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PREFACE-*FOREWORD*

Vie et Milieu is an international journal devoted to studies on ecology, ethology, systematics, biology and life sciences in general. It is usually not dedicated to physiology. However, it seems clear today that many questions asked by ecology involve, or are in some way related to, physiology. We have seen in the past that ecology needed mathematical approaches; modelisation is now an important part of the present studies. Models are constantly compared to, and validated by, observations and measurements achieved in nature. But ecology also needs physiological approaches. The ecological and behavioural trends and patterns of living organisms are affected by the impact of abiotic and biotic factors on populations (ecophysiology) as well as on individual metabolisms and living processes (environmental physiology). Physiological data are more and more frequently used in ecology. The distribution, behaviour, oxygen uptake, feeding activities, development, growth and reproduction of marine and terrestrial species depend on physical abiotic and biotic factors.

This special issue of *Vie et Milieu/Life and Environment* is dedicated to ecophysiology and environmental physiology including mechanisms of adaptation. Adaptation to the surrounding environment is a prerequisite for each species of being able to develop, grow and reproduce. Adaptation means first the need of specific sensors to capture, translate and transmit the external given information to cells, organs or integrative system, and second an appropriate physiological and behavioural response. The eleven following papers tackle some of these aspects in different living groups. Archaea are presented with their extraordinary capacities to develop and live in extreme (very) hot environments. The impact of temperature is also studied in a member of the Polychaeta. Four papers are then dedicated to mollusks, including the effects of food availability in a gastropod, excretion mechanisms in a bivalve, copper accumulation in another bivalve and growth patterns in a cephalopod. The next six papers are dedicated to vertebrates, teleost fish. The first two describe biological aspects of Triglidae from South Africa, and the general problem of stress response in young and adult fish. The next three reviews focus on rhythmical processes, including daily feeding rhythms, photoperiod receptivity and influence on somatic growth and the relationships between otolith and somatic growth.

Probably many manuscripts exposing data and analyses on ecophysiology and environmental physiology will be submitted to *Vie et Milieu* in the future but for the present it was a good opportunity to gather all these studies in the same issue to assess the importance of these aspects in ecology. A high priority of international programmes corresponds to global climatic changes and impacts of human activity on ecosystems and biodiversity. Comparative physiology and ecophysiology will play an increasingly important and relevant role in such scientific approaches.

G. BOEUF

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THERMOPHILES FROM DEEP-SEA HYDROTHERMAL VENTS

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THERMOPHILES
HYDROTHERMAL VENT
ARCHAEA
THERMOSTABILITY

ABSTRACT. – Deep-sea hydrothermal vents represent extreme environments where original microbial communities have evolved during geological times. Of particular interest are the thermophilic and hyperthermophilic microbes thriving in the various microhabitats determined by the geochemical processes at work in the hydrothermal vent fields. In the present review, the general characteristics of the hydrothermal vents as habitats for thermophiles and hyperthermophiles are described. The microbial diversity is discussed on the basis of molecular and cultivation approaches. The most original features of thermophily at molecular level are briefly reviewed. Data gathered during the last two decades have substantially modified our understanding of diversity and evolution of deep-sea hydrothermal vent communities and ecology.

THERMOPHILES
SOURCES HYDROTHERMALES
ARCHAEA
THERMOSTABILITÉ

RÉSUMÉ. – Les sources hydrothermales profondes constituent des environnements extrêmes où des communautés bactériennes originales se sont développées au cours des temps géologiques. Les thermophiles et hyperthermophiles colonisant les divers microhabitats déterminés par les processus géochimiques prévalant dans ces milieux présentent un intérêt particulier. Les caractéristiques principales de ces habitats sont passées en revue. Les connaissances sur la diversité microbienne, estimées à la fois par les approches moléculaires et de culture, sont discutées. Les aspects les plus originaux de l'adaptation à la thermophilie sont brièvement décrits. Les données accumulées au cours des deux dernières décades ont considérablement modifié notre compréhension de la diversité et de l'évolution des communautés microbiennes des sources hydrothermales profondes et de leur écologie.

INTRODUCTION

Since the discovery of microbial life in Yellowstone hot springs by Thomas Brock more than 30 years ago, it has become more and more evident that life is present in a great variety of extreme environments. In an anthropocentric view, most of these extreme environments were unsuitable for microbial life and this discovery opened the way to a new era for microbiologists. Inventorying *a priori* hostile environments demonstrated that most of them were not only able to tolerate some sporadic microbial forms of life, but constitute the selective habitats of a great variety of microorganisms belonging to the 2 domains defined by Woese (1977): Archaea and Bacteria. These microorganisms have been called 'extremophiles'. Considering the parameters controlling their optimal growth, they are named thermophiles (>60°C), hyperthermophiles (>80°C), psychrophiles, acidophiles, alcalophiles, barophiles or halophiles. Not only do they tolerate extreme environments but these extreme conditions are required for proper growth of microorganisms that have been selected over geologic time scale. Generally, they cannot grow in conditions suitable for eukaryotic forms of life. Following the work of Brock on terrestrial hot springs and thermophiles (Brock 1969), the discovery of deep-sea vents at the end of the 1970s boosted the research of the most extreme microorganisms. Among the different teams contributing to the research on extremophiles biodiversity, a remarkable contribution to the description of new genera and new species of hyperthermophiles is due to Stetter and his colleagues at University of Regensburg, Germany (Stetter 1998, Stetter 1999a,b). Besides these recent reviews, the present one focuses more specifically on hyperthermophiles from deep-sea hydrothermal vents.

Hydrothermal vents as habitats for thermophiles and hyperthermophiles

Thermophiles and hyperthermophiles have been isolated from various habitats: terrestrial hot springs, solfatara and volcano acidic hot springs, shallow marine hot springs, submarine vents, deep-sea sediments and deep-sea hydrothermal vents. Moreover, evidence of the existence of microorganisms in the deep terrestrial and oceanic subsurface has been increasing during the last decade (Delaney *et al.* 1998, Summit & Baross 2001). Since the temperature and pressure of subsurface environments increase with depth, they are potentially among the most appropriate habitats for hyperthermophiles. An exhaustive list of thermophiles and hyperthermophiles isolated from these various environments as well as related references can be found in a recent review (Amend & Shock 2001) and on a dedicated web site at http://levee.wustl.edu/~chan/Research/research.html.

Geochemical mechanisms at work on mid-oceanic ridges result from the interaction between seawater and volcanic rocks and from seawater and hydrothermal fluxes of heat and dissolved matter. Since their discovery in 1977 at the Galapagos Spreading Center (GSC) at 2500 m water depth (Hessler & Smithey 1983), it has been shown that seafloor hydrothermal vents constitute, in almost every mid-oceanic ridges investigated, ecosystems supporting important biomass and high productivity in contrast with other deep sea environments. Faunal assemblages, similar to those discovered at GSC, have been sampled at various sites on the East Pacific Rise (EPR) (Lutz & Kennish 1993), Juan de Fuca Ridge (Tunnicliffe 1988), Mid-Atlantic Ridge (MAR) (Van Dover 1995), and the spreading centres of Western and South-Western Pacific Back Arc Basin (Desbruyères *et al.* 1994, Hessler *et al.* 1987). Numerous active sites are still under investigation along the mid-ocean ridges in the Pacific Ocean, the Atlantic Ocean and more recently in the Indian Ocean (Fig. 1). Intensive research has been conducted on different vent fields at East Pacific Rise from 26°S to 21°N and at Mid-Atlantic Ridge between 15 and 40°N. Currently eight vent fields have been described from MAR, including Mount Saldanha (36°N) discovered in 1998 during the Portuguese-French SALDANHA cruise (Desbruyères *et al.* 2000). Most known hydrothermal vents along the mid-ocean ridges are located on young crust where the cooling of hot basaltic material drives hydrothermal flow (Fornari & Embley 1995). Recently an off-axis hydrothermal vent field named "Lost City" has been discovered near the Mid-Atlantic Ridge at 30°N at nearly 15 km from the spreading axis on a 1.5 Myr old crust, demonstrating that hydrothermal venting occurs not only along mid-ocean ridges, but also on old regions of the oceanic crust away from spreading centres (Kelley *et al.* 2001). These last findings indicate that a much larger portion of the oceanic crust may support hydrothermal activity and microbial life than previously thought. On mid-ocean ridges, at depth ranging from 1 500 to 4 000 m, hydrothermal fluids can reach temperatures up to 400°C whereas ambient sea water is around 2°C, producing steep temperature gradients within diffusion structures. Hydrothermal solutions reaching the seafloor contain various products resulting from hot acidic seawater and volcanic rocks: metals (Fe, Mn, Zn, Cu, etc.) and reduced sulphur (Zierenberg *et al.* 2000). Most of the hydrothermal fluids display physico-chemical compositions that are unsuitable for eukaryotic organisms due to their high toxicity. However, active chimneys (white and black smokers) are densely populated, hosting not only microorganisms belonging to Bacteria and Archaea, but also a very rich macrofauna typical of these environments (Desbruyères & Segonzac 1997). Strikingly, fluids of "Lost City" off-axis vent field were found to be relatively cool (40-75°C) and alkaline (pH 9.0-9.8), supporting dense microbial communities including anaerobic thermophiles, but rare macrofaunal assemblages that typify most vent environments (Kelley *et al.* 2001).

Hydrothermal vent ecosystems stability is directly dependent upon the variations in fluid fluxes and chemical composition of the fluids. Microbial blooms are observed at the initiation of magmatic cycle, suggesting that the upper oceanic crust is inoculated with microbial communities (Zierenberg *et al.* 2000). Volcanic events initially are followed by relative increase in fluid temperature and magmatic gases, often accompanied by decrease salinity in hydrothermal fluids as a result of supercritical phase separation in seawater in the subsurface. The high-salinity components of the phase separated fluids reaches the seafloor a few years later (Von Damm 1995). Direct observation of discontinuity in vent emission has been done in different ridge dynamic contexts (Desbruyères *et al.* 2000). High variability in fluid composition at a same vent according to volcanic activity has been reported. In some cases, like at Lucky Strike (MAR 37°N), variability in chemical composition suggests the existence of two different sources (Von Damm *et al.* 1998). As an example, the concentration of most of the major chemical components in MAR vent fluid end-members is given in table I (Desbruyères *et al.* 2000). Despite the important differences between sites, it can be noticed that H_2S, CO_2, NH_4 and CH_4 are present in vent fluids and are available as primary compounds for chemolithoautotrophic microbes essential to the good running of the ecosystems. Another source of variability between different vent fields, acting as a major ecological factor, is the particulate (mineral and organic) content of the fluids. Finally, these

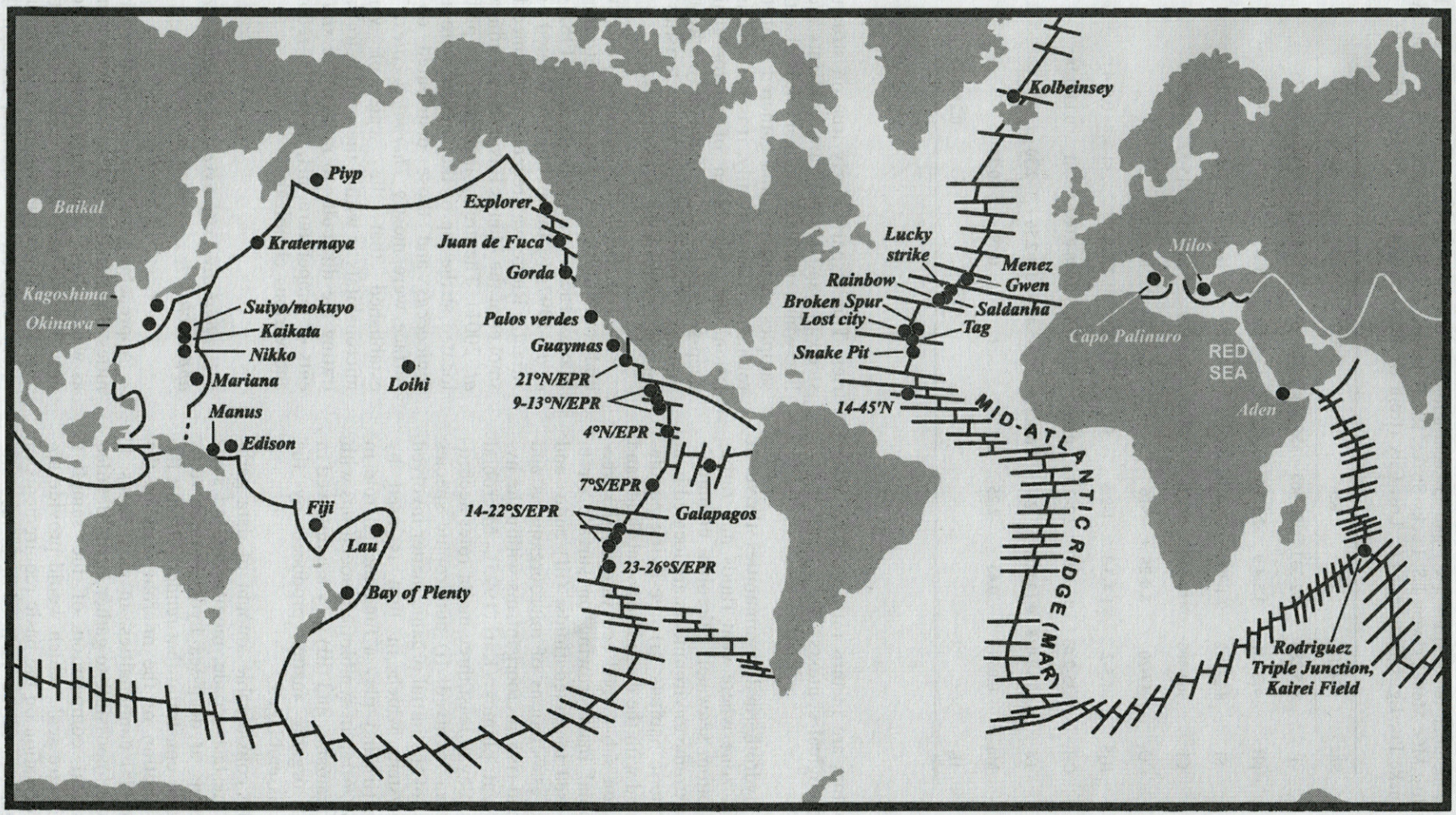

Fig. 1. – Distribution of deep-sea hydrothermal vents along oceanic ridges (from Desbruyères & Ségonzac 1997 modified and completed).

Table I. – End-member temperature (°C) and concentrations of chemical species (mmol kg^{-1}) for the different M.A.R.vent fields: MG: Menez Gwen; LS: Lucky Strike; Rb: Rainbow; BS: Broken Spur; T.A.G.: Trans Atlantic Geotraverse; SP: Snake Pit; Lg: Logatchev; LC: Lost City (from Desbruyères 2000 completed Kelley 2001).

Site	MG	LS	Rb	BS	T.A.G.	SP	Lg	LC
T	265-284	152-333	360-365	356-364	270-363	335-356	>353	40-75
pH	4.2-4.8	3.5-4.9	2.8-3.1		2.5-3.4	3.7-3.9	<3.3	9-9.8
Si	8.2-11.2	9.1-17.5	6.9-8.0		18-22	18-20	7-8.2	9-19
Cl^-	360-400	410-540	>750	469	633-675	550-563	515-522	546-549
CO_2	17-20	8.9-28	<16		2.9-4.1	10.63		
H_2S	1.5-2	1.4-3.3	1-2.5	9.3	2.5-6.7	2.7-6.1	<1	0.064
CH_4	1.35-2.63	0.5-0.97	2.2-2.5	0.065	0.14-0.62	0.046-0.062	2.1	0.13-0.28
Fe	0.002-0.018	0.13-0.86	24	1.68-2.16	1.64-5.45	1.8-2.56	2.50	
Mn	0.068	0.45	2.25	0.26	1	0.49	0.33	
H_2								0.25-0.43

groups of factors are in turn influenced by water depth and geological contexts.

Deep-sea hydrothermal communities thrive in the interfacial zone where vent fluids mix turbulently with bottom seawater. Microbial communities in these extreme environments are located in at least four generic habitats: (i) free-living populations associated with the discharged vent fluids and depending upon sub-seabed ecosystems, (ii) free-living microbial mats growing on surface strata that are exposed to mixed fluids, (iii) endo- and exosymbiotic associations of microorganisms and vent fauna, and (iv) microorganisms within the hydrothermal vent plumes (Karl 1995). Microbial communities play at least three major roles according to the taxa involved: (i) autotrophic species produce most of the initial organic matter involved in the ecosystem which in turn is used by heterotrophs, (ii) they play a fundamental role in detoxification, often in symbiotic associations with eukaryotic organisms, and (iii) they are involved in recycling of organic matter produced by the macrofaunal assemblages.

Despite the considerable amount of data gathered during the last decade, monitoring and sampling conditions at deep-sea hydrothermal vents are still a major constraint that render difficult the accurate description of the microhabitats of the various microbial communities. In most cases, it remains almost impossible to gather *in situ* detailed physico-chemical composition of the sampling sites at centimetre scale which would provide appropriate information on the basic requirements of the microorganisms further isolated in the laboratories. This is particularly true on structures where steep pH and temperature gradients are observed like white and black smokers. It is also the case for samples of macrofauna gathered from these environments. Consequently, it is not surprising if isolation and cultivation of microorganisms from deep-sea vents are still the only way to describe their basic physiological characteristics and to infer their role in the ecosystems.

The first report of occurrence of discrete microbial communities using enrichment procedures and fluorescence *in situ* hybridisation (FISH) (Harmsen *et al.* 1997a,b) for a chimney located on the Mid-Atlantic Ridge was recently confirmed for a black smoker of the Manus Basin (New Guinea) (Takai *et al.* 2001). The archaeal phylotypes predominantly located at the top of the smoker were affiliated to *Ignicoccus* and those distributed on the vent surface were mostly *Thermococcales*. These data established that the scale of variation of microhabitats is within the centimetre range, illustrating the difficulty to measure *in situ* the gradients of temperature, pH, oxidation-redox potential and various chemicals.

Microbial diversity assessed by molecular approach

Approaching the diversity of microbial communities associated with deep-sea hydrothermal vents, as well as from other ecosystems, has long been a very tedious task since only two methods were available: direct microscopic observation and laboratory cultivation. Evidence, through 16S rDNA

sequences, of numerous uncultured microorganisms in natural communities has gained strength during the last decade since pioneering work in that field (Ward *et al.* 1990, Ward *et al.* 1992). Despite specific bias introduced by molecular methods, demonstrated by the possible lack within the pool of amplified 16S rDNA of sequences corresponding to already isolated microorganisms, it is generally admitted that no more than 1 to 5% of the total microorganisms of a natural community are available through isolation procedures. Consequently, the molecular approach is complementary to the standard enrichment and cultivation procedures in assessing the diversity of microbial communities in extreme environment where our knowledge of appropriate isolation and culture techniques is still limited. This approach, based on extraction of total DNA from crude samples, amplification by PCR of 16S rDNA genes by use of universal primers for Bacteria and Archaea, cloning of these genes for isolation prior sequencing, has allowed, besides determination of microbial communities *in situ*, the discovery of entirely new phylogenetic lineages of prokaryotes (Moyer *et al.* 1995, Moyer *et al.* 1998, Takai & Horikoshii 1999). These novel taxa, based on FISH experiments, appear to be major components of the deep-sea vent ecosystems. Microbial mats are widely distributed at most deep-sea hydrothermal vent sites from Guaymas Basin to East Pacific Rise and Mid-Atlantic Ridge. Recently new phylotypes belonging to a group of uncultured ε-*Proteobacteria* have been identified using an *in situ* growth chamber at a MAR hydrothermal vent (Reysenbach *et al.* 2000). The same research group established on a different hydrothermal vent site located on the Southern East Pacific Rise that more than 98% of the sequences of 16S rDNA obtained from a sample of microbial mat were closely related to the previously identified ε-*Proteobacteria*. They concluded from these data and from previous indications that this new clade of ε-*Proteobacteria* may be endemic and widely distributed among deep-sea hydrothermal vents (Longnecker & Reysenbach 2001). Two recent results reinforce this hypothesis. Firstly, DGGE profiles and phylogenetic analysis, conducted on enrichment cultures of samples of hydrothermal fluids, chimney-like structures and tubes and specimens of *Alvinella* spp. collected on a deep-sea vent at 13°N EPR, demonstrated that archaeal sequences were related to the genus *Thermococcus* and the bacterial sequences to uncultured ε-*Proteobacteria*, and *Deferribacter thermophilus*, *Bacillus halodurans*, *Ralstonia pickettii* and *Marinitoga camini* (Slobodkin *et al.* 2001). Secondly, we demonstrated (unpublished results) by ARDRA profiles and phylogenetic analysis, conducted on enrichment cultures, using complex proteinaceous substrates, from white tubes of *Alvinella* spp. collected on 13°N EPR, that most archaeal sequences were related to the *Thermococcus* genus as previously mentioned and that the bacterial sequences belonged to *Clostridiales* (20%) and ε-*Proteobacteria* (30%).

The diversity of metabolic types within the ε-*Proteobacteria* already isolated and cultivated from other environments makes it difficult to hypothesise about the metabolic capabilities of the various strains inhabiting deep-sea vents. Considering the high prevalence of the uncultured ε-*Proteobacteria* in both 16S rDNA gene populations of crude samples and of enrichment cultures and their putative role in sulphur cycling in deep-sea vents environments hypothesised by Longnecker *et al.*, inferred from the white sulphur-like coloration of the mat filaments, it is of considerable interest to succeed in culturing them in order to assess their basic metabolic properties.

Cultivated thermophilic microorganisms from deep-sea hydrothermal vents

Research in this field had two main incentives. Firstly, thermophilic and more generally extremophilic microorganisms represent an unprecedented source of enzymes displaying original properties interesting many industrial activities, and known as extremozymes. Hyperthermostable DNA polymerases from *Thermococcus* and *Pyrococcus* genera such as Deep-Vent from *Pyrococcus* sp. GB-D (New England Biolabs, Boston, USA) and Tfu Pol and Pab Pol respectively from *Thermococcus fumicolans* (Cambon Bonavita *et al.* 2000) and *Pyrococcus abyssi* (QbioGene, Illkirch, F) are examples of extremozymes. Despite the fact that extremozymes properties do not generally fit exactly the industry requirements, extremozymes are a matchless material for directed evolution and protein engineering programs. The second major incentive is related to molecular evolution and the origin of life question. Phylogenetic analyses, based on comparisons of 16S rDNA sequences, place the hyperthermophiles as the most slowly evolving of all forms of life, the first to have diverged from the last common universal ancestor. This suggests that life first emerged on this planet in hyperthermophilic conditions. This statement represents a controversial dogma (Wiegel & Adams 1998) that strongly fuels research on hyperthermophiles and deep-sea hydrothermal vents.

Within the Archaea inhabiting deep-sea hydrothermal vents are found the most extreme of known hyperthermophiles with growth temperature optima above 100°C. *Pyrolobus fumarii,* a facultatively aerobic chemolithoautotroph isolated from chimney walls MAR (Blöchl *et al.* 1997) with an optimum growth temperature of 106°C, measurable growth up to 113°C and ability to remain viable after one hour treatment in the autoclave at 121°C,

has the highest cardinal temperatures for any known microorganism. Next to *P. fumarii* are found the methanogen *Methanopyrus kandleri*, isolated from chimney walls of a black smoker where it reaches numbers of 10^8 per gram of chimney rock (Kurr *et al.* 1991), the obligate anaerobic heterotroph isolated from the Guaymas Basin *Pyrodictium abyssi* (Pley *et al.* 1991) and the barophilic chemoorganotrophic *Pyrococcus* sp. ES4 isolated from Juan de Fuca Ridge (Pledger & Baross 1991) which are able to grow up to 110 °C.

As free living microorganisms, the *Thermococcaceae* appear largely distributed on the various hydrothermal fields whether in shallow water or in the deep-sea. Strains belonging to this family are strictly anaerobic hyperthermophilic fermentative sulphur reducing heterotrophs. They use a variety of carbon substrates such as peptides and/or carbohydrates and are probably involved in recycling organic matter produced by autotrophs and higher organisms belonging to deep-sea vent communities. They are the predominant hyperthermophilic group of microorganisms isolated from deep-sea vents. Three genera are represented: *Paleococcus* with only one species, *Pyrococcus* (3 from the deep-sea among 5 valid species) and *Thermococcus* (10 species from the deep-sea among 21). Optimal temperatures for growth are at between 80-88 °C for *Paleococcus* and *Thermococcus* and between 90-95 °C for *Pyrococcus*. Differentiation of the species within the *Thermococcus* genus requires DNA-DNA hybridisation since they are morphologically and physiologically rather similar to each other. Some of them are barophilic with a direct effect of pressure on growth, like for *Paleococcus ferrophilus* (1-600 bar range, optimum at 300 bar) (Takai *et al.* 2000), *Pyrococcus abyssi* (optimum growth temperature 96 °C at 1 bar, 100 °C at 200 bars) (Erauso *et al.* 1993). *Thermococcus barophilus*, isolated from MAR at a depth of 3550 m on the external layer of a chimney wall and from enrichment cultures conducted at 400 bar, requires a minimum of 150-170 bar for growth in the range of 95-100°C (Marteinsson *et al.* 1999b).

Hyperthermophilic methanogens are also well distributed in deep-sea hydrothermal vent environments. Besides *Methanopyrus kandleri* already mentioned, the genus *Methanococcus* has been documented from MAR (*M. infernus* (Jeanthon *et al.* 1998)), from EPR (*M. vulcanius* (Jeanthon *et al.* 1999)) and from Guaymas Basin, Gulf of California (*M. jannaschii* (Jones *et al.* 1983), *M. fervens* (Jeanthon *et al.* 1999)). Obligate anaerobic, they are chemolithotrophs. They use H_2 and CO_2 as the only substrate for growth and methane production. In the presence of CO_2 and H_2, they reduce elemental sulphur to hydrogen sulphide.

The other Archaea isolated from deep-sea hydrothermal vents are the sulphate reducer *Archaeoglobus profondus* (Burggraf *et al.* 1990), the sulphite reducer *Archaeoglobus veneficus* (Huber *et al.* 1997) and members of the kingdom *Crenarchaeota* including sulphur metabolisers from the genus *Desulfurococcus*, *Staphylothermus* (Fiala *et al.* 1986) and *Ignicoccus* (Huber *et al.* 2000).

Many of these genera and species are also found in other environments characterised by their extreme temperatures. *Thermococcus litoralis* (Neuner *et al.* 1990) initially isolated from a shallow marine hot spring was also isolated from solfatara and subterranean oil reservoirs. The genus *Archaeoglobus* is also found in Alaskan and North Sea oil reservoirs (Stetter *et al.* 1993) as well as from coastal submarine hot springs in Vulcano Island (Stetter 1988). Genera *Thermococcus*, *Archaeoglobus* and to a less extent *Methanococcus* appear to be ubiquitous in marine thermal habitats (Holden *et al.* 2001).

Comparatively, only a limited number of species belonging to the Bacteria domain have been isolated from deep-sea hydrothermal vents. Among described species of the Thermotogales *Thermosipho melanesiensis* (Antoine *et al.* 1997) was isolated from gills of a deep-sea hydrothermal mussel from the Lau Basin in South Western Pacific Ocean, *Thermosipho japonicus* isolated from the Iheya Basin of Okinawa (Takai & Horikoshi 2000). *Marinitoga camini* (optimum growth temperature of 55 °C) a chemoorganotrophic sulphur reducing strain (Wery *et al.* 2001a) and *Caloranaerobacter azorensis*, an anaerobic thermophilic bacterium belonging to the cluster XII of the Clostridiales (Wery *et al.* 2001b) were isolated from a chimney sample collected at MAR. A chemolithoautotrophic sulphur reducing Bacteria, *Desulfurobacterium thermolithotrophum*, with an optimum growth temperature of 70 °C, was isolated from a deep-sea hydrothermal chimney sample collected at the MAR (L'Haridon *et al.* 1998). More recently our laboratory has succeeded in culturing an ε-*Proteobacteria, Caminibacter hydrogenophilus* (Alain *et al.* in press), proposed as the representative strain of a new family of ε-*Proteobacteria* well distributed in deep-sea hydrothermal vent environments as reported by several authors (Longnecker & Reysenbach 2001, Reysenbach *et al.* 2000), on the basis of molecular approaches of microorganisms diversity. This strain was isolated from fragments of chimney rocks and emptied white tubes of *Alvinella pompejana* samples, collected at EPR 13°N. It represents a novel thermophilic, anaerobic, obligate hydrogen-oxidising bacterium and is able to grow chemolithoautotrophically under an atmosphere of H_2/CO_2 with S^o or NO_3^- as electron acceptor and is probably involved in sulphur cycling in deep-sea hydrothermal vent ecosystems.

Aerobic thermophilic strains are less frequently isolated from deep-sea vent environments, proba-

bly due to anaerobic prevalent conditions above 35°C and to unbalanced efforts to uncover new species. However, aerobic Bacteria isolated from vents at 2000 m depth from the Guaymas Basin and MAR at 3500 m and belonging to the genera *Bacillus* and *Thermus* have been reported (Marteinsson *et al.* 1995, Marteinsson *et al.* 1999a).

Molecular adaptations to thermophily

Studies on life at high temperatures are comparatively recent and, despite the results gathered during the last two decades, remain a promising field of interest for topics as diverse as 'origin of life', biomolecules (in)stability, biotechnology and extremozymes, strategies for detection of novel hyperthermophilic organisms in extreme environments on Earth and preparing sampling for life discovery on other planets. All cell components of extremophiles microorganisms have to be heat adapted or at least heat resistant for periods of time compatible with corresponding metabolic constraints. This implies that not only proteins are stable at temperatures above 80, 100 or even 110°C according to the species considered, but that many low molecular weight metabolites and coenzymes, nucleic acids and lipids are either heat resistant or protected against denaturation and/or degradation by specific mechanisms. The upper limit of life at high temperatures evidenced from isolated Archaea might be slightly revised in the future. However, it depends on the stability of biomolecules, availability of energy and thermodynamics, and not the least on the ability of cells to maintain an appropriate proton permeability range of membranes in order to keep electrochemical proton gradients for energy gain (Albers *et al.* 2000).

Different recent reviews deal with the stability of proteins at high temperatures (Daniel & Cowan 2000, Jaenicke 2000, Ladenstein & Antranikian 1998, Scandurra *et al.* 2000). A surprising conclusion emerges from these studies: there are no general rules to achieve protein stabilisation. Each extremophilic protein adopts various strategies and the outstanding adaptation to extreme temperature and solvent conditions is realised through the same weak electrostatic and hydrophobic interactions among the ordinary amino acid residues which are also responsible for the proper balance between protein stability and flexibility in mesophilic proteins. Comparative studies between mesophilic enzymes and their hyperthermophilic counterparts indicate that hyperthermostability is gained through a variable combination of a small number of noncovalent features: minimisation of the surface energy and the hydration of apolar surface groups, increased compactness with a decrease of internal cavities and a high number of ionic interactions. The adaptation of proteins to extreme temperatures appears then to be the result of a compromise between the increased rigidity responsible for thermal stability and the flexibility required for playing their physiological roles. Besides these intrinsic stability factors, protein stability can be enhanced by molecular chaperonins as demonstrated with the thermosome characterised from *Methanopyrus kandleri* (Andra *et al.* 1998). This property is probably critical at the upper temperature border of life where heat-shock proteins could play a key role: cultures of *P. fumarii* with the thermosome fully induced were able to survive 1 h autoclaving at 2 bar and 121°C (Blöchl *et al.* 1997). Recently, it has been shown that when overexpressed in *E. coli* the recombinant small heat shock protein (sHSP) from the hyperthermophile *Pyrococcus furiosus* (involved in adaptation to exposure to temperatures over growth temperature optimum), prevented the majority of *E. coli* proteins from aggregating *in vitro* for up to 40 min at 105 °C. Apparently, the sHSP confers a survival advantage on mesophilic Bacteria by preventing protein aggregation at supraoptimal temperatures (Laksanalamai *et al.* 2001). For more detail, the reader is directed to a recent review on the mechanisms for thermostability of hyperthermophilic enzymes (Vieille & Zeikus 2001).

High temperatures require also a tight control of exchanges at cytoplasmic membrane level. The membrane lipids, as basic components of the thermophilic microbial cell membrane, play a key role in thermophily. The ion permeability of the membrane increases with temperature and is a major factor determining the maximum growth temperature. As demonstrated by Könings, the most important finding is that the proton permeability of most bacterial and all archaeal membranes at their temperature of growth is maintained within a narrow window (H^+ permeability coefficient near 10^{-9} cm.s^{-1}). In contrast, the permeability of the membranes for sodium ions at different growth temperatures was not constant, but was found to increase exponentially with temperature in a similar way for all organisms studied (Albers *et al.* 2000). Changes in lipid composition of membrane is a common strategy of Bacteria and Archaea in response to changes of ambient temperatures. The 'core' lipids of the Archaea are mainly based on saturated isoprenoid chains linked to a glycerol backbone by ether bonds. The increase in cyclization of transmembrane lipids contributes to a better adaptation to high temperatures by reducing membrane fluidity (Gliozzi *et al.* 1983). Increase in growth temperatures of *M. jannaschii* induces an increase in the ratio tetraether/diether lipids. The resulting cyclization of the chains tends to decrease the motion of the lipids and therefore contributes to an acceptable membrane fluidity at elevated growth temperature (Sprott *et al.* 1991).

The maintenance of the genomes and more generally of nucleic acids of hyperthermophiles inhabiting deep-sea hydrothermal vents is also a challenging question since to high temperatures is added a variety of chemical compounds with possible genotoxic effects. Considerable research has been done during the last two decades to analyse the strategies of hyperthermophilic Bacteria and Archaea in DNA and RNA maintenance and summarised in a recent review (Daniel & Cowan 2000). The main problem of DNA stability at high temperatures is thermal degradation due to depurination and subsequent breakage of the phosphodiester bonds (Marguet & Forterre 1994). The DNA of *P. furiosus* was found to be 20 times more resistant to thermal degradation than DNA of *E. coli* on the basis of the number of DNA backbone breaks after incubation of the cells at 105 °C (Peak *et al.* 1995). Less is known about strategies developed to cope with the other harsh conditions found in these environments where the relative instability of fluids composition and of the vents themselves with time imply appropriate molecular adaptations. The case of an other extremophile, *Deinococcus radiodurans*, isolated from canned meat exposed to X rays is of great interest. The complete sequencing of its genome (White *et al.* 1999) demonstrated that all systems for DNA repair, DNA damage export, desiccation and starvation recovery, and genetic redundancy are present in one cell. However, a subsequent analysis of this genome reached the conclusion that the fundamental questions underlying the extreme resistance phenotype of *D. radiodurans* remain unanswered (Makarova *et al.* 2001). Besides features revealed by genome analysis, resistance is probably the result of a very complex combination of modifications of proteins, nucleic acids and other cell components that are not readily inferred from the sequences. The availability of several complete genomes of hyperthermophiles, including 3 different species of *Pyrococcus* (Kawarabayasi *et al.* 1998, Lecompte *et al.* 2001, Robb *et al.* 2001), opens the way for more detailed research on specific metabolic pathways which are involved in genome integrity and cell detoxification. Like *D. radiodurans*, *Pyrococcus furiosus* has an extraordinarily high capacity for repair of radiation-induced double-strand breaks (DiRuggiero *et al.* 1997). Interestingly, the same authors demonstrated that at the sequence level, only a few genes share homology with known bacterial repair genes. Phylogenetic analysis indicates that archaeal recombinases occur in two paralogous gene families, one of which is very deeply branched, and both recombinases are more closely related to the eukaryotic Rad51 and Dmc1 gene families than to the *E. coli* recA gene (DiRuggiero *et al.* 1999). Comparisons of double-strand breaks induced by gamma radiation in genomic DNA of *P. furiosus* and *abyssi*, *D. radiodurans* and *E. coli* have shown that the strong radioresistance of *Pyrococcus* is not related to a specific protection of DNA. It might partly be linked to the smaller size of the *Pyrococcus* chromosome compared to *E. coli* (1.8 Mb vs 4.6 Mb) and to the homologous recombination processes, taking advantage of the existence of several copies of their chromosomes during the log and stationary phases of growth (Gérard *et al.* 2001).

