PREFACE

Larvae are the distinct juvenile form many animals undergo before metamorphosis into adults. Animals with indirect development such as insects, amphibians, or cnidarians typically have a larval phase of their life cycle. In this book, the authors present current research in the study of the morphology, biology and life cycle of larvae. Topics include the morphological changes of marine fish larvae and their nutrition needs; spawning and nursery areas of neotropical fish species; larvae and embryonization principles of ontogeny in arthropods; larvicidal lectins and trypsin inhibitors isolated from different plant tissues; aquaculture production and larval rearing; expression of recombinant proteins in insect larvae; and adaptive plasticity and evolution in larvae with particular reference to the chironomid midge.

Chapter 1 - Fish larvae represent a critical period in their life history because during ontogenetic development, important changes in structure and function occur in body tissues, organs and systems. Fish larval nutrition starts from an endogenous origin where larvae derive nutrition from yolk reserves and subsequently develop into exogenous feeding. In the early feeding stage, fish experience mixed nutrition during the transitional period from endogenous to exogenous nutrition. In mixed feeding, nutrition supply plays an important role in fish survival. Therefore, an understanding of the structural and functional changes of the larval digestive system is essential to select appropriate feed to improve fish survival in larval rearing. In aquaculture, high fish mortality usually occurs in the early stage that coincides with the period of initial exogenous feeding. Specifically, the timing of food introduction is critical because food ingestion in first-feeding larvae determines subsequent fish survival and growth. In this chapter, we use the life cycle of larval fish as a framework to examine internal factors regulating ontogenetic development in fish larvae and environmental factors contributing to fish mortality during early life history.

Chapter 2 - A hypothetical initial pattern of arthropod ontogenesis, the origin and role of the larval stage and significance of metamorphosis in the life cycle are analyzed and discussed. Arthropods are characterized by the following characteristics: (1) a unique chitinous segmented exoskeleton that is replaced by a new one in every moultingmolting process, and (2) by multi-stage ontogenesis, which both should be treated in tight interrelations. The existence of several developmental stages is thought to be defined by two evolutionary processes going in opposite directions: embryonization and de-embryonization. Within a definite arthropod group, these two processes compete. The result of this competition depends on the starting conditions of the whole ontogeny as well as on the ecological and physiological character and background of realization of the entire

ontogenesis, in particular, on the preconditions to the enrichment of the egg in yolk. In its extreme expression, the latter factor may potentially result in non-larval mode of development. Nevertheless, even if the eggs are rich in yolk, only larva with accelerated tissue differentiation and, correspondingly, morphologically different from adult organism, hatches. Consequently, the richness of eggs in yolk provides not the process of "embryonalization" [as identified by Schmalghausen (1982)] but only larval life owing to the remaining embryonic yolk. However, another point of view on the nature of arthropod ontogenesis may be proposed. Initially, deeply embryonized animals with non-larval type of development, i.e., directly capable of reproduction, with a single protostage, in the course of evolution have undergone, first, the superstruction of ontogenesis and, second, de-embryonization, which have both resulted in the appearance of a number of successive postembryonic stages with certain preadaptations and, on the other hand, lead to a wide ecological radiation in different groups. Thus, moultings and changing of stages could have appeared in the ontogenesis of various groups of arthropods not at once but gradually in the course of progressive de-embryonization. The divergence of the developmental ways of the larva and adult animal leads to metamorphosis whichthat, in the most general sense, expresses the transition between the larval and adult developmental programs, particularly if the larval organization possesses not only phyletic features like that of the polychaetous trochophore but also adaptive ones like in maggots.

Chapter 3 - Lectins are hemagglutinating proteins that promote cellular responses due to their interaction with glycosylated molecules. Trypsin inhibitors have been described as endogenous regulators of proteolytic enzymes and as storage proteins. Plant lectins and trypsin inhibitors with insecticidal activity may become an alternative to synthetic insecticides that adversely affect the environment and have promoted the emergence of resistant organisms. These proteins exert deleterious effects on larval survival, weight, feeding ability, and development of insects as well as morphology of larvae, pupae and adults. The digestive processes in larval gut are highly active and their integrity is an essential aspect in insect development. Plant lectins and trypsin inhibitors can modulate the activity of digestive enzymes in larval gut. This chapter reports larvicidal lectins and trypsin inhibitors isolated from different plant tissues (e.g. bark, heartwood, leaves and seeds) with potential applications in the control of numerous insect species, including *Achaea janata*, *Aedes aegypti*, *Anthonomus grandis*, *Callosobruchus maculatus*, *Corcyra cephalonica*, *Ephestia kuehniella*, *Helicoverpa armigera*, *Lacanobia oleracea*, *Ostrinia nubilalis*, *Spodoptera littoralis*, *Spodoptera litura* and *Zabrotes subfasciatus*. The changes in morphology of larvae treated with lectins or trypsin inhibitors, as well as the effect of these proteins on larval α-amylase, β-glucosidase, protease and aminopeptidase activities, are also discussed.

