herring, sardines and anchovies) for understanding the relative dietary value of these forage fish as prey (for piscivorous fish such as cod and hake or for top predators such as birds and mammals) (Fig. 6.13).

6.3   Fish condition as an indicator of climate-driven and anthropogenic changes in the ecosystem

Studies have shown that the decline in the condition of several top consumers in the North Atlantic could be due to climate-driven changes in the pelagic marine ecosystem. Thus, for example, the negative correlations between condition of salmon *Salmo salar* and ocean warming (Todd et al., 2008) could be due to reduced prey availability for pre-adult salmon in connection with climate change. There are also recent observations of breeding failure for European seabirds correlating with ocean warming and reduced (fish) prey availability (e.g., Durant et al., 2003) and of declining somatic condition and lipid content of tuna *Thunnus thynnus* in the northwest Atlantic (Golet et al., 2007).

Here we deal with the monitoring of food supply/nutrition condition of marine fish, because this aspect has been studied in detail. It is important to remember that food is a key aspect that connects living organisms with their habitat. Food supply is not a simple notion and demands detailed explanation (Shulman et al., 2009a,b). The food supply of organisms and populations (F_s) can be described as the ratio between food consumed (F_c) and food required (F_r):

$$F_s = F_c / F_r \tag{6.1}$$

The direct determination of these characteristics in field conditions is very difficult. Indirect determination of organism and population food supply is also confronted with serious problems, as it is dependent on many factors, including some which cannot be defined:

$$F_s = f(Ab, Ab_v, Ab_a, Ac_1, \ldots Ac_n, T, L, \ldots X_1, \ldots X_n) \tag{6.2}$$

where Ab represents the biomass of fodder organisms, Ab_v their nutritional value, Ab_a their availability, $Ac_1, \ldots Ac_n$ the biomass of consumers of own species as well as competitors of other ones, T temperature, L illumination (especially important for aquatic organisms) and $X_1, \ldots X_n$ undefined factors. Nevertheless, there is a rather effective way for estimating food supply, which is determining the result of the feeding process, which reflects nutritional condition such as weight increment (ΔW) and accumulation of energy reserves (ΔE):

$$\Delta W, \Delta E = fF_s \tag{6.3}$$

Weight increment and growth are clearly defined by protein retention, energy accumulation (for many marine fish), and by the formation of fat stores (neutral lipids, triacylglycerols mainly). Determination of the weight (protein) increment for adult fish is not an easy task. For this reason current studies use the RNA/DNA ratio in tissues for estimating the "immediate" rate of protein biosynthesis and consequently the growth rate of larvae and fry fish (Bulow et al., 1981; Thorpe et al., 1982; Nakano et al., 1985; Varnavsky et al., 1991; Bergeron, 2000; Drazen, 2002; Gwak et al., 2003; Smith & Buckley, 2003). Conversely, estimation of fat content is an easy and useful method for evaluating the condition of fish. This process of energy accumulation begins every year almost from "zero" following the spawning period and reaches a peak at the end of the feeding period. The content of reserve lipids at the time of feeding completion is therefore an integral indicator of the conditions under which fish stocks or populations consumed food:

$$\text{Fat} = \int_{t_1}^{t_2} f(F_s)\,dt \tag{6.4}$$

Sometimes this indicator is expressed as an index:

$$I(F_s)i = F_i / F \tag{6.5}$$

where F_i is fat content in fish at the end of feeding in a certain year and F is average fat content at the same time, determined during all observed years. When $I(F_s)i \geq 1$, the food supply/nutrition condition of fish individual or population is adequate or good; when $I(F_s)i \leq 1$ it is inadequate or bad.

Determination of fat (neutral lipids) content is widely used for evaluation fish condition (see reviews by Shulman, 1960a; Lovern, 1964; Love, 1970, 1980; Shatunovsky, 1980; Kreps, 1981; Sidorov, 1983; Ackman, 1989; Minyuk et al., 1997; Adams, 1999; Morris & Culkin, 2000; Kolakovska et al., 2003; Kolakovska, 2010). Unfortunately, however, most of these investigations did not look at the problem from a food supply point of view. The few papers that have been published on the characterization of the feeding history of fish in which the food supply was estimated using lipid parameters include larvae of sardine *Sardina pilchardus* (Fraser et al., 1988), Californian anchovy *Engraulis mordax* (Hakanson, 1989a,b, 1993), the North Sea sprat *Sprattus sprattus* (Hakanson & Coombs, 1994), and Atlantic cod *Gadus morhua* (Lochmann et al., 1995). Recently, interesting data have been published on the relationship between lipid content and nutritional factors in adult demersal fish of the Mediterranean (Levi et al., 2005; Lloret et al., 2005), and in North Atlantic cod (Lambert et al., 2000; Yaragina & Marshall, 2000; Orlova et al., 2002). Good fish condition affects maturation, improving lipid content in eggs and larvae, and promotes high survival and replenishment of populations (Lasker & Theilacker, 1962; Nikolsky, 1965; Guisande et al., 1998; Marshall et al., 1999; Sogard & Olla, 2000; Zenitani, 2001). The example of a relationship between the fat content of female gonads of Black Sea horse-mackerel and fecundity (egg number) and diameter of oocytes has also been observed (Shulman et al., 1970; Shulman & Love, 1999). Assessment of the food supply of several marine fish populations by determination of accumulated fat stores (fat content) has been examined since the second half of the last century. These investigations involved Pacific sardine in the California current (Lasker, 1970), Californian anchovy (Garcia-Franko et al., 1999), and Atlantic cod (Marshall et al., 1999; Orlova et al., 1999).

Our investigations have centered on Azov and Black Sea subspecies of European anchovy (Shulman & Dobrovolov, 1979) and Black Sea sprat (Shulman et al., 1994, 2005, 2009a,b; Minyuk et al., 1997; Nikolsky & Shulman, 2005; Nikolsky et al., 2007, 2011). Anchovy and sprat are two key small pelagic fish species of the Black Sea and have significant effect on the ecosystem. The nutritional spectrum of both species (copepods) is rather similar. Sprat is a cold-tolerant planktivorous fish, spawning in winter and forming local populations. It feeds intensively in spring and early summer (Svetovidov, 1964), achieving considerable fat stores of about 10–15% of body mass. Conversely, anchovy is a warm-tolerant fish, spawning in summer, and undertakes long migrations and feeds intensively, preparing for wintering migration at the end of summer and early fall. Anchovy, like sprat, accumulates fat stores of about 10–15% of body mass, but to the end of the fall (Fig. 6.14). So by determining fat content in sprat populations at the end of the feeding period in summer and in
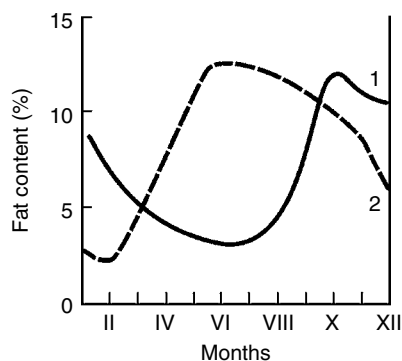

Figure 6.14 Dynamics of fatness in Black Sea anchovy (1) and sprat (2). After Shulman et al. (2009).

anchovy in November–December, we can estimate degree of well-being/nutritional condition of small pelagic fish in the Black Sea during most of the year. Moreover, condition of sprat and anchovy could be an indicator of mesozooplankton biomass (the main food of pelagic fish) in the Black Sea as a whole. Indeed, the monitoring of the nutritional condition of both species in the Black Sea is undertaken on this basis. Monitoring has been carried out on sprat almost every year since 1960, but not on anchovy until more recent years. However, the relationship between fat content at the end of feeding in the fall and features of the wintering migrations of the Azov (Shulman, 1974, 2002) and Black Sea (Chashchin & Axelev, 1990; Nikolsky et al., 2011) subspecies has been studied (see section 5.1), and indeed this relationship is also affected by food supply. There are only a few investigations in which the long-term fat content of fish populations has been monitored: Lasker (1970) on Californian sardine, Garcia-Franko et al. (1999) on anchovy from the same region, and Marshal et al. (1999) and Orlova et al. (2000) on Atlantic cod. Food supply monitoring of Black Sea pelagic fish is necessary not only to estimate their condition but also to assess the pelagic ecosystem as a whole. We aim to show here that this monitoring may clarify the complicated processes involved in the Black Sea ecosystem, but these cannot be revealed without defining food consumption by sprat and anchovy.

It is known that the Black Sea ecosystem has been affected by many factors since at least the 1970s (Vinogradov et al., 1992; Kideys, 2002; Sorokin, 2002; Eremeev & Efimov, 2003). These include eutrophication, pollution, invasion by ctenophore aliens, overfishing, and sea warming (climate change). The data on dynamics of fat content in sprat and anchovy populations is a significant contribution to the study of the Black Sea ecosystem. Of course, the study of fish food supply by lipid characterization must not be limited to indicators of fat content (neutral lipids or triacylglycerols) only. Significant information can also be obtained by comparing fractional and especially fatty acid content of fish lipids and the lipids of their food (Lovern, 1964; Ackman, 1967, 1989, 2005; Sargent, 1976; Sargent et al., 1987, 1999; Sargent & Henderson, 1980; Yuneva et al., 2009, 2011). The aim of this section is to trace the long-term changes in food supply/nutritional condition of the Black Sea sprat and anchovy stocks, based on determination of their fat content dynamics. The objectives of investigation are to reveal how the characteristics studied are connected with (i) the dynamics of sprat and anchovy stock biomass, (ii) the dynamics of biomass of the lower trophic levels (i.e., zooplankton and phytoplankton), and (iii) climate (temperature) changes in the Black Sea.

Dramatic changes in climate have occurred in the last few years in the Black Sea, a semi-closed basin that is very vulnerable due to the peculiarities of its hydrological and hydrochemical regimes (Eremeev & Efimov, 2003). These changes are mirrored by the high trophic levels of the pelagic ecosystem, including the condition of populations and stocks of two small pelagic fish, sprat and anchovy, the mass species that dominate the Black Sea pelagic ecosystem. These species are indicators of the degree of well-being (health) of the ecosystem as a whole, as their condition is the result of processes occurring at preceding trophic levels. Significant characteristics of this condition, together with abundance and biomass, is the level of energy stores (neutral lipids, fat) accumulated to the end of post-spawning feeding, i.e., the condition of their fodder base (Shulman & Love, 1999). The results of more than 50 years' monitoring of fat content in the sprat population (Shulman et al., 1993, 2005, 2007, 2009a,b; Nikolsky & Shulman, 2005; Nikolsky et al., 2009b, 2011) and more modest data in anchovy (Nikolsky et al., 2009a, 2011) have revealed the role of climate change in food supply and nutritive condition of both species. At the same time we have tried to observe the influence on these parameters of fodder competition
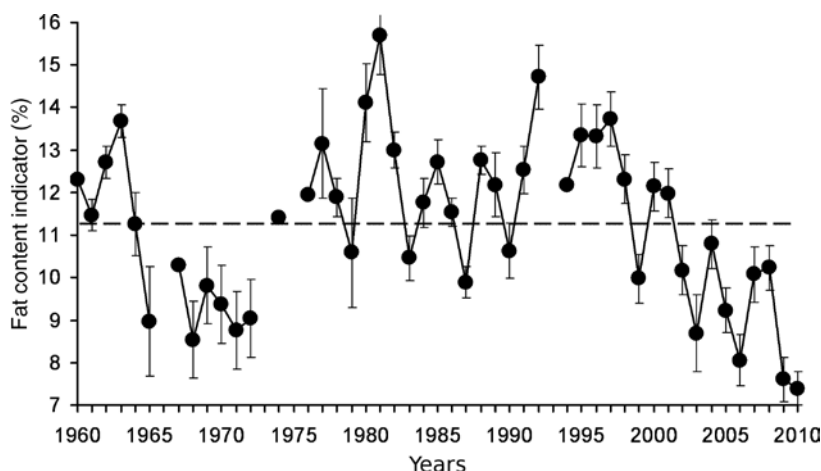
Figure 6.15 Dynamic of fat content in Black Sea sprat at the end of feeding from 1960 to 2011. From Nikolsky et al. (2011).
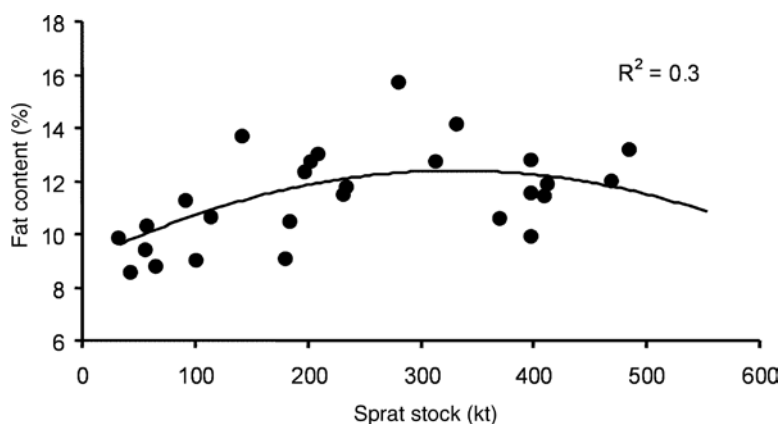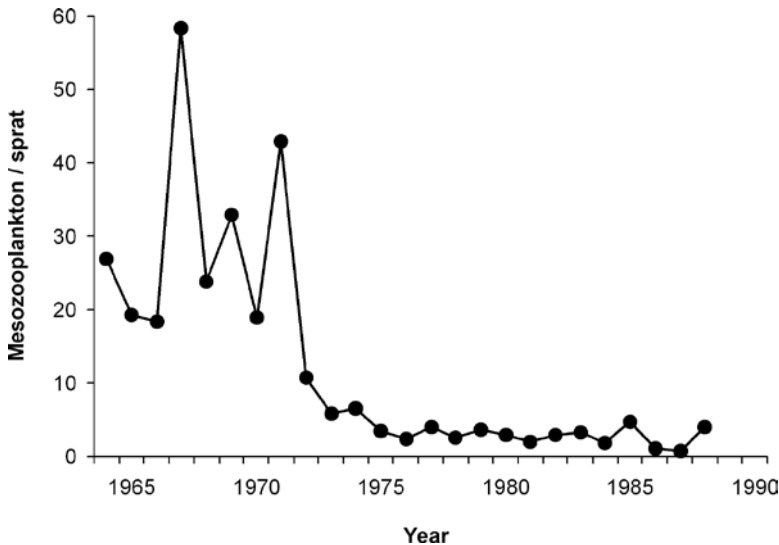