Many low molecular weight metabolites and coenzymes like ATP and NAD(P) used by archaeal hyperthermophiles have short half-lives at their optimum growth temperatures and are unstable at 110°C (Daniel & Cowan 2000). The way these microorganisms have circumvent this instability is still open to research.

CONCLUSIONS

During the last two decades, more and more new thermophiles and hyperthermophiles from deep-sea hydrothermal vents have been discovered despite the high cost and relative difficulties in sampling. Assessing the global diversity of these extreme environments for microorganisms remains a challenging task as demonstrated by the incongruity of the cultivation and molecular retrieval approaches. A combination of both approaches is obviously needed to better characterise the microbial diversity and obtain novel species in culture. Allocating already isolated species and microorganisms evidenced only by their 16S rDNA sequences to a precise microhabitat on chimney structures has long been difficult for technical reasons. In the near future, the improvement of sampling procedures and of *in situ* monitoring of physico-chemical parameters is expected to produce data which are deeply needed to understand the ecology of hyperthermophiles. Coupling microbiology with geochemical and physical dynamics of deep-sea hydrothermal vent structure is also a promising approach. The main interest of the molecular approach was the construction of 16S rDNA libraries from which completely new taxa emerged. From sequences, it is impossible to infer the thermophily of the corresponding microorganisms and much broader temperature ranges have consequently to be tested for enrichment cultures and subsequent isolation procedures. Isolation of novel ε-*Proteobacteria* like *Caminibacter hydrogenophilus* illustrates this approach.

Considerable progress have been made in recent years in the study of molecular mechanisms that enable the proteins of hyperthermophiles to operate under extreme conditions. The major determinants of protein thermostability have been characterised for many different proteins and revealed the lack of

universal law to solve the transition from mesophilic to hyperthermophilic status. Modelling the corresponding processes is consequently almost impossible and directed evolution through DNA shuffling is still the basic method to engineer proteins in order to adjust their properties to industrial requirements. The upper limit of life appears to be less constrained by protein stability, which in many cases could tolerate life up to 150°C, than by limited stability of nucleic acids and low molecular weight compounds. Moreover, most of available data were gathered from *in vitro* experiments and the shortage of data is acute for *in vivo* conditions. The complex interactions between the various cell components and the resulting possible stabilising effects make it difficult to evaluate the true stabilities of these molecules *in vivo*.

The increasing availability of complete genomes, and more specifically genomes from extremophiles isolated from deep-sea hydrothermal vents is an invaluable source of data for the near future. Despite the high percentage of ORFs with undetermined functions, averaging 50% in some cases, the evidence of interkingdom lateral gene transfer (Koonin *et al.* 1997, Nelson *et al.* 1999) and the possible resulting chimeric origin of Archaea are strong incentives for genomic research. Genome comparisons between phylogenetically close species like the 3 *Pyrococcus* have evidenced the high plasticity of these genomes (Lecompte *et al.* 2001, Myllykallio *et al.* 2000). It might also be of considerable interest in reconstructing metabolic pathways and help to design experiments for their validation.

Finally, it might be stressed that the way we see the extreme conditions prevailing at deep-sea vent fields is probably inappropriate. Microorganisms have been thriving for millions or even billions of years in these "extreme" conditions and not only survived, but developed strategies for colonising almost every type of habitats including subterranean reservoirs under the sea flour. These harsh conditions are probably the driving force to molecular adaptations which are still to be uncovered.

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EFFECT OF TEMPERATURE ON DEMOGRAPHY OF *OPHRYOTROCHA LABRONICA* (POLYCHAETA: DORVILLEIDAE)

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POLYCHAETA
OPHRYOTROCHA LABRONICA
TEMPERATURE
DEMOGRAPHY

ABSTRACT. – A laboratory experiment was performed to evaluate the temperature-induced variation in the life history traits of *Ophryotrocha labronica* (Polychaeta). In this species age and size at maturity, survival and fecundity are affected by temperature. In particular in *O. labronica*, low temperatures cause a considerable delay in the attainment of sexual maturity, a reduced number of spawnings and longer intervals between one spawning and the next; fecundity is therefore lower and the animals generally live longer. At high temperatures, the animals mature rapidly, spawn very frequently and at shorter intervals; fecundity is high, even though the animals have a much shorter life span. Temperature determines the age-specific fecundity and survival patterns and hence the demographic characteristics of *O. labronica*; both the net reproductive rate R_0 and the population growth rate λ vary in accordance with the temperature, thus demonstrating the importance of environmental factors in determining not only the reproductive characteristics of the individual animal but also the fitness of the populations as a whole.

POLYCHAETA
OPHRYOTROCHA LABRONICA
TEMPERATURE
DEMOGRAPHIE

RÉSUMÉ. – Les effets de la température sur différents paramètres démographiques de la Polychète *Ophryotrocha labronica* ont été étudiés en conditions contrôlées au laboratoire. Chez cette espèce, l'âge et la taille à la maturité, la survie et la fécondité sont influencés par la température. Les basses températures entraînent un retard important de la maturité sexuelle, un nombre réduit de pontes et des intervalles plus longs entre les pontes, en particulier chez *O. labronica* ; la fécondité est donc plus basse et les animaux vivent généralement plus longtemps. A températures élevées, les animaux atteignent la maturité rapidement, ils pondent plus souvent et à intervalles plus courts ; la fécondité est élevée même si la durée de vie est plus brève. La température détermine l'âge de la maturité et les modalités de survie, et les caractéristiques démographiques de *O. labronica* ; le taux de reproduction net R_0 et le taux de croissance de la population λ varient selon la température, montrant ainsi l'importance des facteurs de l'environnement dans le déterminisme des caractéristiques de la reproduction à l'échelle de l'individu, mais aussi à celle de la population et de l'adaptation.

INTRODUCTION

The effects of temperature on the survival, growth, development and reproduction of polychaetes have long been the subject of research (Bhaud 1988, Yokohama 1988, Prevedelli 1991, 1992, 1994). The role of temperature in the regulation of the reproductive cycles of many annual iteroparous and semelparous polychaetes with markedly-seasonal and highly-synchronized spawning patterns is evident and well documented (Neuhoff 1979, Garwood 1980, Olive 1984, Olive *et al.* 1997, Olive *et al.* 2000). In the case of semicontinuous iteroparous species, which, by definition, lay small groups of eggs at short intervals of time, the effects of temperature, and in particular their impact on population levels, have received less attention. In theory, these species reproduce throughout the year, but in some small-sized species belonging to the fouling communities of harbour environments in temperate regions, marked seasonal variations in population densities have been observed. It is therefore likely that the temperature-induced variations on survival and fecundity, already noted in some small-sized species employing a semicontinuous, iteroparous reproductive strategy, may occasion considerable variations in

population growth rate (Åkesson 1976, Chu & Levin 1989, Levin & Creed 1986, Åkesson & Costlow 1978, 1991).

Ophryotrocha labronica is a semicontinuous iteroparous and geographically widespread species belonging to the Dorvilleidae family, colonizing harbour environments. It is a small-sized worm with an extremely high reproductive capacity. It was first described by La Greca & Bacci (1962) as a proterandric, hermaphroditic species in a population from Leghorn, Italy. Subsequently, gonochoric species collected in the harbours of Leghorn and Naples, Italy (Åkesson 1970, 1972 a, b) and Genoa, Italy (Premoli *et al.* 1996) were also described. *O. labronica* is particularly suitable for studies on environmental control of reproduction because it is easy to breed in laboratory conditions. Moreover, the animals grow quickly, reach sexual maturity in a very short time, reproduce a number of times at intervals of just a few days and produce a large number of eggs (Åkesson 1973). Åkesson (1976) has studied the effects of temperature on the life cycle of a population of *O. labronica*, originally from Leghorn harbour, after breeding in the laboratory for about ten years.

We investigated the effects of three temperature regimes on a population of *O. labronica* collected in Genoa harbour to understand the relationship between temperature and the animals' life history characteristics. Our objectives were to compare the survivorship and reproductive activity of *O. labronica* at different temperatures; to evaluate environment-induced variation in life history traits such as: age and size at first reproduction, age-specific survival, age-specific fecundity and brood size; to perform a demographic analysis to link the individual life history traits to population levels.

MATERIALS AND METHODS

Collection and maintenance: The population analyzed in this experiment came from the harbour of Genoa. The experiment was performed with recently collected specimens. After collection the animals were housed in 10 cm diameter, 5 cm tall beakers containing approximately 200 ml of artificial 35 psu seawater and maintained at a constant temperature of 24 °C in a L:D 12:12 photoperiod. Twice a week, the animals were fed with Tetramin, an artificial food for aquarium fish, slightly in excess of the appetite. The nutritional characteristics of this food are reported in Prevedelli and Zunarelli Vandini (1998). Newly laid egg masses were transferred together with the female to three new beakers, one for each of tested temperature and placed at 15 °C, 22 °C and 30 °C respectively. As soon as the larvae began to hatch, the females were removed, thus ensuring that a large number of individuals of the same age and a known date of birth were selected.

Description of the experiment: The experiment was carried out on couples of one male and one female. In *O. labronica* sex can be clearly distinguished; in fact there is an apparent dimorphism between males and females in the jaw, and the oocytes are visible through the body wall in females with 13-14 setigerous segments. Each couple was placed in a 4.5 cm diameter beaker containing 10 ml of artificial 35 psu seawater. The effects of each temperature were assessed on 20 couples. Food was given twice a week. Couples were checked twice a week to see whether reproduction had occurred. If so, the fecundity and growth rate were recorded. If a male died, he was replaced by a young individual of known age, while if the female died, the couple was eliminated from the experiment. The fecundity of each couple was assessed as: (1) the number of eggs produced at each spawning by the female, (2) the number of spawnings, and (3) the total number of eggs produced by a female during its lifetime. Growth rate was recorded by counting the number of setigerous segments.

Data analysis: The effects of temperature on life history characteristics were assessed by analysis of variance (ANOVA) or the non-parametric Kruskal-Wallis test. Age-specific survivorship and fecundity schedules were obtained for each examined temperature. These life tables data were used to build a complete age-classified population model and the relative projection matrices (Leslie matrices), using a projection interval of 1 week. Since this species exhibits semicontinuous reproductive mode (births occur continuously over the time interval), the survival probabilities (P_i) appearing on the subdiagonal were calculated as:

$$P_i = \frac{l_{(i+1)} + l_{(i)}}{l_{(i)} + l_{(i-1)}}$$

and the age-specific fecundity (F_i) in the first row as:

$$F_i = (l_{(0)} l_{(1)})^{1/2} \frac{(m_i + P_i m_{i+1})}{2}$$

where $l_{(i)}$ is survivorship from zygote to age i and m_i is the average number of offspring female per female in age class i (Caswell 1989).

The finite population growth rate λ was calculated as the dominant eigenvalue of each matrix. The stable age distribution is given by the corresponding right eigenvector (w) and the reproductive value distribution by the corresponding left eigenvector (v). The sensitivities of λ to changes in the matrix entries P_i and F_i were calculated according to Caswell (1989, 2000):

$$s_{Pi} = \frac{\partial \lambda}{\partial P_i} = \frac{w_i v_1}{< w, v >}$$

$$s_{Fi} = \frac{\partial \lambda}{\partial F_i} = \frac{w_i v_{i+1}}{< w, v >}$$

where $<w,v>$ denotes the scalar product between the stable age composition and the reproductive value.

The sensitivity is determined by the life history of the organism as described by P_i and F_i. However since survivorship and fecundity are measured on different scales, absolute values of sensitivities cannot readily be compared among different parameters. To overcome this difficulty the measure of relative sensitivity, know as elasticity, was calculated:

$$e_a = \frac{a}{\lambda} s_a$$

where a denotes the considered life history trait P_i or F_i (de Kroon *et al* 1986).

The effect of each treatment on λ, was decomposed into contributions from each of the age-specific survivorship and fecundity terms using the techniques outlined by Caswell (1989). Let $\lambda(c)$ and $\lambda(d)$ denote the values of λ for treatment c and d, respectively. Then:

$$\Delta\lambda = \lambda^{(c)} - \lambda^{(d)} = \sum_i \sum_j \Delta a_{ij} \frac{\partial \lambda}{\partial a_{ij}}$$

Each term in the summation is the contribution of the difference in the matrix entry a_{ij} of treatment c respect to d on Δλ. In this way it was possible a) to identify the life-history traits that were most responsible for the effects of treatment on λ and b) to compare the contribution of each trait to the Δλ between two treatments.

We also calculated the expectation of life:

$$e^0 = 0{,}5 + \frac{l_1 + l_2 + l_3 + \ldots l_n}{l_0}$$

the net reproductive rate:

$$R_0 = \sum_i F_i \prod_{j=1}^{i-1} P_j$$

and the generation time:

$$T = \frac{\sum_i i F_i \prod_{j=1}^{i-1} P_j}{\sum_i F_i \prod_{j=1}^{i-1} P_j}$$

RESULTS

Life history traits

Many traits in the life history of *O. labronica* vary in relation to temperature. In particular, significant differences have been recorded in the animals' age and size at maturity, in the number of spawnings occurring during the lifetime of a female, in the maximum size reached by both males and females, in fecundity, in the number of eggs laid in each cluster and in the time elapsing between one spawning and the next. The mean values (±2 SE), minima and maxima are reported in Table I. Generally speaking, the animals maintained at 15 °C are slower to reach maturity, spawn less frequently and tend to be smaller and less fecund, although the number of eggs contained in each cluster is greater, on average, than at the other temperatures. The animals maintained at 30 °C, on the other hand, quickly reach sexual maturity, are quite the biggest and most fecund and spawn at brief intervals of time; the number of eggs in each cluster is between that found at the other temperatures and the number of spawnings is lower than at 22 °C.

Table I. – Summary of the life history characteristics of *O. labronica* at tested temperatures.

	Size at maturity (s.s.)			Age at maturity (days)		
	15°C	*22°C*	*30°C*	*15°C*	*22°C*	*30°C*
Mean	16.6	14.8	15.8	56.7	30.4	22.7
2*ES	0.7	0.5	0.6	2.2	1.8	2.0
Min.	14	14	14	53	24	18
Max.	18	17	17	64	35	28
n	10	17	12	10	17	12
χ^2		12.6			30.6	
p		0.002			< 0.001	
	Number of spawnings			Maximum female's size (s.s.)		
	15°C	*22°C*	*30°C*	*15°C*	*22°C*	*30°C*
Mean	3.0	7.6	6.2	18.0	18.2	19.4
2*ES	0.5	1.0	1.5	0.9	0.6	1.9
Min.	2	3	3	15	16	17
Max.	4	10	11	20	20	25
n	10	17	12	10	17	12
χ^2		20.7			0.1	
p		< 0.001			0.947	
	Maximum male's size (s.s.)			Fecundity (eggs/female)		
	15°C	*22°C*	*30°C*	*15°C*	*22°C*	*30°C*
Mean	13.9	14.9	15.9	868.3	942.1	1089.4
2*ES	0.6	0.5	0.8	168.8	109.9	344.5
Min.	13	12	13	567	312	276
Max.	16	16	18	1335	1202	2215
n	10	17	12	10	17	12
χ^2		13.5			1.5	
p		0.001			0.467	
	Eggs/sleeve			Interval between spawnings		
	15°C	*22°C*	*30°C*	*15°C*	*22°C*	*30°C*
Mean	289.4	125.1	176.7	26.1	13.2	8.7
2*ES	31.3	10.3	23.4	4.0	0.7	0.8
Min.	120	23	28	16	7	4
Max.	440	255	387	58	26	19
n	30	128	74	20	111	62
F		56			111.8	
p		< 0.001			< 0.001	

Age-specific survival and fecundity

The survival and fecundity graphs are shown in Fig. 1. The survival graphs are very different at the three temperatures assayed. In particular, survival is highest at 15 °C with about 90% of the animals surviving until the 14th week; thereafter, the animals very gradually begin to die, the longest-lived continuing to survive until the 24th week. At 22 °C the curve is much more linear, but here too the longest-lived survive for 24 weeks. At 30 °C, on the other hand, the survival pattern is very different: as early as the 2nd week only 60% of the animals are still alive, while from the 8th week onwards there is a sharp drop in the survival rate, the longest-lived surviving only until the 17th week.

Fecundity patterns are very different at the different temperatures. The differences relate not only to the total number of eggs produced by one female but also to the time elapsing between spawning and hatching, between birth and the first spawning and

to the span of fecund life, factors which obviously determine overall fecundity. The displacement of the age-specific fecundity curves is due to the time required for the animals to reach sexual maturity, which varies greatly between temperatures. The animals kept at 15 °C reach sexual maturity at the 7th week and stop reproducing at the 20th week of life. During this period the fecundity curve presents three well-spaced and progressively lower peaks. At 22 °C the pattern is very different: the animals start spawning from the 3rd week and, at least in its first part, the curve does not present very pronounced peaks. At 30 °C the pattern is again quite different: at this temperature the animals reach sexual maturity earlier and soon begin to lay a large number of eggs; also, the fecundity curve is characterized by three peaks that are much higher and closer together than those observed at 15 °C.

Demography

The weekly record of survival and fecundity of *O. labronica* has enabled us to draw up life tables and to calculate all the demographic parameters relating to the three temperatures assayed, as follows: net reproductive rate (R_0), generation time (T), expectation of life at birth (e^0) and the population growth rate per time unit (λ). The values of the demographic parameters at the different temperatures are reported in Table II. The net reproductive rate varies considerably with temperature; it is higher at the lowest temperature (R_0 = 540.76 at 15 °C) and gradually drops as the temperature increases (R_0 = 425.80 at 30 °C). Expectation of life at birth also varies greatly with temperature, being decidedly higher at 15 °C and gradually shortening as the temperature increases until, at 30 °C, it is less than half that recorded at 15 °C. Generation time is higher at the lower temperatures: the differences between 15 °C and 22 °C are fairly modest, while at 30 °C it is much lower. The population growth rate is extremely susceptible to temperature: the lowest value (λ=1.87) is found at 15 °C and the highest value (λ=3.44) at 30 °C. The elasticity of λ dependent on the variations in age-specific survival and fecundity is illustrated in Fig. 2 a, b. The elasticity patterns appear more biased to younger age classes at higher temperature because the reduction of the age at maturity; moreover the population growth rate observed at 30 °C seems to be more sensitive to variation in fecundity than at lower temperatures. Thus, the period during which the population growth rate is affected by variations in survival rate and fecundity gradually shortens as the temperature rises; while at 15 °C the elasticity values do not reduce to zero until after the tenth week, at 30 °C they do so as early as the fourth week of life. The contributions made by the variations in age-specific survival and fecundity to the differences in λ are set out in Fig. 3 a, b. The differences between 30 °C and 22 °C are due to the greater fecundity among the first age groups of the animals kept at 30 °C; the higher survival rate, albeit slight, of the animals kept at 22 °C being an advantage only in the first week of life. The differences between 22 °C and 15 °C are also due essentially to fecundity, and in particular to the greater fecundity, from the second week, of the animals kept at 22 °C.

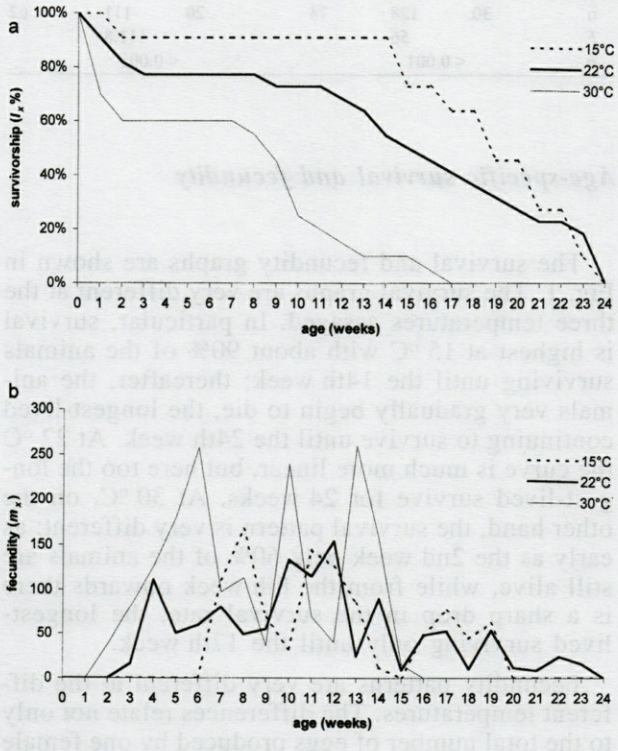

Fig. – 1. a, Survival (l_x) and b, fecundity (m_x) in worms maintained at the tested temperatures.

DISCUSSION

In *Ophryotrocha labronica*, as in many other species of marine invertebrates, numerous traits of

Table II. – Values of demographic parameters in worms maintained at the three tested temperatures.

	15 °C	22 °C	30 °C
λ	1.95	2.84	4.09
r	0.66	1.04	1.40
R_0	540.76	491.80	425.80
e^0	17.59	13.86	6.75
T	11.66	10.25	7.01

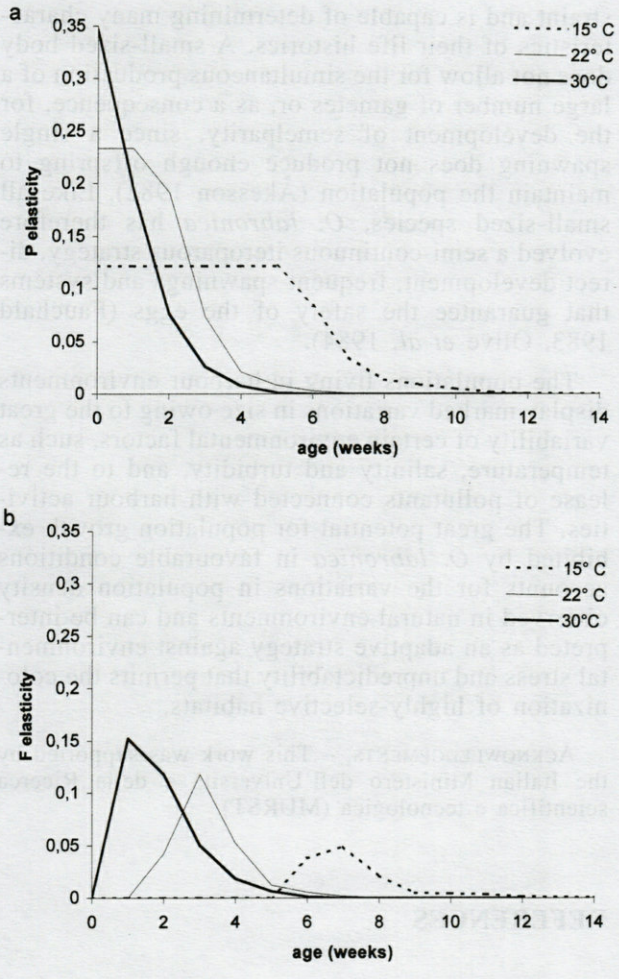

Fig. 2. – Elasticity of a, age-specific survivorship (*P*) and b, age-specific fecundity (*F*) in worms maintained at the tested temperatures.

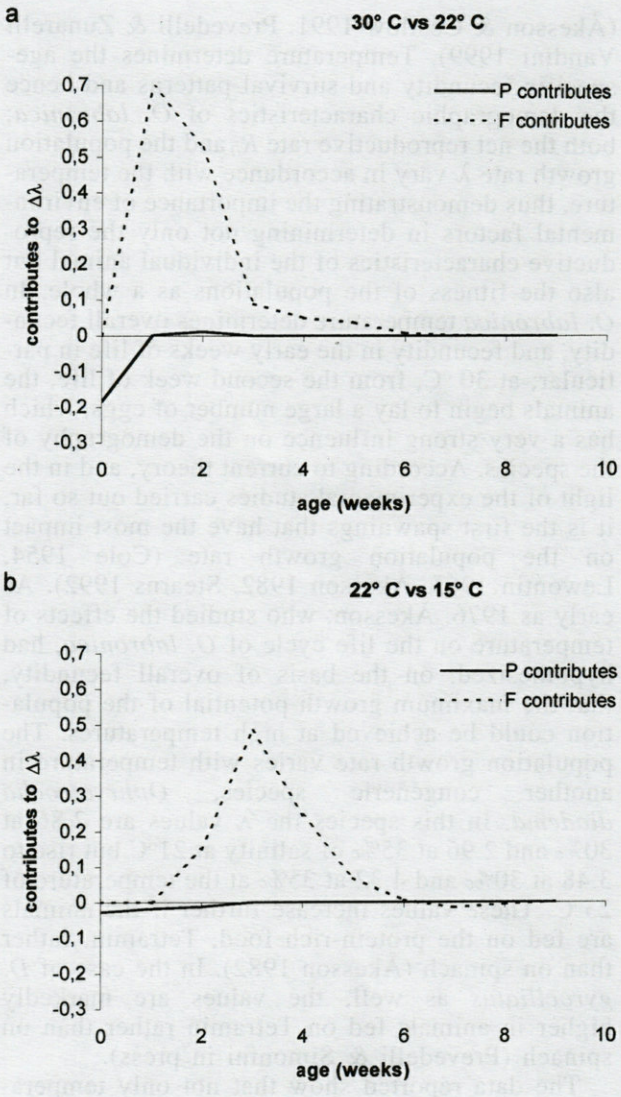

Fig. 3. – Decomposition analysis of temperature effects. Contribution of age-specific survivorship (*P*) and age-specific fecundity (*F*) to the differences in population growth rate (λ): a, 30 °C versus 22 °C; b, 22 °C versus 15 °C.

their life history vary in accordance with environmental factors. Temperature in particular determines not only the age at maturity but also survival and fecundity, with very important consequences for the demography and fitness of the species. The influence of environmental factors on many phases of the life cycle has been demonstrated in numerous species of opportunistic polychaetes characterized by a short life cycle, rapid growth, speedy sexual development and continuous or semi-continuous reproduction (Åkesson 1976, Åkesson & Costlow 1991, Chu & Levin 1986, Gremare *et al.* 1988, Gremare *et al.* 1989 a, b, Levin & Creed 1986, Qian 1984, Qian & Chia 1991, 1992, Tenore & Chesney 1985, Prevedelli & Zunarelli Vandini 1998, 1999, Prevedelli & Simonini 2000). In the case of *O. labronica*, low temperatures cause a considerable delay in the attainment of sexual maturity, a reduced number of spawnings and longer intervals between one spawning and the next; fecundity is therefore low on the whole, even though each cluster averages a greater number of eggs and the animals generally live longer. At high temperatures, on the other hand, the animals mature rapidly, spawn very frequently, fecundity is therefore high, even though the animals have a much shorter life span. The greater and earlier mortality of adults at 30 °C could be related to the greater fecundity seen at this temperature. The greater energy allocated to the production of gametes may be the reason for the shorter lifespan. In iteroparous species, there is a "trade-off" between fecundity and the survival of each single individual: those animals which allocate high levels of energy to germinal tissues potentially risk lower soma survival (Pianka & Parker 1975, Taylor 1991, Stearns 1992). This "trade-off" has been also observed in the small polychaetes *Dinophilus gyrociliatus*

(Åkesson & Costlow 1991, Prevedelli & Zunarelli Vandini 1999). Temperature determines the age-specific fecundity and survival patterns and hence the demographic characteristics of *O. labronica*; both the net reproductive rate R_0 and the population growth rate λ vary in accordance with the temperature, thus demonstrating the importance of environmental factors in determining not only the reproductive characteristics of the individual animal but also the fitness of the populations as a whole. In *O. labronica* temperature determines overall fecundity, and fecundity in the early weeks of life in particular; at 30 °C, from the second week of life, the animals begin to lay a large number of eggs, which has a very strong influence on the demography of the species. According to current theory, and in the light of the experimental studies carried out so far, it is the first spawnings that have the most impact on the population growth rate (Cole 1954, Lewontin 1965, Åkesson 1982, Stearns 1992). As early as 1976, Åkesson, who studied the effects of temperature on the life cycle of *O. labronica*, had hypothesized, on the basis of overall fecundity, that the maximum growth potential of the population could be achieved at high temperatures. The population growth rate varies with temperature in another congeneric species, *Ophryotrocha diadema*. In this species the λ values are 2.86 at 30‰ and 2.96 at 35‰ of salinity at 21°C but rise to 3.48 at 30‰ and 4.32 at 35‰ at the temperature of 25°C. These values increase further if the animals are fed on the protein-rich food, Tetramin, rather than on spinach (Åkesson 1982). In the case of *D. gyrociliatus* as well, the values are markedly higher in animals fed on Tetramin rather than on spinach (Prevedelli & Simonini in press).

The data reported show that not only temperature but also salinity and diet influence the demographic characteristics of the populations. In the other species of polychaetes whose demography has been studied hitherto the values of the basic reproduction rate R_0 as well as of the growth rate λ are decidedly lower, even if one takes into account all the cases of opportunistic species; the λ values in populations of *Streblospio benedicti, Polydora ligni* and *Capitella capitata*, for example range from a minimum of 1.205 to a maximum 1.381 (Levin *et al.* 1987). The differences between these species and *O. labronica, O. diadema* and *D. gyrociliatus* mainly concern the body size. *S. benedicti, P. ligni* and *C. capitata* are much bigger (10-20 mm) than either *D. gyrociliatus*, which is the smallest species of all, being only 0.8 mm long, or *O. labronica* and *O. diadema*, which are at most 4 mm long. The R_0 and λ values of *O. labronica* are greater than those of typically opportunistic species, which are by definition *r*-strategists (Grassle & Grassle 1974). This feature, which is shared by *D. gyrociliatus* and *O. diadema*, is very probably due to the animals' small size. Body size constitutes a very important morphological constraint and is capable of determining many characteristics of their life histories. A small-sized body does not allow for the simultaneous production of a large number of gametes or, as a consequence, for the development of semelparity, since a single spawning does not produce enough offspring to maintain the population (Åkesson 1982). Like all small-sized species, *O. labronica* has therefore evolved a semi-continuous iteroparous strategy, direct development, frequent spawnings and systems that guarantee the safety of the eggs (Fauchald 1983, Olive *et al.* 1984).

The populations living in harbour environments display marked variations in size owing to the great variability of certain environmental factors, such as temperature, salinity and turbidity, and to the release of pollutants connected with harbour activities. The great potential for population growth exhibited by *O. labronica* in favourable conditions accounts for the variations in population density observed in natural environments and can be interpreted as an adaptive strategy against environmental stress and unpredictability that permits the colonization of highly-selective habitats.

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ANNOUNCEMENT

POLYCHAETES AS BIOLOGICAL AND ECOLOGICAL MODELS
From taxonomy to applied research

An advanced international course

Department of Biological and Environmental Sciences and Technologies, University of Lecce, Italy.

9-22 September, 2002

The aim of the course is to re-evaluate taxonomy of polychaetes to better exploit their potential in addressing basic biological and ecological problems, as well as applied research.The Course will be addressed to young scientists who wish to start or to increase their knowledge on polychaetes both for taxonomical and bioecological purposes. The first part of the course will consist of lectures and exercises on preserved and fresh material focusing mainly on taxonomy of polychaetes to give a general picture for the identification of adults at family level and the use of morphological characters in phylogenetic reconstruction. For some representative families also features at generic and, where possible, at species level, will be treated.

Development and reproductive biology as well as molecular markers will be also taken into consideration for phylogenetic analysis. Basic cladistic methodology will be given.

The second part of the course will consist in lectures and open discussions concerning different biological and ecological topics in which polychaetes can be used as model organisms : population genetics and phylogeography, developmental and reproductive patterns, ecophysiology, ecological role and environmental monitoring. Finally, some aspects of the use of polychaetes for applied research (e.g. aquaculture, bioreimediation) will be addressed.

Course organizers

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Maria Cristina Gambi (Zoological Station of Naples, Italy)

Faculty

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Teaching and Technical assistants

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(Dept of Biological and Environmental Sciences and Technologies, University of Lecce, Italy)

General information

The course will be held at the Department of Biological and Environmental Sciences and Technologies of the University of Lecce, at Lecce Italy. It will run for two weeks starting from 9 September 2002. It will be organized to include formal lectures, laboratory and field researches training.

The course will be limited to 25 students (post-graduates and PhD) and 10 auditors (from post-doctors to senior scientists). Only students will participate in all course activities, auditors will attend the lectures and open discussions.

Applications for both participant types and application forms may be obtained from the web site http : //www.polychaeta.net or from the e-mail address provided below.

Applications must be received by 1 April 2002. Notifications of acceptance will be made not later than 30 June 2002. Successful applicants will receive additional information concerning the course, local arrangements, and pre-course material at the time of acceptance.

The course includes two half-day field trips along coastal areas near Lecce for collecting material, plus a visit to an aquaculture farm.

The fee for the two week course is 1000 Euros for students, and 800 Euros for senior students. (including accomodation). Two participation fellowships (1000 Euro each) are available to facilitate participation of students from less developed countries. The fellowship must be required by students in the statement for reason to attend the course, to be added to the application form.

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EFFECT OF FOOD AVAILABILITY ON THE ENERGETICS OF THE INTERTIDAL SCAVENGING GASTROPOD *NASSARIUS FESTIVUS*

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NASSARIUS
GASTROPOD
GROWTH
MAINTENANCE RATION
FOOD AVAILABILITY

ABSTRACT. – Individuals of the intertidal scavenging gastropod *Nassarius festivus* (Powys) were kept at one of the four ration levels in the laboratory for ten weeks. The fastest growth (shell and somatic tissue) was observed when individuals were fed once every two days (high-ration) and followed by those fed once every seven days (medium-ration). Individuals lost weight when fed once every 21 days (low-ration), or unfed throughout the experiment. Maintenance ration was 16.5 J day^{-1} ind^{-1} and could be met by a food ration of one meal every 8.2 days. Positive values of gross growth efficiency (P/C) were obtained for the high- and medium-ration group and were estimated at 4.2 and 2.1%, respectively. As food availability decreased, the proportion of consumed energy expended on respiration (R/C) increased from 6.7% for the high-ration group to 25.1% for the unfed group. Results are discussed with respect to the unpredictable food supply to this obligate scavenger.

NASSARIUS
GASTÉROPODE
CROISSANCE
RATION DE MAINTIEN
RESSOURCES NUTRITIVES

RÉSUMÉ. – Des individus du Gastéropode nécrophage *Nassarius festivus* ont été elevés au laboratoire en recevant une des quatre rations de nourriture pendant 10 semaines. La croissance la plus rapide (coquille et tissu somatique) est observée lorsque les individus sont nourris une fois tous les deux jours (ration élevée), immédiatement suivie par ceux qui sont nourris une fois tous les sept jours (ration moyenne). Les individus nourris une fois tous les 21 jours (basse ration) ou restés à jeun pendant toute la durée de l'expérience perdent du poids. La ration de maintien s'établit à 16,5 J $jour^{-1}$ ind^{-1} et correspond à une ration de un repas tous les 8,2 jours. Les valeurs positives de croissance brute (P/C) sont obtenues pour les groupes ayant reçu les rations forte et moyenne, et sont estimées à 4,2 % et 2,1 % respectivement. Lorsque la nourriture disponible décroît, la part de l'énergie consommée due à la respiration (R/C) augmente de 6,7 % pour le groupe nourri avec la ration la plus élevée et de 25,1 % pour le groupe resté à jeun. La discussion des résultats porte notamment sur le fait que les ressources nutritives de ce nécrophage exclusif sont aléatoires.