Chapter 4 - The anadromous twaite shad, *Alosa fallax fallax*, is a European Union Berne Convention *Natura 2000* species, requiring protection from range states. In Portugal it is classified as "Vulnerable" by the Instituto de Conservação da Natureza using IUCN criteria but still migrates upriver to spawn in several rivers, *e.g.* River Mira. Evidence suggests that this species exhibits homing behavior. This stock-river relationship is important considering that environmental conditions during the embryo-larval period in the freshwater reaches of rivers play an important role in the future of populations, namely through their impact on growth and mortality rates that affect stage duration and survival. Nonetheless, published information on growth and/or mortality of twaite shad larvae is anecdotal. This study was designed to model growth and mortality rates and study their within season variation and

examine to what extent short-term changes in environmental and biological factors affect within-year changes in growth rates and relative survival of larvae of twaite shad in the River Mira.

Plankton samples were collected every other week from 28 March until 7 July 2000, the presumed spawning season, at stations in the upper River Mira. Shad larvae were sorted out of the zooplankton. Microstructure of *sagittae* from *c.* 330 post yolk-sac shad larvae, 7.21 to 20.40 mm standard length, was analysed using an image analysis system and an age-length key was prepared. Growth in length of larvae, g^*, was estimated based on the backcalculated length-at-age using a model-based resampling (bootstrap) approach. Instantaneous daily mortality rates, Z, of larvae were estimated from the ln-transformed form of an exponential model of decline in abundance of adjusted numbers with respect to age (time). The relationship among nine environmental and biological variables, and instantaneous growth and mortality rates of shad larvae was assessed.

Larvae do not grow at the same rate or experience uniform mortality throughout the spawning season. They were estimated to grow at a faster rate earlier in the season when compared to individuals collected later, after mid-May. Early and later-spawned larvae were subject to comparable and declining mortality rates. Seasonal changes in river (water) temperature and rainfall (a proxy of river discharge) as well as variation in crustacean *nauplii* abundance and feeding incidence, coincident with early larval development, are important variables, regulating the growth and mortality of larval twaite shad.

Chapter 5 - Trichinellosis is a zoonotic disease with worldwide distribution caused by intestine nematode of the genus *Trichinella*. Infectivity of this parasite is determined by host immune and inflammatory response to the infection. The murine cellular and humoral immune response to the infection with low doses of larvae of encapsulating (*Trichinella spiralis, T. britovi*) and non-encapsulating (*T. pseudospiralis*) species was studied. Mice were experimentally infected with 10 muscle larvae of the parasite to simulate natural conditions of the infection in rodents, important reservoirs of trichinellosis.

Both *T. spiralis* and *T. britovi* stimulated the proliferation of splenic T and B lymphocytes during the intestinal phase of infection, but *T. spiralis* activated the proliferative response also at the muscle phase, particularly in B cells. Non-encapsulating *T. pseudospiralis* stimulated the proliferation of T and B cells only on day 10 post infection (p.i.) and later at the muscle phase. The numbers of splenic CD4 and CD8 T cells of *T. spiralis* infected mice were significantly increased till day 10 p.i., i.e. at the intestinal phase, and then at the late muscle phase, on day 60 p.i. *T. britovi* infection increased the CD4 and CD8 T cell numbers only on day 30 p.i. Decreased numbers of CD4 and CD8 T cells after *T. pseudospiralis* infection suggest a suppression of cellular immunity. Both encapsulating *Trichinella* species induced the Th2 response (cytokines IL-5, IL-10) at the intestinal phase and the Th2 dominant response at the advanced muscle phase. IFN-γ production (Th1 type) started to increase with migrating newborn larvae from day 15 p.i. till the end of the experiment. IL-5 production was suppressed during the intestinal phase of *T. pseudospiralis* infection. The immune response to *T. pseudospiralis* was directed more to the Th1 response at the muscle phase, the high IFN-γ production was found on day 10 p.i. and it peaked on days 45 and 60 p.i. The low infective dose of *T. spiralis*, *T. britovi*, and *T. pseudospiralis* induced a late seroconversion in infected mice. Both *T. spiralis* and *T. britovi* did not evoke an increased specific IgM response, which is typical for the acute infection. Only *T. pseudospiralis* induced a higher specific IgM level in the intestinal phase of the infection, till

day 30 p.i. *T. spiralis* caused earlier and more intensive specific antibody response (IgG_1, IgG_{2a}, IgG_{2b}), from day 45 p.i, when antigens from newborn and muscle larvae w accumulated, on the contrary to *T. britovi* (IgG_1, IgG_{2a}, IgG_{2b}) and *T. pseudospiralis* (IgG which induced specific antibody response from day 60 p.i.