Figure 6.16 Relationship between fat content and biomass of sprat in the Black Sea.

and regional anthropogenic factors, including biogenic river inflow, pollution, aliens, and fisheries.

Results of sprat fat dynamics during maximal accumulation (June–August) from 1960 to 2011 are shown in Fig. 6.15. Data include populations in the northwestern Black Sea, as well as Crimea, Caucasus, and Bulgarian coasts. Fat content in sprat exhibits large interannual variations. These changes are associated with sprat biomass either positively, when biomass is not large, or negatively, when biomass is large (according to the principle of self-regulation) (Fig. 6.16). It is notable that there is a positive relationship between sprat fat content and phytoplankton biomass (Fig. 6.17). More remarkable is the relationship between sprat fat content and mesozooplankton biomass, the main food of this fish. This is demonstrated by comparison of Fig. 6.15 and Fig. 6.18, which shows the dynamics of mesozooplankton per sprat catch (landing) in the Black Sea from the 1960s to the 1990s. That is obviously caused by ingestion of the fodder base by sprat itself, as well as by other forms inhabiting this trophic

(a)

(b)
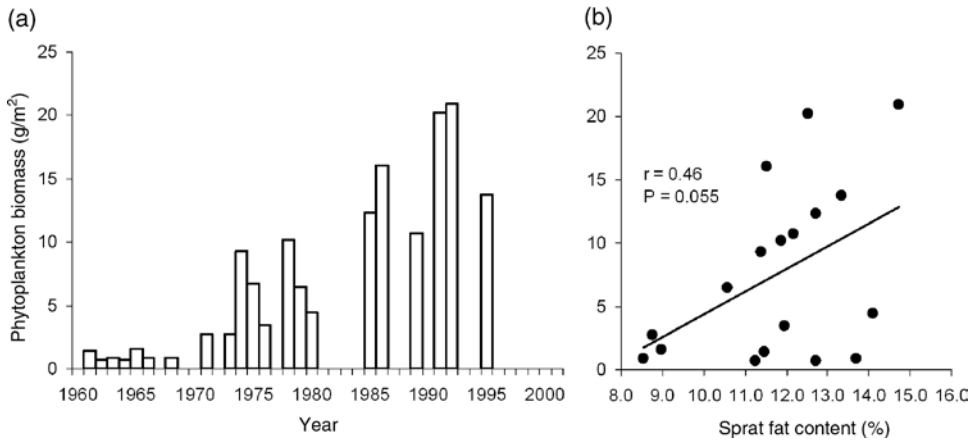
Figure 6.17   (a) Dynamics of phytoplankton biomass in the Black Sea (Mikaelyan, 1997) and (b) relationship between phytoplankton biomass and sprat fat content (Shulman et al., 2005).

Figure 6.18   Dynamics of mesozooplankton (per sprat biomass) in the Black Sea in the 1960s to the 1990s. After Kovalev et al. (1998) and Shulman et al. (2005).

level (e.g., anchovy). Comparison between fat content in sprat populations (Fig. 6.15), its catch biomass (Fig. 6.19), and biomass of phytoplankton (Fig. 6.17) reveals an approximately 9–11 year period where these variables exhibited conjugate fluctuations (Table 6.3). This table also shows the dynamics of surface water temperature in the Black Sea (Fig. 6.20) (Nikolsky et al., 2009a,b, 2011). The environmental factors that control the dynamics of sprat fatness (Fig. 6.21) show temperature increasing in the 1960s and especially the years since 1999 in comparison with average years. In the 1970s, 1980s and 1990s annual temperatures were below the average long-term values (negative anomalies). Higher temperatures predominated first, coinciding with a decrease in fat content in sprat and phytoplankton biomass. In the last few years up to the present, these processes exhibit an especially dramatic
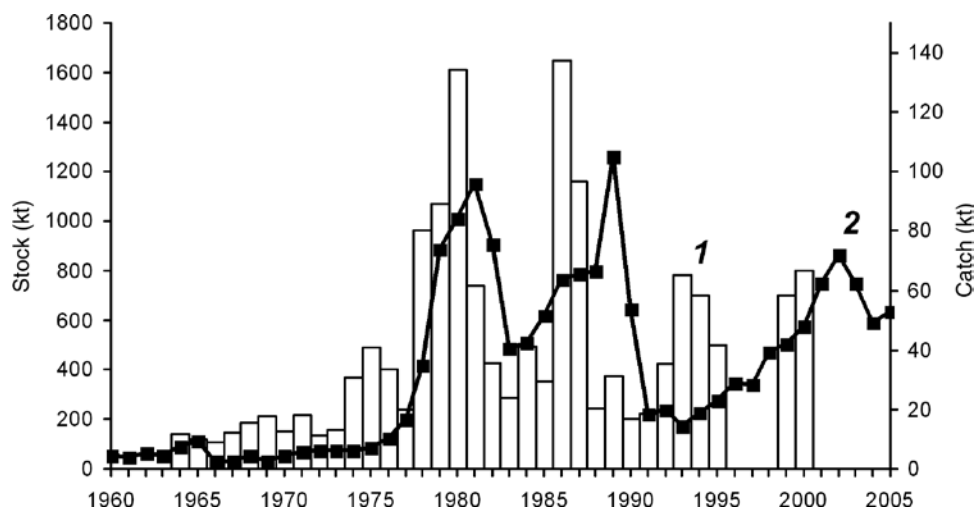
Figure 6.19 Biomass (1) and catch (landing) (2) of sprat. After Shulman et al. (2007).

Table 6.3 Decadal changes in the analyzed indicators for the period 1960–2005

	Decades				
Indicators	**1960s**	**1970s**	**1980s**	**1990s**	**Beginning of the 21st century**
Surface water temperature	High	Low	Low	Increasing	High
Phytoplankton biomass	Decreasing	Increasing	High	High	Low
Sprat fat	Decreasing	Increasing	High	High	Low
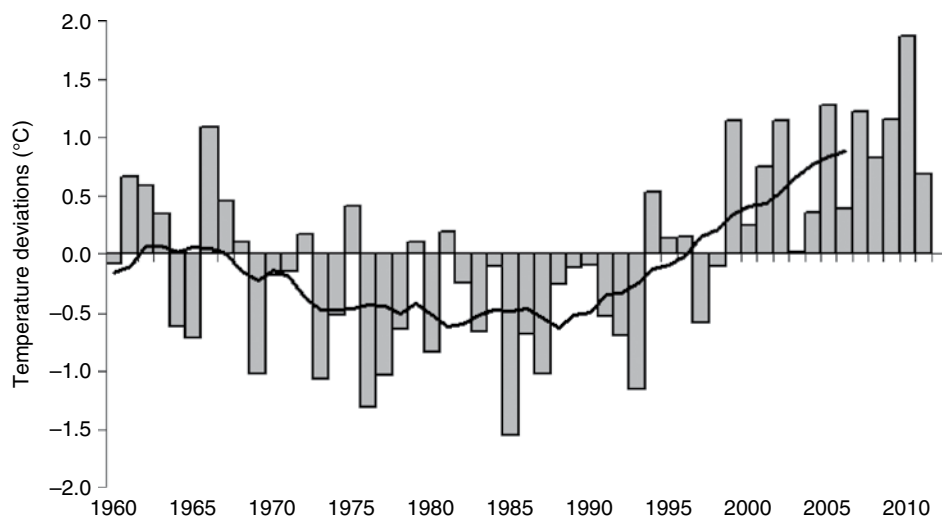
Figure 6.20 Dynamics of water surface annual temperature in the Black Sea. From Eremeev & Efimov (2003) and Nikolsky et al. (2011).
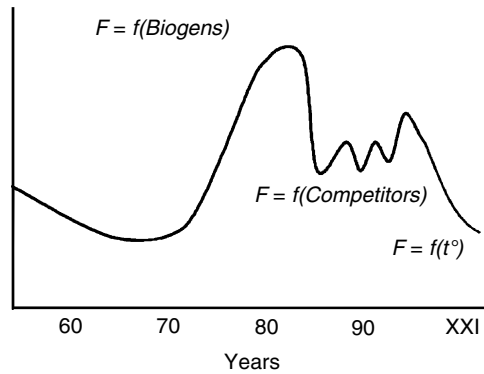
Figure 6.21 Scheme of environmental factors controlling sprat fat dynamics. After Shulman et al. (2007).

character. A constant increase in water temperature has been accompanied by a sharp decline in the biomass of the cold-tolerant complex of phytoplankton (Yunev et al., 2009, 2011) and in fat content in sprat body. Data in 2012 show that sprat fatness at the end of the feeding period decreased to the lowest value ever observed (about 5%).

How are these processes connected to each other? The negative relationship between temperature change and condition of sprat stocks (level of accumulated fat stores and sprat biomass) may be indirectly due to the primary fodder base (i.e., phytoplankton). In cold winters more intensive upwelling of water masses occurs and this increases the inflow of nutrients to the photic layer used by phytoplankton (Yunev et al., 2011). Because of the trophic chain involves phytoplankton to zooplankton to fish, the latter are indirectly influenced by climate, of which sea temperature is an indicator. It is known that the average annual temperature of surface water is determined mainly by water temperature during the the colder months (February–March). However, in our study, average annual temperatures are the best indicators of sea warming (Fig. 6.20). A lower water temperature can also have a direct effect on cold-tolerant sprat, increasing its habitation area and promoting fodder base utilization. At low temperatures, spawning area, intensity and efficiency of reproduction, and survival of eggs and larvae also increase, and this positively affects stock recruitment of this species. In addition, conditions are also conducive to the increasing abundance and biomass of cold-tolerant forms of zooplankton (copepods of the genus *Calanus* and *Pseudocalanus*), the favorite food of sprat. Clearly, this situation has promoted considerable abundance of sprat in the Black Sea since the second half of the 1970s (Shulman et al., 2011). Conversely, warmer than average winter temperatures due to a decrease in the influx of water masses has negatively influenced primary productivity (and thus secondary productivity). As a result of this, sprat have exhibited a dramatic fall in fat content, especially in latter years, and sprat abundance and biomass have obviously decreased in comparison with values in the 1980s. Thus the effect of climate change on phytoplankton biomass and fat content in sprat populations has a compound character due to the multiple processes occurring in marine basins. This develops more often than not in the same year, although sometimes there is a large delay for reasons which we cannot yet explain. Nikolsky (2005), on the basis of correlations, calculated the regression between sprat fat content in given year FCI (t) and in previous ones FCI (t − 1), FCI (t − 3) with average annual surface temperature with 4 years delay SST(t − 4) in the form:

$$FCI(t) = a0 + a1\ FCI(t-1) + a2\ FCI(t-3) + a3\ SST(t-4) \qquad (6.6)$$

where a0, a1, a2 and a3 are coefficients, with values recounted every year as new data is received.