INTRODUCTION

The neogastropods Nassariidae are one of the largest families of the Gastropoda with more than 300 species and most of them are presumed exclusively scavengers of carrion (Cernohorsky 1984). Intertidal species of this family commonly inhabit eulittoral soft shores having moderate to broad tidal ranges and low exposure to wave energy. As food supply to these animals is sporadic, these gastropods may undergo starvation for long periods of time. Four intertidal species of *Nassarius* are found in Hong Kong with *Nassarius festivus* being the dominant species (Britton & Morton 1992). When food is available, *N. festivus* moves towards food rapidly and consumes large quantities of it quickly, about 50-60% of the body weight (Morton 1990, Cheung 1994). In the face of hunger, however, *N. festivus* can survive >100 days without food (Morton 1990) and reduce energy expenditure by reducing activity (Cheung 1994). This animal, however, abandons a meal in the face of the risk of predation when food availability is equal to or more than one meal every 14 days. A further decrease in the food availability increases the risk of starvation to such an extent that the animal will feed, despite the risk of being consumed (Morton *et al.* 1995).

Hong Kong is facing an overfishing problem and nutrient enrichment in the surrounding waters. Such environmental perturbations are suggested to be responsible for the decrease in benthic species diversity which is reflected in a reduction of neogastropods and an increase in the number of scavenging gastropod species of Buccinidae and Nassariidae, e.g. *N. festivus* (Morton 1993). These scavengers now assume an important ecological role as cleaners which help in preventing further degradation of the marine environment (Morton 1993). For *N. festivus*, energy expenditure on individual activities has been reported by Cheung (1994) and the effect of food availability on fecundity by Cheung & Lam (1999). The objectives of this study included the investigation of the effect of food availability on the energetics of *N. festivus* and the determination of the maintenance ration for this species.

MATERIALS AND METHODS

Maintenance of the animals: Individuals of *Nassarius festivus* (shell length: 9.02-1.60 mm) were collected from the sandy beach at Lok Wu Sha, Tolo Harbour, Hong Kong and acclimated in laboratory conditions for one week (salinity: 30‰, temperature: 20°C) prior to experimentation. During acclimation, individuals were allowed to feed to satiation everyday on excised short-necked clams, *Tapes philippinarum* collected from the same site. The individuals of *N. festivus* were then exposed to one of the three ration levels with food provided once either every 2, 7 or 21 days which were considered as the high-, medium- and low-ration group, respectively. The fourth treatment group was unfed throughout the experiment, except at the start of the experiment and was labelled as the unfed group. Ten individuals of *N. festivus* from each treatment group were placed in two plastic vials with five individuals each. Plastic vials of all the four treatment groups were then placed in a fish tank. Three fish tanks were prepared to act as replicates. Therefore, there were thirty individuals of *N. festivus* for each treatment group and the experiment lasted for ten weeks.

Food consumption: Five individuals of *N. festivus* from each treatment group were allowed to feed to satiation on excised *Tapes philippinarum*. The time spent feeding was calculated as the time between when the proboscis was everted and retracted (Cheung 1994). The number of individuals fed and the time spent feeding by each individual was recorded. The initial and final body wet weight (after consumption by *N. festivus*) of *T. philippinarum* was measured to the nearest 0.1mg. As *T. philippinarum* loses weight after immersion in water, 3 individuals were placed in water for 15 minutes, which was about the time an individual of *N. festivus* spent feeding, and the weight change was used to adjust the computed consumption rate. The mean consumption rate per meal per individual was then calculated by dividing the obtained values by a factor of five. The experiment was repeated twice.

The wet weight of *T. philippinarum* consumed by *N. festivus* was converted into the dry weight using a linear regression relating wet weight (WW) and dry weight (DW): $DW = 0.22 \times WW + 0.01$ ($r^2=0.96$, $P < 0.005$) (Cheung 1994). Energy gained from consumption of *T. philippinarum* was calculated using the mean calorific value of the tissue of *T. philippinarum*, i.e. of 20.46 KJ g^{-1} dry wt (Cheung 1994).

Shell growth: The shell length of each of the 30 individuals of *N. festivus* from each treatment group was measured to the nearest 0.01 mm every seven days for ten weeks using vernier calipers. To calculate the amount of energy allocated to shell production, the measured increase in shell length was converted into shell weight by a regression equation relating shell weight to shell length. The equation was computed from 20 individuals with shell lengths of between 6.90 and 14.10 mm and was calculated as Log shell weight (g) = 2.64 × Log shell length (mm) – 3.64 ($n = 30$, $r^2 = 0.97$). The organic content of the shell was determined by drying 4 empty shells in an oven at 80°C for 48 h and then ashing in a muffle furnace at 500°C for 8 h. The percentage weight lost was considered as the organic content of the shell which was determined as 3.23% ± 0.23 (1SD). Using the calorific value of the organic matter of shell, which was 20.82 KJ g^{-1} (Edwards & Welsh 1982), the energy allocated to shell growth was calculated.

Somatic growth: The body wet weight of each of the 30 individuals of *N. festivus* from each treatment group was measured every 7 days for 10 weeks. The body wet weight was determined by an electronic balance to the nearest 0.1 mg after being blotted dry. The weight of the body tissue was obtained by subtracting the shell weight, which was predicted by the regression, from the body wet weight. The water content of the body tissue of *N. festivus* was found to be 75% (unpublished data), this value was used for converting tissue wet weight into tissue dry weight. The calorific value of body tissue was determined by drying the body tissue of five individuals of *N. festivus* at 105 °C for 48 h. The dried tissue of each individual was then ground into powder, mixed with benzoic acid and pressed into pellets. The pellets were then burnt using the Parr semi-micro bomb calorimeter and the mean calorific value of body tissue was estimated at 16.61 KJ g^{-1} dry wt. The energy allocated to tissue growth was computed from the change in the tissue weight and the calorific value of the tissue.

Oxygen consumption: The oxygen consumption rate was measured on nine occasions, at 7-day intervals. Twelve individuals of *N. festivus* from each treatment group were used. Because of the small size of the individuals, two individuals were placed in each container filled with 50 ml seawater, so there were six measurements for each treatment group. Three containers without animals were used as controls. To minimize disturbance, individuals were allowed to stay in the containers for 30 min before the experiment started. The seawater inside containers was then renewed and the containers were sealed for 90 min. Initial and final oxygen levels in each container were measured using a Clarke-type pola-

rographic oxygen meter. The decrease in oxygen tension was less than 25% of full saturation. Oxygen consumption rate (mg O_2 hr^{-1} ind^{-1}) was then calculated after correction for the control. Energy expended on respiration was calculated from the amount of oxygen consumed and the conversion factor of 13.98 J mg^{-1} O_2 (Ivlev, 1934).

Statistical analysis: As no significant difference in the above measurements was found among the three replicates of each treatment group, data of the three replicates were pooled and differences among treatment groups were compared using two-way repeated measure ANOVA. Considering the number of meals taken by each treatment group was different, the effect of time on each parameter was tested separately for each treatment group by Kruskal-Wallis one-way ANOVA. Percentage data were arcsine transformed prior to analysis.

RESULTS

Food consumption

In this 10-week experiment, all individuals from the low-ration group fed on all the occasions whereas the percentage number of individuals fed for the medium-ration group ranged from 96.7 to 100% and that for the high-ration group ranged from 73.3 to 100%. The mean percentage number of individuals that fed in this 10-week experiment was 92.0 ±7.7% (1SD), 99.3 ± 1.4% and 100 ± 0% for the high-, medium- and low-ration group, respectively. The percentage number of individuals that fed did not change significantly with time for all the three ration groups as tested by Kruskal-Wallis one-way ANOVA ($P > 0.05$). Since the total number of meals taken by different treatment groups was different, the percentage number of individuals that fed for the three ration groups were compared by two-way repeated measures ANOVA, using data from weeks when all the three ration groups fed. The percentage number of individuals that fed was significantly lower for the high-ration group as compared with the medium- and low-ration group, the effect of time and the interaction between time and ration, however, were not significant ($P < 0.05$).

The mean time spent feeding for the high-, medium- and low- ration group was 12.2, 20.9 and 22.4 min, respectively. The effect of time, ration and the interaction between ration and time were statistically significant ($P < 0.05$) with longer time spent feeding as food availability decreased (Fig. 1).

Food consumption per meal did not vary significantly ($P > 0.05$) with time for all treatment groups (Fig. 2). The mean food consumption per meal throughout the experimental period was 22, 30 and 24 mg wet wt $meal^{-1}$ ind^{-1} for the high-, medium- and low-ration group, respectively, and was neither significantly affected by ration nor the interaction between ration and time (two-way repeated measure ANOVA, $P > 0.05$). The accumulated dry

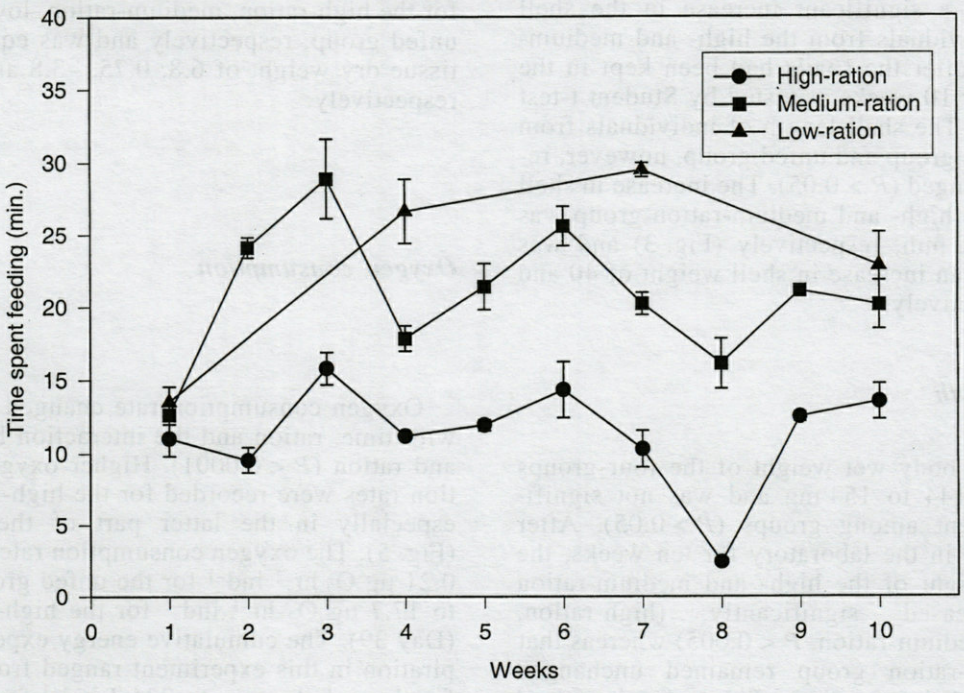

Fig. 1. – *N. festivus*. Mean time spent feeding (± S.E.) for individuals fed at one of the four ration levels.

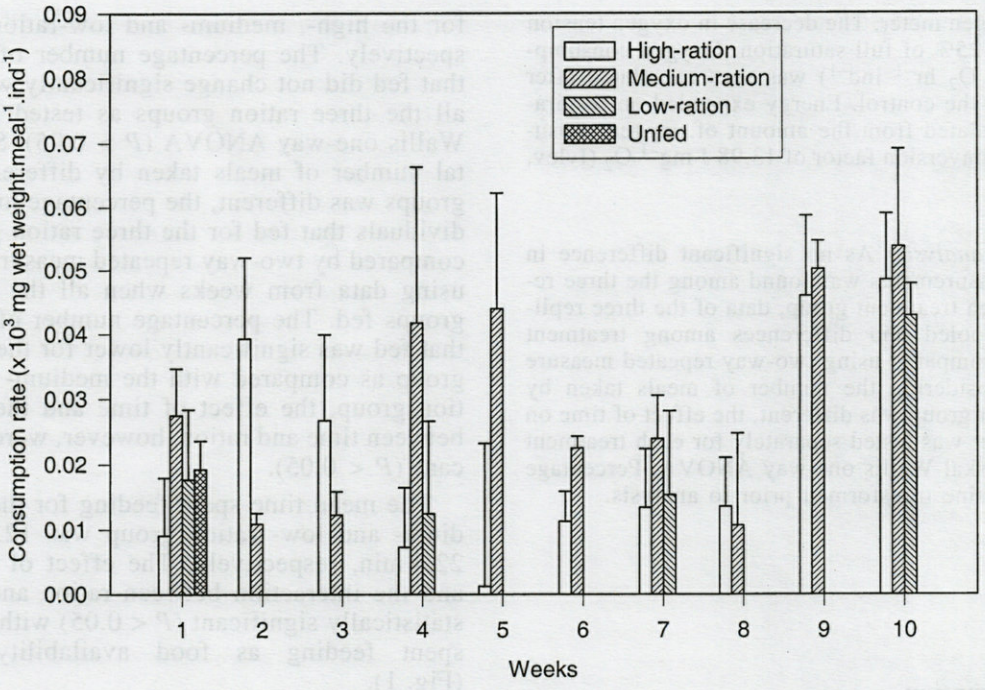

Fig. 2. – *N. festivus*. Food consumption rate (± S.E.) for individuals fed at one of the four ration levels. The * indicates data of the high-ration group obtained on day 9 instead of day 8.

weight of clam tissue consumed by an individual of *N. festivus* in 10 weeks was 164, 75, 29 mg for the high-, medium- and low-ration group, respectively.

Shell growth

There was a significant increase in the shell length of individuals from the high- and medium-ration group after the snails had been kept in the laboratory for 10 weeks as tested by Student t-test ($P < 0.0001$). The shell length of individuals from the low ration group and unfed group, however, remained unchanged ($P > 0.05$). The increase in shell length for the high- and medium-ration group was 1.38 and 0.80 mm, respectively (Fig. 3) and was equivalent to an increase in shell weight of 40 and 22 mg, respectively.

Somatic growth

The initial body wet weight of the four groups varied from 144 to 154 mg and was not significantly different among groups ($P > 0.05$). After keeping them in the laboratory for ten weeks, the body wet weight of the high- and medium-ration group increased significantly (high-ration, $P < 0.001$; medium-ration, $P < 0.005$) whereas that for the low-ration group remained unchanged ($P > 0.05$) and that for the unfed group decreased ($P < 0.0005$). The final body wet weight increased significantly with ration (Fig. 4) as tested by one-way ANOVA followed by Student-Newman-Keuls (SNK) multiple range test (F=23.1, *df*=3,116, $P < 0.0001$).

After subtracting the shell weight from the body wet weight, the change in the tissue wet weight at the end of the experiment was 9, 1, –5 and –23 mg for the high-ration, medium-ration, low-ration, and unfed group, respectively and was equivalent to a tissue dry weight of 6.8, 0.75, –3.8 and –17.3 mg, respectively.

Oxygen consumption

Oxygen consumption rate changed significantly with time, ration and the interaction between time and ration ($P < 0.0001$). Higher oxygen consumption rates were recorded for the high-ration group, especially in the latter part of the experiment (Fig. 5). The oxygen consumption rate ranged from 0.21 µg O_2 hr^{-1} ind^{-1} for the unfed group (Day 18) to 17.7 µg O_2 hr^{-1} ind^{-1} for the high-ration group (Day 39). The cumulative energy expended on respiration in this experiment ranged from 73 J ind^{-1} for the unfed group to 224 J ind^{-1} for the high-ration group (Table I).

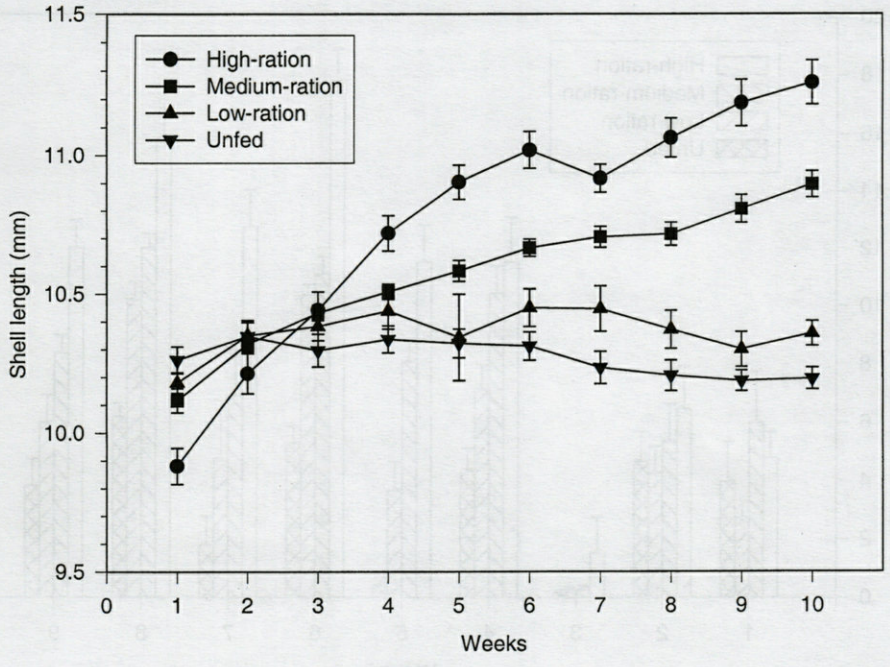

Fig. 3. – *N. festivus*. Growth in shell length (± S.E.) for individuals fed at one of the four ration levels.

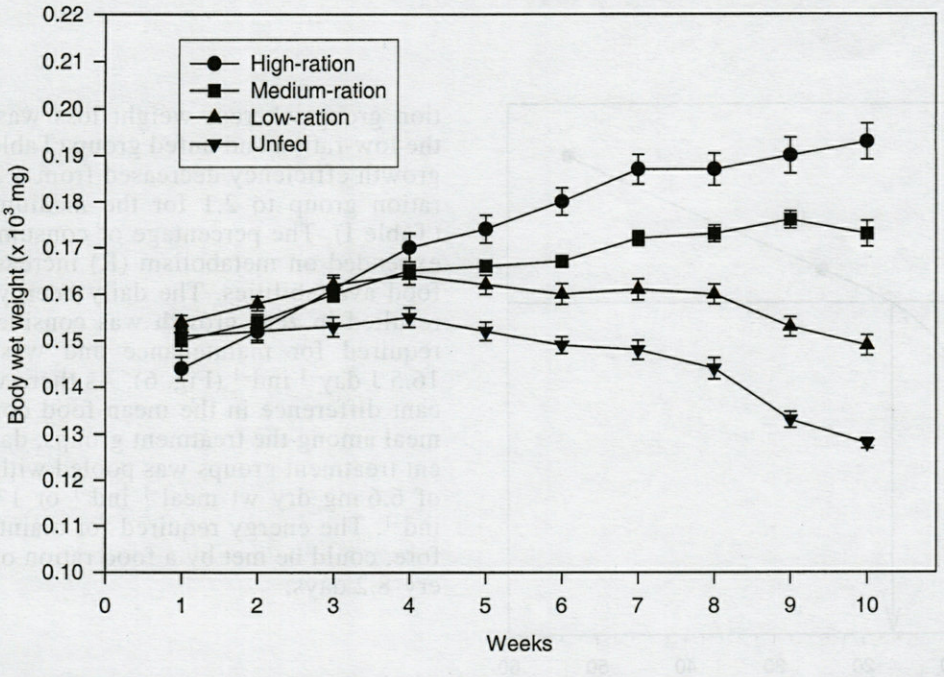

Fig. 4. – *N. festivus*. The change in the body wet weight (± S.E.) for individuals fed at one of the four ration levels.

Table 1. – Energy budget of *N. festivus* (J individual $^{-1}$) in terms of consumption (C), production of soma (Pg) and shell (Psh), and respiration (R).

Food ration	C (J)	Psh (J)	Pg (J)	R (J)	Psh + Pg (J)	(Psh + Pg)/C x 100 (%)	R/C x 100 (%)
High	3353	27	113	224	140	4.2	6.7
Medium	1543	15	17	163	32	2.1	10.6
Low	602	0	-63	117	-63	---	19.4
Unfed	291	0	-287	73	-287	---	25.1

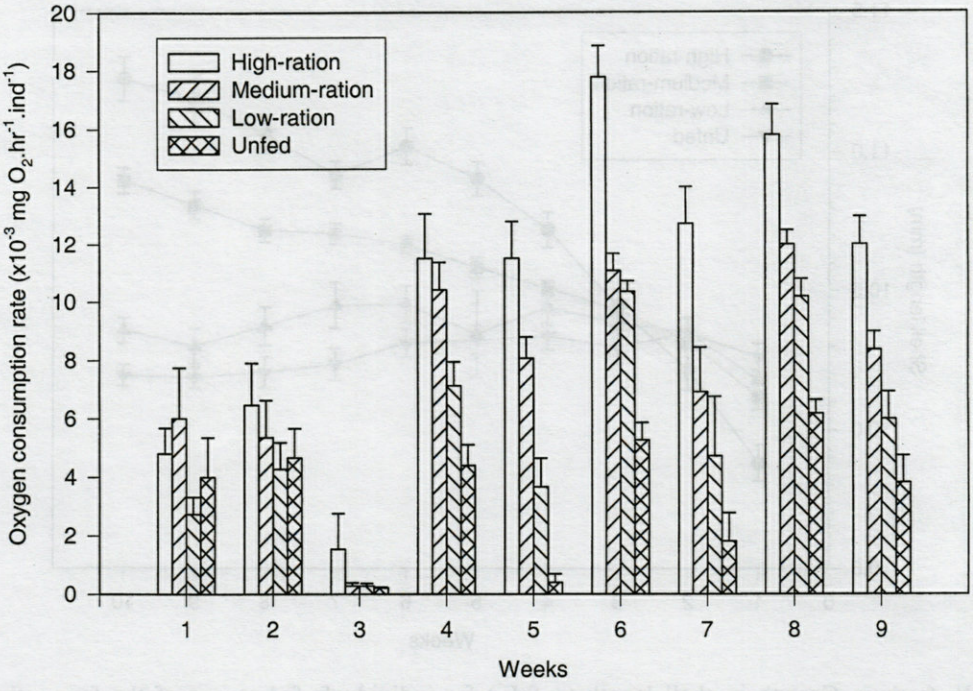

Fig. 5. – *N. festivus*. Mean oxygen consumption rate (± S.E.) for individuals fed at one of the four ration levels.

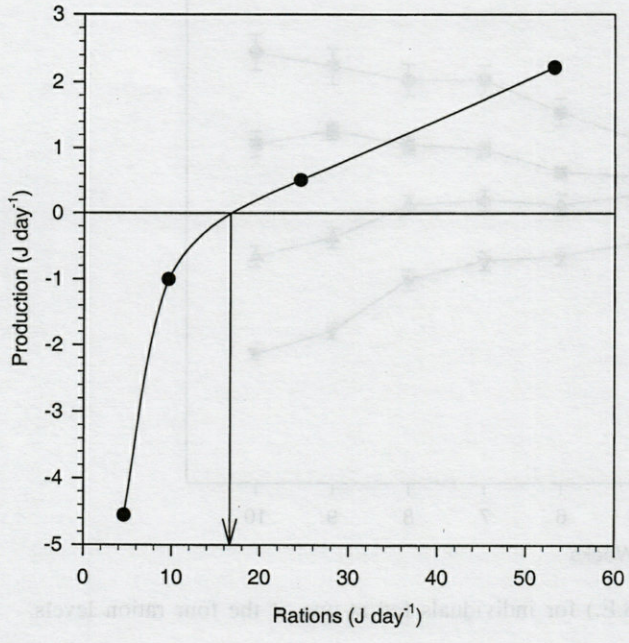

Fig. 6. – *N. festivus*. Production as a function of ration. Maintenance ration is considered as the ration level that results in zero growth, i.e. production = 0 J day^{-1}.

Maintenance ration

In this 10-week experiment, positive energy balance was obtained for the high- and medium-ration group whereas weight loss was observed for the low-ration and unfed group (Table I). The gross growth efficiency decreased from 4.2 for the high-ration group to 2.1 for the medium-ration group (Table I). The percentage of consumed energy (C) expended on metabolism (R) increased at reduced food availabilities. The daily energy intake which resulted in zero growth was considered as energy required for maintenance and was estimated at 16.5 J day^{-1} ind^{-1} (Fig. 6). As there was no significant difference in the mean food consumption per meal among the treatment groups, data from different treatment groups was pooled with a mean value of 6.6 mg dry wt meal^{-1} ind^{-1} or 135.04 J meal^{-1} ind^{-1}. The energy required for maintenance, therefore, could be met by a food ration of one meal every 8.2 days.

DISCUSSION

A common response during periods of reduced food availability is a reduction in the rate of oxygen consumption (Bayne & Newell 1983) so as to conserve energy. This may be due to a depression in the level of activity, a depletion of energy reserves, or a loss of material from the gut. However, for predators or scavengers that must seek out their food, a decline in standard (or resting) rate of metabolism may be offset against higher activity as the search for food is increased (Bayne & Newell

1983). The present study showed that oxygen consumption rate of the unfed group did not decrease with time of starvation. Higher oxygen consumption rates obtained for individuals maintained at higher rations were largely attributed to the growth of the animals. A similar independence of oxygen consumption from starvation was reported for *Thais lamellosa* (Stickle & Duerr 1970) and *N. festivus* (Morton 1990). The oxygen consumption rate of the latter remained constant when it was starved for 45 days and was reduced to about one third as periods of starvation increased to 60 days or more. For scavengers to conserve energy, spending a longer time in the sand when starved and coming out immediately once food is available should be a better strategy than reducing standard (resting) metabolism. This also explains why the proportion of consumed energy allocated to respiration in this study increased from 6.7% for the high ration group to 25.1% for the unfed group. Such an increase in R/C following stress was also observed in predatory gastropods *Polinices alderi* (Ansell 1982) for which the values increased from 11.1% under optimal conditions of growth and reproduction to 32.7% where growth was poor and no reproduction occurred.

The amount of food consumed per meal by *N. festivus* did not vary significantly with rations and is probably attributed to the limit set by the size of the gut. Individuals maintained at lower rations, however, spent a longer time feeding. Similar results were reported for the burrowing scavenging prosobranch *Bullia digitalis* (Stenton-Dozey *et al.* 1995) and the predator *Thais lapillus* (Bayne & Scullard 1978) and may be due to physiological deterioration that leads to a reduction in the functioning efficiency of the proboscis (Hughes 1986, Stenton-Dozey *et al.* 1995). The feeding time of field populations of *N. festivus* was reduced significantly when the number of conspecifics increased from 10 to 20 (Morton & Yuen 2000). Some *N. festivus*, unable to penetrate the cluster of feeding individuals gathering on dying clams in the field, were observed to turn away (Cheung 1994). With competition from conspecifics in the field, a slower feeding rate of *N. festivus* following starvation would ultimately affect meal size. Another constraint on feeding time in the field is the risk of predation. Morton *et al.* (1995) reported that *N. festivus* would abandon a meal in the face of the risk of predation when food availability was equal to or more than one meal every 14 days, although a further decrease in the food availability would increase the risk of starvation to such an extent that the animal would feed, despite the risk of being consumed. The maintenance ration of *N. festivus* was estimated at 16.5 J day^{-1} ind^{-1} which could be met by having one meal every 8.2 days. This, however, was determined under laboratory conditions where individuals of *N. festivus* were free from competition and predation and they were allowed to feed to satiation. As predation and competition are two constraints on feeding time, the maintenance ration in the field could only be met by having a meal at 8.2 day intervals. Nevertheless, the calculated maintenance ration helped to explain why *N. festivus* individuals are willing to risk their life for food for periods of starvation more than 14 days as reported by Morton *et al.* (1995) because individuals are now in negative energy balance.

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GLUTAMATE DEHYDROGENASE ACTIVITY IN *MYTILUS EDULIS*: THE EFFECT OF HYPERAMMONIA

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EXPOSURE
HYPERAMMONIA
GDH ACTIVITY
ADDUCTOR MUSCLE
MYTILUS EDULIS

ABSTRACT. – The effect of high levels of ammonia on glutamate dehydrogenase activity (GDH) in *Mytilus edulis* was evaluated. Adductor muscle GDH activity of 3.629 + 0.011 µmoles min^{-1} was found to be similar to that published for other species of bivalves and crustaceans. GDH activity showed a complete dependence on its co-factor (NADH) because no detectable activity was measured in the absence of NADH. The observed excitatory effect of ADP on the reductive activities of adductor muscle GDH indicated a possible regulatory link between amino acid production (for energy metabolism) and protein synthesis (for growth). The insignificant change ($P > 0.05$) in haemolymph protein levels of *M. edulis* exposed to hyperammonia found in this study, together with increased haemolymph free amino acids (FAA) levels found under the same conditions in an earlier investigation, suggest the involvement of GDH in the assimilation of exogenous ammonia into amino acids. It is concluded that enhanced GDH activity measured under hyperammonia, allows mussels to utilise transient increases in ammonia availability by assimilating most of the nitrogen present. Persistently high ammonia levels, however, may involve alternative metabolic pathways because GDH activity remains constant under prolonged exposure to hyperammonia. It would appear that a complex set of biochemical strategies have evolved to enable mussels to resist high ambient ammonia levels.

EXPOSITION
HYPERAMMONIA
ACTIVITÉ GDH
MUSCLE ADDUCTEUR
MYTILUS EDULIS

RÉSUMÉ. – L'effet des taux élevés de l'ammoniaque sur l'activité du glutamate déhydrogénase (GDH) de *Mytilus edulis* a été évalué. L'activité du GDH du muscle adducteur est égale à 3,629 ± 0,011 µmoles min^{-1}, celle-ci s'avérant semblable à celles trouvées pour d'autres espèces de Bivalves et de Crustacés. L'activité du GDH a montré une dépendance complète à l'égard de son cofacteur (NADH) parce qu'aucune activité discernable n'a été mesurée en l'absence du NADH. L'effet excitatoire observé de l'ADP sur les activités réductrices du GDH du muscle adducteur a indiqué un lien régulateur possible entre la production d'acide aminé (pour le métabolisme énergétique) et la synthèse de protéine (pour la croissance). Le changement non significatif ($P > 0.05$) des taux de protéine de l'hémolymphe de *M. edulis* exposé à un milieu hyper-ammoniacal (trouvé dans cette étude), ainsi que les taux accrus des acides aminés libres de l'hémolymphe (FAA) trouvés dans les mêmes conditions lors d'une première recherche, suggèrent la participation du GDH dans l'assimilation de l'ammoniaque exogène dans des acides aminés. On conclut que l'activité accrue du GDH mesurée sous hyperammonia, permet aux Moules d'utiliser des augmentations passagères d'ammoniaque en assimilant la majeure partie de l'azote présent. Un taux élevé d'ammoniaque peut cependant impliquer des voies métaboliques alternatives car l'activité du GDH est restée constante au cours de l'exposition prolongée des Moules à un milieu hyper-ammoniacal. Il semble qu'un ensemble complexe de stratégies biochimiques est impliqué pour permettre aux Moules de résister aux taux ambiants élevés d'ammoniaque.

INTRODUCTION

Glutamate dehydrogenase (GDH) is a key enzyme in carbohydrate and amino acid metabolism in animal cells which catalyses the amination of carbon skeletons and the deamination of amino acids (Lehninger 1976). GDH is specific to glutamate, 2-oxoglutarate (α-ketoglutarate), and NH_4^+ and at physiological pH 7.4, the activity of GDH with other substances is relatively low (Goldin & Frieden 1971). GDH is localised in mitochondria and has physiological significance in terms of free amino acid (FAA) metabolism despite its relatively low activity (Burcham *et al.* 1983, Moyes *et al.* 1985).

Several roles for the GDH system have been established, particularly in marine, euryhaline invertebrate species subjected to environmental changes. For example, GDH probably plays a major role in cell volume regulation by controlling the level of the intracellular FAA pool which, in bivalves, contributes 20-60% of the intracellular osmotic pressure (Hayashi 1987, Chew *et al.* 1994).

Changes in the kinetic properties of GDH under anoxia have also been reported. Ip *et al.* (1994) found a significant increase in glutamate affinity of GDH in the sipunculid *Phascolosoma arcuatum* under anoxia, which resulted in increased deaminating activity at physiological concentrations of glutamate. However, other species exposed to anoxic conditions such as *M. edulis* (Zurburg & De Zwaan 1981) and the mudskipper, *Periothalmodon schlosseri* (Peng *et al.* 1998) anoxia showed a significant increase in GDH aminating activities which led to enhanced tissue FAA levels which presumably detoxified excess ammonia accumulated during anaerobiosis.

It was shown earlier (Sadok *et al.* 1995) that *M. edulis* has the ability to take ammonia up from the external medium but, apart some marine invertebrate-alga associations (Rees *et al.* 1994, Roberts *et al.* 1999) no data are available on *M. edulis* metabolism under hyperammonia, although these animals have been used as biofilters in water with high ammonia levels (Enander & Hasselstrom 1994).

The present investigation was directed towards an attempt to elucidate better the results of hyperammonia on *M. edulis* described in an earlier study (Sadok *et al.* 1995) and, since mussels are able to withstand relatively high external ammonia levels, to investigate the potential role of GDH in ammonia detoxification. Consequently, only the reductive properties of GDH (i.e., glutamate formation) have been considered here.

MATERIAL AND METHODS

Collection and maintenance of M. edulis: Mussels were obtained from the intertidal zone at Filey, N. Yorkshire, UK. In the laboratory, shells were scrubbed clean of all epibiota and stock mussels were kept for two weeks in an aerated, circulating natural, seawater system (10 °C, salinity 32-34‰) equipped with a biofilter. Mussels were initially fed *Phaeodactylum tricornutum,* but 1 week prior to the experiments the mussels were not fed. For experiments in which mussels were exposed to hyperammonia, they were maintained in oxygenated and ammonia-enriched (3 mM) seawater which was prepared by making appropriate dilutions of a stock solution of ammonium sulphate. Groups of mussels (n = 6 each) from this medium were removed after 2h, 24h, and 4 d for protein and GDH activity determinations. The ammonia-enriched seawater was replaced each day to ensure its constancy, and dissolved ammonia levels monitored at regular intervals using flow injection analysis (FIA) (Hunter & Uglow 1993). No significant ammonia changes were found up to 24 h of exposure. On each sampling occasion, six mussels from the control tank were taken in addition to the six treated mussels.

Enzyme extract preparation: Enzyme extracts were prepared by homogenising a weighed adductor muscle sample in the presence of aluminium oxide (to disrupt the cells) in 50 mM Tris maleate, pH 6.5. The resultant homogenate was clarified by centrifugation at 16,000 g for 30 min, and the supernatant was filtered through Sephadex G-25 to eliminate the endogenous ammonium ion and some metabolites.

Enzyme assays: GDH was estimated using the methods of Ruis Ruano *et al.* (1985) by measuring the rate of decrease (as only the GDH-NADH reaction was considered in this study) at an absorbance of 340 nm using Unicam 86 25 UV/VIS spectrophotometer connected to a Chessel chart recorder. GDH activity in the reductive step was estimated from the oxidation of NADH to NAD^+ (ε = 6.22 cm^{-1} / µmole at 340 nm).

The reaction mixture, in a final volume of 3 ml contained 100 mM potassium phosphate pH 8, 100 mM ammonium chloride, 5 mM ADP, 6.5 mM α-ketoglutarate (αKG), and 0.04 mM NADH. The reaction was initiated by the addition of the muscle extract and the GDH activity was determined from the slope of the initial velocity (Trevor 1985) recorded at 340 nm at 25° C. Results were expressed as international units (I.U. = µmole NADH oxidized min^{-1}).

Protein determination: Following denaturation, total protein was measured using the Biuret method. The copper ions in the Biuret reagent react with the peptides to form a purple colour with an absorbency maximum at 540 nm. The intensity of the colour is proportional to the total protein concentration. The protein in the supernatant was denatured using the method of Gallagher *et al.* (1984), by adding 50 µl of a 4 N NaOH solution to 1 ml to the aliquot sample, this mixture was left for 1 h then centrifuged at 3000 g for 6 min. Following this extraction, the protein was analysed with a SIGMA diagnostics kit (Procedure No. 541). The enzymatic activity

was expressed as μmole min^{-1} mg^{-1} protein (Bergmeyer 1974).