Knowledge of the functional consequences of species species-specific parasite infecti may be used in immunodiagnosis of trichinellosis as well as immunologist immunolo strategies to control the infection.

Chapter 6 - The production of animals through aquaculture practices has narrowed do the dependence on fisheries-derived products. Aquaculture production yields are increasi but the majority of farmed aquatic animals are still represented by freshwater fish such carp and tilapia. On the other hand, the production of marine organisms is dominated several species of fish, crustaceans and molluscs; which have been successfully breed captivity through their whole life cycles. These advances have been in part supported applied research that has led to development of well-established rearing practices. T continued growth of the aquaculture industry requires high numbers of good quali postlarvae and juveniles produced in commercial hatcheries. Very often, the larval rearing marine species represents the main bottleneck of the whole aquaculture production proce and this is highlighted by the present situation with the production of marine fish. Althou hundreds of species of marine fish are viable for farming, the current production accounts only 3% of the global production of farmed aquatic animals. This figure is in part explain by the current lack of knowledge on the digestive physiology and nutrient requirements f most marine fish larvae. While mollusc larvae entirely depend on phytoplankton, m economically important species of marine crustaceans and fish are strongly dependent zooplankton during the larval stage. The intrinsic difficulties in producing large amounts specific live feeds to support the larval rearing of many marine species represents one of t main problems that marine farmers have to cope with. Therefore, intensive research on t ontogeny and physiology of the larval digestive tract is continuously conducted in order have a better understanding of the larval digestive processes. New findings on the biology an physiology of marine larvae rapidly impacts technical production aspects such as the shap and size of the rearing vessels, the establishment of optimal larval rearing conditions and t larval feeding protocols used to supply live and inert feeds. New research findings assi nutritionists to formulate diets that can successfully replace live preys needed during t critical larval rearing stages.

Chapter 7 - All metamorphosing insects pass through four consecutive stages befo reaching their adult form and these stages are known as the egg, the larva, the semipupa an the pupa. The larval stage comprising individuals who have completely different morpholog of the other three, since the body is devoid of external appendages and with internally adipos tissue. From the larval stage begins the growth of individuals who shed their skins sever times and depending on the insect considered the substages vary from 3 to 5. In bees general there are five larval stages called L1 to L5, interspersed with each other for fo periods of change ending in larval and pupal changes when successful-L5 larvae enter t pupal stage, where individuals have had adult characteristics. Since ants have about fo larval stages (L1, L2, L3, L4), but this number can vary from 3 to 6 depending on the species

In the larvae of ants there are morphological peculiarities in certain species that set the apart from most reported in the literature so far. An interesting fact was observed in t Argentine ant *Linepithema humile*, an invasive species, since they present a dors

protuberance in the sixth abdominal segment found exclusively in their larvae. Researchers have tried to explain the social function of this structure. One function described was the nutrition. However the morphological results obtained in this study suggested that the dorsal protuberance has no secretory function, since ducts or pore channels were not found, only small folds in the cuticle, which does not indicate a secretion release function. The secretion released is just for the cuticle compounds and does not synthesize other one which would be important in social behavior. Therefore the unique social function that can be attributed to the dorsal protuberance was mechanical or serving like a facilitator making it easier for the workers to transport the immatures through the different environments in the colony.

Chapter 8 - Larvae in the genus *Thitarodes* (Lepidoptera: Hepialidae) are host to *Ophiocordyceps sinensis,* a unique entomophagous fungal parasite that occurs principally in the alpine meadow environment of the Tibetan Plateau and for centuries a widely known and valuable invigorant in traditional Chinese medicine. *T. pui* is mainly distributed at an altitude of 4100 ~ 4650 m in the alpine meadows and alpine shrub meadows of Mt. Segyi La in Tibet. Larvae feed on plants herbaceous roots and humus fragments. Larval development lasts three to four years (about 1095 ~ 1460 d), including 41 ~ 47 d for the egg, 990 ~ 1350 d for the larva, 35 ~ 41 d for the pupa, and 3 ~ 8 d for the adult. Larval growth involves seven to nine instars. Larval head capsule width is the principal parameter to distinguish each larval instar. The ratio of head capsule width to body length and weight decreased with increasing age. The 7^{th} instar pupates into male adults, the 9^{th} instar linto female adults, and the 8^{th} instar larvae into both males and females. Larvae showed an aggregated distribution where larval densities had their greatest concentration between altitudes of 4100 ~ 4650 m. Pupation occurred in the end of April to early May. Adults emerged during the day at any time between sunrise and sunset from late June to mid July. Courtship occurred between 21:00 to 21:30 h followed by mating last from about one to several hours. The ratio of males to females was 1.5:1. The average oviposition was 768 ± 206 eggs per female. The life table of an experimental *T. pui* population was constructed through laboratory rearing. The total survival rate of the experimental population was 2.6%, and the population trend index was 7.95 which indicated that the population size of the next generation will be 7.95 times greater.