Returning to Fig. 6.15, it can be seen that during the 50 years of monitoring, the environment of sprat habitat in the Black Sea has changed considerably. At the end of the 1960s, there was a change in the direction of temperature process in the Black Sea basin (Eremeev & Efimov, 2003). This was part of the transformation in all the world's oceans caused by global climate change. At the end of the 1990s, a sharp warming started that has continued to the present. The same cyclic processes occur in fish species habitat and fisheries when examined in "mega-scopes" of the world's oceans (Jennings et al., 2001; Klyashtorin & Lyubushin, 2007).

Together with the observed local influence of climate on Black sea sprat condition, regional factors also have an effect. In some cases these strengthen, and in others weaken, this influence. The condition of the Black Sea ecosystem as a whole and its separate components during the 1970s and 1980s were seriously affected by anthropogenic inputs. Primarily this was river inflow into the Black Sea of phosphates, nitrates, and ammonium compounds, the products of intensive agriculture (Konovalov & Murray, 2001; Yunev et al., 2007) and which increased especially during the so-called "green revolution." In the beginning this process strengthened primary production in the sea (eutrophication), increasing sprat food supply (nutritional condition) and stock biomass. However, the increase in primary productivity, and thus biomass of phytoplankton and mesozooplankton, improved the fodder base for other consumers (medusa *Aurelia aurita*, anchovy, etc.). This caused increasing food competition, leading to sharp fluctuations in sprat fatness in the 1980s. The increased inflow of biogens (nitrogen and phosphorus) during the 1970s and especially the 1980s coincided with severe and growing pollution caused by the rise in industrial and agricultural production in the countries of the Black Sea basin and the consequent flow to the sea of harmful chemical substances (heavy metals, pesticides, etc.) of terrestrial origin. This weakened the capacity of the Black Sea ecosystem to resist harmful influences and, as result, mass invasion of the predator ctenophore *Mnemiopsis leidyi* occurred at the end of the 1980s; this negatively affected the biomass of mesozooplankton and destroyed the fodder base of small pelagic planktivorous fish. Nevertheless, during the 1990s, coinciding with the economic crises in the countries of the Black Sea basin, pollution of the sea decreased considerably and as a result ecosystem condition improved and consequently the condition (fat content) and biomass of sprat.

The final anthropogenic factor is fisheries. Because short-cycle small pelagic fish quickly restore their numbers and biomass, some authors consider that a decline to 30% of biomass does not negatively affect stocks (Ivanov & Beverton, 1985). We also consider that the observed ecological and anthropogenic factors (temperature, biogenic outflow, food competition, pollution), as well as the invasion of *Mnemiopsis* and its predator the alien ctenophore *Beroe ovate*, could affect sprat stock condition more than the fishery itself. However, fisheries have a strong influence on the demographic structure of anchovy stock through effects on length and age. In conclusion, while global climate impacts on the Black Sea sprat are obvious, regional factors are also relevant, their influence on sprat condition being firstly positive but then negative (see Fig. 6.21). During recent years the influence of temperature has became especially strong, and this has had a clear negative effect on the sprat population, worsening their food supply and level of energy stores accumulated to the end of feeding
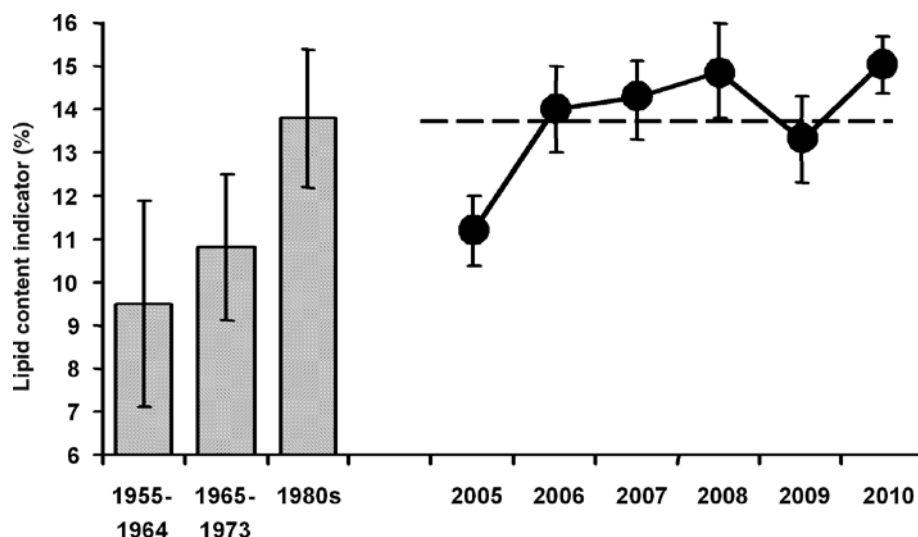
Figure 6.22 Dynamics of neutral lipid (fat) content in the Black Sea anchovy. After Nikolsky et al. (2011).

and thus nutrition condition. Ultimately, this can very seriously affect the abundance and biomass of this species in the Black Sea.

The dynamics of neutral lipid (fat, triacylglycerols) content in the Black Sea anchovy can be observed from the data obtained during 1955 to 1964 (Danilevsky, 1964; Taranenko, 1964; Shulman, 1972a, 1974), 1965 to 1973 (Shulman & Dobrovolov, 1979), the 1980s (Chashchin & Axelev, 1990) and 2005 to 2011 (Nikolsky et al., 2009a, 2011) (Fig. 6.22). These data were obtained in different regions of the Black Sea: northwestern part, and Caucasus, Bulgarian and Turkish coasts. Unfortunately, these results are not as representative as those obtained on sprat, but can provide significant information about condition of the most important mass pelagic fish of the basin. We see that energy content in anchovy at the end of the autumn feeding migration and the start of wintering in the 1980s and later years is higher than that in 1965–1973. The 1980s were years of maximal eutrophication (see above) whereas recent years are characterized by a steady increase in temperature: both these factors are very positive for development of the fodder base (copepods) of warm-tolerant anchovy. At the same time stock biomass and catch of anchovy in the Black Sea reached maximal values during the 1980s as well as in recent year (FAO, 2011).

To end, it is important to note that the energy content of anchovy near the Turkish coast is, as a rule, higher than at the same time in the northwestern Black Sea (Nikolsky et al., 2011). It is known that anchovy populations from the northwestern region migrate for wintering to the Turkish region (Chashchin, 1998). This migration starts with those anchovies which have accumulated more energy stores. Anchovies that have accumulated less energy probably stay in the northwest over winter. This reinforces our ideas about the significance of energy accumulation in preparation for wintering migrations. It is interesting to compare data on the nutritional condition of the Black Sea anchovy and sprat. During most years of monitoring, these processes develop synchronously. Energy accumulation in both species increased during the 1970s, reaching a maximum in the 1980s. The same changes occurred with stock values and catches during these decades (FAO, 2011) and were similar in the
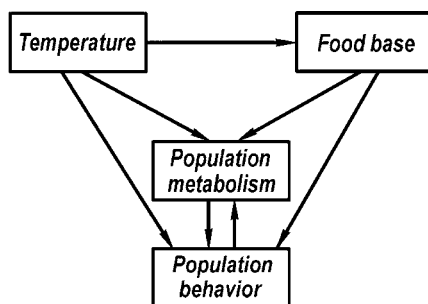
Figure 6.23 Scheme of the interaction between temperature and food supply. After Shulman & Urdenko (1989) and Shulman & Love (1999).

1990s too. However, in the first decade of the 2000s (and up to the present), energy stores in sprat fell and those in anchovy rose. This shows that only during the last few years did temperature became the dominant factor behind the divergence of energy accumulation in the two species. In all the preceding years, eutrophication and food competition played dominant roles, causing similar changes in food supply dynamics. The relationship between temperature and fodder base for both species, and presumably for other fish, may be illustrated in general by the scheme shown in Fig. 6.23.

It is a pity that there are so few data regarding the monitoring of energy stores for different fish species in other regions of the world. This precludes assessment of the dynamics of their food supply, although data on the dynamics of stock biomass and catches can help understanding of the situation in other regions. Shulman et al. (2005) noted that variability of sprat *Sprattus sprattus mediterraneus* biomass in the Adriatic (data for 1976–1996, Azzali et al., 2001) followed a similar temporal pattern as fat content and catches of Black Sea sprat. In both cases increasing values from the 1970s to the 1980s were followed by considerable interannual fluctuations during the 1980s and a strong decrease at the end of the 1980s and start of the 1990s, with restoration during the the 1990s. Data on anchovy *Engraulis encrasicolus mediterraneus* (Sinovcic, 2000; Bombace, 2001) showed a similar trend. Further, biomass of phytoplankton (Vucetic, 1988; Caddy, 1990; Marasovich et al., 1998) and temperature (Caddy, 1990; Halim, 1992; Konovalov, 1995) fluctuated in the Adriatic in a similar manner. Similar trends in stocks and catches of anchovy were observed in other regions of Mediterranean (Caddy, 1990; Lleonart & Recasens, 1996; Fiorentini et al., 1997). This includes the anchovy stock biomass and catches in the Ionic and Aegean seas (Stergiou, 1988), the Tyrrhenian Sea (Arneri, 1996; Bombace, 2001), and Lyons Bay, Catalonia Region and Bolear and Andaluse seas (Lopez-Cazorla & Sanchez, 1986; Pertierra & Lleonart, 1992). In the Catalan Sea, a relationship between anchovy catches and primary production was reported (Tudela, 1992). Similar interannual dynamics are observed for many species in other seas of the Atlantic Basin: for Benguela anchovy *Engraulis meridionalis* (Lluch-Belda et al., 1992) and Southern African anchovy *Engraulis capensis* (Shelton et al., 1993), for Iceland herring *Clupea harengus harengus* (Bakken, 1983), and Iceland and Barents Seas capelin *Mallotus villosus villosus* (Yakobsson, 1991). Remarkably, Californian anchovy *Engraulis mordax* and Peruvian anchoveta *Engraulis ringens* also show the same regularity, as do California sardine *Sardinops caerulea* and Japanese sardine *Sardinops sagax melanostica* (Lluch-Belda et al., 1992). In all these cases, a rise in stock biomass and catches during the 1970s and 1980s was followed by a large fall

at the end of the 1980s and recovery (in those cases where data are available) in the 1990s. Undoubtedly, these long-term fluctuations have a global character as was suggested by Nierman et al. (1999), who analyzed the dynamics of plankton biomass in world oceans, and by Klyashtorin and Lyubushin (2007), who studied the dynamics of catches and stock biomass of marine fish in connection with global climate (temperature) fluctuations. A significant amount of information on the regularity of climatic changes in the oceans that is relevant to the dynamics of fish stocks can be found in Levitus (1994), Doumenge (1997), Issar (1997) and Lalli and Parsons (1997).