Normally-distributed data ($P > 0.05$, K-S Lilliefors test) of GDH activity were subjected to analysis of variance (one way ANOVA) using SPSS (Subprogram of the statistical Package for the Social Sciences) on a PC. The level of significance for the F test used in conjunction with ANOVA variance data was at the 95% level of confidence.

RESULTS

Although the method of Ruis Ruano *et al.* (1985) was used in these studies, it was necessary to determine the validity of its use with the mollusc adductor muscle assays made here, as compared with the hepatopancreatic tissue used by Ruis Ruano and co-workers. Figure 1A shows the relationship between pH and GDH activity assayed in the reductive direction. The pH optimum of the reaction in glutamate formation was in the range 7.3 – 8.1. The effect of ADP, a GDH activator, on the glutamate-forming activities was found (Fig. 1B) to exhibit a hyperbolic kinetic pattern, with maximal activity occurring in the presence of 5mM ADP.

Optimal conditions of the extract assay for the NADH-dependent GDH activity were obtained using phosphate buffer. Enzyme saturation was obtained with 40 μM NADH but enzyme activity decreased at NADH concentrations > 200 μM (Fig. 2A). Figure 2B gives a graphical Michaelis-Menten plot for α-KG. The enzyme saturation by α-KG was obtained with 6.5 mM concentration of substrate. From the results described above, standard assay conditions were determined as those described the Methods section.

The mean reductive activity of GDH measured in extracts of adductor muscles in the glutamate-forming direction of the control group was 3.62 ± 0.011 μmoles $min^{-1}mg^{-1}$. protein under the extract assay conditions (n = 6). A summary of the enzyme activity of the control, 2 h, 24 h and 4 d ammonia-treated groups is given in Table I. A significant ($P < 0.05$) increase in the glutamate-forming GDH activity was found after 2 h of the hyperammonia treatment. The 24 h-treated group showed a slight, non-significant ($P > 0.05$) change in GDH activity. The GDH activities of the control groups showed no changes ($P < 0.05$) over the 4 d period. The initial induced, elevated GDH activities of the treated groups (*ca* 43.1% higher than those of the control group) were maintained for 4 days and may reflect acclimation of the animals to the contamination of their environment.

Although the hyperammonia-treated groups showed significant alteration of the muscle reductive GDH activities, their muscle protein concentrations (13.84 ± 1.92% wet wt) did not show

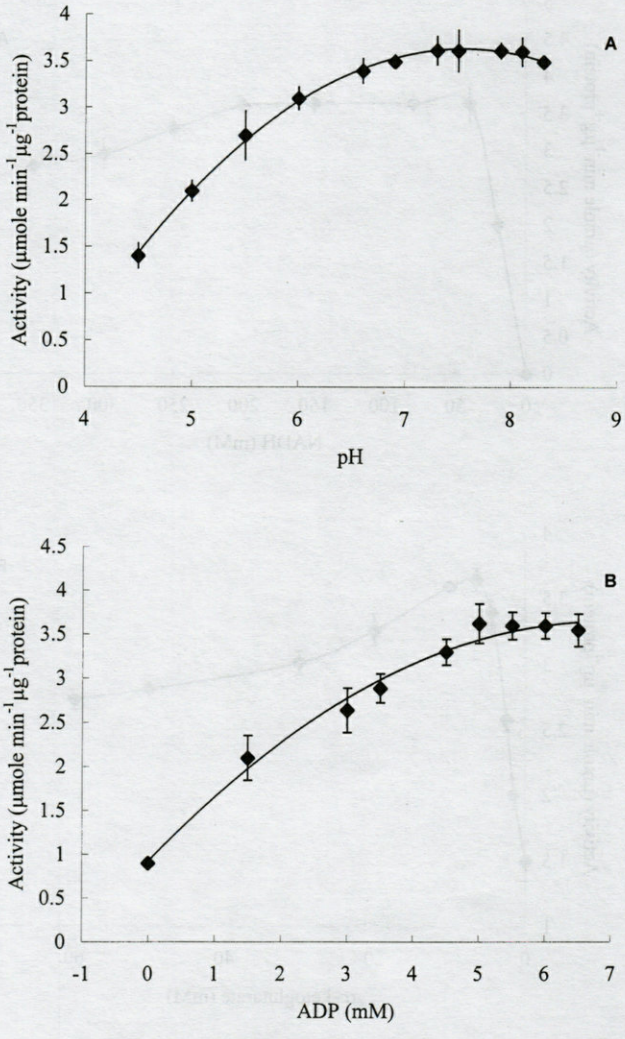

Fig. 1 – *Mytilus edulis*. A, pH and, B, ADP concentration effects on the reductive animation of α-ketoglutarate catalysed par GDH for the adductor muscle (vertical bars = SE, n = 6, each).

Table I. – *Mytilus edulis*. Glutamate dehydrogenase (GDH) activity in the adductor muscle of control and hyperammonia-exposed mussels. Values in column 3 are given ± SE (similar; a / different; b letters comparisons = $P > 0.05$ / $P < 0.05$, One Way ANOVA).

Exposure time to Ammonia enriched Seawater (3 mM)	GDH activity μmole min. $^{-1}mg^{-1}$ pr	SE (n=6)
(Control) 0h	3.629[a]	0.011
2h	6.890[b]	0.415
24h	7.102[b]	0.095
4 days	7.352[b]	0.162

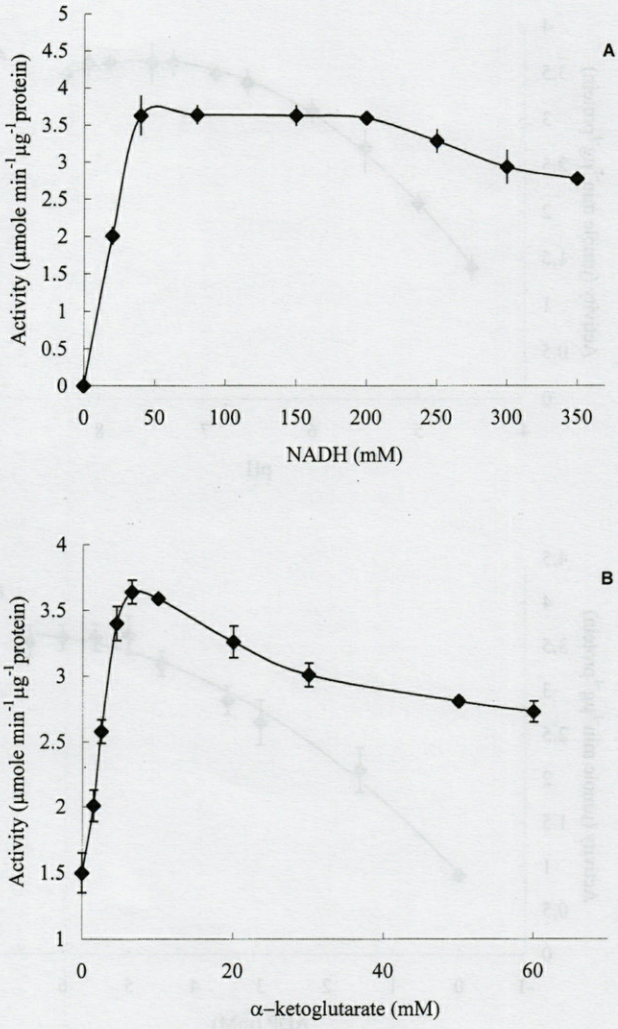

Fig. 2 – *Mytilus edulis*. A, Effects of NADH and, B , α-ketoglutarate concentrations on GDH activity in the adductor muscle (vertical bars = SE, n = 6, each).

significant ($P > 0.05$) changes over the experimental period, despite a lower mean protein concentration measured in the 4 d-exposed group (12.03 ± 0.87% wet wt).

DISCUSSION

The levels of GDH in *Mytilus edulis* adductor muscles measured here (Table I), and those measured in other bivalves, are low in comparison with those of cephalopod tissues (Bishop *et al.* 1983).

Although GDH is considered to be a key enzyme associated with nitrogen metabolism (Batrel & Le Gal 1983) and ammonia represents the bulk of nitrogen excreted (Hawkins & Bayne 1992), few data are available concerning the enzymes associated with nitrogen metabolism in *M. edulis*. Abundant information about GDH activities in other invertebrates is available and a significant relationship exists between ammonia excretion and GDH activity in some crustacean (e.g. *Praunus flexuosus*, Bidigare & King 1981) and annelids (e.g. *Arenicola marina*, Batrel & Le Gal 1983). The paucity of information on *M. edulis* GDH may be related to the relatively low levels of this enzyme in the tissues of that species.

The regulation of GDH is complex (Frieden 1976). The enzymes of some invertebrates utilise either NAD^+ or $NADP^+$ as co-factors (Male & Storey 1983) and others are either $NADP^+$- (Male & Storey 1983) or NAD^+-specific (Reiss *et al.* 1977). Ruis Ruano *et al.* (1985) used different buffers, nucleotides and metabolic ions to demonstrate that *in vitro* preparations of *M edulis* hepatopancreas showed GDH-NAD^+/H activity but no detectable GDH-$NADP^+$ activity. The present findings on the effects of NADH on *M. edulis* adductor muscle GDH activity reveal a total dependence of this enzyme on its co-factor, as no activity was detectable in the absence of NADH (Fig. 2A).

The present study also reveals that ADP exerts an activity effect on the reductive reaction of GDH. The concentrations used lie within the physiological range of this nucleotide and, therefore, are possible physiological regulators. This agrees with the results found for the hepatopancreas of *M. edulis* (Ruis Ruano *et al.* 1985) and with the data found for some crustaceans (Regnault 1989). The role of nucleotides such as ADP on GDH activity has been interpreted as a regulatory link between amino acid production and protein synthesis (Bidigare & King 1981, Dieter *et al* 1981). Hence an activity effect of ADP could be related to energy metabolism, because low energy levels correspond to high ADP concentrations which would stimulate GDH activity and amino acids could be used for energy metabolism. Conversely as energy levels increase, GDH would be inhibited and protein synthesis stimulated (Dieter *et al.* 1981). This implies a role for GDH in the regulation of energy production and growth (Bidigare & King 1981)

Inhibition of GDH by the substrate α-KG occurred at relatively high (> 7.8 mM) concentrations of the substrate (Fig. 2B), which suggests initially high substrate levels in the tissues. These levels in turn may be related to the high activities of various transaminases such as glutamate-oxalacteate and glutamate-pyruvate transaminases as has been shown in *Mya arenaria* mantle tissue (Moyes *et al.* 1985). Under normal conditions, high tissue levels of α-KG would thus reflect periods of GDH inhibition and intense amino acid synthesis.

In the present study, *M. edulis* adductor muscle GDH activity was maximal in the pH range shown

in fig. 1A and these results are in accordance with those of Ruano *et al.* (1995). The *in vitro* pH found for *M. edulis* adductor muscle is close to the internal physiological pH (Zange *et al.* 1990) indicating that GDH is likely to be of functional significance in these bivalves. It was previously shown that some acidification occured in the tissues of mussels under hyperammonia (Sadok *et al.* 1995) which would probably be accompanied by a drop in intracellular pH. The decreased pH would favour ammonia fixation because the optimum *in vitro* pH values for the aminative activities of GDH were lower than those for its oxidative deamination activities (Ruis Ruano *et al.* 1985).

The lack of studies on the effect of hyperammonia on bivalve physiology (Sadok *et al.* 1995), has meant that the activities of enzymes involved in nitrogen metabolism have been largely overlooked. Controversy, evoked by the relative contributions of host tissues to measured ammonia uptake by some bivalve-symbiont associations (Rees 1991), has been largely resolved, because recent work has demonstrated the ability of host tissue to take up ammonia (Fitt *et al.* 1993) and the involvement of enzymes in the regulation of the metabolism of enhanced ammonia in host tissues (Rees *et al.* 1994, Lee *et al.* 1999). These last authors showed that the assimilation of exogenous, inorganic nitrogen by the host tissues, which showed significant activities of the ammonia-assimilating enzyme, glutamine synthetase (Rees *et al.* 1994). Rees *et al.* (1994) showed that, under ammonia enrichment, host tissue had a significantly lower glutamine synthetase activity than the control group in normal seawater, and concluded that ammonia played a role in regulating glutamine synthetase activity. On the other hand, these authors did not detect any GDH activity in the host tissue. The present study revealed a relatively low GDH activity in the adductor muscles of *M. edulis* kept in normal seawater, and the involvement of this enzyme in the assimilation of exogenous ammonia, because significantly higher GDH aminating activities were found in the tissues of mussels exposed to hyperammonia (Table I). In some pelagic and benthic bacteria, hyperammonia also induces a significant increase in the aminating activities of GDH (Hoch 1992). However, Anmuth *et al.* (1982) found a lowered GDH activity in the wood-boring and mud-burrowing bivalves, *Lyrodus pedicellatus* and *Solemya velum*, following long-term incubation in 50 µmole l^{-1} NH_4–N medium.

The reaction involving the α-KG, the NADH and NH_4^+ is also catalysed by inorganic ions (Hochachka & Somero 1973). This reaction could increase the production of glutamate in mussels exposed to ambient ammonia and, *via* the transaminase reactions, glutamate could give rise to other amino acids (such as alanine and glycine) because the requisite sets of aminotransferases have been found in *M. edulis* (Bishop *et al.* 1983). This may explain the increase in haemolymph amino acids (measured as ninhydrin positive substances, NPS) levels found in hyperammonia-treated mussels (Sadok *et al.* 1995). Moreover, the insignificant ($P > 0.05$) drop in protein content measured here during the ammonia treatment period (5.89 ± 0.52% wet wt, after 4 d), emphasises this assumption as, in this case, catabolism of protein did not contribute to the observed rise in NPS levels.

Given the higher GDH activity found under hyperammonia it is likely that, in the event of a short-term increase in ammonia availability, mussels would have the potential to assimilate most of the nitrogen present. However, in the persistent presence of high ammonia levels, other metabolic pathways may be used, as GDH activity remained constant under hyperammonia. Furthermore, several mechanisms may operate simultaneously within an individual in order to maintain physiological homeostasis. Such diversity emphasises the complex nature of strategies which have evolved in mussels in order to enable them to withstand high levels of ammonia in their environment.

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A COMPARISON OF THE ECOPHYSIOLOGICAL RESPONSE ON COPPER IN BALTIC CLAMS FROM DIFFERENT POPULATIONS IN EUROPE

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BIOACCUMULATION
METAL
COPPER
METALLOTHIONEIN
STRESS SENSITIVITY
BIVALVE
MACOMA BALTHICA
ARCTIC

ABSTRACT. – Differences in performance and bioaccumulation of copper, metallothionein-like protein (MTLP) levels and resistance in Baltic clams, *Macoma balthica*, from Arctic, subarctic and temperate areas were determined during a stress period caused by starvation and exposure to copper. Although the conditions at the start were different, the losses of weight and mortality rates were in general comparable in clams from all areas. In contrast to expectation, the accumulation of copper in (sub)Arctic clams was much faster than in temperate specimens, whereas the level of MTLP in all populations hardly increased. Copper was primarily accumulated in insoluble form: MTLP has in clams no major role in copper sequestration. It is suggested that the differences in copper accumulation rates between populations might be related to genetic (racial) differentiation.

BIOACCUMULATION
MÉTAL
CUIVRE
MÉTALLOTHIONÉINE
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MACOMA BALTHICA
ARCTIQUE

RÉSUMÉ. – La performance et la bioaccumulation du cuivre, les niveaux de protéines type métallothionéine et la résistance des Bivalves *Macoma balthica* ont été comparées dans des spécimens de zones Arctique, subarctique et tempérée durant une période de stress dûe au jeûne et à l'exposition au cuivre. Bien que leur condition soit initialement différente, les pertes de poids et les taux de mortalité sont en général comparables dans les Bivalves des trois zones. Contrairement à ce qui était attendu, l'accumulation du cuivre dans les Bivalves subarctiques est plus rapide que dans les spécimens de la zone tempérée tandis que le niveau de PTM dans toutes les populations augmente peu. Le cuivre est principalement accumulé sous forme insoluble : les PTM n'ont pas un rôle majeur dans la séquestration du cuivre chez ces Bivalves. Il est suggéré que les différences interpopulationnelles dans les taux d'accumulation du cuivre pourraient être liées à une différenciation génétique (raciale).

INTRODUCTION

In a series of field surveys and experiments with the Baltic clam *Macoma balthica*, covering its total European distribution range from south (SW France; Bachelet 1980) to north (N. Russia; Hummel *et al.* 1997a) (Fig. 1), the sensitivity to stressors was determined. It was found that different types of stress, as e.g. starvation, extreme temperatures or copper exposure, could have the same impact (Hummel *et al.* 1995, 1996). Due to a stressor the performance of the clams was hampered in a similar way when expressed in ecophysiological terms as growth or survival. Even changes in the genetic constitution, e.g. out-selection of some specific genotypes, were similar under different types of stress.

Strong differences in the reactions to stress were obtained when testing clams from different regions in Europe. These differences were caused by comparing different genetic ecotypes (Fig. 1) or comparing specimens from a place central in the species distribution with those living at the limit of their distribution. Animals at the limits of their distribution are often thought to be more sensitive to disturbances than their conspecifics from areas more central in their distribution (Conover 1978, Hoffmann & Parsons 1991). Such is the consequence of living at the limits of a species' adaptation capacity, whereby the performance, such as growth or fitness, becomes poor. It was found that this hypothesis was valid too for the Baltic clam, at least at its southern distribution limit (Hummel *et al.* 1995, 1996, 1997a, 1998). However, in the northern (subarctic and Arctic) area the animals were equally or less sensitive to disturbance by heavy metals (copper) or by starvation than those from central places (Hummel *et al.* 1997a, 1998). Differences in metabolic rate, genetic constitution, or sequestration of metals in the animals were suggested as an explanation of the low sensitivity to stress for (sub-)Arctic animals.

Support for a low metabolism in Arctic specimens was found in their very low annual growth, but long lifespan (Hummel *et al.* 1998). Could it be that such a low metabolism is also reflected in slow accumulation kinetics of metals in (sub-)Arctic clams and thereby in a low sensitivity to copper exposure?

Additionally, Arctic specimens were shown to have different genetic traits from clams of temperate areas, being a different subspecies, whereas those of subarctic areas could be called a different race (Hummel *et al.* 1997a). Could it be that the different genetic constitutions coincide with different biochemical defence strategies as with regard to e.g. metallothioneins (MTs)? For the sequestration of metals, clams from temperate areas probably lack a well-developed physiological regulatory mechanism to control copper concentrations over the short term (weeks), although with regard to the presence of metallothioneins (MT) in clams conflicting results have been obtained (Langston & Zhou 1987, Bordin *et al.* 1994, 1997, Mouneyrac *et al.* 2000).

Yet, it might be that latitudinal gradients in the presence/absence of MT in clams could be the reason for the observed differences in sensitivity to contaminants.

On basis of the above suppositions it was hypothesized that when stressing Baltic clams by starvation and/or copper exposure, the specimens from temperate areas would show a better performance, e.g. lower mortality and a lower decrease of the weight, than Arctic specimens, whereas during a period with copper exposure Arctic clams would show lower copper accumulation (rates) and/or a more enhanced increase of MT-like proteins than temperate specimens. For subarctic specimens performance and intermediate copper accumulation (rates) are expected, since they belong to the same race as the temperate specimens (Hummel *et al.* 1997a), yet, live under almost similar extreme climatic conditions as the Arctic specimens.

Therefore, in this comparative study we tried to assess simultaneously for clams from Arctic, subarctic and temperate areas the differences in performance under stress (starvation and/or copper exposure) and differences in bioaccumulation strategies and metallothionein levels at copper exposure.

MATERIAL AND METHODS

Sampling and experimental exposure: In the framework of an INTAS mission (International Association for the Promotion of Cooperation with Scientists from the Independent States of the former Soviet Union; project 94-391), Baltic clams, *Macoma balthica*, were taken alive from the Arctic (29-7-1997; Khaypudyr, Pechora Sea; 68° 39.8' N, 59° 50.7' E; temperature 9 °C, salinity 32; Cu 1.6 ppm in sediment (Amiard *et al.* 1998)), the subarctic (11-8-1997; Kartesh, Chupa Bay, White Sea; 66° 0.2' N, 33° 39.0' E; temperature 12 °C, salinity 25; Cu 10.6 ppm in sediment (Amiard *et al.* 1998)) and a station in temperate areas (13-8-1997; Paulina, Westerschelde, the Netherlands; 51° 21.6' N, 3° 42.7' E; temperature 19 °C, salinity 15; Cu 7.9 ppm in sediment) (Fig. 1). During transport to the Netherlands Institute of Ecology at Yerseke, the animals were kept in wet tissue-paper at a temperature of 0 to 5 °C in a transportable cooling unit till the start of the experiment. On 21-8-1997 the bivalves were introduced into aquaria of 1 l with sea-

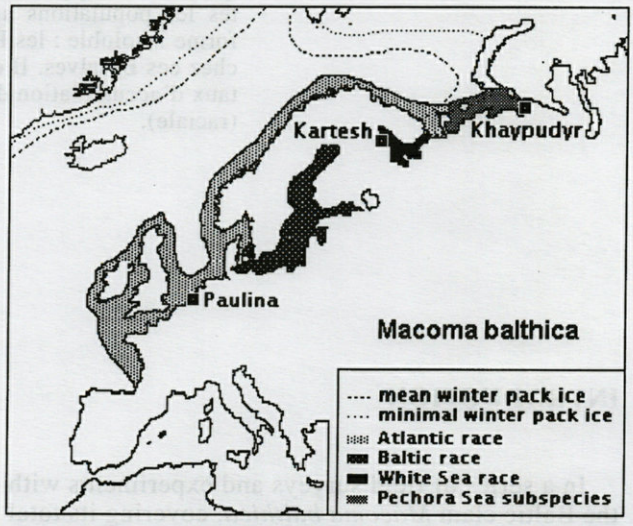

Fig. 1. – Distribution of the different ecotypes of the Baltic clam *Macoma balthica* in Europe and the location of sampling stations for animals used in the experiment on Cu accumulation and metallothionein.

Table I. – Sampling and experimental exposure to copper (Sm: clams sampled when reaching 50% mortality, S: clams sampled before 50% mortality was reached; in the controls no mortality occurred; 50, 100: exposure to 50 and 100 ppb Cu).

Exposure period (days)	Date	Paulina (temperate)			Kartesh (subarctic)			Khaypudyr (Arctic)	
	29/07/97							sampling	
	11/08/97				sampling				
	13/08/97	sampling							
	Starvation period	8 days			10 days			23 days	
0	21/08/97	control	50	100	control	50	100	control	100
		↓	↓	↓	↓	↓	↓	↓	↓
13	2-4/09/97	S	S	S	S	S	Sm	S	Sm
		↓	↓	↓	↓	↓			
18	8-9/09/97	↓	↓	Sm	S	Sm			
		↓	↓		↓				
28	18/09/97	S	Sm		S				

water of 30 ppt salinity at a constant temperature of 12 °C, and 6 hours later copper was added at nominal concentrations of 50 and 100 ppb to test the animals for their resistance to copper (Table I). The water in the aquaria was changed twice a week. No food was administered to prevent too strong complexation of copper. Due to complexation, e.g. adsorption to the walls, the average copper concentration in our aquaria is 50 to 75% of the nominal concentration (Hummel *et al.* 1995, 1997a).

The rather high Cu exposure concentrations were chosen since in preliminary experiments the sub-Arctic specimens were shown to survive more than 3 to 4 weeks at concentrations ranging from 25 to 75 ppb Cu.

Fifty specimens originating from the subarctic Kartesh and temperate region (Paulina) were exposed to each treatment in 2 duplicate aquaria. Due to the restricted number of specimens, twenty clams from the Arctic Khaypudyr were exposed to the highest dose only and the treatment was not duplicated. Identical groups of clams were kept under the same laboratory conditions as controls. Moreover, ten specimens from each site were taken at the start of the exposure period (Table I).

Mortalities were registered daily. No mortality occurred in controls. For each treatment and each geographical site, the experiment was stopped at the moment when 50% mortality was reached (Table I). Additionally some specimens were taken in the different experimental groups far before some mortality was found in order to unravel the copper accumulation kinetics (Table I). The samples were lyophilized till further determinations.

Compartmentation: The length of the shell, down to the nearest 0.1 mm by means of a caliper, and the dry weight of soft tissues, after 3 days of lyophilization, was determined in all the individuals. Soft tissues of bivalves were individually homogenized in 50 mM Tris-NaCl (pH = 8.6) buffer (25:1, v:w) in an ice bath. The soluble (S1) and insoluble fractions (P1) were separated by centrifugation at 25 000 g for 1 hr at 4 °C. Tris contained 10^{-5} mM ß-mercaptoethanol to avoid the formation of disulfide bridges between MT molecules. An aliquot of the supernatant was submitted to heat denaturation (75 °C for 30 min) and then centrifuged (15 000 g for 10 min at 4 °C). The supernatants S2 recovered after heat denaturation were stored (at least 1 week) at –20 °C until MTLP quantification.

Metallothionein-like protein (MTLP) analysis: The amount of MTLP has been determined by differential pulse polarographic analysis (DPP). This technique is based on –SH compound determination according to the Brdicka reaction (Brdicka 1933) as described by Thompson & Cosson (1984). The PAR Model 174 analyser, the PAR/EG&G Model 303 static mercury drop electrode (SMDE) and an X-Y recorder (RE 0089) were used. Metallothionein (MT) of rabbit liver (Sigma Chemical Co., St. Louis, MO) was used to carry out the calibration according to the method of standard additions. The system consisted of a bevelled capillary mercury working electrode, a platinum counter electrode and an Ag/AgCl reference electrode. The polarographic determination in heat-denaturated cytosol is an analytical procedure based on several characteristics of MTs but does not allow the assertion that the studied molecule is a true MT since purification and sequencing have not been carried out. Thus later on, the terminology of metallothionein-like protein (MTLP) will be preferred and results will be expressed as concentrations: mg of MTLP per gram dry weight of homogenized tissue.

Metal analysis: Nalgene bottles were used to store all reagents. All labware was soaked in 10% hydrochloric acid, rinsed 3 times with deionized water and dried in a desiccator sheltered from atmospheric dust. Pellets (P1) were heated (65 °C, 24 h) with nitric acid 65% (RPE Carlo Erba). After digestion, the volume was adjusted to 5 mL with deionized water. The supernatant was digested with nitric acid (1 mL per 1 mL S1) at 100 °C for 1h then at 120 °C till drying was completed. The residue was solubilized in 0.1 mL nitric acid 65% + 0.4 mL deionized water. Metal levels in these acid solutions were determined by flame (clams experimentally exposed to Cu) or flameless (controls) atomic absorption spectrophotometry using Zeeman effect (HITACHI Z 8 200). The laboratory is involved in procedures of internal quality controls based on standard reference materials (CRM 278 Mussel tissue, SRM 1566 Oyster tissue) and in external intercalibrations under the I.A.E.A.

Statistical treatment: Differences between stations or treatments were evaluated by analysis of variance (ANOVA) and post hoc comparisons assessed by the Multiple Ranges Test of Scheffé. These tests as well as paired t-tests and linear regressions were carried out using standard statistical packages (Systat (Wilkinson 1988) and StatView SE + Graphics^{tm}).

RESULTS

Mortality

Clams experienced different starvation periods, depending on the period of transport (Table I): Arctic (Khaypudyr) 23 days, subarctic (Kartesh) 10 days and temperate region (Paulina) 8 days. As it became clear from previous experiments (Hummel *et al.* 1995, 1996) different stressors may yield the same impact on the performance of Baltic clams, and therefore it might be that the starvation period during transport and the period of copper exposure should be added.

At 100 ppb copper the mean lethal time (MLT) of the animals from the Arctic Khaypudyr was 13 days (35 days including the starvation period), for those from the subarctic Kartesh 12 days (22 days including the starvation period), and from the temperate region (Paulina) 17 days (25 days including the starvation period). The survival period thus does not differ strongly between locations, being slightly higher at Paulina when excluding the starvation period, and higher at Khaypudyr when including the starvation period.

At 50 ppb the MLT of the subarctic Kartesh specimens was 18 days (28 days including the starvation period), and of those from the temperate region (Paulina) 28 days (36 days including the starvation period) (no animals from the Arctic Khaypudyr were available). At the lower copper concentration, the temperate specimens thus showed a slightly better performance.

Biometric data

Clams originating from the temperate region had a higher shell length than specimens taken from Arctic (Khaypudyr) and subarctic (Kartesh) sites (Fig. 2). The intersite differences were enhanced when the weight of soft tissues was considered (Fig. 2). Yet, a proper comparison should be made on basis of the weight-index, i.e. the weight per volume ratio (estimated by DW / (length)^3; Hummel *et al.* 1996, 1997b), which is a better indicator of the (differences in) condition of an animal. The weight-indices of the clams differed significantly between stations (Table II; $p < 0.001$). Clams from the temperate region showed the highest weight index, whereas subarctic and Arctic specimens had a 40% lower weight index (Fig 2).

With an increase of the experimental period the weight-index significantly decreased (Fig. 2; Table II). With exposure to copper, the weight-index showed a significant stronger trend to decrease (Fig. 2, Table II). However, the degree of change in the weight-index is not different between clams from different locations.

Table II. Significance of the effects of origin (=station), exposure to copper (=copper) and exposure periods (=period) on the length, weight-index, soluble and insoluble copper and metallothionein concentrations in clams (ANalysis Of VAriance, General Linear Model (Wilkinson 1988); –: non significant, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$).

	Stations	Copper	Period
Length	***	–	–
Weight-index	***	**	***
Soluble copper	***	***	***
Insoluble copper	***	***	***

Bioaccumulation of copper

Whatever the geographic origin, exposure to copper induced a bioaccumulation of this element in soft tissues. Copper concentrations reached in Arctic and subarctic samples were approximately three times higher than in temperate clams (Fig. 3). Such can not have been caused by the difference in weight index, which was in (sub-)Arctic specimens 40% lower; thus would have accounted for only 1.7 times higher copper concentrations (and not 3 times higher).

When exposed to a twofold higher copper concentration in the external medium (100 vs 50 ppb Cu), the subarctic clams showed after 13 days the same ratio in their soft tissues (167 vs 86 ngCu.mg^{-1}), whereas in specimens originating from the temperate region, copper accumulation under both regimes remained limited (45 vs 31 ngCu.mg^{-1})(Fig. 3).

A preferential storage of copper in insoluble form was observed in clams originating from all three sites (Fig. 4; at start as well as in controls and in specimens exposed to copper; paired t-tests, $p < 0.001$, in controls $p = 0.01$).

Both soluble and insoluble copper concentrations in clams differed significantly between stations (Table II). Concentrations were significantly higher in subarctic (Kartesh) clams compared to specimens from the temperate site (Paulina) or the Arctic site Khaypudyr (Fig. 4; as well as during the start as in controls and in copper exposed animals; $p < 0.001$ for both Cu doses and both physico-chemical forms of storage). Only when exposed to 100 ppb copper, the Arctic clams even reached the same insoluble copper concentrations as subarctic clams (for both being significantly higher ($p < 0.001$) than temperate specimens) (Fig. 4).

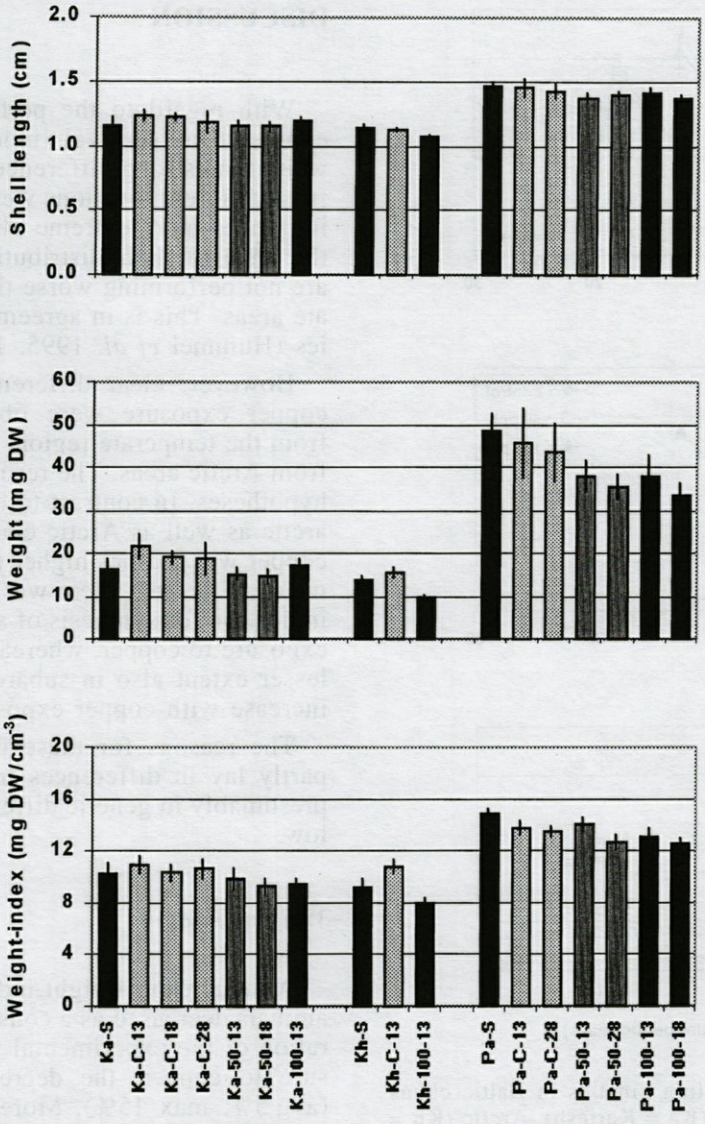

Fig. 2. – Mean shell length, lyophilized soft tissue weight and weight-index of Baltic clams (standard error) originating from subarctic (Ka = Kartesh), Arctic (Kh = Khaypudyr) and temperate areas (Pa = Paulina), and exposed or not to copper (C = control, 50 = exposed to 50 ppb Cu, 100 = exposed to 100 ppb Cu) for different periods (S = start = specimens lyophilized at the beginning of the experiment, 13,18,28=days of experimental exposure).

MTLP levels

As for copper concentrations, MTLP levels differed significantly between stations (Table II), and the subarctic clams (Kartesh) were characterized by the highest levels of MTLP (Fig. 5).

For all the three studied sites, MTLP levels at start were significantly higher than the level determined in controls maintained under experimental conditions over the different durations (Fig. 5; $p < 0.001$).