Chapter 9 - This chapter provides information on ontogenetic patterns of neotropical fish species distribution in tributaries (Verde, Pardo, Anhanduí, and Aguapeí rivers) of the Porto Primavera Reservoir, in the heavily dammed Paraná River, Brazil, identifying key spawning and nursery habitats. Samplings were conducted monthly in the main channel of rivers and in marginal lagoons from October through March during three consecutive spawning seasons in 2007-2010. Most species spawn in December especially in Verde River. Main river channels are spawning habitats and marginal lagoons are nursery areas for most fish, mainly for migratory species. The tributaries have high diversity of larvae species: a total of 56 taxa representing 21 families, dominated by Characidae. Sedentary species without parental care are more abundant (45.7%), and many long-distance migratory fish species are present (17.4%). Migrators included *Prochilodus lineatus, Rhaphiodon vulpinus, Hemisorubim platyrhynchos, Pimelodus maculatus, Pseudoplatystoma corruscans, Sorubim lima,* two threatened migratory species: *Salminus brasiliensis* and *Zungaro jahu*, and one endangered migratory species: *Brycon orbignyanus*. Most of these migratory species are vital to commercial and recreational fishing, and their stocks have decreased drastically in the last decades, attributed to habitat alteration, especially impoundments. The fish ladder at Porto Primavera Dam appears to be playing an important role in re-establishing longitudinal

connectivity among critical habitats, allowing ascent to migratory fish species, and thus access to upstream reaches and tributaries. Establishment of Permanent Conservation Units in tributaries can help preserve habitats identified as essential spawning and nursery areas, and can be key to the maintenance and conservation of the fish species in the Paraná River basin.

Chapter 10 - In Biotechnology, the expression of recombinant proteins is a constantly growing field and different hosts are used for this purpose. Insects from the order Lepidoptera infected with recombinant baculovirus have appeared as a good choice to express high levels of proteins, especially those with post-translational modifications. Lepidopteran insects are extensively distributed in the world. Species like *Bombyx mori* (silkworm) have been explored in Asian countries to produce a great number of recombinant proteins for academic and industrial purposes. Several recombinant proteins produced in silkworms have already been commercialized. On the other hand, species like *Spodoptera frugiperda, Heliothis virescens, Rachiplusia nu* and *Trichoplusia ni* are widely distributed in the occidental world and Europe. The expression of recombinant proteins based on Lepidoptera has the advantage of its low cost in comparison with insect cell cultures. A wide variety of recombinant proteins, including enzymes, hormones and vaccines, have been efficiently expressed with intact biological activity. The expression of pharmaceutically relevant proteins, including cell/viral surface proteins and membrane proteins using insect larvae or cocoons, has become very attractive. This chapter describes the methods for scaling up the protein production using insect larvae as biofactories.

Chapter 11 - In aquaculture fish larvae rearing still represents a considerable challenge because fish larval nutrition is not yet fully understood. The initial larval stages still rely on live feed to survive and growth and live feed have substantial nutritional imbalances. It is crucial to determine the amino acids and fatty acid requirements of fish larvae in order to formulate suitable inert diets. It is possible to estimate larval amino acids requirements by determining the AA profiles from fish larvae carcass. Although this is just a first step it can give a preliminary indication of possible fish AA requirements. Also, if the AA profiles of fish are determined for several ages during fish larval ontogeny is possible to associate certain changes to specific events of fish development such as metamorphosis. Fatty acids are another extremely important nutrient during the first larval stages. Fatty acids are the most important energetic substrate and are crucial to the development of certain organs such as the eye. A change in the fatty acid profile of fish larvae may indicate a higher requirement of a specific fatty acid or its preferential spare. This study aims to compare the differences between the fatty acid and amino acid profiles of two different marine species and associate these changes to certain events occurring during fish larval ontogeny.

Chapter 12 - The theory of evolution is arguable the most influential theory shaping our thinking about biology. Yet it is almost exclusively a theory of adults. It virtually ignores the immature stages of organisms. Here I attempt to make a contribution toward redressing this imbalance, principally by reference to the aquatic larvae of a ubiquitous insect, the chironomid midge. These larvae occur in wide variety of inland waters, significantly including extreme habitats such as transient pools and hence provide good models to illustrate the adaptive response of larvae to a set of identifiable selective pressures. Importantly, the selective pressures experienced by larval stages are quite different to those experienced by adults and hence lead to divergent evolutionary trajectories.