However, it must be noted that not all species follow the same trends. Biomass and catches of sardine *Sardina pilchardus* in the Mediterranean show opposite fluctuations to those of anchovy (Caddy, 1990; Arneri, 1996; Cingolani et al., 1998; Jukic-Peladic et al., 2001; Bombace, 2001). Also, catches of Peruvian anchoveta and sardine *S. sagax* in the Humboldt current region show opposite cycles (Lluch-Belda et al., 1992). This asynchronicity obviously relates to the different relationship of these fish with temperature and fodder organisms. Apart from these examples, there are no similarities in interannual changes in catches of Black Sea sprat and *Sprattus sprattus* in Baltic and Northern seas (Biryukov, 1980; Kolakovska et al., 2003). All these examples show the complexity of processes that affect condition of pelagic fish in different regions of the world.

Overall, the research on fish condition allows consideration of (i) the central role of global climatic factors in regulating condition of small pelagic fish and (ii) monitoring of energy (neutral lipid) content in fish populations as a significant "indicator" for estimating food supply/nutrition condition of key components of the high trophic level in marine ecosystems. Obviously temperature is the most important global factor by its effect on the intensity of water mass circulation and on production processes of different trophic levels, and subsequently on food supply and nutrition condition of pelagic fish, since food supply influences abundance (number and biomass) of populations and condition (health), maturation, spawning, survival of earlier stages, and elimination of adult fish.

Particularly interesting is the relationship between small pelagic fish stock value and biomass of phytoplankton as an indicator of the Black Sea pelagic ecosystem. We believe that indicators and monitoring of fish condition can signify the condition of the sea ecosystem as a whole. In the following paragraphs we will try to demonstrate this using the example of stock value dynamics of summary data of small Black Sea pelagic fish (sprat and anchovy).

It is natural to consider that there must be a positive relationship between primary fodder base of lower trophic levels and higher ones in a marine ecosystem, especially in the upper trophic levels. In the Black Sea pelagic system, the upper trophic levels are mainly represented by small pelagic fish, because at present the significance of large predator fish, dolphins and seabirds is not great. Thus the pelagic ecosystem of the Black Sea can be considered as a cybernetic "black box" where primary production (phytoplankton) is the input and small pelagic fish the output. We can find an example of such a relationship in Jennings et al. (2001). We have data from the Azov-Black Sea Research Institute of Marine Fisheries and Oceanography (AzcherNIRO) (Chashchin et al., 1996) for biomass of sprat and anchovy stocks in the Black Sea for 1967–1994 and data for aggregate and separate catch (landings) from these species from 1967 to the present (FAO, 2011). There is very good relationship between both parameters (Fig. 6.24). As an indicator of phytoplankton biomass dynamics, we used data for the most productive pre-Danube region of the northwestern Black Sea basin (Mashtakova & Roukhiyainen, 1979; Moncheva & Krastev, 1997; Black Sea Data Base, 2003; Yunev et al., 2009, 2011) (Fig. 6.25). This part is considered the
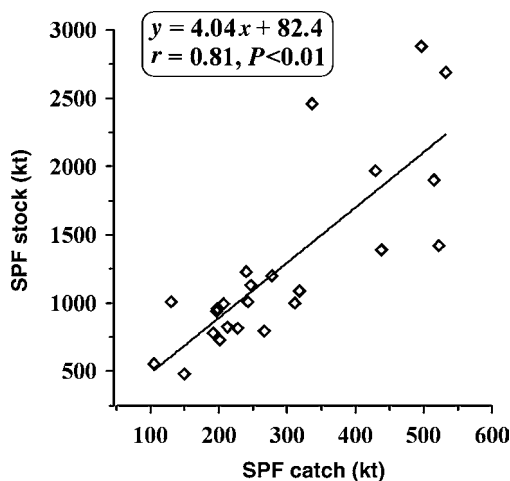
Figure 6.24 Relationship between total stock abundance and total catch of small pelagic fish (SPF, anchovy and sprat) in the Black Sea. After Yunev et al. (2009).
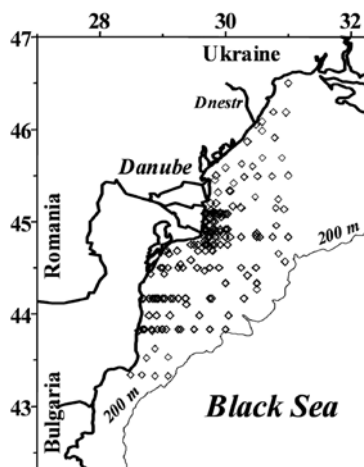
Figure 6.25 Stations in area of pre-Danube region used for estimation of phytoplankton abundance. After Yunev et al. (2009).

main region for reproduction and feeding of sprat and anchovy in the Black Sea. The relationships are shown in Fig. 6.26 (explanations in the figure legend). In addition to these characteristics, we show the relationship between summary stock values of both species and biomass of phytoplankton in the Danube region (Table 6.4 and Fig. 6.27). We have named this relationship the Q-factor, and this can be used as an indicator (and only as an indicator) of pelagic ecosystem condition (Yunev et al., 2009, 2011): low Q-factor values are a proxy for poor ecosystem condition and vice versa. With regard to Q-factor, ecosystem condition improved in the 1970s due to the increase in eutrophication (we have shown that the positive effect of eutrophication on stock value is considerably greater than the negative effect of catch); ecosystem condition reached its highest level in the 1980s and 1990s, and is at intermediate levels in the 2000s. A dramatic fall in Q-factor during 1988–1992 was caused
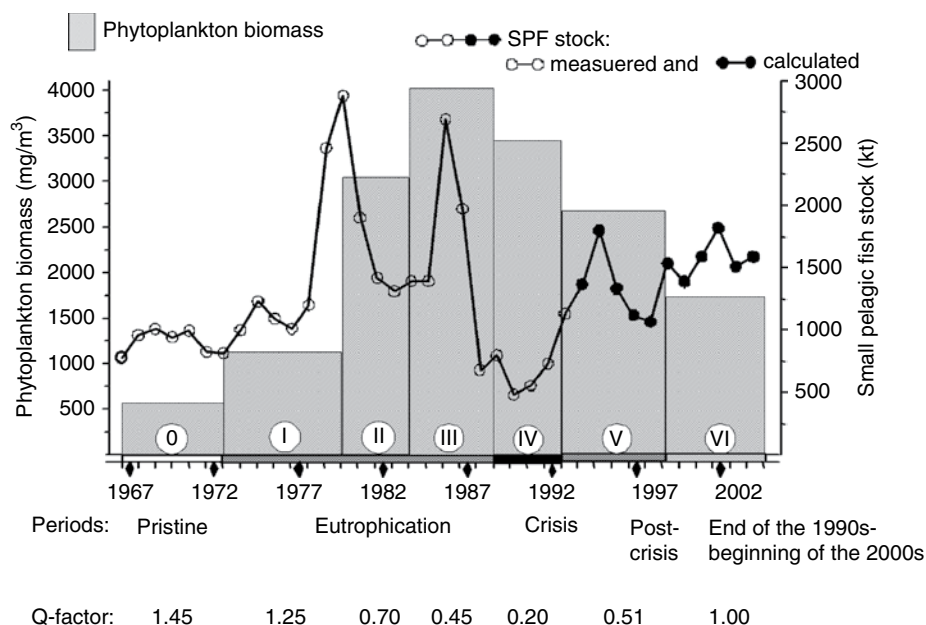
Figure 6.26 Dynamics of phytoplankton abundance (biomass) in pre-Danube region and biomass of small pelagic fish stocks in the Black Sea. After Yunev et al. (2009).

Table 6.4 Characteristics of the pelagic ecosystem of the Black Sea (mean data for interannual periods)

	Interannual period						
	0	**I**	**II**	**III**	**IV**	**V**	**VI**
Parameter	**1967–1972**	**1973–1979**	**1980–1983**	**1984–1988**	**1989–1992**	**1993–1998**	**1999–2004**
Stock of small pelagic fish (kt)	570	1258	1877	1624	640	1300	1570
Phytoplankton biomass (kt)	397	1004	2740	3602	1805	1415	1574
Q-value	1.44	1.25	0.69	0.45	0.35	0.92	1.00

Q-value represents relation between average value (biomass) of the stock of small pelagic fish and phytoplankton biomass.
Source: after Yunev et al. (2009, 2011).

by the mass invasion of the alien ctenophore *Mnemiopsis leidyi*. The complete effect of eutrophication during the 1970s, 1980s and the beginning of the 1990s on small fish stock biomass, as well as on the ctenophore predator, is well observed (compare with fat monitoring). During most of the 1990s, due to economic crises in West European countries, eutrophication decreased considerably, and this caused a decrease of *Mnemiopsis* predation on small pelagic fish fodder base and, as a consequence, a restoration of their stock values. Another significant factor contributing to decreased predation pressure on the fish fodder base was the invasion into the Black Sea by the alien predator ctenophore *Beroe ovata*,
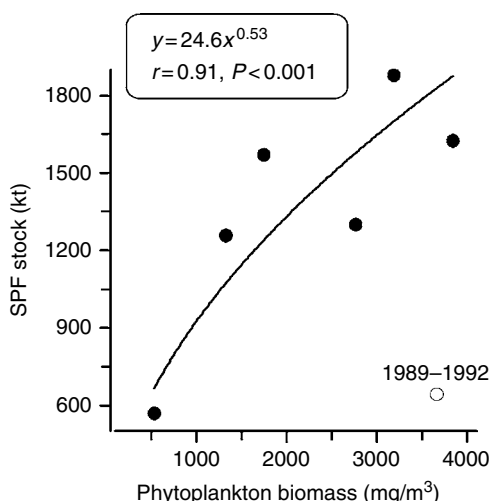
Figure 6.27 Relationship between phytoplankton biomass in pre-Danube region and total small fish stock biomass in the Black Sea. After Yunev et al. (2009).

which feeds intensively on *Mnemiopsis*. In 2000, a new environmental factor – strong warming – developed, and this has had a positive role for catches (and fatness) of anchovy and a negative or yet indefinite role for catches (and fatness) of sprat. Overall, the Black Sea pelagic ecosystem has not returned to the pre-eutrophication level (1960s) or to the high level of the 1980s, and at present is at an intermediate level (lower than in the 1970s).

Particularly important is the monitoring of fatty acid composition of sprat and anchovy. As previously discussed, the content of polar lipids in fish body remains relatively stable, but the content and composition of fatty acids changes greatly. Especially important are the changes in content of unsaturated omega-3 fatty acids (EPA and, primarily, DHA). We have obtained some data on the dynamics of these acids in phospholipids in comparison with triacylglycerols in sprat and anchovy bodies (Yuneva et al., 2011). Data for 2005–2009 show that intensity of feeding and fodder composition of sprat and anchovy changed considerably. These show that feeding condition in 2006 differs significantly from feeding condition in the following years due to the dominance (after 2006) of fodder containing smaller amounts of DHA and in some cases EPA.

## 6.4   Indicators of interspecific and intraspecific differentiation

Up to now very few studies have employed a unified genetic and ecological approach to the study of intraspecific differences (Tsuyuki et al., 1965; Altukhov, 1974, 2003; Lukyanenko et al., 1991; Peng et al., 2003). Although the results of all these studies are impressive, it is a pity that they have only a formal character, because they do not reveal phenotypical eco-physiological mechanisms of fish adaptations to the environment. Without doubt the integration of genetic and ecophysiological approaches will have great success in the future, but have not been well developed yet. A good example of an ecophysiological study was
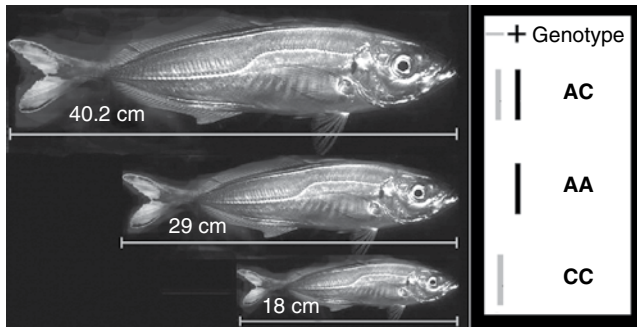
Figure 6.28 Three subspecies of horse-mackerel: small form (*Trachurus mediterraneus ponticus*); medium-size form (*T. m. mediterraneus*); hybrid (?) form. AA, CC and AC, muscle isozymes of aspartate aminotransferase. After Dobrovolov (1980).

provided by the investigations of Ushakov (1963) on the species specificity of temperature resistance of isolated tissues and proteins from different forms of animals including fish. All studied species have specific adaptations to the temperature environment that affects molecular and tissue structure and functions, and as such temperature resistance can be a good indicator of species characteristics. However, Ushakov considered that temperature resistance has a universal significance. Species that adapt not to temperature but to other environmental factors have corresponding functional features that can be used as indicators of species specificity. For example, the degree of lipid unsaturation in Sea of Azov gobies may be an indicator of a species in the specific ecological niche that they occupy (in this case, degree of oxygen saturation of sea water).