Significant higher MTLP levels were found in specimens exposed to either of the copper doses than in the controls (Fig. 5; Table II). These results were partly corroborated by the relationships between copper and MTLP concentrations in the animals (Fig. 6). In the specimens from the temperate region (Paulina) (apart from the specimens measured at the start) a strong positive correlation ($n = 72$, $r = 0.41$, $p < 0.001$) was shown. Higher MTLP levels coincided with higher internal copper concentrations. Specimens from the subarctic Kartesh showed only a weakly significant relation between copper and MTLP tissue levels ($n = 62$, $r = 0.26$, $p = 0.05$) (Fig. 6). Yet, the slightly higher MTLP level for specimens from the subarctic Kartesh at Start, in comparison to the level of

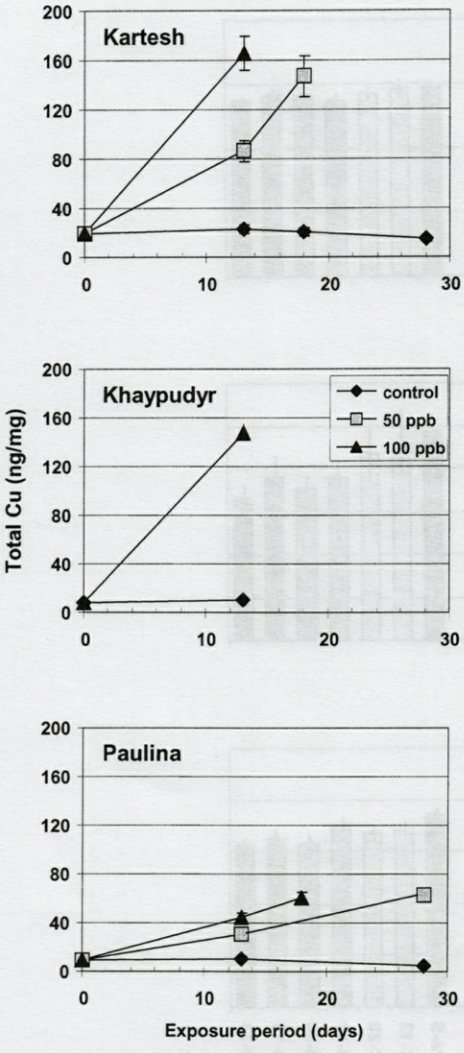

Fig. 3. – Copper accumulation kinetics in Baltic clams originating from subarctic (Ka = Kartesh), Arctic (Kh = Khaypudyr) and temperate areas (Pa = Paulina), and exposed or not to copper (means and standard errors).

specimens from the other locations (Fig. 5), may thus be related to the higher copper concentration in those clams (Fig. 3). Such a slightly higher copper concentration for animals from the area of Kartesh was already observed by Amiard-Triquet *et al.* (1998) and Regoli *et al.* (1998), and could be contributed to a higher copper concentration in the sediment (see also M&M) as a consequence of moderate industrial wastewater discharges in the near neighbourhood.

Specimens from the Arctic Khaypudyr showed a non-significant trend-line between copper and MTLP tissue levels ($n = 16$, $r = 0.32$, $p > 0.10$).

Irrespective the increase due to copper exposure, in all cases the increased MTLP levels due to copper exposure remained far below the MTLP level in the specimens directly at Start.

DISCUSSION

With regard to the performance during stress, expressed as survival (mortality) or (changes in) weight, no strong differences between Baltic clams from different locations were found. Thus even being under more extreme climatic conditions, or at the edge of their distribution, (sub-)Arctic clams are not performing worse than clams from temperate areas. This is in agreement with previous studies (Hummel *et al.* 1995, 1996, 1997a, 1998).

However, clear differences in the reaction to copper exposure were observed between clams from the temperate region, those from subarctic or from Arctic areas. The results were opposite to our hypotheses. In contrast to our expectations in subarctic as well as Arctic clams the accumulation of copper was 3 times higher than in temperate specimens. Moreover, there was in Arctic specimens no indication for synthesis of additional MTLP during exposure to copper, whereas in temperate, and to a lesser extent also in subarctic, specimens a slight increase with copper exposure was observed.

The reasons for these unexpected results may partly lay in differences in the weight index and presumably in genetic differences, as explained below.

Weight-index

Although the weight-index of the experimental animals decreased as a consequence of both the duration of the experimental period as well as exposure to copper, the decrease remained minimal (avg 5%, max 15%). Moreover, the weight index did not reach the level observed in clams living at the limits of their adaptation capabilities (Hummel *et al.* 1995, 2000). A weight index of 5 mg DW/cm^3 was suggested as the minimum value below which the metabolic energy balance of Baltic clams becomes negative, and clams die. In all cases the weight-index remained 8 mg DW/cm^3 or higher. The observed subtle changes in the weight index during the experiment therefore do not help to explain differences in Cu accumulation.

Copper accumulation and MTLP

The lowest bioaccumulation rates of both cytosolic and insoluble copper were determined in clams originating from the temperate site. Similar low copper accumulation (and elimination) rates in Dutch (= temperate) Baltic clams, lasting for several weeks before reaching equilibrium, were observed by Absil *et al.* (1996). At the other hand, the (sub-)Arctic clams showed higher accumulation rates.

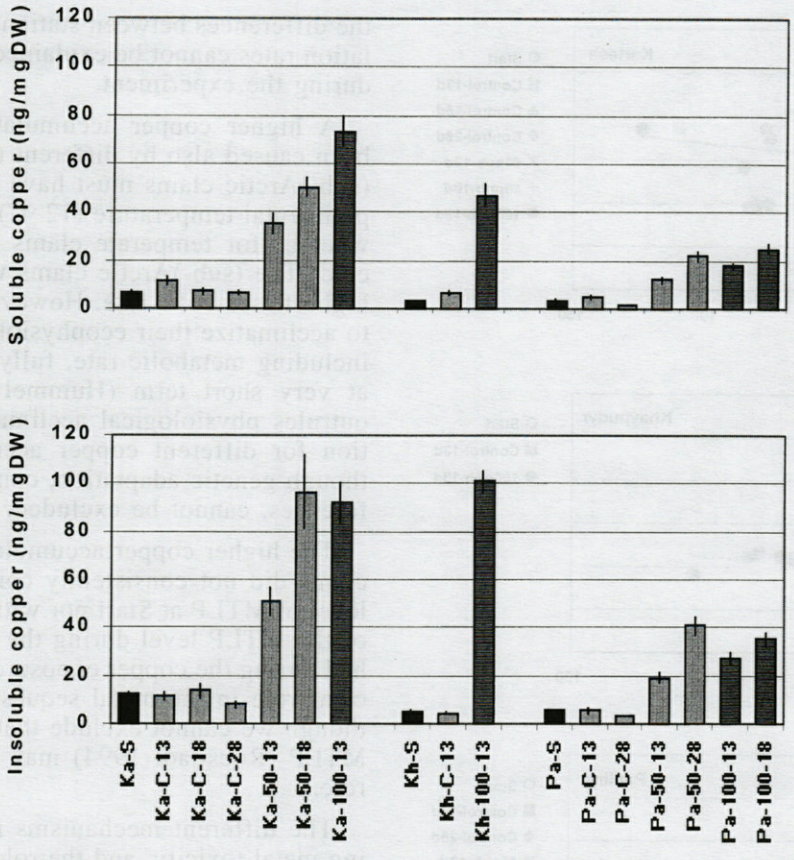

Fig. 4. – Concentrations of soluble and insoluble copper ($ng.mg^{-1}$) in soft tissues of Baltic clams originating from subarctic (Ka = Kartesh), Arctic (Kh = Khaypudyr) and temperate areas (Pa = Paulina), and exposed or not to this metal (abbreviations as in Fig. 2).

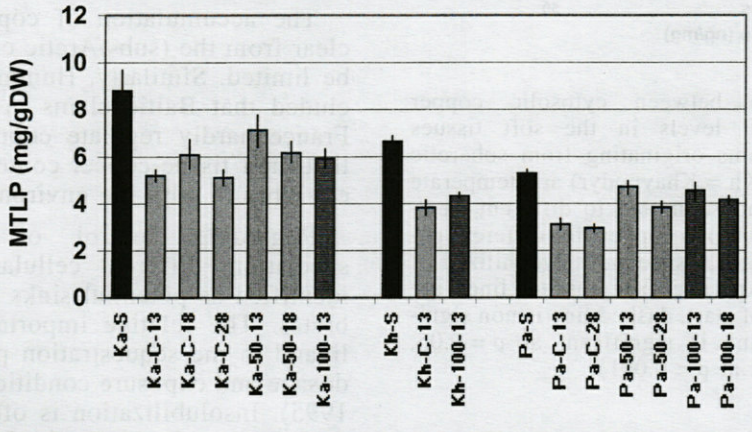

Fig.5. – Metallothionein-like-protein concentrations (MTLP; $mg.g^{-1}$) in soft tissues of Baltic clams originating from subarctic (Ka = Kartesh), Arctic (Kh = Khaypudyr) and temperate areas (Pa = Paulina), and exposed or not to copper (abbreviations as in Fig. 2).

It might be thought that the higher accumulation rates of (sub-)Arctic clams could be related to a higher mortality rate. However, for such we thus have no indication. Similarly, in Baltic clams originating from the Bay of Somme and the Loire estuary (France), specimens that survived Ag or Hg exposure at LT50 did not protect themselves against metal toxicity by accumulating a significantly lesser amount of these metals than clams that did not survive metal stress (Boisson *et al.* 1998).

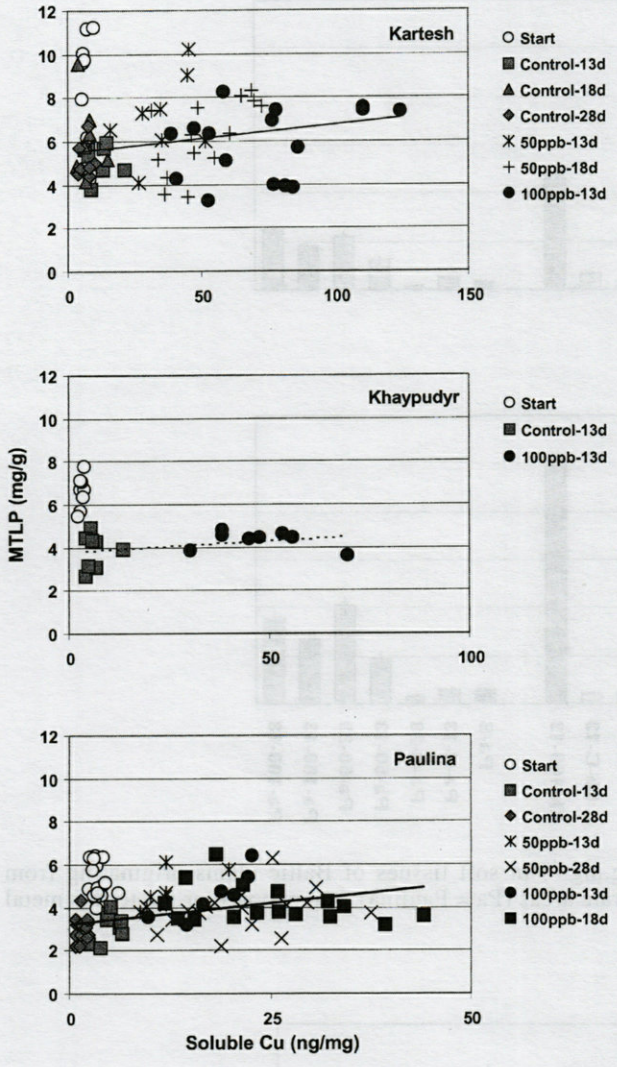

Fig. 6. – Relationship between cytosolic copper (ng/mgDW) and MTLP levels in the soft tissues (mg/gDW) of Baltic clams originating from subarctic (Ka = Kartesh), Arctic (Kh = Khaypudyr) and temperate areas (Pa = Paulina), and submitted to different treatments (control, 50 and 100 ppb copper) for different periods (13,18,28 days; start = specimens lyophilized at the beginning of the experiment)(regression lines are calculated without data of start, dashed line is non significant trendline, thin line is significant at $p = 0.05$, strong line is significant at $p < 0.001$).

In the short-term (within days or some weeks), changes in tissue copper concentrations may also be caused by changes in the weight-index. Meagre specimens were shown to have a higher Cu concentration, and heavier specimens lower Cu concentrations, in such a way that the Cu body burden in clams from a certain population was similar when viewed within a short period (Hummel *et al.* 1997b). However, because in this study changes in the weight-index remained minimal for all stations, the differences between stations in copper accumulation rates cannot be explained by weight changes during the experiment.

A higher copper accumulation rate may have been caused also by different metabolic rates. The (sub-)Arctic clams must have experienced the experimental temperature (12 °C) as relatively warm, whereas for temperate clams it would have been cold. The (sub-)Arctic clams would have then the highest metabolic rate. However, clams are shown to acclimatize their ecophysiological performance, including metabolic rate, fully to a new condition at very short term (Hummel *et al.* 2000). This outrules physiological acclimation as an explanation for different copper accumulation rates, although genetic adaptation, connected to racial differences, cannot be excluded.

The higher copper accumulation in (sub-)Arctic clams did not consistently coincide with a higher level of MTLP at Start nor with a stronger increase of the MTLP level during the experiment. MTLPs had during the copper exposure experiment thus no clear role in the metal sequestration of clams, although we cannot exclude that a rapid turnover of MTLP (Roesijadi 1994) may have obscured their role.

The different mechanisms involved in preventing metal toxicity, and the role of metallothionein, have been reviewed before (Mason & Jenkins 1995, Amiard & Cosson 1997) and fall broadly into one of two basic strategies: limiting metal accumulation and controlling intracellular metal speciation.

The accumulation of copper, as was at least clear from the (sub-)Arctic clams, seems hardly to be limited. Similarly, Hummel *et al* (1997b) concluded that Baltic clams in the Netherlands and France hardly regulate copper accumulation and that their tissue copper content is in (partitioning) equilibrium with the environment.

Regarding control of intracellular metal speciation, different cellular ligands have been identified as potential sinks for metals in invertebrates. The relative importance of each type of ligand in the sequestration process can vary with dosage and exposure conditions (Mason & Jenkins 1995). Insolubilization is often the major way of detoxication. In this study the storage of copper in clams was also mainly in the insoluble fraction of the soft tissues. This coincides with the general observation that in European clams MTs seem not to play a substantial role in metal complexation (storage) in clams, whereas the major part of the incorporated metal becomes associated with high molecular weight proteins or mineral granules (Langston & Zhou 1987, Hummel *et al.* 1997b). Lately the presence of excess copper in lysosomes of clams from France exposed to copper showed that these organelles are able to play a role in detoxifying

ions in excess in the medium through precipitation (Jeantet, pers Comm). Yet, in a previous study on Arctic bivalves a relationship between MTLP and copper levels was shown, suggesting that a detoxication process was at work in the studied species, including *M. balthica* (Amiard-Triquet *et al.* 1998). Moreover, in Baltic clams originating from the same site in the Netherlands and exposed to a mixture of 100 ng $Cd.mL^{-1}$, 100 ng $Cu.mL^{-1}$ and 600 ng $Zn.mL^{-1}$, Bordin *et al.* (1994, 1997) showed increased concentrations of metal-binding proteins displaying several of the main characteristics of MTs. The conditions differed from those used in the present study by the presence of noticeable amounts of Cd and Zn besides Cu but the duration of exposure was considerably shorter (2 days). At longer exposure the threshold level of metals to induce effects generally decreases (Amiard *et al.* 1987). Yet, in our study with a much longer exposure period and still a similar amount of copper as used by Bordin *et al.* the increase of MTLP levels was still modest.

Thus, although MTLP production by clams has been shown also to occur in our study, as in some other studies, the minor role of MT(LP)s in the sequestration of copper remains most apparent. The fact that MTLP levels at Start were even higher than those exposed to copper for 4 weeks contributes to this thought.

The decrease of MTLP concentrations in the control animals, when compared to the concentrations at start, may have been caused by the long period of transport and the subsequent submersion in clean seawater. Indeed, MTLP is reported to be formed also in response to general stress factors including injuries, handling, starvation and laboratory manipulation (George 1990, Roesijadi 1992, Kägi 1993). During the transport, being out of the water, intracellular processes and the excretion of (MTLP) degradation products will have been hampered. Only during the course of the experiment, by submersion in water, the animals might have eliminated the stored MTLP again. Similarly, Wrench (1978) had shown that controls of the oyster *Ostrea edulis* submerged in water had significantly reduced soluble protein level of both gills and digestive gland. Therefore, the long transport of the clams used in our study by itself may have generated a higher MTLP level.

CONCLUSION

When taking the starvation (= transport) period into account, the results showed that the mortality and changes in weight-index during the copper exposure experiment were comparable for temperate as well as subarctic and Arctic clams. Such is in accordance with our hypothesis. However, in opposite to our hypotheses, copper is accumulated faster in (sub-)Arctic clams than in temperate clams and MTLPs have no major role in the sequestration of copper. At all stations, copper is accumulated primarily in insoluble form. Since we could not exclude genetic adaptation as a differentiating mechanism, we conclude that racial differences between the populations, as observed by Hummel *et al.* (1997a), may be causative for the comparable performance of (sub-)Arctic clams irrespective of a much higher copper accumulation rate. Further studies are needed to unravel the ecophysiological (detoxification and tolerance) mechanisms that differentiate the temperate and (sub-)Arctic clams.

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TIME, SPACE AND THE ECOPHYSIOLOGY OF SQUID GROWTH, LIFE IN THE FAST LANE

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SQUID GROWTH
STATOLITHS
AGE VALIDATION
METABOLISM
TELEMETRY

ABSTRACT. – Squids are important components of many marine ecosystems and continue to come under increasing commercial fishing pressure. In some heavily fished regions, squid have replaced their teleost competitors. They achieve this through rapid growth and short life spans. Valuable insights have been made regarding squid life histories by both statolith ageing studies and culture experiments where growth could be observed in the laboratory. These studies continue to reveal that most species of squid live for a year or less and there is only evidence from a small number of species for life spans longer than a year. Small warm water species can complete their life spans in just a few months. While there has been some recently published statolith increment validation experiments, there is a need for increased work in this area. Squids appear to have fast growth rates and short life spans due to: (1) a combination of efficient digestion with a protein based metabolism; (2) the ability to sustain continued growth by a combination of both an increase in muscle fibre size (hypertrophy) along with continual recruitment of new muscle fibres (hyperplasia); (3) efficient use of oxygen and (4) low levels of antioxidative defense. Heavy fishing pressure on large late maturing fishes may have irreversibly tipped the balance of the ecosystem in favour of the fast growing short-lived squids. Detailed studies of marine protected areas (MPA's) including the use of telemetry technology will help clarify if and how overfished ecosystems can be brought back to their original 'balance'.

CROISSANCE DU CALMAR
STATOLITHES
DATATION
MÉTABOLISME
TÉLÉMÉTRIE

RÉSUMÉ. – Les Calmars représentent une partie importante de nombreux écosystèmes marins et sont soumis à des pressions croissantes de la pêche commerciale. Dans certaines régions où la pêche est intensive, les Calmars ont remplacé leurs compétiteurs, les Poissons Téléostéens. Cette réussite est due à une croissance rapide et à un cycle de vie court. L'étude de la croissance du Calmar en laboratoire et de la datation par les statolithes a permis d'approfondir les connaissances sur son cycle de vie. La durée de vie de la plupart des espèces est de un an ou moins et quelques espèces seulement ont un cycle de vie supérieur à un an. Certaines espèces tropicales de petite taille complètent leur cycle de vie en quelques mois seulement. Des expériences de validation sur la croissance évaluée à partir des statolithes ont été publiées récemment. Cependant, il est nécessaire d'étendre les connaissances dans ce domaine. Les Calmars semblent avoir une croissance rapide et un cycle de vie court pour plusieurs raisons : (1) ils combinent une digestion efficace et un métabolisme protéinique ; (2) ils sont capables de maintenir une croissance continue en combinant un accroissement de la taille des fibres musculaires (hypertrophie) avec l'addition continuelle de nouvelles fibres (hyperplasie) ; (3) ils utilisent l'oxygène de manière efficace et (4) leur niveau de défenses antioxidantes est bas. La pêche intensive des espèces de Poisson à maturité tardive pourrait avoir modifié de manière irréversible l'équilibre de l'écosytème en faveur d'espèces à croissance rapide et à cycle de vie court. Des études détaillées utilisant notamment les techniques de télémétrie dans les zones marines protégées (ZMP) permettront de vérifier si les écosytèmes surexploités peuvent être ramenés à leur équilibre initial.

Considerable progress has been made in the last decade on the growth dynamics of squid. Studies continue to reveal elements of their life history that point to life spans that are short, growth rates that are rapid and populations that turnover quickly. Our best understanding of the dynamics of squid growth has arisen as a result of both controlled culture experiments (e.g., Lee *et al.* 1994, Forsythe *et al.* 2001) and statolith based ageing studies (e.g., Jackson *et al.* 1997). As a result we have better data on how squid grow and how this can impact the environment where they live.

Evidence now suggests that as longer lived finfish stocks have been depleted, they have been replaced by cephalopods (Pauly & Christensen 1995, Caddy & Rodhouse 1998). Circumstantial evidence strongly suggests that increased cephalopod abundance in Tunisian waters, the Adriatic Sea and the Gulf of Thailand is due to a decrease in the standing stock of groundfish competitors by half or more along with a decrease in predators (Caddy & Rodhouse 1998). Furthermore, it is likely that oceanic squid stocks may have increased due to a reduction in predators. In consideration of tuna consumption alone, Caddy & Rodhouse (1998) pointed out how tuna landings have risen from 2 to 4 million t y^{-1}. Given that tuna diet is approximately 25% oceanic squids and consumption is about 10% body weight d^{-1}, this 2 ton difference accounts for an extra 20 million t of squid in the world's oceans in recent years.

The reduction in traditional groundfish landings has resulted in squid stocks coming under increasing fishing pressure. While total world catch of groundfish has remained stable or decreased in recent years the catch of cephalopods has increased dramatically (Caddy & Rodhouse 1998). The growth dynamics of squid populations appear to be well suited to filling niches once their teleost competitors have been removed. They are essentially 'weeds of the sea' O'Dor (1998), filling spaces just as fast growing weeds quickly colonise an area of ground after a forest is felled. Rapid population turnover also provides challenges to those needing to sustainably manage squid fisheries (Murphy & Rodhouse 1999).

So where do we stand now with regard to our understanding of the population dynamics of squids? A comprehensive review of squid growth based on statolith ageing was compiled by Jackson (1994) and statolith-based loliginid studies were reviewed by Jackson (1998). The Jackson (1994) review summarised what ageing work had been carried out and how this data was used to model growth. To some extent, many of the studies covered in that review were simply a preliminary application of statolith increment counts. Since then work has expanded on both the mechanisms and dynamics of squid growth. This paper intends to provide a status report of where we are in our understanding of squid growth.

Statolith validation

An important area of research has been continuing work on statolith validation (e.g., Estácio *et al.* 1999) to verify the periodicity of statolith increments. Jackson (1994) reported validation studies for 11 squid species and one sepioid (*Idiosepius pygmaeus*). Since 1994 work has continued with validation studies (Table I). Especially noteworthy is the study of Lipinski *et al.*(1998a) as this study is the only one thus far to document directly, daily periodicity of adult squid in the wild. The number of studies is small which indicates the problems associated with maintaining squid successfully under experimental conditions. The results continue to support daily increment periodicity in statoliths and reveal the need for further work to be carried out with more species of squid and especially with oceanic and deep sea oegopsid squids.

The study by Arkhipkin *et al.* (1996) on *Berryteuthis magister* reveals that other indirect methods of age verification such as following modes may work for cold water species with discrete cohorts. However, trying to identify modes for many species in relation to age is not possible due to the extreme plasticity in squid growth and the poor relationship between size and age (Jackson *et al.* 2000a). Research by Villanueva (2000a) showed that increment periodicity was daily in *Loligo vulgaris* regardless of the culture temperature. However, this was not the case for *L. vulgaris* embryos (Villanueva 2000b). Work by Yastsu & Mori (2000) who compared known age paralarvae raised in culture to aged field-captured specimens indicated agreement in the form of growth for both groups which suggested that the statolith based field estimates were realistic descriptors of growth in paralarvae and young juveniles.

Statoliths as ageing tools

A number of papers continue to use statoliths for routine ageing. The precision of statolith increment counts continues to be refined (Arkhipkin *et al.* 1998a, Durholtz & Lipinski 2000, Gonzalez *et al.* 1998, 2000, Jackson & Moltschaniwskyj 1999) and the technique is becoming more widespread and incorporated into large scale studies (e.g., Arkhipkin 2000, Arkhipkin *et al.* 1998b, Bower 1996, Macy & Brodziak 2001). Since the review of Jackson (1994) there have been a number of studies that have undertaken a comprehensive ageing analysis for a variety of squid species (Table II)

An update of statolith based loliginid life history studies (Jackson 1998) included 17 species from

Table I. – Studies published since Jackson (1994) that have used validation techniques for statolith increment periodicity in squids.

Species	Number of individuals	Technique	Reference
Loligo vulgaris reynaudii	8	Tetracycline staining in field	Lipinski et al. 1998a
Loligo vulgaris	31	Tetracycline staining in culture	Villanueva 2000a
Loligo vulgaris embryos	36	Tetracycline staining in culture	Villanueva 2000b
Loliolus noctiluca	6	Tetracycline staining in culture	Dimmlich & Hoedt 1998
Lolliguncula brevis	43	Tetracycline staining in culture	Jackson et al. 1997
Sepioteuthis lessoniana	5	Alizarin red staining in culture	Balgos & Pauly 1998
Sepioteuthis lessoniana	11	Tetracycline staining in culture	Jackson & Moltschaniwskyj 2001a
Gonatus onyx	4	Counting increments from capture stress check	Arkhipkin & Bizikov 1997
Gonatus borealis	2	Counting increments from capture stress check	Arkhipkin & Bizikov 1997
Gonatus magister	1	Counting increments from capture stress check	Arkhipkin & Bizikov 1997
Galiteuthis phyllura	1	Observing increment from check	Arkhipkin 1996a
Eogonatus tinro	4	Counting increments from capture stress check	Arkhipkin & Bizikov 1997
Berryteuthis magister	88	Comparing statolith increments to gladius increments	Arkhipkin et al. 1996
Berryteuthis magister	60	Comparing increment number to elapsed days between 2 cohorts	Arkhipkin et al. 1996

around the world. Ages ranged from less than a hundred days for small warm water and tropical species (*Lolliguncula brevis* Jackson *et al.* 1997; *Loligo duvauceli* Chotiyaputta 1997) to around a year for more temperate species. However, of the 17 loliginids reviewed in Jackson (1998) only three had life spans of over a year (*Loligo vulgaris* Arkhipkin1995, *Heterololigo bleekeri* Kinoshita 1989, & *Loligo vulgaris reynaudii* Lipinski 1991). More recent work (Table II) also supports a life span > 1 yr for *L. vulgaris* (Raya *et al.* 1999) and possibly for *L. forbesi* as well (Rocha & Guerra 1999). The majority of loliginids however, appear to have life spans of less than a year.

Oegopsid squids also appear to not have extensive life spans (Table II). Only 7 species from recent studies have reported life spans of < 1 yr (*Berryteuthis magister*, *Nototodarus sloanii*, *N. gouldi*, *Martialia hyadesi*, *Gonatus fabrici*, *Ancistrocheirus lesueurii*, and *Architeuthis*) and only *Gonatus fabricii* has a life span of > 22 months. More surprising are the extremely short life spans of small tropical species such as *Pterygioteuthis gemmata* with a life span of < 3 months and *Abralia trigonura*, *Abraliopsis pfefferi* and *Loliolus noctiluca* with life spans < 6 months (Table II). Small tropical species appear to have an extremely rapid population turnover.

The majority of squid ages reviewed in Table II are based on assumed daily periodicity of statolith increments, as many have not been validated (Table I, see also Jackson 1994). However, advances in culture techniques provide a means to directly observe squid growth and life spans. There is now a substantial body of information available for a single squid species: the Indo-Pacific squid *Sepioteuthis lessoniana* which allows for a direct comparison of growth between culture experiments and field-based statolith ageing studies.

Statolith ageing and validation studies of *S. lessoniana* include Jackson (1990), Jackson & Choat (1992), Jackson *et al.* (1993), Jackson & Moltschaniwskyj (2001a) and, Balgos & Pauly (1998). Furthermore, a comprehensive seasonal/geographical ageing study of *S. lessoniana* was carried out by Jackson & Moltschaniwskyj (2001b) and reproductive strategies of *Sepioteuthis* were examined by Pecl 2001. In all these studies the post-hatching life cycle of *S. lessoniana* was less than 250 d. Moreover, there have been extensive culture experiments with this species both in Japan (Tsuchiya 1982, Segawa 1987), Texas USA (Lee *et al.* 1994, Forsythe *et al.* 2001) and in Thailand (Nabhitabhata 1995, 1996). All the culture studies indicate a life history of < 1 yr with growth to as much as 2 kg. The growth information for this species based on validated statolith age estimates and direct observation of growth of cultured individu-

Table II. – Studies published since Jackson (1994) that have used statolith increment counts to determine age and life spans of squids. See also Jackson (1998) for other studies of loliginid age and growth. The asterisk indicates pen length rather than mantle length.

Species	Estimated maximum age (days)	Mantle length (mm)	Location	Comments	Reference
Loligo vulgaris	361 (F) 382 (M)	255 (F) 383 (M)	North-west Spain	Some seasonal variation in growth	Rocha & Guerra 1999
Loligo vulgaris	294 (F) 308 (M)	285 (F) 534 (M)	Saharan Bank	Tropical	Raya et al. 1999
Loligo vulgaris	335 (F) 396 (M)	290 (F) 498 (M)	Saharan Shelf	Tropical	Arkhipkin 1995
Loligo vulgaris	~253 (F) ~288 (M)	~308 (F) ~311 (M)	Southern Portugal	Warmwater	Bettencourt et al. 1996
Loligo gahi	366 (F) 339 (M)	~146 (F) ~169 (M)	Patagonian Shelf	Seasonal variation in growth	Hatfield 2000
Loligo opalescens	238(F) 243(M)	~128(F) ~138(M)	California	No differences between males or females	Butler et al. 1999
Loligo pealei	~275 (F) ~295 (M)	~213 (F) ~295 (M)	North-west Atlantic	Growth variable depending on season	Brodziak & Macy 1996
Lolliguncula brevis	172 (F) 150 (M)	72 (F) 61 (M)	Gulf of Mexico	Growth variable depending on season	Jackson et al. 1997
Sepioteuthis lessoniana	173 (F) 224 (M)	276 (F) 256 (M)	Australia, Thailand	Subtropical, tropical, seasonal/geographical differences in growth rates	Jackson & Moltschaniwskyj 2001b
Sepioteuthis lessoniana	~186 (F) ~174(M)	~174 (F) ~212 (M)	Australia	Tropical	Semmens & Moltschaniwskyj 2000
Photololigo sp.	~91	~102	Northeastern Australia	Tropical	Moltschaniwskyj 1995
Photololigo sp. 1	158 (F) 119 (M)	115 (F) 87 (M)	Northwest Shelf of Australia	Tropical	Jackson & Yeatman 1996
Loliolus noctiluca	~250 (F) ~256 (M)	~ 69 (F) ~54 (M)	Eastern Australia ~38°S	Temperate	Dimmlich & Hoedt 1998
Loliolus noctiluca	148 (F) 129 (M)	80 (F) 52 (M)	Eastern Australia ~33°S	Temperate	Jackson & Moltschaniwskyj 2001c
Loliolus noctiluca	121 (F) 107 (M)	54 (F) 61 (M)	Eastern Australia ~19°S	Tropical, seasonal variation in growth	Jackson & Moltschaniwskyj 2001c
Loligo forbesi	514 (F) 480 (M)	322 (F) 400 (M)	North-west Spain	Some seasonal variation in growth	Rocha & Guerra 1999
Abralia trigonura	188 (F) 182 (M)	~36 (F) ~31 (M)	Hawaii	Abundant species in mesopelagic boundary community	Young & Mangold 1994
Abraliopsis pfefferi	154 (F) 127 (M)	33 (F) 25 (M)	Central East Atlantic	Tropical, small, short life span	Arkhipkin 1996c
Ancistrocheirus lesueurii	609 (F) 360 (M)	423 (F) 90 (M)	Central-East Atlantic	Females strikingly larger and older than males	Arkhipkin 1997b
Pterygioteuthis gemmata	77 (F) ~73 (M)	30 (F) ~26 (M)	Central East Atlantic	Tropical, extremely short life span < 3 months	Arkhipkin 1997a
Onychoteuthis banksi	261 (F) 224 (M)	130 (F) 77 (M)	Atlantic, Pacific and Indian Oceans	Females not mature, full life span not known	Arkhipkin & Nigmatullin 1997
Moroteuthis ingens	358 (F) 393 (M)	544 (F) 382 (M)	New Zealand	Sexually dimorphic with females bigger	Jackson 1997
Gonatus fabricii	~644 (F) 654 (M)	~205* (F) 182* (M)	Norwegian Sea	Arctic, suggested 2 year life cycle	Arkhipkin & Bjørke 2000
Berryteuthis magister	473 (M) 479 (F)	295 (M) 369 (F)	Bering Sea	Coldwater	Arkhipkin et al. 1996
Architeuthis	294 (M) 375 (M) 422 (M)	1028 (M) 975 (M) 1084 (M)	Off Ireland	All mature males	Lordan et al. 1998
Illex illecebrosus	~247 (F) ~216 (M)	~280 (F) ~246 (M)	Newfoundland	Growth variable depending on season of hatch	Dawe & Beck 1997
Illex illecebrosus	~198	~205	Nova Scotian shelf		Arkhipkin & Fetisov 2000
Illex coindetii	286 (F) 233 (M)	300 (F) 203 (M)	Western Sahara	2 groups (young 0.5 yr and older ~1yr maturing squid)	Arkhipkin 1996b
Illex coindetii	242 (F) 189 (M)	190 (F) 140 (M)	Sierra Leone	Tropical	Arkhipkin 1996b

Table II. – (*continued*).

Illex coindetii	176 (F) 191(M)	~159 (F) ~124(M)	Central Mediterranean	Warmwater	Arkhipkin et al. 1999a
Illex coindetii	240 (F) 230 (M)	197 (F) 143 (M)	Central Mediterranean	Warmwater	Arkhipkin et al. 2000
Illex coindetii	~422 (M) 477 (F)	~164 (M) ~201 (F)	Spanish Mediterranean	Warmwater, summer population appeared to grow faster than winter population	Sánchez 1995
Illex coindetii	~442 (F) ~380 (M)	~377 (F) ~243 (M)	NW Spain	Seasonal variation in growth	González et al. 1996
Todarodes sagittatus	409 (F)	473 (F)	Irish & Scottish waters	Larger squid were deeper suggesting ontogenetic downward migration	Lordan et al. 2001
Todarodes sagittatus	262 (F) 231 (M)	319 (F) 201 (M)	Western Sahara	Tropical	Arkhipkin et al. 1999b
Todaropsis eblanae	220 (F)	139 (F)	North West African Shelf	Tropical, suggested 1 year life span	Arkhipkin & Laptikhovsky 2000
Nototodarus sloanii	374 (F)	406 (F)	New Zealand	Seasonal variation in growth	Uozumi 1998
Nototodarus gouldi	373	376	New Zealand	Seasonal variation in growth	Uozumi 1998
Nototodarus hawaiiensis	195 (F) 192 (M)	183 (F) 164 (M)	North West Slope of Australia	Tropical	Jackson & Wadley 1998
Martialia hyadesi	357 (F) 330 (M)	~343 (F)	Patagonian Shelf	Cool, no mature females	González et al. 1997
Martialia hyadesi	399 (F) 354 (M)	398 (F) 295 (M)	South-west Atlantic	Coldwater	Arkhipkin & Silvanovich 1997
Martialia hyadesi	330 (F) 360 (M)	330 (F) 314 (M)	South Georgia	Coldwater	González & Rodhouse 1998
Ommastrephes bartramii	306 (F)	454 (F)	North Pacific	Small sample size	Yatsu et al. 1998
Ommastrephes bartramii	~306 (F) ~306 (M)	~458 (F) ~348 (M)	North Pacific	Some seasonal and geographical differences in growth rates	Yatsu et al. 1997, see also Yatsu 2000
Ornithoteuthis antillarum	182 (F) 173 (M)	117 (F) 83 (M)	Central-east Atlantic	tropical	Arkhipkin et al. 1998c
Thysanoteuthis rhombus	305 (F) 309 (M)	750 (F) 770 (M)	Eastern tropical Atlantic/ Southwest Pacific	One of fastest growing squid species	Nigmatullin et al. 1995
Cranchia scabra	166 (F)	118 (F)	Central East Atlantic	Tropical, fast growing, life span unknown only immature individuals	Arkhipkin 1996d
Liocranchia reinhardti	146 (M)	183 (M)	Central East Atlantic	Tropical, fast growing, life span unknown only immature individuals	Arkhipkin 1996d

als throughout their life cycle is unambiguous (see also Jackson *et al.* 2000a). We thus have a high degree of confidence in the growth rate and life span data for this loliginid. The synopsis of the research over the last several years along with earlier reviews (Rodhouse & Hatfield 1990, Jackson 1994, 1998) suggests that in fact it is difficult to find many squid species older than a year. Furthermore, extensive studies have revealed that growth of squid is very plastic and growth rates vary according to changes in temperature (Forsythe 1993, Forsythe *et al.* 2001, Hatfield 2000, Jackson & Moltschaniwskyj, 2001b, 2001c).