A good example of the importance of a unified genetic and ecological approach in the study of intraspecific differences is provided by horse-mackerel in the Black Sea and Mediterranean (Fig. 6.28). While the Black Sea horse-mackerel is a small fish (15–20 cm) and the Mediterranean horse-mackerel can reach 30 cm, hybrids can grow up to 50 cm and even larger (Dobrovolov, 1980). This is an example of heterosis, the phenomenon where progeny of diverse varieties of a species or crosses between species exhibit greater biomass, speed of development, and fertility than both parents. In addition, there are large differences in protein growth and fat accumulation between subspecies of European anchovy in the Mediterranean, Black and Azov seas (Fig. 6.29; Shulman & Urdenko, 1989). The differences in their genetic content are well known (Dobrovolov, 1992), but do not convey information about the mechanisms behind their different adaptations to the environment in the three different sea basins. For anchovy, differences in temperature and particularly food supply are the main triggers of the observed differences in condition. Since zooplankton is more abundant in the Sea of Azov, fish spend less energy obtaining food and therefore can "save" more towards fat accumulation. In the Mediterranean, zooplankton abundance is the lowest, so fish spend more energy obtaining food and therefore fat accumulation is minimal. Black sea anchovy are in an intermediate position. Protein and weight (mass) growth "conflict" with fat accumulation because fat accumulation reduces protein growth. Because of this, Mediterranean anchovy can grow to larger sizes than Azov anchovy. In addition, the time available for feeding and growth processes is shorter in the Sea of Azov than in the Mediterranean. Yuneva et al. (2011) showed that DHA in phospholipids of Black Sea anchovy is higher than that in Azov anchovy (Table 6.5). In our opinion, the reason is the difference in food supply. Because Black Sea anchovy must spend more energy to obtain
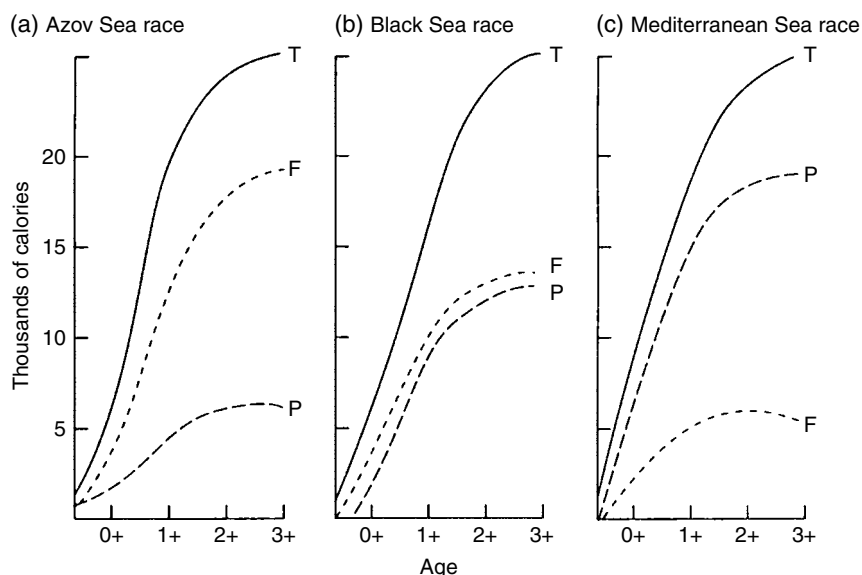
Figure 6.29   The races (subspecies) of anchovy: (a) *Engraulis encrasicolis maeoticus*; (b) *E. e. ponticus*; (c) *E. e. mediterraneus*. P, protein; F, fat; T, total energy equivalent. From Shulman & Urdenko (1989) and Shulman & Love (1999).

Table 6.5   DHA (22:6ω3) content (% of total fatty acids) in phospholipids of two anchovy subspecies at the end of feeding period, November–December.

Years	Sea of Azov anchovy		Black Sea anchovy	
2006	$n=3$	14.32 ± 1.97	—	—
2007	—	—	$n=3$	22.14 ± 1.44
2008	$n=3$	15.37 ± 2.94	$n=3$	26.85 ± 0.36
2009	$n=3$	14.49 ± 0.32	$n=4$	22.87 ± 1.43
2010	$n=2$	15.82	$n=7$	25.89 ± 0.78
2011	$n=3$	12.48 ± 1.06	—	—

n, Trawl numbers.
*Source*: after Yuneva et al. (2011).

food, it has higher locomotory activity and higher concentrations of DHA to support this. By the way, DHA content in muscle phospholipids of the Black Sea anchovy in the summer spawning period is higher than that in winter (Fig 6.30). The Black Sea cold-tolerant sprat also has a higher DHA content during the summer feeding period than in winter (Fig. 6.31). These are examples of an uncorrected "temperature paradigm" that relates change in DHA (particularly in ectotherms) to habitat temperature. DHA content can be used for identifying both subspecies in different regions of the Black Sea during the fall migration and wintering and therefore complements genetic analyses. In this case, former ideas about the distribution of the Azov and Black Sea anchovy during the wintering period in the Black Sea must be revised (Fig. 6.32). As Yuneva et al. (2011) showed, the C22 content of anchovy phospholipids in the Turkish region are often similar to those of the Azov subspecies.
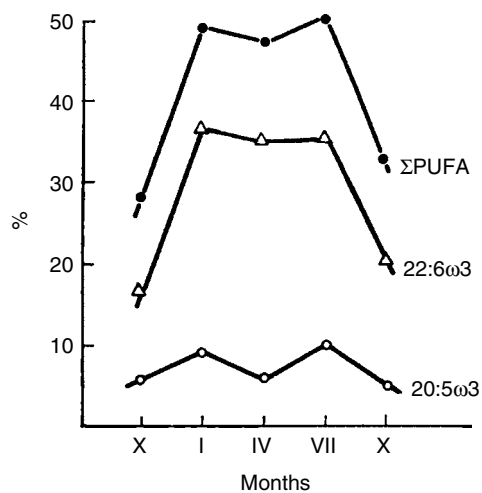
Figure 6.30 Dynamics of DHA (22:6ω3), EPA (20:5ω3) and total polyunsaturated fatty acids (ΣPUFA) in phospholipids of the Black sea anchovy during the annual cycle. After Yuneva et al. (1990).
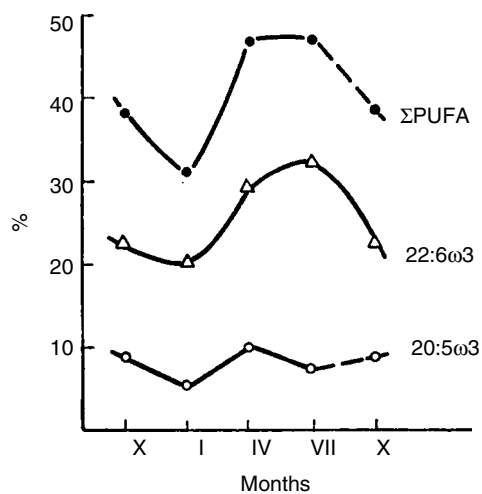
Figure 6.31 Dynamics of DHA (22:6ω3), EPA (20:5ω3) and total polyunsaturated fatty acids (ΣPUFA) in phospholipids of the Black sea sprat during the annual cycle. After Yuneva et al. (1990).
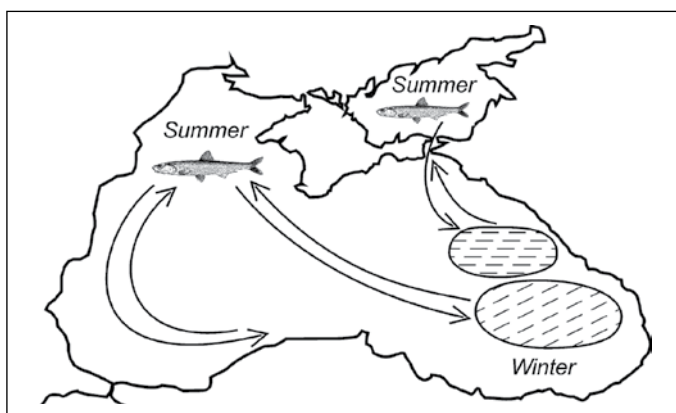
Figure 6.32 Former view on anchovy distribution in the Black Sea. After Chashchin (1996, 1998).

Research from other oceans has reported differences in lipid and glycogen content, as well as growth of different stocks of cod, haddock, herring, flounder, Arctic char, and other species in the North Atlantic including the North Sea, Baltic Sea, Norwegian Sea, Barents Sea, and White Sea (Shatunovsky, 1980; Love, 1970, 1975, 1988; Jamieson, 1974; Love et al., 1974, 1975; Cloout et al., 2002). Some of these results were discussed in our previous book (Shulman & Love, 1999). Similarly with anchovy, in these northern species there is an inverse relationship between growth rate and fat accumulation; because of their longer lifespan, the opposite dependence between growth rate, start of reproduction, and duration of life cycle is better revealed. Northern fish spend around 4–10% of total energy on maturation, while southern fish of the same species (e.g., sprat and herring) spend around 20–40% on maturation (Koshelev, 1984).

Intrapopulation (individual) differences also have an ecological foundation. When environmental conditions for populations worsen, individual variation in physiological parameters increases whether it is lipid and glycogen content in North Atlantic cod (Black & Love, 1986) or growth rate of White Sea flounder (Shatunovsky, 1980). We have shown some other examples in this book and in the previous one (Shulman & Love, 1999).

7 Use of fish condition indicators to improve stock assessment and fisheries management

Summary: This chapter shows how fish health indicators in marine ecosystems constitute a valuable tool that can be used in stock assessment and management. Furthermore, the implications of the spatial (interannual and seasonal) and temporal variability in condition for fisheries (not only sustainability but also the attainment of maximum yields and maximum quality) is reviewed. Based on the examples provided, long-term monitoring of fish condition is recommended.

Key words: stock assessment, fisheries management, product quality, maximum yield, seasonal and spatial closure, monitoring

Population and individual parameters such as stock abundance, biomass, growth, mortality, maturity, and recruitment reflect the productivity of fish stocks and are necessary for evaluating the status of an exploited fish population. Knowledge of these parameters is increasingly recognized as an important criterion for effective management and conservation (reviewed by Young et al., 2006). Apart from a few cases, such as anchovy in the Black Sea (Shulman & Love, 1999), fish condition has never been taken into account for stock assessment and management. Although condition data alone is of little help to fishery managers, when combined with other information (abundance, biomass, size structure, exploitation, etc.), condition data will likely help fisheries biologists make correct assessments of population dynamics and environmental and anthropogenic influences.

Although the assessment of fish health is playing an increasing role in fishery management and environmental monitoring policy in freshwater ecosystems (see for example Goede & Barton, 1990; Sutton et al., 2000; Ebener & Arts, 2007; Brenden et al., 2010), the monitoring of fish health indicators in marine ecosystems is scarce and the use of these indicators in stock assessment and management is practically nil. Typically, stock assessment and fisheries management of marine species have focused on specific individual and population parameters (e.g., growth and natural mortality), with no integration of fish health information. In particular, the relationship between health status and natural mortality of fish is relevant

because natural mortality M is one of the most influential parameters in fisheries stock assessment and management (Beverton & Holt, 1957; Vetter, 1988). Estimates of natural mortality relate directly to the productivity of stock yields, sustainable exploitation rates, management quantities, and reference points. Unfortunately, in many oceans around the world, fish health is not regularly monitored and the linkages between population dynamics and fish health have been largely overlooked (Lloret et al., 2012).

7.1   Fish condition and fishing mortality

Declining condition may decrease the metabolic and swimming capacities of fish and hence their ability not only to avoid predation but also to avoid being fished. Thus, fishing mortality could potentially be impacted if fish with lower condition have a lower probability of escape from some active gears (e.g., trawl) because of a reduced swimming ability (Marshall et al., 2004). Further, immediate and delayed mortalities can occur in fish escaping from fishing gear such as trawls due to physical damage or stress incurred during capture and escape, particularly for immature forms (Chopin & Arimoto, 1995). Thus improving selectivity without reducing damage or stress incurred during capture and escape may not be the most appropriate way of protecting immature fish. The analysis of fish condition can therefore be used to estimate the unaccounted fishing mortality associated with fishing gear damage, and to propose measures other than gear selectivity changes to reduce such mortalities. Finally, it is important to consider the negative impacts of bycatch and catch & release practice (usually attributed to the commercial and recreational fisheries, respectively). Released fishes in both cases often display lethal and non-lethal injuries caused by fishing practice (reviewed by Lenin et al. 2006 and Cooke and Schramm, 2007). Then, the evaluation of the physiological response and condition of fishes that are released would improve the estimation of fishing mortality values and therefore the fish stock assessments.

7.2   Spatial and temporal variability: implications for fisheries

Spatial and temporal variability in condition of exploited fish resulting from the intrinsic and extrinsic factors described previously can influence stock biomass and also the potential economic benefit of fisheries, as a consequence of changes in yield and quality of fish products (see for example Sylvia et al., 1996; Larkin & Sylvia, 1999; Mello & Rose, 2005). Particularly for pelagic fish, in which lipids concentrate mostly in the edible part (muscle), a reduction in fat content of the fish will presumably also reduce the value of the catch for processing for fishmeal and/or oil.

7.2.1   Seasonality

Seasonal biological cycles in fish condition could be used as templates for management strategies that promote fisheries conservation and economic and human health benefits. Although some studies have linked seasonal variability in fish condition with the quality and market value of harvested fish (see for example Mustac & Sinovcic, 2012), only a few attempts have been made to optimize harvesting strategies (e.g., timing of fishing) in relation to reproduction/condition cycles or to address the related economic benefits (Larkin & Sylvia, 1999; Mello & Rose, 2005). This would be very important in those fisheries that are
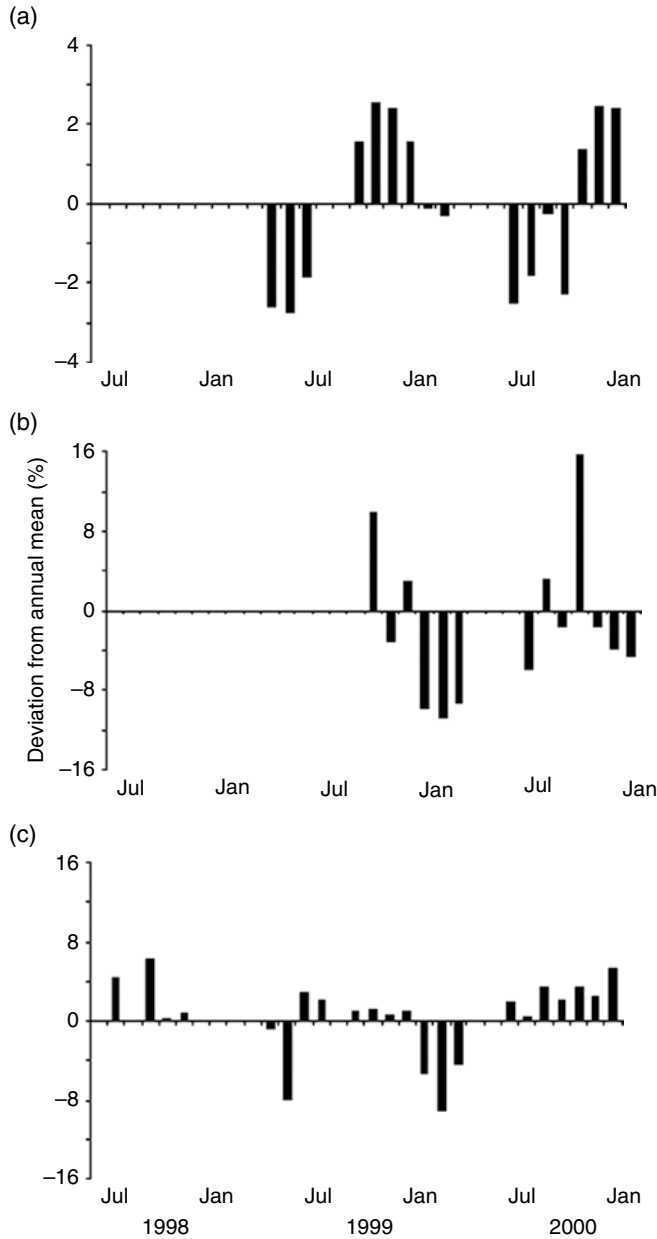
Figure 7.1 Temporal changes in (a) commercial package yield, (b) block percent, and (c) grade A percent from cod (*Gadus morhua*) processed by National Sea Products fish plant in Arnold's Cove, Newfoundland. Commercial indices defined in text. Temporal variability is expressed as the percentage difference between the monthly and annual mean indices. From Mello & Rose (2005).

prosecuted on spawning fish such as cod trawl fishery in the North Atlantic or European hake long line fishery in the northwest Mediterranean (Lloret et al., 2012). For example, cod exhibits strong seasonality in weight and condition, reaching highest levels in late fall and lowest in spring during spawning (Mello & Rose, 2005). In this study, commercial package yield was lower in spring (when Fulton's *K* condition factor and hepatosomatic index,

HSI were lower coinciding with the spawning season) and higher in fall (when K and HSI were at a maximum) (Fig. 7.1). Hence, an industry index based on package yield was positively and significantly correlated with K and HSI (Mello & Rose, 2005). Historically, cod fisheries have been prosecuted during all seasons, but simulations indicated that a fall fishery resulted in an 8–17% decrease in the number of cod removed from the stock while maintaining the same weight-based quotas, and profiting from maximum yield and better product quality (Mello & Rose, 2005). Conversely, spring and summer fisheries resulted in lower yield (6%) and quality (5–26%) of fish products by weight. This is explained by the fact that the fall is the period of peak physiological condition, whereas spring and summer (spawning season) are the periods of weak physiological condition. Hence, Guderley et al. (1996) reported that wild October cod had higher condition factors, hepatosomatic indicators, white muscle lactate dehydrogenase activities, sarcoplasmic protein concentrations, and buffering capacities than June cod. In fact, in June the cod were losing mass (–0.18% body mass/day) whereas in early October they were increasing their mass (+0.83% body mass/day). In consequence, during the spawning season, cod are in their poorest somatic condition, and commercial quality and yield will be poorer than during the fall period.

Similar, Sylvia et al. (1996) and Larkin and Sylvia (1999) used bioeconomic models to evaluate the impact of seasonal quality variation of Pacific whiting *Merluccius productus* on optimal management strategies and economic benefits. These studies recommended that a delay in the timing of harvest until the end of the feeding season (historically more than 50% of the annual quota is harvested shortly after the end of the spawning season) would result in more than doubling the net revenue from the fishery because of the improvement in flesh quality.

On the other hand, the evaluation of seasonal cycles in fish total lipid, particularly the omega-3 fatty acids, could also be important for achieving the maximum health benefits from a given stock because of the beneficial effects of fish omega-3 fatty acids on human health (reviewed by Lloret, 2010). This aspect is clearly demonstrated in studying the traditional diet in Mediterranean countries (so-called "Mediterranean diet"), which has been consistently shown to be associated with favorable health outcomes and a better quality of life (reviewed by Lloret, 2010). Several epidemiological and observational studies suggest that this type of diet traditionally followed by Mediterranean peoples may protect against chronic diseases and mortality, with Mediterranean nations presenting lower rates of cardiovascular disease and cancer in comparison with other nations (reviewed by Lloret, 2010). The long-chain omega-3 (or n-3) fatty acids (eicosapentaenoic and docosahexaenoic fatty acids) found in seafood, which are important components of the Mediterranean diet, have been identified as the main elements responsible for this protection against cancer and cardiovascular disease in Mediterranean populations. In addition to the cardiovascular and cancer protective effects of fish consumption, fish intake in the Mediterranean has also been associated with less severe depressive symptoms in adults and less development of asthma and respiratory allergies in children. Omega-3 fatty acids also mediate the inflammatory process and influence the general health status of the skeletal system. However, not all studies found positive effects of omega-3 consumption and human health. Rizos et al. (2012) found that omega-3 supplementation was not associated with a lower risk of all-cause mortality, cardiac death, sudden death, myocardial infarction, or stroke based on relative and absolute measures of association.

In this sense, fishing effort could then be shifted to the seasons when fish total lipid content is at a maximum, in order to obtain the greatest health benefits for the consumers. For example, by fishing for anchovy in late spring in Greek waters, the highest lipid content for the stock and the diet can be obtained (Zlatanos & Laskaridis, 2007). Also, in order to obtain the maximum fish oil from *Sardinella aurita* populations on the Tunisian coast (Mediterranean), fisheries should be carried out all year (lipid could reach 20% wet weight) except in the summer

Figure 7.2 Fish exposed at a Mediterranean fishmarket. Photo by Sílvia Vila.

months (lowest lipid content around 2.5% wet weight in July; Ben Rebah et al., 2010). In particular, the seasonal variation in *n*-3 fatty acid content, and the differences between species, should be considered for human health purposes. For example, anchovy and sardine show the highest *n*-3 fatty acid content in different months of the year (Zlatanos & Laskaridis, 2007). However, the fat content of sardine is always (all year round) higher than that of anchovy, and therefore sardine appears to be the richest source of omega-3 fatty acids during the whole year. Therefore, the analysis of lipid and fatty acid composition of edible parts (muscle, roe, liver, etc.) of fish and seafood consumed is useful in assessing the benefits of fish consumption recommendations.

All these examples support the fact that the biological seasonality of a number of exploited species in temperate ecosystems may directly influence the harvested quantities and qualities of fish and the economics of the fishery, through seasonal variations in product yield and quality (Mello & Rose, 2005). Condition indicators may be useful in identifying periods of increased stock productivity, yield, and quality of fish products (Fig. 7.2). Therefore, the evaluation of seasonal cycles in fish health could also contribute to achieving the maximum sustainable yield from a given stock and the maximum nutritional benefits for the consumers (Lloret et al., 2012). Fishing during the peak physiological condition can result in a decrease in the number of individuals removed from the stock while profiting from maximum yield and quality of the product.

Notwithstanding these facts, concentration of fishing effort on particular seasons may be neither economically feasible nor healthy for the stocks in question (if, for example, the peak in condition coincides with the spawning season, which is however rarely the case). Harvesting strategies cannot be based solely on seasonal cycles in fish condition, but must also consider (from a biological point of view) other factors such as temporal variation in abundance or the impact of fishing on spawners (for those fisheries targeting the spawning stock biomass).

The high degree of intraspecific seasonal variability in lipid content in the muscle obliges us to reflect that some species considered traditionally to be fatty may in particular seasons have the same lipid concentration as fish considered to have little lipid. Thus for example, in the fall the jack mackerel *Trachurus trachurus*, a pelagic species commonly considered to

have a lot of fat, has lower lipid content in the muscle than hake *Merluccius merluccius* has in the same region of the Mediterranean (Soriguer et al., 1997).

It is important to note that the amplitude of the seasonal variations in energy reserves display the greatest range in the temperate-boreal zone – the North Pacific and North Atlantic oceans – because of the large swings in temperature and resulting unevenness of the food supply typical of this climatic zone (Shulman & Love, 1999). In temperate zones, encompassing the Mediterranean, Black, Azov and Caspian seas, the temperature regime and nutritive base are more stable, and so is the seasonal variability in fish condition. Further stability is shown in the tropical zone, where fish need not store much lipid because their spawning season is more extended than in temperate seas and because food supply does not much vary seasonally. Although this general pattern should be kept in mind for fisheries management purposes, a specific study must be implemented case by case because there are notable exceptions. Thus, for example, some tropical pelagic fish such as horse-mackerel and sardine have clearly marked periods of intensified lipid accumulation and expenditure (Shulman, 1974). Further, cold-water species such as sprat inhabiting temperate-warm waters such as the Mediterranean exhibit great seasonal variation in total lipid content which contrasts with the weak seasonality of the warm-water anchovy in the same area (Shulman, 1987a).