Mechanisms responsible for squid growth

Squids successfully compete with their teleost counterparts. Their strategy is to complete their life span quickly (i.e., life in the fast lane). The life history of squids would be a fraction of many of their teleost counterparts. Their major strategy appears to be a protein-based metabolism that converts energy into growth rather than storage (O'Dor & Webber 1986, Lee 1994, Moltschaniwskyj & Semmens 2000). Their high metabolic rates and growth rates are in fact higher than pokilothermic vertebrates and as high as mammals (Pörtner & Zielinski 1998, Zielinski & Pörtner 2000).

Squid also appear to sustain continued growth by a combination of both an increase in muscle fibre size (hypertrophy) along with continual recruitment of new muscle fibres (hyperplasia) (Moltschaniwskyj 1994, Preuss *et al.* 1997, Pecl & Moltschaniwskyj 1999). While teleost fish have both mechanisms hyperplasia eventually ceases with age.

Cephalopods rapidly digest (Boucher-Rodoni *et al.* 1987) and efficiently use protein, however, they appear to not handle lipids well and it has been recently suggested that the digestive gland is used for dumping excess lipid that cannot be metabolised or

stored (Semmens 1998). Furthermore, the combination of jet pressure locomotion that passes water directly over the gills in association with cutaneous respiration (which might be extremely high in squid; Pörtner 1994, Pörtner & Zielinski 1998) provides a mechanism for efficient oxygen consumption (O'Dor & Hoar 2000). O'Dor & Hoar (2000) have even suggested that the thin mitochondria-rich fin musculature may be independent of the circulatory system. Thus squids appear to use oxygen efficiently despite the limitations of their hemocyanin based respiratory transport system (Hochachka 1994, Pörtner & Zielinski 1998).

We now have clues as to why squids have such short life spans. They may in fact be under biochemical constraints. Zielinski & Pörtner (2000) have recently shown that cephalopods have a low enzymatic antioxidative status despite their high metabolic rate. Their low level of enzymatic antioxidant defense correlates with an increased level of oxidative damage, reflected by very high levels of malondialdehyde (MDA) and lipofuscin which indicates oxidative stress is higher in older specimens. Zielinski & Pörtner (2000) have pointed out that this low antioxidative status is in line with short cephalopod life expectancies. They further pose the question 'why isn't antioxidative defense brought to a higher level to prolong cephalopod life?' Their explanation is that antioxidative protection is set to a level just high enough to allow for a 'sufficient life span'.

Antioxidative defense appears to be an exciting area of future research across a number of squid species with varying life spans and perhaps even within species that show considerable differences in life histories with season or location (eg., Jackson & Moltschaniwskyj 2001a,b). The tradeoff between high oxygen concentrations in tissues to sustain high activity and the need for antioxidant protection could be a factor in cephalopod associations with the oxygen minimum layer (e.g., *Stenoteuthis oualaniensis* in the Arabian Sea, Nesis 1993 and *Gonatus onyx* in the deepsea off California, Hunt & Seibel 2000). It could also be a factor in the ontogenetic descent commonly seen as cephalopods age and mature (e.g *Moroteuthis ingens* Jackson 1993, 1997,2001, Jackson *et al.* 2000b, *Gonatus fabricii* Bjørke *et al.* 1997, Arkhipkin & Bjørke 1999, 2000, *Gonatus onyx* Seibel *et al.* 2000, *Berryteuthis magister* Arkhipkin *et al.* 1996, *Galiteuthis glacialis* Nesis *et al.* 1998).

Four important features therefore stand out with regard to mechanisms responsible for squid growth and life span. These are: (1) protein based rapid metabolism and digestion (2) continual recruitment of new muscle fibres (hyperplasia) (3) efficient utilisation of oxygen and (4) low levels of antioxidative defense. These unique features of squid growth set them apart from their main teleost competitors.

Where to from here?

As world finfish stocks continue to be depleted there is likely to be increasing attention given to cephalopod resources. We thus have a pressing need to understand the dynamics and physiology of squid growth and to develop the essential elements needed for successful squid fishery management (O'Dor 1998, Lipinski 1998, Lipinski *et al* 1998b).

A critical unknown is whether the traditional ecosystems based on large, slow-growing, late-maturing fishes will ever recover. Worldwide, governments are establishing marine protected areas (MPAs) to assist recovery and maintenance of fished populations. Even if extensive MPAs are established, it is possible that a new dynamic balance of faster growing squids and fishes has already been established. MPAs will provide a safe haven for large, old fishes, but the overall stability of ecosystems managed with this new tool will depend on the interaction of relative production: biomass ratios in protected areas and areas that are still under heavy fishing pressure.

Ultrasonic telemetry is a new and exciting means to study activity and metabolism of both squid (O'Dor *et al.* 2001a) and fish (O'Dor *et al.* 2001a, Webber *et al.* 2000) in real time and to determine essential elements of energetics and ecophysiology *in situ*. Just as Lipinski *et al* (1998a) has been able to take statolith validation out of the laboratory and into the field environment, remote telemetry allows the researcher to study squid activity and metabolism in the field in a way that is otherwise impossible. Using ultrasonic tags it is possible to telemeter information back on a whole suite of biological parameters of individuals in the field (eg., O'Dor *et al.* 1994, Dewar *et al.* 1999, Webber *et al.* 1998).

Figure 1 shows an example of parallel studies of squid and fish using radio-acoustic positioning telemetry (RAPT) in an MPA at Lizard Island, Australia (O'Dor *et al.* 2001a). Such studies may not give the complete picture but will certainly represent an important component. While the data presented in Fig. 1 simply shows distribution and movement data, two of the species (one squid and one fish) show movement across the MPA boundary. Such technology provides necessary data for studying the dynamics and interactions of fish and squid in both fished and non-fished areas. Future larger scale studies could utilise the deployment of fixed hydrophone monitors (e.g., Voegeli *et al.* 2001) for tracking movement of individuals over very large distances (10's –1000's of kilometers).

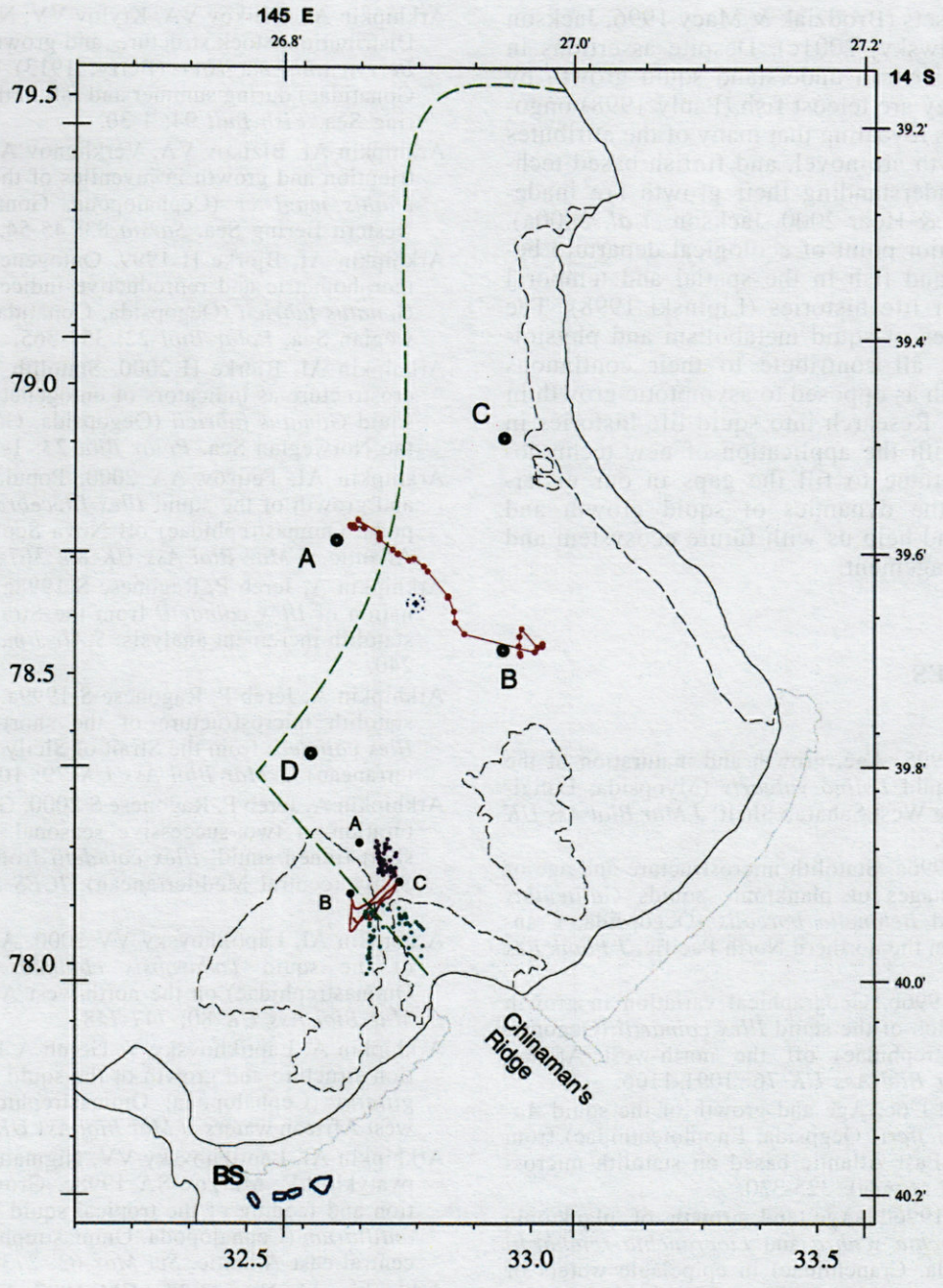

Fig. 1. – RAPT systems deployed in Watson's Bay, Lizard Island, Australia. The map gives both kilometre grid and latitude and longitude references, and the heavy dashed line encloses a no-take MPA zone. The large four-buoy diamond was linked to the base-station (BS) at the Lizard Island Lodge, and produced the illustrated 3h track of the tropical squid *Sepioteuthis lessoniana* crossing the boundry. The smaller triangle recorded territories over 24 h for a stripey *Lutjanus carponotatus* (smaller home range, purple dots) and a coral trout *Plectropomus leopardus* (divided home range, green dots), also showing trans-border movement. The red line is a track of a diver who undertook an underwater survey (From O'Dor *et al.* 2001a).

Currently, much of our understanding regarding squid growth and ecophysiology is imprecise. Even though we know their life histories are short and growth is plastic, we really lack many of the specifics. Continuing work on basic biology and the development of life tables would allow the investigation of age-specific mortality (Wood & O'Dor 2000). We still face difficulties with modelling squid growth due to the extreme plasticity in size-at-age and the rapid response in growth rate due to changes in ambient temperature. Recent work with separating squid samples into seasonal cohorts and using the Schnute model for analysing size-at-age data may be a useful technique for dealing with the

difficult data sets (Brodziak & Macy 1996, Jackson & Moltschaniwskyj 2001c). Despite assertions in the past that we can understand squid growth by pretending they are teleost fish (Pauly 1998) ongoing research is revealing that many of the attributes of squid growth are novel, and finfish based techniques for understanding their growth are inadequate (O'Dor & Hoar 2000, Jackson *et al.* 2000a). There is a major point of ecological departure between squid and fish in the spatial and temporal scales in their life histories (Lipinski 1998). The unique features of squid metabolism and physiology probably all contribute to their continuous form of growth as opposed to asymptotic growth in most teleosts. Research into squid life histories in association with the application of new technologies will continue to fill the gaps in our understanding of the dynamics of squid growth and physiology and help us with future ecosystem and fisheries management.

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ASPECTS OF THE BIOLOGY OF THE CAPE GURNARD, *CHELIDONICHTHYS CAPENSIS* (SCORPAENIFORMES: TRIGLIDAE) ON THE AGULHAS BANK, SOUTH AFRICA

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AGE AND GROWTH
REPRODUCTION
MATURITY
MORTALITY
CHELIDONICHTHYS CAPENSIS

ABSTRACT. – We present estimates of the population structure, reproduction, age, growth and mortality of the Cape gurnard, *Chelidonichthys capensis,* based on data from commercial and research trawls over the Agulhas Bank, South Africa. The mean total length for males (366 mm) was significantly smaller than that for females (411 mm). The sex ratio was close to parity with males being more dominant in commercial trawls, and less dominant in research trawls. Gonad maturation and gonadosomatic indices demonstrated that this species has an extended spawning period with peaks in reproductive activity during September, January and April. First approximations of size and age at 50% maturity revealed that the males matured at a younger age than the females, and at significantly smaller sizes (males: 299 mm TL/3.6 years; Females: 343 mm TL/4.6 years). Sagtital otolith growth marks were validated as annuli using marginal zone analysis. The maximum age estimated was 16 years for a female of 675 mm TL, and recruitment to the commercial fishery was estimated as taking place in the fifth year of growth for both males and females. First approximation of fishing mortality for the commercial trawl fishery (0.52 $year^{-1}$) was higher than that of the natural mortality (0.085 $year^{-1}$), and therefore indicates a degree of fishing pressure on this species on the Agulhas Bank.

AGE ET CROISSANCE
REPRODUCTION
MATURITÉ
MORTALITÉ
CHELIDONICHTHYS CAPENSIS

RÉSUMÉ. – La structure de la population, la reproduction, l'âge, la croissance et la mortalité de *Chelidonichtys capensis* à partir de données commerciales et d'échantillonnages scientifiques au chalut sur l'Agulhas Bank en Afrique du Sud sont présentées. La longueur totale moyenne des mâles (366 mm) est significativement inférieure à celle des femelles (411 mm). La sex-ratio est proche de la parité, avec les mâles plus nombreux dans les chalutages de pêche commerciale et moins dominants dans les chalutages scientifiques. La maturation des gonades et les indices gonadosomatiques montrent que cette espèce présente une période de ponte longue avec des maxima de l'activité reproductrice en septembre, janvier et avril. Les premières approximations de la taille et de l'âge à une maturité de 50 % révèlent que les mâles atteignent la maturité plus jeunes que les femelles, et à une taille significativement inférieure à celle des femelles (mâles : 299 mm TL/3,6 ans ; femelles : 343 mm TL/4,6 ans). La croissance en longueur de l'otolithe est étudiée en validant les stries de croissance observées dans la zone marginale. L'âge maximum estimé est de 16 ans pour les femelles à 675 mm TL, et le recrutement pour la pêche commerciale est estimé avoir lieu dans la 5^{e} année de croissance pour les deux sexes. La première approximation de la mortalité (0,52 an^{-1}) due à la pêche commerciale au chalut est plus élevée que la mortalité naturelle (0,085 an^{-1}), et indique ainsi une certaine pression de pêche de cette espèce sur l'Agulhas Bank.

INTRODUCTION

The Cape gurnard *Chelidonichthys capensis* is an endemic Southern African triglid species found on the continental shelf between the Orange and Umfolozi rivers (Van der Elst 1993). Gurnards represent a significant proportion of the by-catch reported from many of the offshore trawl and line fisheries in Southern Africa (Smale & Badenhorst 1991, Japp *et al.* 1994). Indeed, *C. capensis* is the largest and most frequently caught gurnard, and is

regarded by Van der Elst (1993) as one of the six most important fish trawled off the Eastern Cape coast. Nevertheless, to date the majority of the *C. capensis* caught is discarded with only the larger specimens retained and sold locally. While there may be the potential to establish a fishery based upon the Cape gurnard, credible management and marketing strategies need to be developed.

To date, the majority of the research undertaken on this species has focused on its distribution (MacPherson & Mas Riera 1987, Konchina 1989, Meyer & Smale 1991) and feeding biology (Hecht 1977, Konchina 1989); only Hecht (1977) has addressed age, growth and reproductive issues. While Hecht's (1977) study provides the basis for the development of a management strategy, the data sets are over twenty years old. Furthermore, the data originated from an area that has experienced a rapid increase in fishing effort over this period, thus comparisons between the parameter estimates from the two studies may provide useful indicators of the impacts that fishing has had on the resource. Thus, prior to the development of a management plan, there is clearly a necessity to investigate the current biological status of the species. With this in mind, this study was designed to investigate aspects of the life history of *C. capensis* such as the population structure, age, growth, mortality and reproductive biology.

MATERIALS AND METHODS

Sampling was undertaken between August 1995 and January 1997 on the Agulhas Bank between Port Alfred and Cape Agulhas (Fig. 1). Samples were obtained from either commercial inshore (75 mm stretched mesh) or research (25 mm stretched mesh) demersal otter trawls. Individual fish were weighed (1g), measured (total length to 1 mm) and sexed. The gonads were removed and weighed (0.01g). Gonadosomatic indices were calculated using the formula:

$$GSI = \frac{\text{gonad mass (g)} \times 100}{\text{whole body mass (g)}}$$

Each gonad was macroscopically examined, and a gonad maturation index (GMI) awarded according to the classification outlined in Table I. Gonads were used to determine the temporal variability in reproduction, and length and age at sexual maturity.

The GMI values were used to establish the size at sexual maturity by determining the proportion of reproductively active individuals (stages 2, 3 and 4) in each size class. In order to minimise bias, immature animals were excluded from the calculation. Thus, those animals displaying GMI values of 1 were excluded as they were classed as "virgin or resting". It should however be noted that the exclusion of GMI 1 animals from the calculation introduces a degree of bias as some of the animals will be mature, but in the "resting" reproductive stage. Furthermore, the exclusion of GMI 1 animals may also

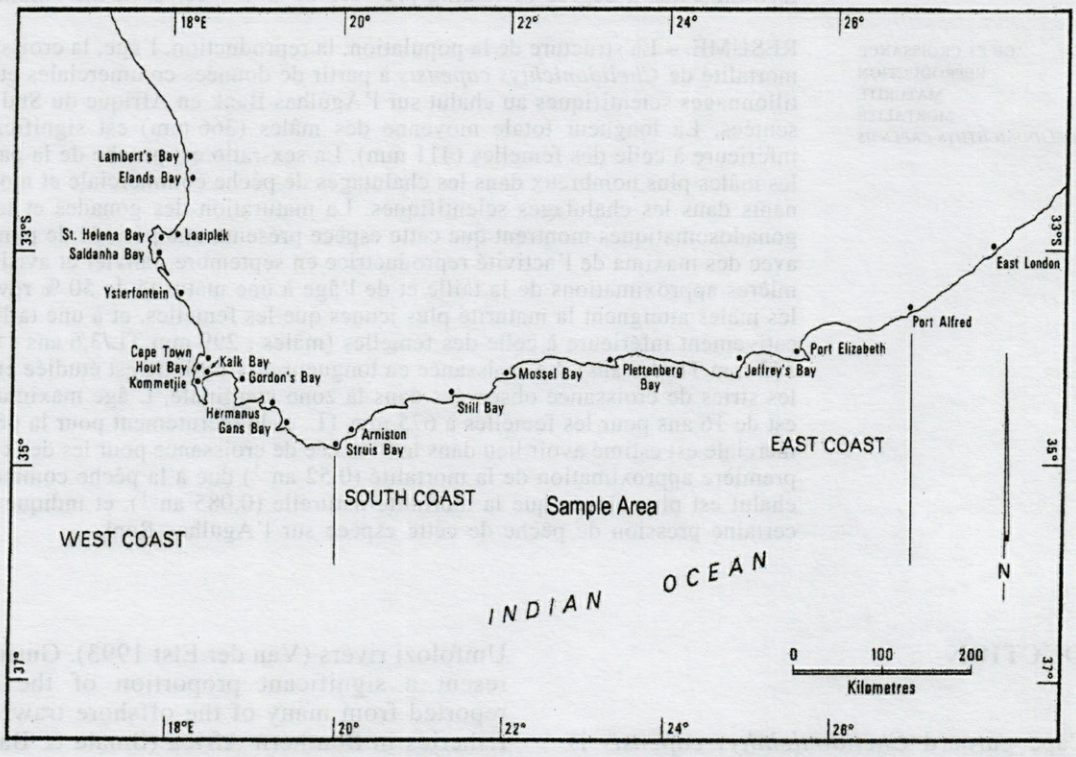

Fig. 1. – Map of the South African coast depicting the division area used for sampling by the Sea Fisheries Research Institute (Cape Town), and the position of the major commercial linefish landing ports.

Table I. – Classification of maturity stages for *Chelidonichthys capensis* (modified from Laevastu 1965, Nikolski 1978 and Buxton & Clarke 1986).

GMI	Stage	Description
1	Virgin and resting	Ovaries and testes small and lying close under the vertebral column. Testes thread-like, opaque and white. Ovaries pinkish and translucent. No eggs visible to the naked eye.
2	Developing	Gonads larger, especially on long axis, and blood vessels present. Testes pale opaque yellow-grey and thick. Ovaries red-orange with opaque eggs visible to the naked eye.
3	Ripe and running	Testes pinkish grey. Sperm runs freely out of sperm duct if pressure applied. Ovaries orange in colour, very large and swollen. Translucent eggs visible.
4	Spent	Testes hard with frilly appearance, pale pink in colour. Ovaries flaccid and much decreased in size, reddish orange and bloodshot.

cause bias as "slow developers" would be excluded from the analysis. Nevertheless, as the potential for bias from these sources may be considered minimal, they are unlikely to affect the L_{50} calculation. The GMI 1 animals were excluded from the sample by applying the following logistic ogive:

$$P_{(L)} = \frac{1}{1+\exp^{-(L-L_{50})/\delta}}$$

where $P_{(L)}$ is the proportion of mature fish at size L, L_{50} is the length at which 50% of the sample was found to be mature, and δ is the width of the ogive. The data was fitted using Newton's non-linear minimisation procedure (Zar 1996). The average length at sexual maturity was taken as the size at which 50% of the population was mature (King 1995). A likelihood ratio test was used to test for differences in the ages of maturity between males and females when all the data were combined, and when research and commercial data were treated independently.

Sagittal otoliths were removed, cleaned and stored dry in paper envelopes. The left sagittae were lightly burnt over a methanol flame to enhance annuli checks. The otoliths were embedded in clear casting resin and sectioned through the nucleus to 0.2 – 0.5 mm using a double-bladed diamond edged saw. The sections were mounted on microscope slides using DPX mountant, and the annuli counted under transmitted light (Campana & Neilson 1985). Each otolith was read on three occasions at weekly intervals. If two out of the three values agreed, this estimate was taken as the age of the fish. If the values did not agree, but did not differ by more than two years, the mean was used, otherwise the otolith was rejected.

Length-weight relationships between male and female fish were compared using Analysis of Covariance (ANCOVA). Prior to analysis, the length-weight data was linearized using natural-log transformations. Tukeys' multiple range test was used to test for differences between slopes.

Growth curves were fitted (least squares) to the length-at-age data using the 3 parameter Von Bertalanffy growth model:

$$L_t = L_\infty (1-e^{-K(t-to)})$$

where L_t is the length at time t. L_∞ is the predicted asymptotic length. K is a measure of the rate at which the length L approaches L_∞, t is the age, and t_0 is the age at zero length (Ricker 1975). Growth was modelled for males, females and pooled data. The best fit was obtained by minimising the squared differences between the observed and fitted data using the absolute-error model (Punt & Butterworth 1993). Von Bertalanffy parameter estimates were determined using Newton's non-linear minimisation procedure (Zar 1996). A likelihood ratio test was used to determine whether there was a difference between the growth models that had been fitted to male and female data sets (Draper & Smith 1966).

Length frequency data was transformed to age frequency distributions using an age-length key (Butterworth *et al.* 1989). Age at recruitment was estimated by fitting a logistic ogive – using non-linear minimisation of the residual sum of squares – to the percentage cumulative age frequency data. The logistic was described by the equation:

$$P_{(L)} = \frac{1}{1+\exp^{-(L-L_{50})/\delta}}$$

where $P_{(L)}$ is the proportion of recruited fish at size L. L_{50} is the estimated length at 50% recruitment, and δ is the width of the ogive. Age at recruitment was taken as the age at which 50% of the population was recruited to the fishery (Punt & Japp 1995). A likelihood ratio test was used to establish differences in the ages of recruitment between the males and females.

First approximations of the total annual mortality (Z) were obtained from the generated catch curves (Butterworth *et al.* 1989). The negative of the slope of the linear regression line, fitted to points greater than the age at full recruitment, provided an estimation of Z (King 1995). Analysis of Covariance was used to test for differences between the regression slopes. Natural mortality

Table II. – Top, mean observed total lengths (mean ± std.), maximal total lengths and weights of *Chelidonichthys capensis* on the Agulhas Bank. Bottom, growth parameters of the von Bertalanffy growth equation as determined by non-linear minimisation of the residual sum of squares for C. *capensis*, including the parameter ϕ', sampled on the Agulhas Bank from August 1995 to January 1997.

Group	Total Length (mm)	Maximum Total Length (mm)	Maximum Wet Weight (g)	*n*
Females				
Total	396 ± 103	676	3300	821
Research	391 ± 134	676	3300	386
Commercial	417 ± 77	625	2835	362
Males				
Total	360 ± 78	582	1100	766
Research	367 ± 79	581	1100	462
Commercial	361 ± 86	582	1061	262

Group	L_∞	K	t_0	ϕ'	n	Age range
Males	754.94	0.084	-2.527	6.17	144	1-12
Females	803.38	0.104	-1.619	6.51	239	1-16
All	894.23	0.079	-2.043	6.45	383	1-16

(M) was estimated from Pauly's (1980) empirical model. For this calculation, the mean annual seawater temperature (°C at which the species lives), and the L and K parameters from the Von Bertalanffy equation were employed. The mean annual seawater temperature was taken as 12 °C (Schumann & Beekman 1984). Fishing mortality (F) was obtained by substitution (F = Z-M). It was noted that Pauly's (1980) empirical formula for estimating natural mortality (M) falls into the realms of "qualified guesses". Inherent in the formula are the assumptions that the ambient water temperatures are high, and that small fish and those with fast growth rates experience high mortalities. In addition, processes influencing M such as reproductive physiology, predation and schooling behaviour, are regarded as "random noise about the regression line". As such, these processes may lead to biased estimates for those species in which they play an important role. Thus, while Pauly's equation has been shown to provide realistic mortality estimates for long-lived species (Buxton 1987), a second method, that of Rikhter and Efanov (1977) was also used to estimate M:

$$M = \frac{1.521}{tm^{0.72}} - 0.155 yr^{-1}$$

where $tm^{0.72}$ is the age at which 50% of the population is mature.

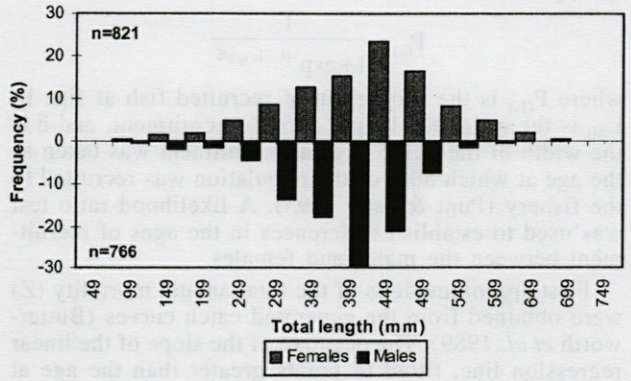

Fig. 2. – Size frequency histograms of male and female *C. capensis* sampled on the Agulhas Bank, South Africa.

RESULTS

Population structure

Size frequency distributions for males and females were found to be significantly different (student *t*-test, $P < 0.001$) (Fig. 2). Mean and maximum lengths are presented in Table II. Males were found to be smaller than females (student *t*-test, $P < 0.001$). The mean size of females in the commercial landings (417 mm) was higher (student *t*-test, $P < 0.05$) than that from the research catches (319 mm), but there was no difference in the sizes of males caught from the two sampling regimes (student *t*-test, $P > 0.05$). Male/female sex ratios of 1:0.9 and 1:1.43 were observed in the commercial and research trawls respectively.

Age and growth

The results of the length weight relationships are presented in Fig. 3. As a function of length, there was no difference between the slopes of the male and female weight regression models ($F = 2.892$, $d.f = 2,1334$, $P > 0.05$). In both cases, the value of b was approximately 3.

Marginal zone analysis provided indirect evidence that hyaline and opaque zones are deposited annually. Hyaline zone formation occurred from September to March and opaque zone deposition from March to August. Of the 382 otoliths analysed, 31 (8%) were rejected as unreadable. The remaining otoliths were used to construct an age-length key. The data from the key were used to estimate growth parameters using the von Bertalanffy growth equation. The parameter values that were applied to the equation were calculated from combined, research and commercial data, and separately for males and females (Table II). In order to compare overall growth performance it was necessary to compare the parameters K and L_∞. However, as these parameters are statistically dependant, an additional index – phi prime (ϕ') (Pauly & Munro 1984) – was required. Phi prime (ϕ') was calculated using the formula:

$$\phi' = 2\ln L_\infty + \ln K$$

The higher the ϕ' values, the larger the maximum size attained and the faster the growth rate.

Growth curves corresponding to the growth equations for each sex are shown in Figure 4. A likelihood ratio test established that there were differences between male and female growth models ($F = 38.58$, $d.f. = 3,376$, $P < 0.05$). Mean observed and calculated length-at-age figures are presented in Table III.

Size at maturity

The observed and expected proportions of mature male and females plotted against length are presented in Fig. 4. Estimated total length and age at 50% maturity (L_{50} and t_m respectively) are presented in Table IV for males and females. A likelihood ratio test revealed that in comparison with the females, the males matured at significantly smaller lengths and at younger ages ($F = 5.32$, $d.f. = 2,32$, $P < 0.05$; females: 343 mm TL/4.6 years; males: 299 mm TL/3.6 years). The rates of maturation (δ) for both males and females were similar (2.12 and 2.04 year^{-1} respectively).

Age at recruitment and mortality estimations

Age frequency distributions derived from the normalised catch length frequency data, the estimated age-at-recruitment and catch curves are presented in Fig. 5. The modal age for both males and females was 5 years. The estimated ages at recruitment to fishery for males and females were 4.9 and 5.2 years respectively (Table IV). The age at recruitment for females (5.2) was significantly higher than that observed in the males (4.9) ($F = 19.31$, $d.f. = 2,52$, $P > 0.05$). First approximations of total mortality (Z), fishing mortality (F) and natural mortality (M) are presented in Table IV (bottom). While the estimates of Z and F are similar for both males and females, the estimates for M were higher for the females than for males.

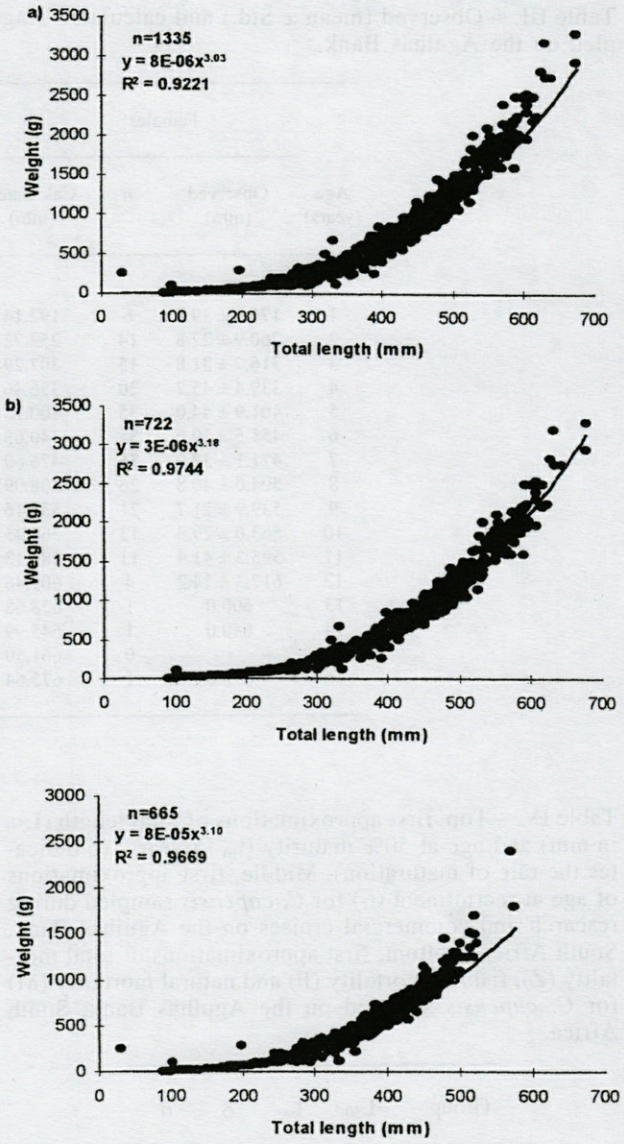

Fig. 3. – Length weight relationship for *C. capensis* sampled on the Agulhas Bank, South Africa. a, all samples; b, females; c, males.

Table III. – Observed (mean ± Std.) and calculated length-at-age for female and male *Chelidonichthys capensis* sampled on the Agulhas Bank.

	Females			Males		
Age (years)	Observed (mm)	*n*	Calculated (mm)	Observed (mm)	*n*	Calculated (mm)
1	176.3 ± 39.8	6	192.14	175.0 ± 32.9	7	195.00
2	260.9 ± 22.8	14	252.72	255.9 ± 30.0	11	238.50
3	316.7 ± 31.8	15	307.29	284.0 ± 26.5	8	279.09
4	339.4 ± 45.7	20	356.46	318.9 ± 38.9	27	316.94
5	401.9 ± 44.0	35	400.75	356.1 ± 36.2	32	352.26
6	451.5 ± 39.6	35	440.65	384.9 ± 26.8	27	385.20
7	471.1 ± 37.9	36	476.60	408.5 ± 29.0	12	415.93
8	504.0 ± 40.8	26	508.99	456.0 ± 40.2	6	444.60
9	539.9 ± 21.7	21	538.16	454.7 ± 5.7	3	471.34
10	563.0 ± 29.4	13	564.45	492.3 ± 26.2	6	496.29
11	595.3 ± 41.4	11	588.13	513.0 ± 12.1	3	519.56
12	617.3 ± 14.2	4	609.46	550.5 ± 44.6	2	541.27
13	600.0	1	628.68			
14	640.0	1	645.99			
15	-	0	661.59			
16	675.0	1	675.64			

Table IV. – Top, first approximations of total length (L_{50} in mm) and age at 50% maturity (t_m in years) (δ indicates the rate of maturation). Middle, first approximations of age at recruitment (t_r) for *C. capensis* sampled during research and commercial cruises on the Agulhas Bank, South Africa. Bottom, first approximations of total mortality (Z), fishing mortality (F) and natural mortality (M) for *C. capensis* sampled on the Agulhas Bank, South Africa.

Group	L_{50}	t_m	δ	*n*
Males	299	3.6	2.12	632
Females	343	4.6	2.04	692

Group	t_r	δ	*n*
Males	4.9	1.076	683
Females	5.2	1.462	744

Group	Z	log M	M	F
Pooled	0.56	-1.07	0.085	0.52
Males	0.53	-1.01	0.097	0.44
Females	0.54	-0.96	0.110	0.43

Reproductive biology

Changes in gonad maturation indices and the seasonal variations in these indices are presented in Fig. 6-7 (Fig. 6 and 7 (top) for males; Fig. 6 and 7 (bottom) for females). Peaks in the numbers of spent females occurred in November 1995, March and May 1996, and from October 1996 to January 1997 (Fig. 6, bottom). Peaks in the GSI values occurred just before these periods (Fig. 7, bottom), and therefore indicate the periods during which the females were spawning. A similar picture emerges for the males (Fig. 6,7). Thus, peaks in the numbers of spent males are preceded by peaks in GSI values (Fig. 7, top). Male GSI values were considerably lower than those calculated for the females. A Multiple comparison of mean monthly gonadosomatic indices using Tukey's multiple range analysis (95% confidence), suggests an extended spawning period with peaks of reproductive activity in August, September and January.