Overall, seasonal cycles in fish health could be used to refine management strategies that should promote fisheries economic, conservation and human health and nutritional benefits by harvesting fish during periods when biological impacts are minimal and economic and human health returns maximal.

7.2.2   Interannual variability

From a fisheries perspective, probably the most interesting feature is that of interannual variability in condition typical of many fish species, which can explain some of the interannual changes in the productivity of stocks. The interannual variability in condition of cod is a good example. Pardoe et al. (2008) showed strong interannual and spatial variation of mean HSI and relative condition factor (Fig. 7.3) of immature and mature cod in shallow and deep waters from Iceland (Pardoe et al., 2008). Also, northeast Artic cod display strong interannual variability in predicted weight and liver weight at a given length (Marshall et al., 2004). In Greenlandic waters, the annual mean condition factors of cod also showed clear interannual variations (Lloret & Rätz, 2002). In the northwest Mediterranean, several groundfish species have displayed strong interannual fluctuations in their condition (Fig. 7.4; Lloret et al., 2002). In the Black Sea, Shulman et al. (2005) revealed long-term variability of fat content of *Sprattus sprattus*. All these interannual changes are expected to have implications for trophic interactions, ecosystem functions, fisheries, and global protein and lipid supply, and therefore need to be well evaluated.

In particular, it is important to detect declining condition over years that have experienced a number of fish stocks, which sometimes preceded or coincided with steep decreases in the stock size. For example, the collapse of the northwest Atlantic cod fisheries in the early 1990s coincided with a significant decrease in the condition of the species (Lambert & Dutil, 1997b). The reduced condition of cod may have led to an increase in natural mortality, particularly for adults that have to invest energy reserves in reproduction, thus possibly contributing to the drastic decline of this stock (Lambert & Dutil, 1997a,b). In northeast Artic cod stock, Marshall et al. (2000) also revealed a negative trend in total lipid energy contained in the livers of mature females since the mid-1970s, paralleling a decline in spawner abundance and suggesting that the reproductive potential of the stock was in decline since then.
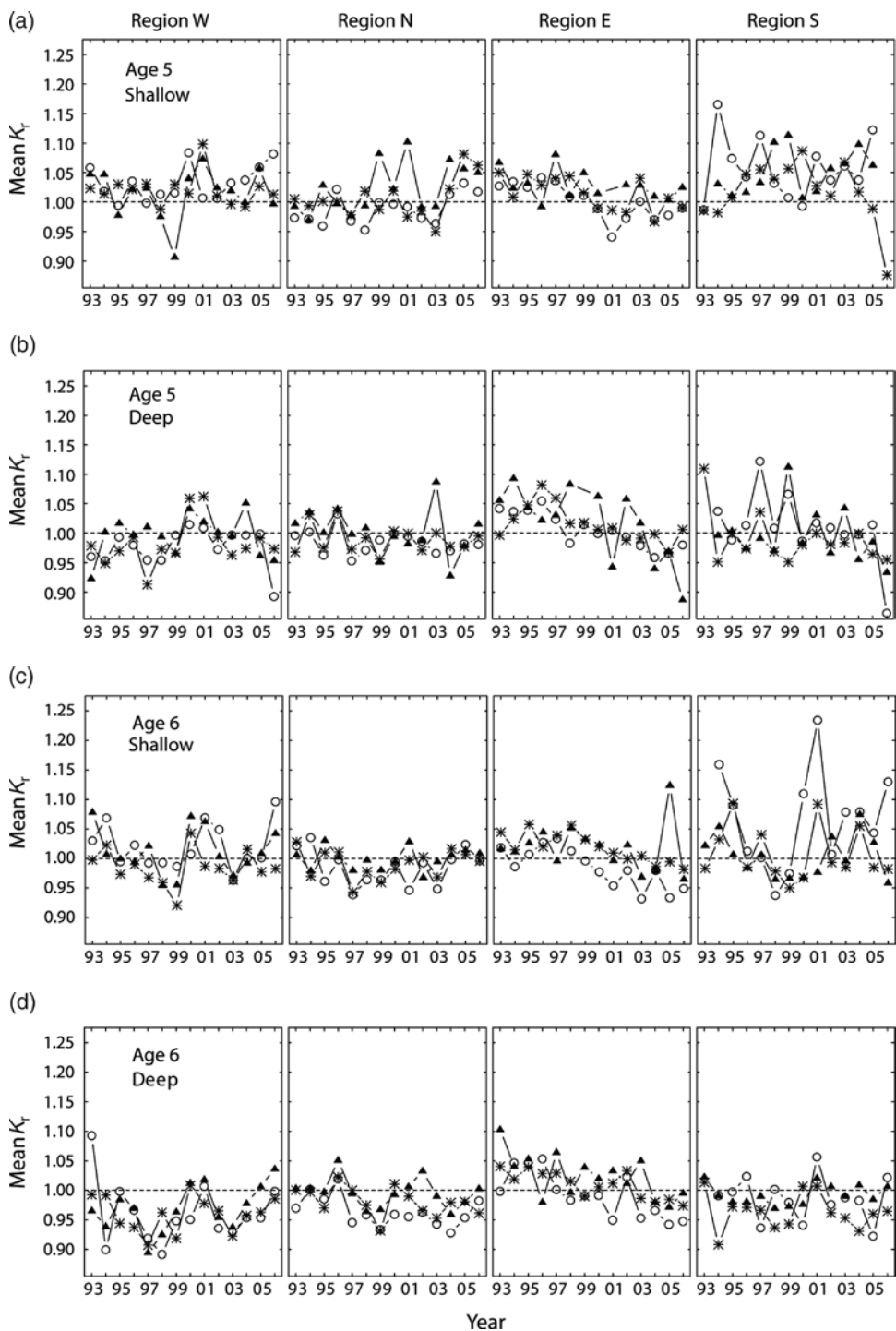
Figure 7.3 Cod *Gadus morhua* off Iceland. Interannual (1993–2006) and spatial variation of the relative condition factor (K_r) of age 5 year immature and mature cod in (a) shallow (<200 m) and (b) deep (>200 m) water, and age 6 year immature cod in (c) shallow and (d) deep water. The horizontal dashed line shows the overall mean for the age present in each plot. Open circles, both sexes combined; triangles, mature females; asterisks, mature males. From Pardoe et al. (2008).
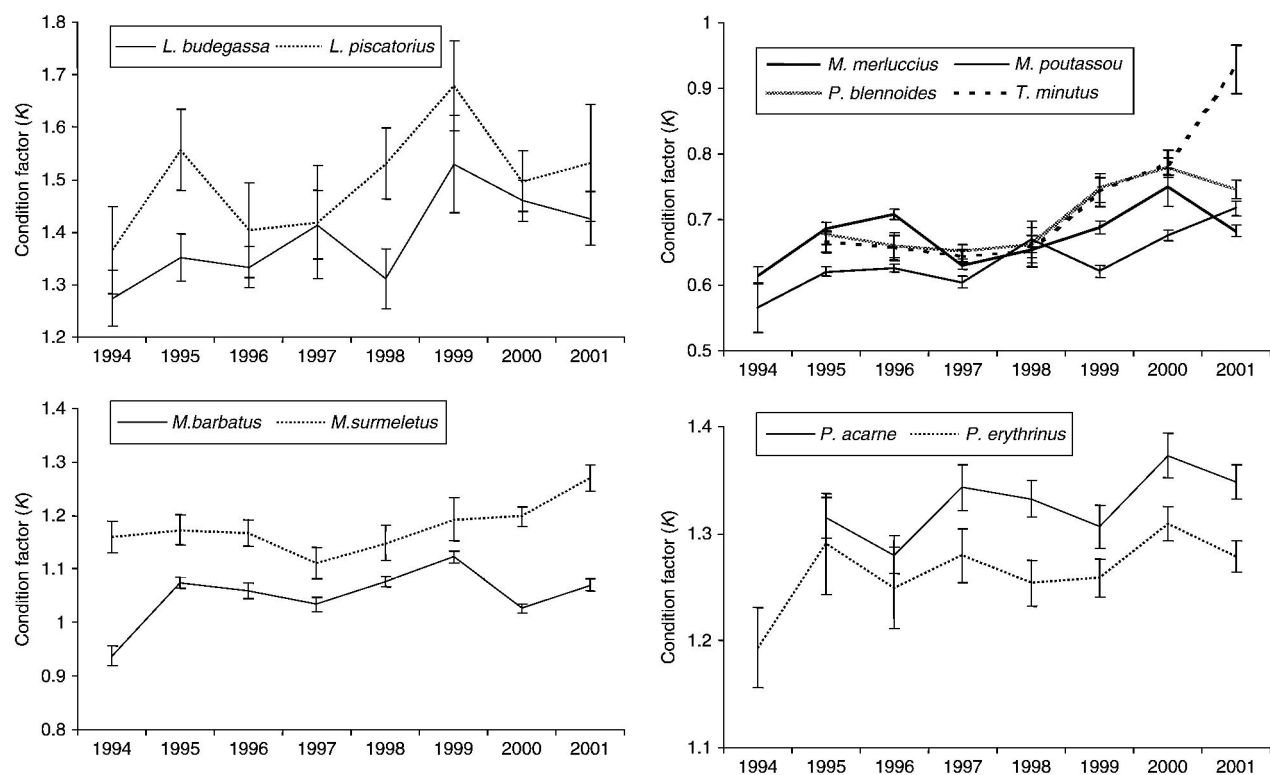
Figure 7.4 Interannual variations (1994–2001) in Fulton's *K* condition factor of several demersal species in the western Mediterranean. Modified from Lloret et al. (2002).
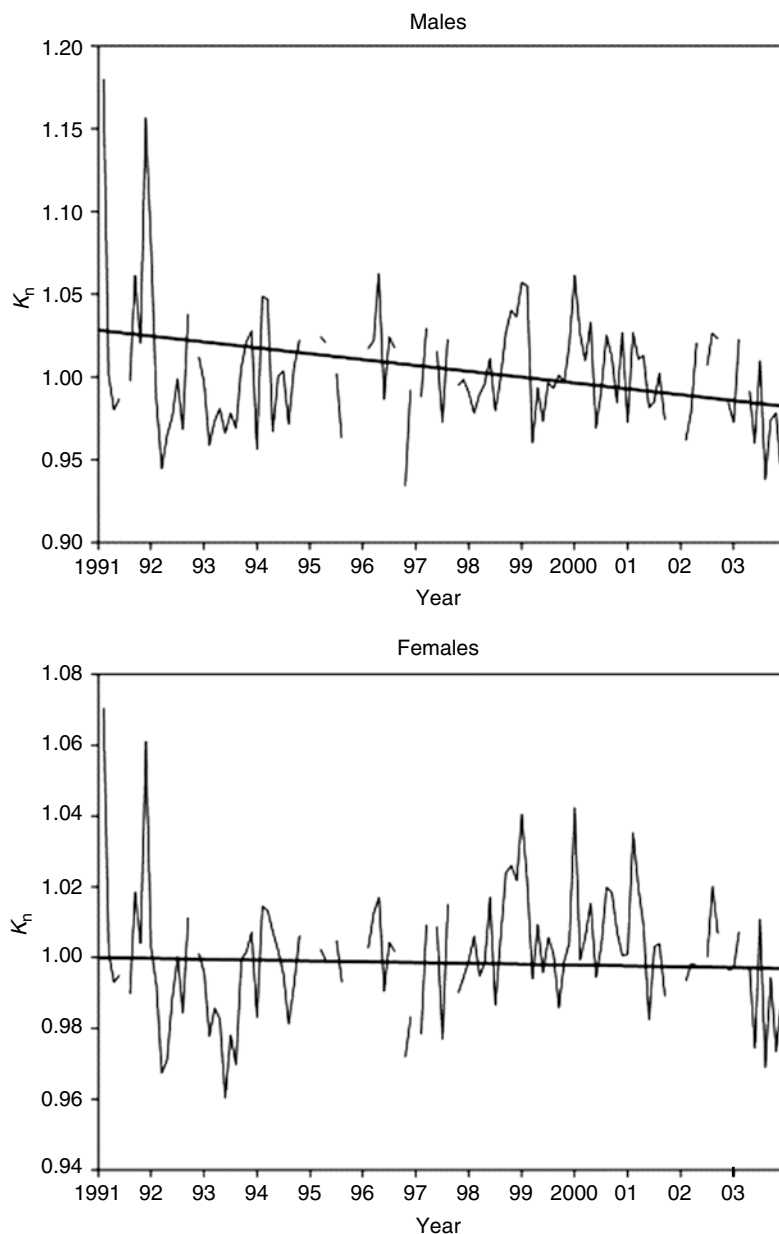
Figure 7.5 Monthly relative condition index (K_n) of red shrimp *Aristeus antennatus*, 1991–2003, for males and females. Missing values are due to gaps in the monthly sampling. The straight line represents the linear trend. From Carbonell et al. (2008).