DISCUSSION

The von Bertalanffy parameter estimates derived for *C. capensis* suggest that in common with other triglid species (McEachran & Davis 1970, Elder 1976, Hecht 1977, Papaconstantinou 1984, Booth 1997), *C. capensis* is relatively long-lived and fast growing. Indeed, the oldest fish recorded was a

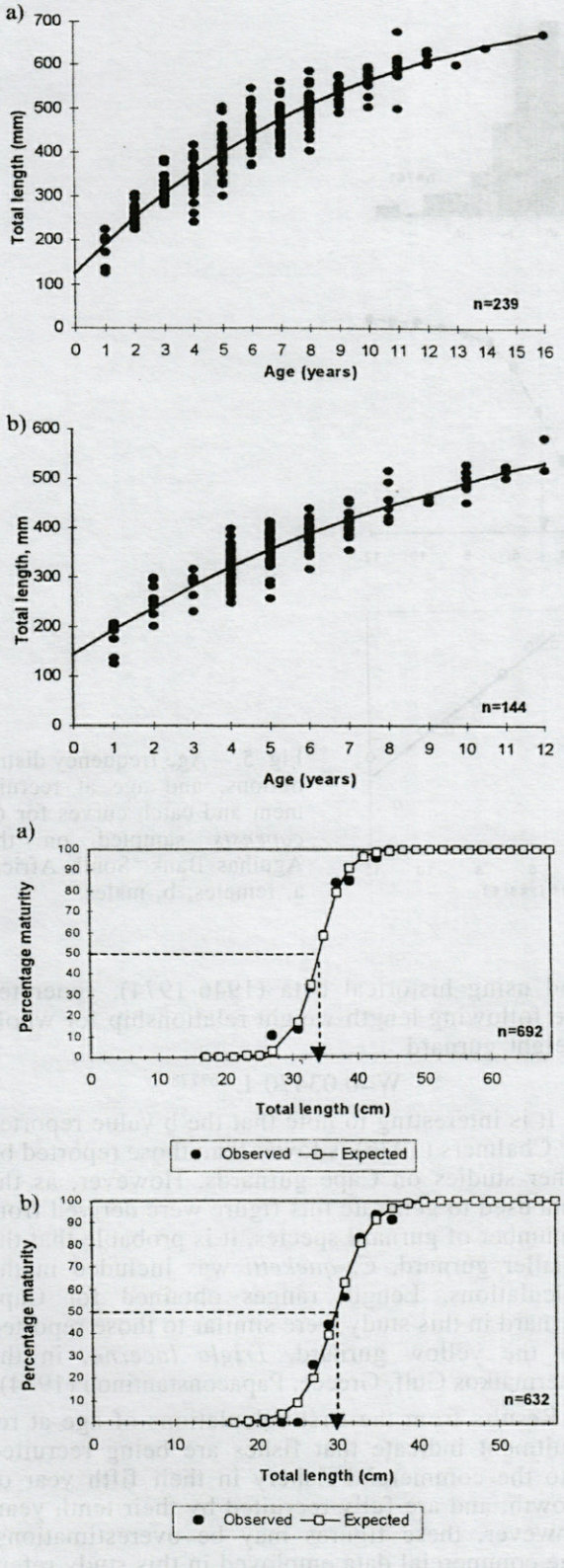

Fig. 4. – Top, growth curve fitted using the von Bertalanffy growth model for *C. capensis* sampled on the Agulhas Bank. a, females; b, males. Bottom, percentage frequency of mature *C. capensis* in different length classes sampled on the Agulhas Bank, South Africa. The curve was fitted using a 2-parameter logistic ogive. a, females; b, males.

16 years old female. As large Brody growth coefficients (K) generally indicate a fast growth rate, it was established that the females grew at a faster rate (K = 0.104) than the males (K = 0.084). A similar result was reported by Hecht (1976). Nevertheless, as K values are dependant upon the maximal asymptotic length, growth rate comparisons between populations, stocks or species cannot be interpreted independently (Pauly & Munro 1984). Thus, valid comparisons may only be undertaken once the growth performance index phi prime (ϕ') has been applied. A comparison of the phi prime values generated for both male and female fish confirmed the initial finding and established that, in contrast to *C. queketti* (Booth 1997), the females grew at a significantly faster rate than the males (ϕ'= 6.51 and ϕ'= 6.17 respectively).

The Cape gurnard is one of the larger gurnard species. The largest male and female recorded in this study were 582 and 676 mm TL respectively. These lengths are not inconsistent with either those previously recorded by Hecht (1976) (514 and 612 mm TL for males and females respectively), or those recorded by Bianchi *et al* (1993) and Smith & Heemstra (1986) (750 and 700 mm TL respectively). Sexual dimorphism is common amongst the larger triglids, and has previously been reported for *C. capensis* (Hecht 1976, Trunov & Maelvanyy 1974), *C. kumu* (Elder 1976), *Trigla lucerna, Eutrigla gurnardus* and *Aspitrigla cuculus* (Baron 1985). In all cases, females were larger than males.

Inclusion of all the available age data generated unrealistically large L_∞ values (female – 803; male – 759), and illustrates one of the difficulties associated with fitting Von Bertalanffy growth models to data sets that are deficient in individuals at the extremes of their size range. Small fish are often under-sampled by the fishing gear, whereas samples from heavily exploited populations may underestimate the larger size classes of fish. As a result, the L_∞ values that relate to the extreme upper limits of the growth curve represent extrapolations beyond the range of the sampled data. The same holds true for t_0 values. Commercial data obtained by Hecht (1977) generated more realistic values. Thus, it may be more appropriate to use L_∞, values of 702 mm TL for all fishes combined, and 714 and 586 mm TL for females and males respectively. The values of t_0 in this study were closer to zero, ranging between –0.086 and –0.413.

Analysis of Covariance revealed no significant difference in the length/weight relationship between males and females. Thus, as male and female growth (length and mass) was proportionally equivalent, the data can be pooled for use in stock assessment models. Hecht (1977) reported a value of 3.0151 for b in the length/weight equation, a value not dissimilar to that obtained in this study (3.0271). Chalmers (1976) studied weight conversion factors of several South African trawl species,

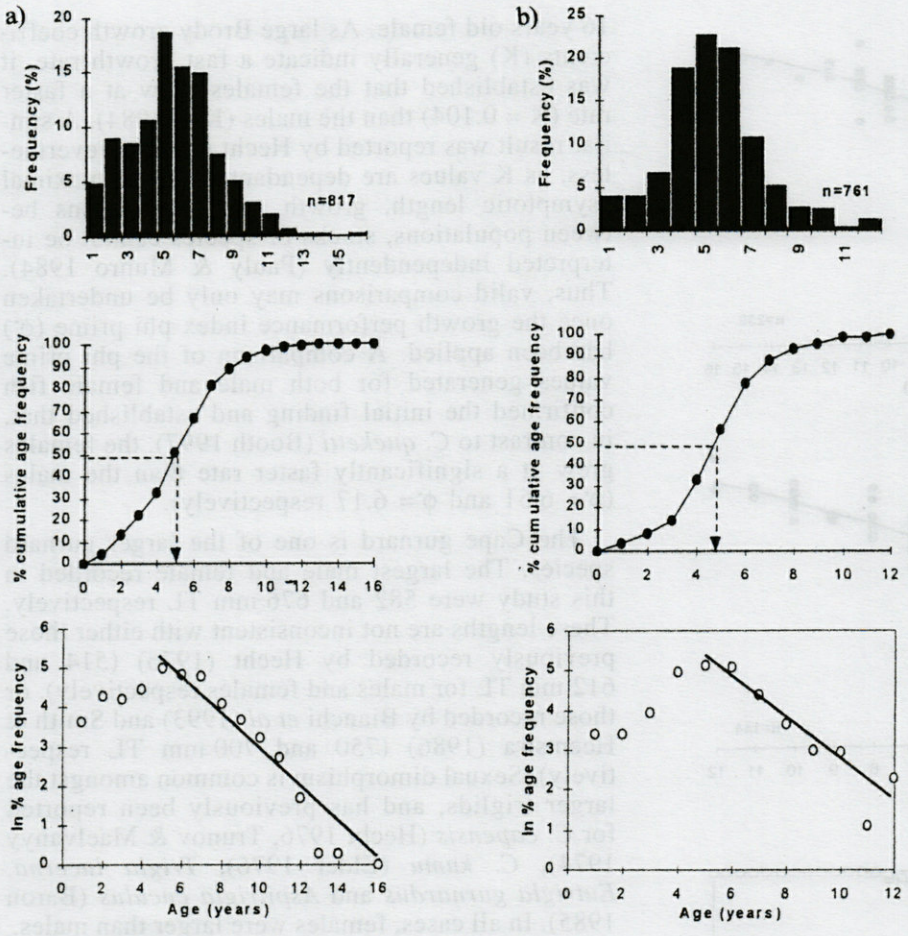

Fig. 5. – Age frequency distributions, and age at recruitment and catch curves for *C. capensis* sampled on the Agulhas Bank, South Africa. a, females; b, males.

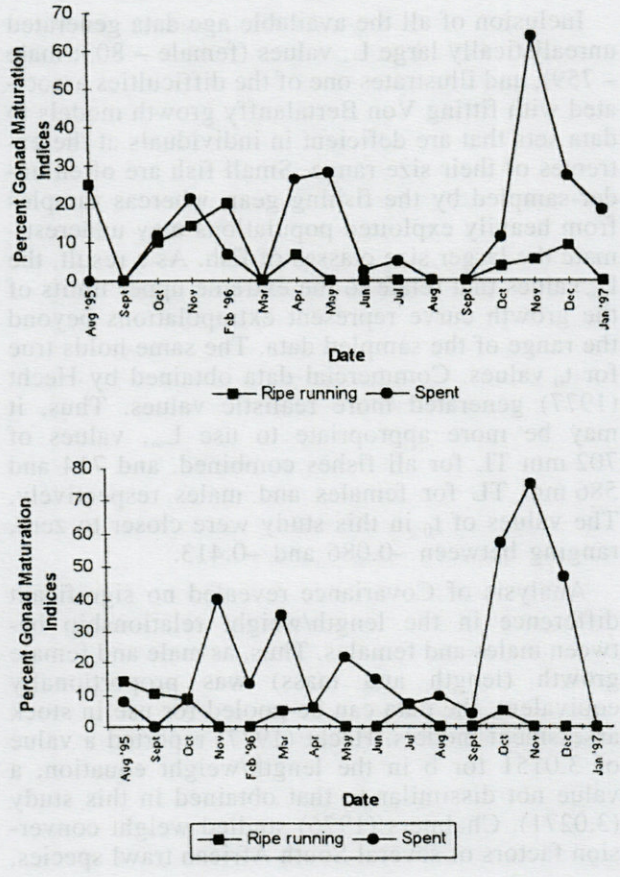

Fig. 6. – Monthly frequency distribution of the testes (top) and of the ovaries (bottom) of *C. capensis* in the ripe running and spent stages on the Agulhas Bank, South Africa.

and using historical data (1946-1974), generated the following length-weight relationship for whole weight gurnard

$$W=0.03470\ L^{2.67778}$$

It is interesting to note that the b value reported by Chalmers (1976) is lower than those reported by other studies on Cape gurnards. However, as the data used to generate this figure were derived from a number of gurnard species, it is probable that the smaller gurnard, *C. queketti* was included in the calculations. Length ranges obtained for Cape gurnard in this study were similar to those reported for the yellow gurnard, *Trigla lucerna*, in the Thermaikos Gulf, Greece, Papaconstantinou (1984).

Results from the first calculations of age at recruitment indicate that fishes are being recruited into the commercial fishery in their fifth year of growth, and are fully recruited by their tenth year. However, these figures may be overestimations. The commercial data employed in this study refers

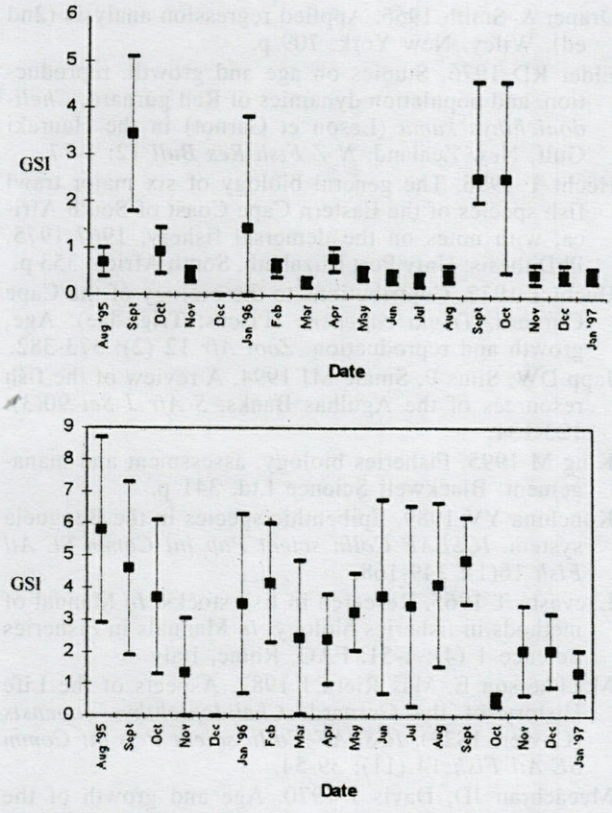

Fig. 7. – Mean monthly gonadosomatic indices (GSI) for male (top) and female (bottom) *C. capensis* sampled on the Agulhas Bank, South Africa. The error bars represent 1 standard error and deviation.

to landings and not catches. Fisheries observer data (Hart, unpubl data) has indicated that 29% of the gurnard catch is discarded at sea. Furthermore, as these fish do not command a high retail price, it is not unreasonable to assume that the smaller fish are selectively discarded. Thus, while further investigation is required, it is probable that the animals are being recruited to the fishery at an earlier age than this study would suggest.

The fishing mortality estimate (0.52 year^{-1}) for the pooled data was higher than the natural mortality estimate (0.085 year^{-1}), and therefore indicates that there is fishing pressure on *C. capensis* on the Agulhas bank. The total (0.56 year^{-1}) and natural mortality estimates (0.085 year^{-1}) for the pooled data recorded in this study were lower than those values obtained by Booth (1997) for *C. queketti* (0.73 and 0.38 year^{-1} respectively).

Male/female sex ratios of 1:0.9 and 1:1.4 were observed in the commercial and research data respectively. Similar sex ratios (approx. 1:1) are not uncommon in many fish species (Nikolsky 1978), and have been reported for other gurnard species (Elder 1976, Booth 1997). Hecht (1977) reported a male/female sex ratio of 1:1.28 for Cape gurnard sampled from commercial trawlers on the east coast of South Africa, the results from the current study suggest that fewer females are now present in the population. The change in the observed sex ratio could be an indication of increased fishing pressure that has selectively targeted the larger females. Alternatively, it could be the result of discard practices in which the cut-off size of discarded fish creates a change in the sex ratios of the retained catches.

The gonad maturation indices suggest that the *C. capensis* has an extended reproductive season. Peaks of reproductive activity were observed during January/February and August/September. A result confirmed by egg and larval distribution studies undertaken at Tsitsikamma National Park, that have demonstrated that the number of *C. capensis* larvae are highest during February, August and October – months directly following those of peak reproductive activity observed in this study (Wood, Rhodes University, pers. comm.). An extended spawning period is common amongst gurnards, and has previously been reported in *C. capensis* (Hecht 1977). In common with many species (Baylis 1981), male gonadosomatic indices were considerably lower than those of the females, and thus indicate that with respect to energetic investment, the males reproductive effort is lower than the females. With respect to the males, the combination of faster growth and reproductive effort associated with the females is difficult to reconcile. It is probable that there are sex specific energetic costs that are associated with the males. For example, the males may have to expend considerable energy maintaining a territory. However, such issues were beyond the scope of this study, and therefore require further investigation.

Generally, Triglid males mature at a younger age and smaller size than their female counterparts (Baron 1985, Papaconstantinou 1984). *C. capensis* was no exception with males and females maturing at 299 mm (3.6 yrs) and 343 mm TL (4.6 yrs) respectively. Nevertheless, Hecht (1977) reported 50% maturity at 340 mm TL (4 yrs) and 305 mm TL (3yrs) for males and females respectively. Although the ages at maturity are similar, the female length at maturity reported in this study (343 mm) was considerably higher than that reported by Hecht (1977). In contrast, the male length at maturity reported in this study (299 mm) was considerably lower than that reported by Hecht (1977). Changes in the relative lengths at maturity may be attributed to a number of factors such as selective targeting, or alternatively, increases in fishing pressures precipitating a reduction in the biomass; and consequently promoting alterations in the density dependent pressures such as intraspecific competition for food. Increases in food availability may alter growth and maturation rates as more resourses

become available for somatic and reproductive development. The maturation rates (δ) for the females (2.04 year^{-1}) and males (2.12 year^{-1}) indicated that there was little difference in the rates of maturation between the sexes.

In conclusion, the results of this study demonstrate that *Chelidonichthys capensis* is a long-lived, fast growing, r-selected generalist species with an extended reproductive season and correspondingly high reproductive rate. It is small at first breeding, and has a sex ratio of almost unity. The current levels of fishing do not appear to be negatively impacting the resource, and thus the potential to develop a fishery based on this species is promising. In particular, incorporation into the by-catch management plan that is currently being formulated for the South African fishing industry will promote the sustainable exploitation of this species.

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STRESS IN VERY YOUNG AND ADULT FISH

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ADAPTATION PHYSIOLOGY
STRESS AXIS
EARLY DEVELOPMENT
IMMUNOLOGY
COPPER

ABSTRACT – This paper reviews aspects of stress physiology of (young) fishes and the perspectives of this knowledge for aquaculture practices and ecophysiology. We will describe our present ideas about endocrine stress axes in fish. Particular attention will be paid to new insights in the bi-directional communication between the neuro-endocrine stress axis and the immune system and the potential modulation of stress axis activity by the immune system as well as to stressful experiences during early development, with a focus on waterborn copper as a modulator of stress axis activity.

PHYSIOLOGIE DE L'ADAPTATION
AXE DU STRESS
DÉVELOPPEMENT PRÉCOCE
IMMUNOLOGIE
CUIVRE

RÉSUMÉ – Une synthèse des aspects de la physiologie du stress chez les jeunes Poissons et des perspectives liées à ces connaissances en aquaculture et en écophysiologie est dressée. Nous présentons nos idées actuelles au sujet du stress endocrinien chez les Poissons. Une attention particulière est accordée aux nouveaux aperçus en matière de communication bidirectionnelle entre l'axe du stress neuro-endocrinien et le système immunitaire, à la modulation potentielle de l'activité de l'axe du stress par le système immunitaire, ainsi qu'aux expériences inductrices de stress au cours du développement précoce, avec notamment l'action du cuivre véhiculé par l'eau en tant que régulateur de l'activité de l'axe du stress.

INTRODUCTION

Our insight in the endocrinology of (stress in) fish is progressing rapidly due to modern molecular biological technologies with ever increasing specificity and sensitivity. The specificity requirement is particularly crucial to fish research considering the phenomenal number of species and their genetic variability. Recent estimates for the number of species of fish come to 35.000 (T. Iwamoto, California Academy of Sciences, pers comm). As a consequence, only a very limited number of species has been studied and thus our knowledge concerning fish endocrinology can only be fragmentary. More important, however, is the notion that an essentially unlimited wealth of adaptation strategies is found within this largest group of the vertebrates. Fish are vertebrates and demonstrate homeostasis of their "milieu intérieur", which differs only in details from the situation as we encounter in terrestrial vertebrates and that we often consider the standard. Fish do so essentially and independently of their habitat. It is relevant to realise oneself that fish live in an aqueous environment (there are of course exceptions found in e.g. lungfishes, mudskippers and annual fish). Living in water imposes an imminent threat of disturbance of homeostasis of the fish, as the animal is in a very close contact with this environment via gills. In gills an enormous surface is exposed to the water. Gills are in most fishes covered by a delicate and fragile epithelium designed for gas exchange; in addition, the epithelium of fish gills harbours the so-called chloride cells (after their initially determined function of chloride extrusion in seawater fish), ion transporting cells (further called ionocytes) that govern ion exchange phenomena and ion balance of the fish. Key in the functioning of these cells is an abundance of ion-pumps (H^+-ATPase, Na^+, K^+-ATPase, Ca^{2+}-ATPase, Na^+/Ca^{2+}-exchange, Na^+, K^+, Cl^-- and Na^+, Cl^--cotransporters etc.) that are associated with an elaborate(d) basolateral plasma membrane compartment; of all the transporters the H^+-ATPase and Na^+, K^+-ATPase are nowadays considered to provide the primary driving forces

for the multiple ion movements seen (Flik *et al.* 1995). As ion transport processes related to gas exchange are linked to those meant to guarantee ion balance it follows that ion strength, ion species (e.g. Na^+, K^+, Cl^-, H^+, HCO_3^-), gas content (O_2, CO_2) as well as physical conditions (e.g. absolute pressure with its consequences for partial gas pressures, water temperature, mixing zones) affect a multitude of interlinked physiological processes. Another important consequence of the thin, non-keratinising lining of the branchial apparatus is the inherent risk of invading organisms (uni- and multicellular parasites) and antigens (when lipophilic or in the event of epithelial damage), for which fish had to develop a strong and dependable innate as well as acquired immunity to successfully exploit and explore all the aquatic habitats where we now find fish. As we now know, the immune system and the neuroendocrine system share signals and receptors and communicate with one another (Verburg-van Kemenenade *et al.* 2001, Weyts *et al.* 1999) and thus understanding stress physiology requires insights in both systems and their signals. In very young fish the development of the immune system is assumed to follow (in time) that of the neuroendocrine system. In carp thymus and headkidney (albeit without lymphoid cells) as recognizable structures are detected no earlier than 3 days post fertilisation and lymphoid cell do not appear in carp headkidney but after two weeks of development (Romano *et al.* 1997). It would seem then that carp larval stages may provide natural models of fish with a certain time window to study the functioning of the neuroendocrine system independently of signals from the immune system. However, molecular biological approaches (Dr Verburg-Van Kemenade, pers comm) have allowed detection of immune parameters much earlier and independently of the existence of the organs proper as seen in adults (thymus and headkidney; thus cellular components later found in these organs may be around and cover the functions associated with these organs in later stages). The carp then, with its rapid development (hatching between 48 and 72 hours post-fertilization at 24° C) may be a less suitable model to test this hypothesis, but a multitude of fish species with slower developmental rate are at hand and we will focus the research in our laboratory on this topic in the near future.

Indeed, fishes inhabit every thinkable (aquatic) niche on earth: ion-rich – hyper-ionic compared to the blood plasma – waters like seawater, concentrated seawater as in evaporating tide pools or inland basins like the Dead Sea in Israel or the Salton Sea basin in southern California, ion-poor waters – hypo-ionic to the blood plasma – as most fresh waters, alkaline (soda lakes of East Africa) and naturally acid (e.g. Amazonian) or acidified waters (resulting from anthropogenic or natural sources of pollution), cold, even supercooled (Arctic and Antarctic seas), and warm waters (tropical streams and lakes). Considering that fish inhabit all strata of lakes, seas and oceans it follows that a volume is taken by fish many times larger than that of the terrestrial niche. Remarkably, fishes such as lungfishes, mudskippers, annual fishes and eels (and many others) have developed mechanisms to survive for prolonged times outside the water; lungfishes and mudskippers leave the water to bask in the sun, annual fishes survive periods of drought in mud and clay burrows, the eel encapsulates its gills in a "bag of mucus" and migrates over land when necessary. Imagine the fantastic array of adaptive capacities that fish must have developed already early in evolution to cope with a great variety in environments and that underlie their evolutionary success. In addition, fish were among the first vertebrates to develop complex social structures and behaviours (schooling, symbiosis, hierarchical populations etc.) that must have further contributed to their ecological radiation.

Stress axes in fish

The hypothalamic-pituitary-adrenal (HPA) axis plays a central role in the ability of vertebrates to respond to stressors and give an appropriate adaptive response. Physiology needs to be adjusted to new situations imposed by stressors and to this end an only partly understood neuroendocrine signalling system is activated. In fact, adjustments of physiology are continuously going on, often without being noticed, and whether we score an adjustment as 'adaptation to a stressor' or stress response may only be a matter of definition of gradual differences in the modulation of the HPA/stress axis. The "classical" (i.e. mammalian) hormonal signals that characterise the stress axis are corticotrophin releasing hormone (CRH) produced by neuroendocrine cells in hypothalamic nuclei near the optic chiasm and third ventricle, adrenocorticotropic hormone (ACTH) from the ACTH cells in the pars distalis of the pituitary gland and eventually cortisol or corticosteron from the zona fasciculata of the adrenal cortex. In fish, the end product of this particular axis (called HPI axis as fish have steroid producing interrenal cells in their headkidney rather than adrenal glands) is cortisol. Interestingly, cortisol has both gluco- and mineralocorticoid functions in fish (Wendelaar Bonga 1997, Fig. 1). It is tempting to predict then that all prime targets for the glucocorticoid (liver, muscle, immune system) *and* mineralocorticoid (gills, intestine, kidney) actions of cortisol and the resulting interactions should be considered in the response of the fish to stressors. The first, albeit still circumstantial, evidence that cortisol may work via both glucocorticoid and mineralocorticoid

receptors was very recently published by Sloman & coll (2001), who showed that the proliferation of chloride cells in trout gills is inhibitable by the mineralocorticoid receptor blocker spironolactone.

As in other vertebrates, synthesis and release of cortisol by fish interrenal cells is under primary and acute control of ACTH produced in, and released by distinct cell clusters in the pars distalis of the pituitary gland. In fish though, not every acute stressor evokes an ACTH response preceding cortisol production: seabream exposed to air for 3 min (a typical aquaculture-related stressor) show rises in plasma cortisol but not ACTH (and this contrasts with confinement by increased density that does evoke an ACTH response, Arends *et al.* 1999) and thus other pathways must be active in the stimulation of the interrenal steroid producing cells under particular conditions. Evidence is accruing that in the headkidney of fish the splanchnic nerve runs with pre- and postganglionic fibers through the interrenal compartment; in addition ganglionic cells are found on or in the headkidney that project to the interrenal components (Gallo *et al.* 2001). Thus a neural, sympathetic control of the interregnal cells may explain the cortisol response seen in the absence of an ACTH-surge (Fig. 1).

In addition to the ACTH-cells of the pars distalis, the pars intermedia of the pituitary gland is a source of corticotropic signals, for which α-melanocyte-stimulating hormone (α-MSH) and N-terminally acetylated β-endorphin (N-Ac-β-END) are likely candidates in tilapia, as demonstrated both *in vivo* and *in vitro* (Balm *et al.* 1995, Lamers *et al.* 1992, Lamers *et al.* 1994). The work of Lamers & coll (1994) further suggests a particular role for Thyrotropin Releasing Hormone (TRH) driven α-MSH as a mild corticotrope in adaptation to a long-lasting, unavoidable stressor (low water pH in that study). Clearly, the functions of the hypothalamic and pituitary signals do not obey the role their classical vertebrate name suggests (i.e. being releasing hormone for the pituitary thyrotropes and stimulating hormone for dermal melanophores).

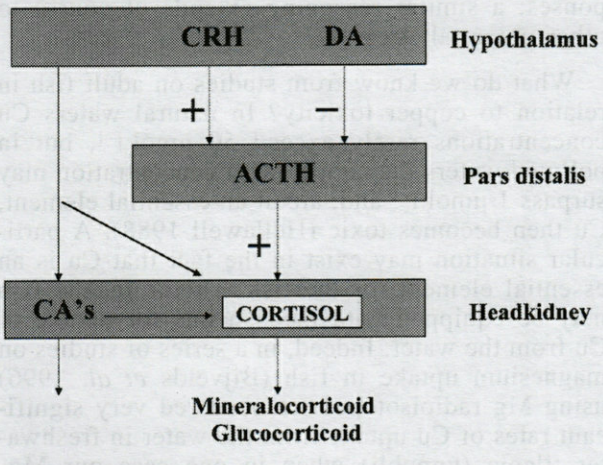

Fig. 1. – Scheme of stress axis activity during acute stress. In the acute stress response catecholamines (CA's) are released via neural pathways. Sympathetic activation leading to CA release may indirectly, via paracrine pathways, and independently from ACTH surges, stimulate cortisol release. The endocrine responses to stressors involve CRH release and inhibition of dopamine (DA) release at the level of the hypothalamus. The stress-induced ACTH-surge from the pituitary corticotropes is a powerful releaser of cortisol from the headkidney steroid producing cells. Cortisol has both glucocorticoid and mineralocorticoid actions, its effects being determined by the steroid receptor make-up of the targets.

An important implication of the particular arrangement of the headkidney – here neural, endocrine, and haematopoietic tissues are found closely together – is that paracrine (Gallo *et al.* 2001) catecholamine release from nerves or neurons following exposure to a stressor may not only activate the chromaffin and steroid producing cells but also the immune system components of this organ (Verburg-van Kemenade *et al.* 2001). And the immune system produces signals such as interleukin-1, tumor necrosis factor ∝ interleukin-6 that affect the neuroendocrine system and thus we can no longer appreciate the stress response of a fish unless we include this notion (Verburg-van Kemenade *et al.* 2001). Evidence was provided that cortisol has immunomodulatory actions on the immune system of carp, e.g. enhancing apoptosis of peripheral lymphocytes but protecting granulocytes from apoptosis (Weyts *et al.* 1998a, Weyts *et al.* 1998b). Such observations substantiate the communication between the neuroendocrine system and the immune system. Very recently, we have found the first in-vitro evidence that carp interleukin 1 stimulates release of MSH and acetylated endorphins from the pars intermedia (Verburg-Van Kemenade *et al.* 2001) and thus the communication between the neuroendocrine and immune system is truly bi-directional (Fig. 2). The mere arrangement of tissues found in fish headkidneys should intrigue and stimulate those involved in comparative studies in the fields of stress physiology and immunology.

Stress in very young fish: a special case for copper as stressor

There is consensus that early life stages of vertebrates are more vulnerable to disturbances than adult stages and this certainly holds true for fishes (von Westerhagen 1988). In fact, nanomolar concentrations of Cu in soft fresh water already disturb the hydromineral balance and the skeletal development of carp larvae (Stouthart *et al.* 1996); adult specimens become intoxicated only when Cu levels

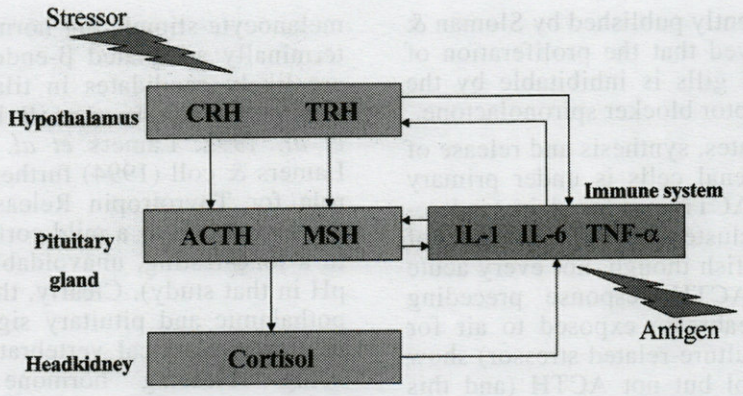

Fig. 2. – Scheme of the stress axis in fish and interaction with immune system.
The CRH, ACTH, cortisol axis (acute responses) and the TRH, MSH, cortisol axis (responses to mild long-lasting and unavoidable stressors) communicate bidirectionally with the immune system, that produces, among others, interleukin-1β (Il-1β) and interleukin-6 (Il-6) and tumor necrosis factor-α (TNF-α) upon stimulation with antigen. The components of the immune system that secure the innate immunity (macrophages, neutrophilic granulocytes, monocytes) are of prime importance for the fish to respond to acute threats. In very young fish this signalling pathway and modulator of the stress axis may be absent for designated time periods.

in the water exceed the micromolar level. With regard to Cu, to date only in *adult* fish an endocrine response to waterborne Cu was demonstrated (Pelgrom *et al.* 1995). The disproportionate sensitivity of fish larvae to toxicants could be attributed to their high surface-to-volume ratio and relatively high metabolic activity. Knowing that hatchlings of fish depend on a large population of integumental (skin, developing gills, yolk sac membrane) chloride cells with a turnover time of around 4 days or less (van der Heijden *et al.* 1999) and considering their crucial role in ion transports and gas exchanges described above for the adult situation, it follows that simply having these cells and their sensitivity to disturbing substances (pollutants, internal metabolites, stress signals) may be the primary cause of the sensitivity of fish early developmental stages to stressors. Alternatively, this sensitivity could relate to an only partly developed HPI-axis in early life stages and incomplete stress response. However, we have recently shown (Stouthart *et al.* 1998) that mere handling of carp eggs elevates the cortisol content. Apparently the embryo already responds with steroid production to handling and it thus follows that the steroid can affect embryonic stages as early as 50 h post-fertilization both in a beneficial and disadvantageous way. It remains to be determined *when* during development the HPI axis of this species becomes fully operational. An important gap in our knowledge exists where it concerns the first occurrence and species of cortisol receptors, as the effects of the steroid eventually depend on the activity of these transcription factors and their specific actions (mineralocorticoid and glucocorticoid receptor actions in fish). Studies on effective feedback by cortisol in early development may be an important tool for the physiologist to get insight in this process.

Knowing now that embryonic stages of fish already give a cortisol response to mechanical (handling) as well as to chemical (Cu) stressors, we have to consider that early exposure to the metal may be a stressful event, even when it is present in concentrations that do not evoke directly visible responses; a similar reasoning extends of course to other potential stressors.

What do we know from studies on adult fish in relation to copper toxicity? In natural waters Cu concentrations rarely exceed 50 $nmol.l^{-1}$, but in polluted waters the copper (Cu) concentration may surpass 1 $\mu mol.l^{-1}$ and, albeit an essential element, Cu then becomes toxic (Hellawell 1988). A particular situation may exist in the fact that Cu is an essential element for the fish. Therefore, the fish may be equipped with mechanisms for uptake of Cu from the water. Indeed, in a series of studies on magnesium uptake in fish (Bijvelds *et al.* 1996) using Mg radioisotopes we observed very significant rates of Cu uptake from the water in freshwater tilapia (unpubli) when in one case our Mg-probe appeared to be contaminated with Cu isotope (this isotope could be discriminated using a Ge/Li detector system). As this uptake was very rapid and constant it occurred in all likelihood via the gills. The chloride cells then would be the prime site to search for the Cu uptake mechanisms as these cells are equipped with the machinery to drive active and regulated ion transports. This being the case, Cu would be a "normal" element for the fish and toxic actions anticipated only at unnaturally high concentrations of Cu; moreover this particular situation would leave a window of Cu concentrations that the researcher could choose to mildly activate

the stress axis. This indeed seems to be possible (see below).