Similar, Golet et al. (2007) revealed a decline in condition (fat and oil content and fish shape) of northern bluefin tuna *Thunnus thynnus* in the Gulf of Maine that could diminish allocations to growth and gamete production and have detrimental consequences for rebuilding the western Atlantic population. Fish landed in the month of June 2004 arrived in poorer condition than they did in the early 1990s. For example, the probability of landing a poor-quality fish in June 1991 was 30% compared with 70% in 2004.

Also, Carbonell et al. (2008) observed a decreasing trend in red shrimp male condition over years in the Balearic Islands (Fig. 7.5). There exists a good relationship between the condition of males and recruitment of red shrimp (Carbonell et al., 2008), which indicates that males play a key role during the whole reproductive process of the species. In this sense, the condition of red shrimp males could have an effect on the quantity and quality of their sperm and fertilization success. Hence, the observed decreasing trend in red shrimp male condition over the years in the Balearic Islands raised concerns about the future reproductive potential of that population.

It is also worth noting the declining trend in relative condition factor that has been observed in several groundfish in the Mediterranean such as European hake *Merluccius merluccius* in the Gulf of Lions and the south and central Tyrrhenian Sea, common sole *Solea solea* in the northern Adriatic, or sardine *Sardina pilchardus* in the Aegean Sea (Scientific, Technical and Economic Committee for Fisheries, Scientific, 2010).

Declines in the condition of fish have also been documented in freshwater ecosystems, for example in the Great Lakes lake whitefish *Coregonus clupeaformis* (Rennie et al., 2009). Although declines in the growth and condition of this population have been largely attributed to food web disruptions caused by invasive dreissenid mussels, Rennie et al. (2009) revealed that environmental change contributed significantly but alone was not sufficient to explain declines in lake whitefish growth after dreissenid establishment, whereas biological variables such as food availability and density dependence effects could account for the majority of growth and condition changes observed in this population. Also in the Great Lakes, an analysis of long-term (1970s to 2008) temporal trends in lipid content of 10 fish species suggests that, overall, lipid content significantly decreased in eight of the ten species in at least part of one or more Great Lakes (Neff et al., 2012).

7.2.3   Spatial variability

A comparative analysis of the health of different populations (for the same species) could be used to refine the parameters used in stock assessment. Rätz and Lloret (2003) established a positive relationship between the estimated biological management reference points F_{med} and the mean cod condition factors of 10 cod stocks in the North Atlantic (Fig. 7.6). F_{med} is one of the precautionary reference points generally accepted as a long-term measure of stock management in terms of safe exploitation rates that a stock can sustain with a low risk of collapse. This implies that the stocks in better condition can sustain higher levels of exploitation in the long term than stocks in poor condition (Rätz & Lloret, 2003).

7.3   Fish condition and stock assessment and management

Lloret et al. (2012) reviewed the importance of fish condition studies for fish stock assessment and management. The authors suggested that stock assessment working groups, fishery agencies, and research centers in the Mediterranean and elsewhere should incorporate simple fish health indicators such as energy reserves and parasitic infection into their routine assessment and research programs, at least for the most important target species. Despite the fact that the links between fish health and productivity (mortality, recruitment and growth) cannot always be clearly demonstrated, implementing long-term monitoring programs of stock health could support stock assessment and sustainable management of profitable fisheries, particularly in stocks where data shortage prevent standard stock assessments, and help understand how exploited fish stocks are responding
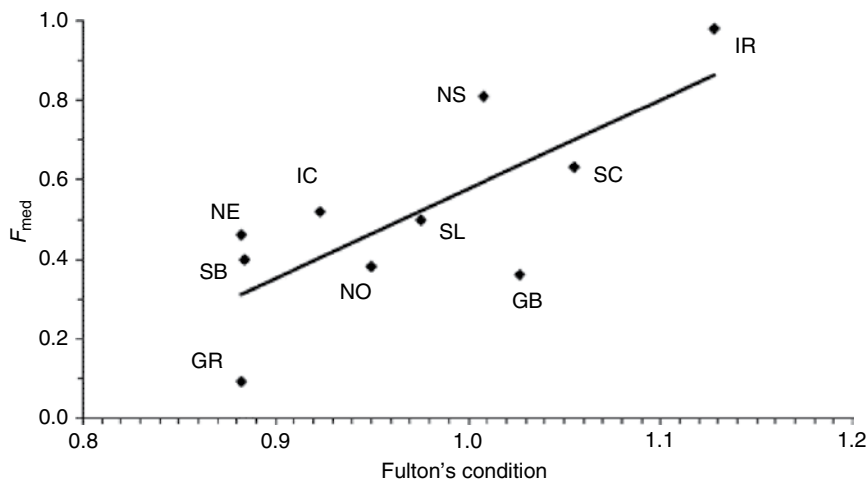
Figure 7.6 Linear regression between F_{med} and Fulton's condition factor for 10 north Atlantic cod stocks. Stock abbreviations: Greenland (GR), Georges Bank (GB), S. Gulf of St Lawrence (SL), Southern Grand Bank (SB), Northern Cod (NO), North-east Arctic (NE), NW Scotland and Ireland (SC), Irish Sea (IR), North Sea (NS), Iceland (IC). From Rätz & Lloret (2003).

to environmental and anthropogenic impacts (Lloret et al., 2012). Condition of fishery species should then be monitored regularly, at least during the pre-spawning period, to detect interannual changes in the health of fish populations that can be used to estimate interannual variability of natural mortality (M) for use as input in stock assessments and forecasts. Moreover, the monitoring of simple fish health indicators could complement the standard population dynamic procedures commonly used by international scientific and management bodies, and also as an indicator of stock status in data-poor situations where standard assessments (e.g., VPA or XSA) cannot be carried out. This approach will therefore provide a means for detecting negative trends in the health of a fish population over time, or anomalous poor health status at a particular time. By monitoring the condition of exploited fish, fisheries biologists should be able to give early warning of trouble. Whenever there are indications of poor health status, such as high parasitic load, low lipid content or lower than average relative condition index, input values of stock assessment models should be updated accordingly, using slower growth rates and higher natural mortalities than average (Lloret et al., 2012). Alternatively, if pre-spawners are found to be in extremely poor health during a particular year, fishery managers could adopt adaptive measures such as a time closure or a reduction of the fishing effort during the reproductive period in order to minimize the likelihood of poor recruitment resulting from the weak paternal stock condition. Managers should be particularly aware of declining trends in the average health status of individuals within a stock such as those detected in several groundfish in the Mediterranean including European hake *Merluccius merluccius* in the Gulf of Lions and the south and central Tyrrhenian Sea, common sole *Solea solea* in the northern Adriatic, sardine *Sardina pilchardus* in the Aegean Sea (Scientific, Technical and Economic Committee for Fisheries, 2010), red shrimp (particularly males) in the Balearic Islands (Carbonell et al., 2008), as well as several stocks in the North Atlantic including cod *Gadus morhua* in Newfoundland waters (Lambert & Dutil, 1997a,b) and bluefin tuna *Thunna thynnus* in the Gulf of Maine (Golet et al., 2007).

Poor fish condition can also be a key factor impairing the recovery of overfished stocks. For example, after reviewing a number of studies from a spawning ground of cod in coastal Newfoundland, Rose et al. (2008) concluded that large fish in good condition are necessary for large reproductive potential in order to allow the stock to rebuild. This lesson, according to the authors, is applicable to the management of rebuilding stocks in general.

For migratory species (e.g., anchovy, eel, and tuna), it is also important to evaluate the health of individuals before the seasonal migration because migrations require a substantial energetic cost (Harden Jones, 1984), with the distance being linked to available lipid stores (Nøttestad et al., 1999). For example, if fishery managers detect lower than average condition (e.g., low energy reserves and high parasitism) before migration for a migratory species, the fishing effort directed to these species could be reduced, or the spatial distribution of the fishing effort may be modified. In particular, there is empirical evidence that biochemical assessments during critical periods of the life cycle of fish are essential in understanding the population dynamics of coastal upwelling ecosystems and in developing a more solid basis for stock management and conservation (Rosa et al., 2010).

Furthermore, the estimation of fatness can be used in certain species to improve the evaluation of catchability because it permits forecasting the start of the migration. For example, the critical level of fatness will trigger migration by the Azov anchovy at a particular range of water temperatures. Knowing the weather forecast and the fatness of the main stocks of anchovy in the Sea of Azov in September, it is possible to predict the time and character of the migration run through the Kerch Strait. This enables fishing boats to gather at the right time and place the anchovy (Shulman & Love, 1999).

Similarly, if artificial sources of light are used for catching sprats, they respond much better to the light if their fatness is low (Gusar et al., 1987); sated fish are no longer interested. In this case, the lipid content indicates whether the fisherman should use lights to attract the sprats (Gusar & Getmansev, 1985) since this method of capture works only on fish with low reserves. Also Azov and Black Sea anchovy form wintering stocks of different density depending on stored lipid content, occupying different depths and developing different mobility rates. Thus, knowledge of this again helps the tactics of the fishermen (Shulman & Love, 1999).

Finally, it is worth noting that management intervention through commercial fishing practices and other procedures may also be needed to ensure healthy fish stocks. For example, viscera of some Mediterranean groundfish species such as catshark *Scyliorhinus canicula* and *Lophius* spp. are often discarded at sea prior to reaching the fish market. In light of the very high parasitic load of the discarded viscera of catshark, Lloret et al. (2012) proposed banning offloading of fish viscera because this practice may result in heavier infections in fish which feed on the discarded viscera (McClelland, 2002). Finally, the potential spread and impact of farm-origin parasites on the survival of coastal wild fish populations must be taken into account (Krkosek et al., 2006).

Conclusions

We have tried to show in this book the possibilities and utility of using structural, metabolic, and functional indicators for estimating the condition (or health) of marine fish (individuals, populations, stocks, and species). We have examined indicators of adaptations to environment, biodiversity, life history, abundance dynamics, behavior, and distribution. We have also considered interannual monitoring and interspecific and intraspecific differences. However, significant aspects of condition indicators related to productivity, constituents and energy balance (budget) have not been considered in this book, as they were examined in detail in the book by G.E. Shulman and R.M. Love *The Biochemical Ecology of Marine Fishes* (Advances in Marine Biology, vol. 36, 1999, Academic Press). We have focused on the normal condition of fish that promotes their success in the ecosystem, and on the condition of wild populations from an ecological and fisheries approach, paying less attention to the problems of marine aquaculture, the effects of pollution, fish disease, and the importance of fish food in human nutrition and medicine. All these important topics have been considered in other publications. We can cite the extensive reviews on: marine aquaculture by Jobling (1993), Sargent et al. (1995), Navarro et al. (1995), Tucker (1998) and Stickney (2011); fisheries ecology by Natochin (1988), Jenning et al. (2001), Garcia-Charton et al. (2004) and Karamushko (2007); marine fish deseases by Mikryakov et al. (2001); effect of pollution on marine fish by Adams et al. (1988), Grubinko and Leus (2001), Vannder Oost et al. (2003), Depledge and Galloway (2005) and Maria (2009); significance of marine fish unsaturated fatty acids for medicine by Ackman (1989, 2005), Li and Suzuki (2000), Arts et al. (2003), Masuda et al. (2003), Njinkone et al. (2002) among others. We hope that this book will make a contribution to marine fish ecology.

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