Unfortunately, fresh waters are often polluted, and fish exposed to toxic levels of Cu. As reflected by very significant increases in cortisol release (Pelgrom *et al.* 1995), Cu then apparently acts as a stressor and this is further substantiated by the fact that Cu disturbs the Na^+- and Ca^{2+}-homeostasis of the fish. It does so by a rather specific interaction with the chloride cells (Li *et al.* 1998) and this is, in addition to the reasoning above, another reason to believe that the chloride cells normally pass Cu from water to blood. Waterborne Cu promotes necrosis and apoptosis of branchial chloride cells and respiratory cells (Li *et al.* 1998, Mathiyalagan *et al.* 1996). An important notion in our understanding of Cu toxicity in fish is that fish exposed to waterborn Cu experience an unavoidable and persisting stressor. Fortunately, exogenously administered cortisol enhances the ion transport capacity of fish gills, as it increases the number of chloride cells and elevates the activity of both the Na^+, K^+-ATPase (McCormick 1995) and Ca^{2+}-ATPase (Flik & Perry 1989, Flik *et al.* 1995) activities in these cells. One may anticipate, therefore, that cortisol will ameliorate or even counteract effects of Cu on these cells, at least partly. Furthermore, cortisol protects chloride cells against necrosis as a gill filament culture pre-incubated with 0.3 $\mu mol.l^{-1}$ cortisol exhibits a 75% reduction in chloride cell necrosis when the tissue is exposed to 100 $\mu mol.l^{-1}$ Cu (Bury *et al.* 1997). Thus, prevention of ionoregulatory disturbances through actions on chloride cells appears to be a major function of the fish's endocrine stress response evoked by Cu, or any stressor that interferes with ion regulation and impinges on chloride cell functioning. Cu is in this respect an interesting stressor to study as its targets and effects are becoming increasingly clear.

When exposed to Cu, ACTH and cortisol contents in very early stages (48 hours post fertilization) of carp rise; this demonstrates that at least the peripheral components of the hypothalamo-pituitary-interrenal (HPI-) axis are active in embryonic stages (Flik *et al* 2001). This observation is in perfect agreement with an earlier study (Stouthart *et al.* 1998), where it was shown that pre-hatch stages of carp (50 hours post fertilization) produce cortisol in response to handling. We thus may conclude that the pituitary-interrenal axis becomes responsive and probably functional in this species before hatching. The very early response may be typical of carp as in some other species of fish cortisol production could be demonstrated only in later stages of development, i.e. as off 24 hours post hatching (DeJesus *et al.* 1991, DeJesus & Hirano 1992, Hwang *et al.* 1992, Hwang & Wu 1993, Barry *et al.* 1995, Sampath-Kumar *et al.* 1995). For handling of eggs and early hatchlings in aquaculture settings it may be beneficial to know

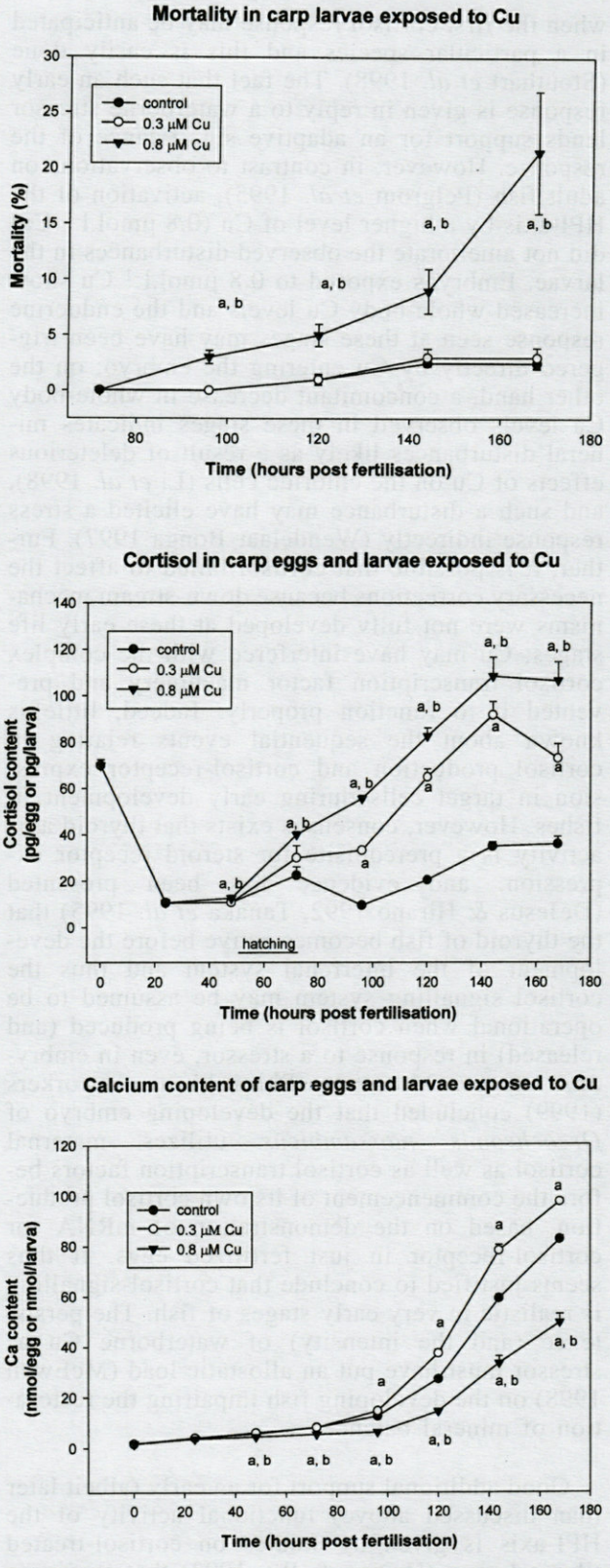

Fig. 3. – Mortality (a), cortisol (b) and Ca content (c) in carp larvae exposed to two levels of Cu (0.3 and 0.8 $mol.l^{-1}$). Data are expressed as means of 6 experiments and bars indicate SEM. Letters signify differences among groups at the 5% level, a compared to controls, b compared to the group exposed to the low concentration of Cu (data taken in modified form from Flik *et al.* 2001).

when the first cortisol response may be anticipated in a particular species and this is easily done (Stouthart *et al.* 1998). The fact that such an early response is given in reply to a waterborne stressor lends support for an adaptive significance of the response. However, in contrast to observations on adult fish (Pelgrom *et al.* 1995), activation of the HPI-axis by a higher level of Cu (0.8 $\mu mol.l^{-1}$ Cu) did not ameliorate the observed disturbances in the larvae. Embryos exposed to 0.8 $\mu mol.l^{-1}$ Cu show increased whole-body Cu levels and the endocrine response seen at these stages may have been triggered directly by Cu entering the embryo; on the other hand, a concomitant decrease in whole-body Ca levels observed in these stages indicates mineral disturbances likely as a result of deleterious effects of Cu on the chloride cells (Li *et al.* 1998), and such a disturbance may have elicited a stress response indirectly (Wendelaar Bonga 1997). Further, it is possible that cortisol failed to affect the necessary corrections because down-stream mechanisms were not fully developed at these early life stages; Cu may have interfered with the complex cortisol transcription factor machinery and prevented it to function properly. Indeed, little is known about the sequential events relating to cortisol production and cortisol-receptor expression in target cells during early development of fishes. However, consensus exists that thyroid axis activity is a prerequisite for steroid receptor expression, and evidence has been presented (DeJesus & Hirano 1992, Tanaka *et al.* 1995) that the thyroid of fish becomes active before the development of the interrenal system and thus the cortisol signalling system may be assumed to be operational when cortisol is being produced (and released) in response to a stressor, even in embryonic stages. Moreover, Shiraishi an coworkers (1999) concluded that the developing embryo of *Oreochromis mossambicus* utilizes maternal cortisol as well as cortisol transcription factors before the commencement of its own cortisol production, based on the demonstration of mRNA for cortisol-receptor in just fertilized eggs. It thus seems justified to conclude that cortisol-signalling is realistic in very early stages of fish. The persistence (and the intensity) of waterborne Cu as stressor must have put an allostatic load (McEwen 1998) on the developing fish impairing the restoration of mineral balance.

Good additional support for an early (albeit later than discussed above) functional activity of the HPI-axis is given by studies on cortisol-treated tilapia larvae (Hwang & Wu 1993) that survive a transfer from fresh water to 27.5 ‰ seawater better than did larvae which did not receive exogenous cortisol. Exogenous cortisol given to 1-day old tilapia enhances growth and development (Mathiyalagan *et al.* 1996). But one cannot exclude that exogenous cortisol impairs, by feedback, the ACTH release normally seen in response to a stressor and thus that an early cortisol response was artificially suppressed. The fact that growth and development were not disturbed in the experiments of Mathiyalagan and colleagues lends support for a physiological effect of cortisol in their experiments. Carefully chosen concentrations of Cu (0.3 $\mu mol.l^{-1}$; 0.8 $\mu mol.l^{-1}$ being already too high) elicit an cortisol response in very young carp without disturbance of mineral balance an example that cortisol/stress responses can be ameliorative and need not be deleterious (Flik *et al.* 2001). Larvae exposed to 0.3 $\mu mol.l^{-1}$ Cu had an elevated whole-body Ca content suggesting a stimulatory effect of cortisol on chloride cell activity as these cells are primarily involved in calcium uptake from the water. The observation that 0.3 $\mu mol.l^{-1}$ Cu does not affect the Na content of carp larvae suggests that sodium and calcium uptake are at least partly dissociated or that sodium uptake mechanisms associated with the chloride cells are more vulnerable to Cu exposure (Na^+ transport mechanisms being stimulated by cortisol and inhibited by Cu, resulting in net unaltered activity). This would be in line with conclusions based on in-vitro (Li *et al.* 1996) as well as in-vivo data (Li *et al.* 1998). It follows then that the *higher* Cu concentration (0.8 $\mu mol.l^{-1}$) poisoned chloride cells, inhibited both calcium and sodium uptake mechanisms and prevented a potential ameliorative action of cortisol leading to a decrease in whole body calcium content. Taken together these results indicate that cortisol is involved in the defence against Cu toxicity inflicted upon the chloride cells of the integument. Cortisol was shown to induce synthesis of the heavy metal-sequestering protein metallothionein in chloride cells specifically; this forms part of the adaptive response to toxic effects of high levels of Cu and again points to the chloride cells as important targets in the actions of cortisol (Dang *et al.* 1999).

The question arises how the (H)PI-axis becomes activated, in particular at lower Cu concentrations. Rainbow trout avoid water containing 2 $nmol.l^{-1}$ Cu (Folmar 1976), a concentration which is below that reported in some natural waters (Hellawell 1988) and not considered toxic at all. After hatching larvae are in direct contact with the ambient water and olfactory perception may allow the newly-hatched larva to trigger the HPI-axis, provided the connections between hypothalamus and pituitary have become functional to pass on the olfactory messages. One cannot exclude however, that as yet unknown signals produced by chloride cells or other mediator cells (macrophages etc.) become systemic and activate the stress axis. Cu *per se* can activate the genes coding for metallothionein and it could, therefore, trigger extracellular signals from these cells as well, an option open for future research.

Although exposure to low levels of Cu (0.3 $\mu mol.l^{-1}$) does not seem to disturb embryonic or larval physiology acutely, one cannot exclude the possibility that artificially increased cortisol levels near hatching can not have adverse effects on the subsequent development of the fish. In mammals prenatal stress alters offspring behaviour, morphology and physiology (Weinstock 1997) and modifies the stress response of the young (Meaney *et al.* 1996). Prenatally stressed human infants and rats show attention deficits, hyperanxiety and disturbed social behaviour during later life (Williams *et al.* 1998). Considering the sensitivity of early life stages, it is likely that stress experienced during embryonic and larval stages in fish has implications for stress-responsiveness during later life. This may be a concern for those involved in fish aquaculture and ecophysiological research.

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DAILY FEEDING RHYTHMS AND FISH PHYSIOLOGY

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FEEDING RHYTHMS
HUNGER
SATIATION
FOOD INTAKE REGULATION

ABSTRACT. – There is a considerable body of evidence demonstrating that hormones or metabolites involved in feeding, growth and energy partitioning show significant daily fluctuations suggesting that fish are in different physiological states at different times of the day. As such, they should respond differently to food depending on the time of feeding. It has been also demonstrated that the act of feeding periodically induce a pre-prandial locomotor activity. Thus, feeding time might have an influence on the phase or amplitude of some of the endocrine cycles involved in the physiological regulation of feeding. Nevertheless, data pertaining to the entraining effect of feeding time on endocrine cycles are scarce and results are equivocal. Although it is believed that feeding is required for the maintenance of a rhythmic pattern of circulating hormones and metabolites, the evidence for an effect of feeding time on the plasma profiles of hormones and metabolites involved in somatic growth is limited. It is concluded that daily rhythms in feeding activity may reflect adaptive responses to food availability and predators in the wild. It also depends upon endogenous mechanisms, and one might suppose that feeding activity occurs when the fish is physiologically best prepared to use nutrients efficiently. However the examination of plasma hormone profiles may not be particularly suitable for gaining information about the mechanisms involved in the effect of feeding time on growth of fish. Investigation of hormonal receptors, gene expression or enzymatic activity might provide more pertinent information to elucidate how feeding time affects metabolism and nutrient utilisation.

RYTHMES ALIMENTAIRES
FAIM
SATIÉTÉ
RÉGULATION DE L'INGESTION

RÉSUMÉ. – De nombreux travaux décrivent les fluctuations plasmatiques d'hormones et de métabolites impliqués dans la régulation de l'appétit, de la croissance et du métabolisme énergétique au cours du nycthémère. Ceux-ci suggèrent que les Poissons passent chaque jour par différents états physiologiques, et que l'utilisation des nutriments ingérés est affectée par l'heure d'alimentation. Lorsque les Poissons sont conditionnés à manger à heure régulière (une fois par jour), une activité locomotrice pré-prandiale quotidienne apparaît. L'heure des repas pourrait alors avoir une influence sur la phase et l'amplitude de certains cycles endocriniens impliqués dans la régulation de l'ingestion. Cependant les travaux portant sur la capacité de l'heure des repas à modifier des cycles endocriniens sont rares, et leurs résultats contradictoires. Bien qu'il soit largement reconnu que l'ingestion d'aliments participe au maintien des cycles des métabolites et de certaines hormones, la démonstration que l'heure d'ingestion affecte leurs profils reste peu probante. Les rythmes circadiens d'activité alimentaire sont probablement une des réponses adaptatives de l'animal aux cycles de disponibilité de la nourriture et de la présence des prédateurs dans le milieu naturel. Ils sont sous le contrôle de mécanismes endogènes, et on peut supposer que le pic d'activité alimentaire est synchrone avec la préparation physiologique à utiliser au mieux les nutriments ingérés. Cependant l'étude des récepteurs hormonaux, de l'expression de certains gènes ou des activités enzymatiques pourrait être plus pertinente que l'analyse des variations du profil plasmatique en hormones dans l'analyse des mécanismes impliqués dans l'effet de l'heure des repas sur la croissance.

INTRODUCTION

Fish are confronted, like other animals, to cyclical fluctuations of their environment. They have to be able to predict and respond to repetitive events, in brief, to develop capacities to evaluate cyclic changes in their environment and to adapt their behaviour. The capacity of the fish to respond behaviourally to these cyclic changes in a rhythmic way is well demonstrated for long (for review see Thorpe 1978), and the existence of rhythms of different periodicity (ultradian, circadian, tidally-synchronised, lunar, etc...), as well as the endogenous origin of some behavioural rhythms were discussed extensively in Ali (1992). In brief, in fish the suprachiasmatic nuclei of the hypothalamus, known as a major circadian oscillator in mammals, has never been identified but the circadian system, which is composed of circadian oscillators, includes the pineal organ and the lateral eyes (Falcon *et al.* 1992).

Feeding rhythm is a particular type of rhythmic behaviour. The first demonstration of the existence of a feeding rhythm under controlled conditions in fish was by Hoar (1942). He demonstrated that two salmonids, Atlantic salmon, *Salmo salar*, and brook trout, *Salvelinus fontinalis*, were eating less when feed was offered during the night than when it was offered during the day. Since then, rhythmic patterns of feeding activity have been described in many fish species, and the existence of not only circadian, but also seasonal rhythms in feeding activity is now largely recognised. In brief, fish are not eating all the time, and the temporal organisation of their feeding activity is under the influence of various factors of exogenous and endogenous origins:

Exogenous factors. It is evidenced that the light dark alternation is the main exogenous factor, but it should be reminded that any environmental factor, of either physico-chemical (t^o, O_2, turbidity, etc...) or biotic nature (predators, competition, etc...), may induce important changes in the profile of the feeding activity rhythm (for review see Spieler 1992, Boujard & Leatherland 1992a, Boujard & Luquet 1996, Boujard 1999, Bolliet *et al.* 2001b, Madrid *et al.* 2001).

Endogenous factors. Unequivocal results concerning the endogenous nature of the feeding activity rhythm in fish were obtained in European sea bass, *Dicentrarchus labrax* as well as goldfish, *Carassius auratus* (Sanchez-Vazquez *et al.* 1996), rainbow trout, *Oncorhynchus mykiss* (Sanchez-Vasquez & Tabata 1998, Bolliet *et al.* 2001a), and European catfish, *Silurus glanis* (Bolliet *et al.* 2001a) submitted to constant lighting conditions. The majority of the individuals studied displayed a free-running rhythm of feeding activity with tau (τ = the period of the biological rhythm) comprised between 22:15 h and 28:45 h. (for review see Madrid *et al.* 2001, Sanchez-Vasquez & Madrid 2001). The ability of fish to anticipate the time of feeding when food is given on a regular basis has also been recently demonstrated, but the location of a food-entrainable oscillator remains unknown (Sanchez-Vasquez *et al.* 2001).

It is of interest to investigate the link between these rhythms and the fluctuations over time of some metabolic and physiologic parameters. In this paper, we aim at reviewing the relationships between feeding and physiological rhythms in fish. In other words, one might wonder i) if some metabolic and physiologic parameters do also fluctuate in a rhythmic manner, and if so, ii) does the act of feeding influence these rhythms, and iii) is there any consequence of the time of feeding on the physiological state of the animal.

The cycle of hunger/satiety and the circadian rhythms of feeding

It is self-evident that the act of feeding has a physiological basis. This means that feeding activity should be triggered by internal signals, and the amount of food ingested within a period of time should be adapted to the metabolic needs of the organism.

The capacity of fish to adjust their feed intake in relation to the energy content of the diet has been demonstrated (Boujard & Médale 1994, Paspatis & Boujard 1996). It was therefore of interest to determine if circadian rhythm of feeding activity is influenced by the dietary energy levels. To that end, groups of European sea bass were fed on demand by means of self feeders, under Light: dark (LD) and constant light (LL) conditions, with a fixed or an unlimited amount of feed with variable lipid contents (Boujard *et al.* 2000a). Daily total feed intake, but not the feeding rhythm, was adjusted in relation to the energy content of the diet regardless of the lighting conditions (Table I). It was concluded that a satiation mechanism was likely responsible for the regulation of feed intake in relation to the dietary fat content but was not acting in itself on the mechanisms that drive the free-running rhythms of feeding activity. The same conclusion was also drawn from the study of Bolliet *et al.* (2001a), where rainbow trout and European catfish displayed free-running rhythms of feeding activity whether feed demand was rewarded by a distribution of food or not.

It is known in mammals that when the organism detect hunger signals to initiate another meal, the level of plasmatic free fatty acids increases rapidly and a short period of hypoglycemia occurs (Geiselman 1996). The existence of an increase in fatty acids in plasma of rainbow trout just before the time of feeding is demonstrated (Boujard &

Table I. – Voluntary feed intake (VFI, mean ± SD), voluntary energy intake (VEI, mean ± SD), and period lengths (τ, $P < 0.05$, values for each of the 3 replicates) of the circadian feeding rhythm of groups of sea bass fed on demand unrestricted amounts of diets with low (L), medium (M) or high (H) lipid content and submitted to LL conditions (from Boujard *et al.* 2000a).

		L	M	H	*p*
VFI (% biomass.24h^{-1})		1.06 ± 0.04	0.99 ± 0.05	0.80 ± 0.03	< 0.001
VEI (kJ.kg^{-1} fish.24h^{-1})		206 ± 8	214 ± 11	196 ± 8	not significant
τ (h)	*replicate # 1*	25:40	22:20	22:40	not significant
	replicate # 2	22:40	26:00	arrhythmic	
	replicate # 3	arrhythmic	21:20	22:40	

Leatherland 1992c, Boujard *et al.* 1993). There is also an increase in plasma concentrations of fatty acid in the late afternoon when fish are allowed to eat only in the morning (Fig. 1). It is well known that in fish, after the ingestion of food, plasma glucose concentrations increase during several hours (Bergot 1979, Brauge *et al.* 1995, Médale *et al.* 1999), and return slowly to their pre-prandial level. It has been shown recently that pre-prandial level of plasma glucose was negatively affected, and the plasma free fatty acids was positively affected, by the duration of feed deprivation. There was a correlation between these two parameters and the subsequent growth performance (Boujard *et al.* 2000b).

One might conclude that superimposed to the rhythm of feeding is the hourglass system regulating the cycle of hunger/satiety. In the homeostatic feedback model, hunger signals are generated when a critical level of depletion is detected for some of the regulated variables. Then, when the animals are eating, there is a monitoring of the total energy and the nutrients ingested until a critical level of repletion is reached (satiation) (See Geiselman 1996 for review on the control of food intake in mammals).

Although it seems reasonable to think that these cyclic changes in nutrient flow can act more or less directly as signals for hunger, satiation and satiety in the hypothalamus, as it is suggested in the glucostatic, lipostatic and homeostatic theories (see Geiselman 1996), it is also known that several peptides and hormones are involved in the regulation of feeding activity (Table II).

Among the peptides involved in the control of satiety, Cholecystokinin (CCK) is probably the most studied and can serve as an example in this review. CCK is found in peripheral and central neurons and in endocrine cells throughout the gut in numerous species, including fish (Aldman & Holmgren 1987). Two pathways have been suggested for the inhibitory effect of CCK on food intake: the action at peripheral sites, mediated by CCK-A-type receptors, and the action at central

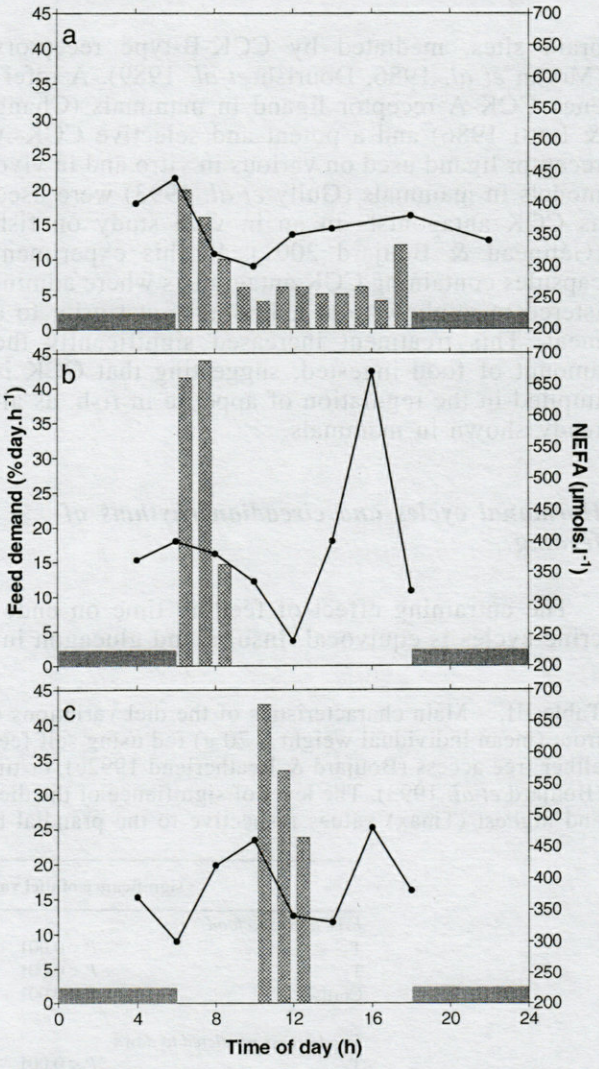

Fig.1. – Patterns of plasma non esterified free fatty acid concentrations (NEFA, full circles) and diel profile of feeding activity (histograms) in rainbow trout when food is available (a) 24h/24h, (b) between dawn and dawn +3h, and (c) between 12h and 15h. Redrawn from Boujard & Leatherland (1992c) and Boujard *et al.* 1993.

Table II. – Main hormones and peptides involved in the stimulation and in the suppression of food intake (see Geiselman 1996, LeBail & Bœuf 1997, DePetro & Björnsson 2001 for details).

Stimulation	Suppression
Aldosterone	Anorectin
Dynorphin	Bombesin
Beta-endorphin	Calcitonin
Beta-casomorphin	Cholecystokinin
Corticosterone	Corticotropin-releasing factor
Galanin	Enterostatin
Growth hormone-releasing hormone	Gastrin-releassing peptide
Insulin (short term)	Glucagon
Neuropeptide Y	Insulin (long term)
Peptide YY	Neurotensin
Thyroid hormones (long term)	Oxytocin
	Somatostatin
	Thyrotropin-releasing hormone
	Vasopressin

brain sites, mediated by CCK-B-type receptors (Moran *et al.* 1986, Dourish *et al.* 1989). A reference CCK-A receptor ligand in mammals (Chang & Lotti 1986) and a potent and selective CCK-A receptor ligand used on various in vitro and in vivo models in mammals (Gully *et al.* 1993) were used as CCK antagonists in an in vivo study on fish (Gélineau & Boujard 2001). In this experiment capsules containing CCK antagonists where administered to rainbow trout held singly just prior to a meal. This treatment increased significantly the amount of food ingested, suggesting that CCK is implied in the regulation of appetite in fish, as already shown in mammals.

Hormonal cycles and circadian rhythms of feeding

The entraining effect of feeding time on endocrine cycles is equivocal. Insulin and glucagon influence nutrient metabolism, and plasma concentrations are affected by feed intake (for review see Mommsen & Plisetskaya 1991, Le Bail & Bœuf 1997). Thyroid hormones (triiodothyronine [T_3], thyrosine [T_4]) are thought to play a permissive role in growth, by potentiating the effect of other anabolic hormones (Sumpter 1992). Growth hormone (GH) is considered to be a major hormone contributing to the regulation of somatic growth in teleosts (Björnsson 1997, DePedro & Björnsson 2001). Consequently, one might expect to find rhythms of these hormonal secretion that parallel those of the feeding activity. On the other hand, feeding time might have an influence on the phase or amplitude of some of these endocrine cycles, thereby affecting processes involved in energy use, and in nutrient partitioning and storage.

An effect of feeding time on circulating insulin has been reported in sea bass fed 2h or 7h after the onset of light (05:45) (Perez *et al.* 1988). A peak in plasma insulin concentration was observed around 15:00, but fish fed in the morning had their lowest plasma insulin concentration around midday, and those fed in the afternoon exhibited their lowest plasma insulin concentration around midnight. In addition, the fish fed early in the photophase had significantly lower plasma insulin concentrations than those fed later. However, according to the authors, the differences in hormonal levels might have been the result of quantitative differences in feed intake rather than to a direct effect of feeding time.

In rainbow trout fed using self-feeders, under different photoperiod regimes (Boujard & Leatherland 1992c), plasmatic concentrations in T_4 and cortisol was at their lowest level at dawn, and reached their highest values 2 to 6 hours after dawn. Plasmatic concentrations in T_3 was also very low at dawn but reached high values only 8 to 18 h

Table III. – Main characteristics of the diel variations of plasma content in T_4, T_3 and Cortisol measured in rainbow trout (mean individual weight = 70 g) fed using self feeders. The self-feeders are computer controlled in order to give either free access (Boujard & Leatherland 1992c), or time-restricted access to the food (3h/24h) at dawn or at midday (Boujard *et al.* 1993). The level of signifiance of the diel fluctuations is given, as well as the time of the lowest (Tmin) and highest (Tmax) values respective to the prandial time. n.s = not significant.

	significance of diel variations	Tmin	Tmax
Free access to food			
T_4	$P < 0.001$	Pre-prandial	Post-prandial
T_3	$P < 0.001$	Pre-prandial	Post-prandial
Cortisol	$P < 0.001$	Pre-prandial	Post prandial
Food access restricted to dawn			
T_4	$P < 0.001$	several	Post-prandial
T_3	n.s.	n.s.	n.s.
Cortisol	$P < 0.01$	several	post-prandial
Food access restricted to midday			
T_4	n.s.	n.s.	n.s.
T_3	n.s.	n.s.	n.s.
Cortisol	$P < 0.01$	pre-prandial	several

after dawn (Table III). There was no clear trend in plasma GH fluctuations.

In another study the relative importance of the time of feeding and the light/dark alternation as putative synchronisers of endocrine parameters were investigated in rainbow trout (Boujard *et al.* 1993). The self-feeders were programmed in such a way that they could deliver food only at certain times of the day, i.e. during the first 4 hours of the photophase or between dawn + 4 h and dawn + 7 h. A significant effect of the time of feeding was observed. This effect was not only a shift in the acrophase of the measured parameters, but mainly a decrease in the amplitude of the rhythm in the animals fed in the middle of the photophase compared to the animals fed at dawn. As an example, the diel profile of plasmatic concentration in T_4 was similar in the fish fed at dawn in comparison with those fed on demand without time restriction, but in the fish allowed to feed only in the middle of the photophase the diel variations in plasma T_4 concentrations were not significant anymore (Table III).

In a study on the effect of nocturnal vs diurnal feeding in rainbow trout (Gélineau *et al.* 1996), a clear effect was found with higher plasmatic T_3 and lower plasmatic GH concentrations in fish fed at dawn than in fish fed at midnight (Fig. 2). However this result was not confirmed in another trial performed in order to characterise more in details the diel profile of GH and thyroid hormones in rainbow trout (Gomez *et al.* 1996, 1997), in relation with the time of feeding. In this additional study fish were sampled at one hour intervals during 24 consecutive hours the plasma of catheterised individuals held single. On average, two peaks of GH, three peaks of T_4, and no peaks of T_3 were observed per 24 h. These peaks had very irregular patterns, thought more frequent during the scotophase in the case of GH, and they were not synchronised with the time of feeding.

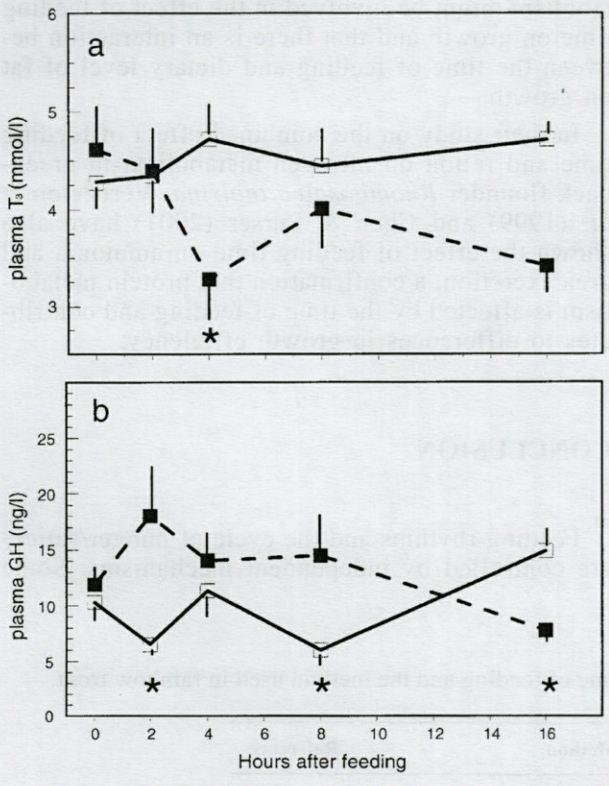

Fig. 2. – Post-prandial patterns of (a) plasma T_3 and (b) plasma GH concentrations in rainbow trout when food is distributed at dawn (full line and open squares) or at midnight (broken line and full squares). Stars indicate when differences are significant at $P < 0.05$ level and vertical bars = 1 SEM. Redrawn from Gélineau *et al.* 1996.

Apart from the studies presented above, only in the work done by Reddy & Leatherland (1994, 1995) was observed a phase shift of the post-prandial peak of circulating GH related to meal timing. Studies on the diel variations of thyroid hormones are more numerous. In some species, plasma T_4 concentrations have been shown by other authors to have diel periodicity. For example, plasma T_4 concentrations in goldfish exhibited a peak at 16:00 h (Spieler & Noeske 1981). By shifting the light/dark alternation and feeding the fish always at the same time, these authors showed that the T_4 peak always appeared during the late photophase regardless of the feeding time even when fish were not fed during the sampling period (Spieler & Noeske 1984). In fact, with the exception of the study reported by Osborne and co-workers (1978), in which plasma T_4 peaked during the scotophase there seems to be a diurnal acrophase of circulating hormone regardless of feeding time.

Cook & Eales (1987) found in rainbow trout that while plasma T_4 concentration did not show a significant diel change when the fish were fasting, a diurnal acrophase was present when the fish were fed at dawn or at the middle of the photophase. However, when the same species was fed four times during the photophase, there was no evidence for a plasma T_4 diel rhythm (Leatherland *et al.* 1977). Other examples are also found in sea bass, sea bream, *Sparus auratus*, and red porgy, *Pagrus pagrus* (Pavlidis *et al.* 1997, 1999a).

If the feeding activity rhythms are driven by thyroid hormone activity rhythms, one might expect T_3 changes to be the more significant than those of T_4, because T_4 is generally considered to be the precursor hormone for the biologically active T_3. An effect of feeding time on plasma T_3 was reported in the goldfish (Spieler & Noeske 1981). Fish fed in the afternoon had a highly significant rhythm of circulating hormone, the highest concentration occurring at 16:00, whereas fish fed in the morning did not show any significant rhythm. Others studies in which diel profiles of plasma T_3 have been investigated in rainbow trout have revealed

either low or no fluctuations, and no effect of feeding time on the profile (Eales *et al.* 1981, Holloway *et al.* 1994, Reddy & Leatherland 1994, 1995).

Daily changes in plasma cortisol concentrations in fish appear to be related strongly to the feeding schedule in goldfish, with the peaks preceding the time of feeding by approximately 4 hours (Spieler & Noeske 1981) but not in Common dentex (*Dentex dentex*, Pavlidis *et al.* 1999b). A phase shift of light/dark alternation did not affect the timing of the peak, but it did affect the amplitude of the peak (Spieler & Noeske 1984). In rainbow trout, plasma cortisol concentrations peaked during the scotophase, but secondary peaks corresponded to the time of feeding (Bry 1982, Rance *et al.* 1982, Laidley & Leatherland 1988). A similar post-prandial peak in plasma cortisol concentrations was reported in brown trout, *Salmo trutta* by Pickering & Pottinger (1983). In brief, a peak in plasma cortisol has been observed 4h before feeding in the goldfish (Spieler & Noeske 1981, 1984), at feeding time in rainbow trout (Bry 1982, Rance *et al.* 1982, Laidley & Leatherland 1988, Boujard & Leatherland 1992c), and several hours after feeding in brown trout (Pickering & Pottinger 1983) and rainbow trout (Boujard *et al.* 1993). In goldfish and rainbow trout, it has been suggested that fluctuations in plasma cortisol concentrations might be entrained by both feeding and photoperiod (Spieler & Noeske 1984, Boujard *et al.* 1993). However, when synchrony is observed between the cortisol peak and feeding time it is difficult to know whether this is a response to feeding *per se* or whether it is an expression of stress resulting from competition for food (Boujard & Leatherland 1992c).

Time of feeding and nutrient metabolism

A series of experiments were performed in rainbow trout with the aim of studying the effect of the time of feeding on nutrient metabolism (Boujard *et al.* 1995, Gélineau *et al.* 1996, 1998, Bolliet *et al.* 2000). The better feed efficiency and nutrient retention observed in fish fed in phase with their natural feeding activity appeared to be related to protein synthesis and retention (Table IV). This was because ammonia excretion, thought to result from a rapid oxidation of exogenous amino-acids (Brett & Zala 1975), was higher in trout fed at midnight than in those fed at dawn (Gélineau *et al.* 1998). Trout fed at dawn seemed to have a higher capacity for protein synthesis (assessed as RNA:DNA ratio, *cf.* Bulow 1987) in the liver than those fed at night (Gélineau *et al.* 1996). A reduced capacity for protein synthesis amongst fish fed at night would be expected to lead to amino acid deamination thereby leading to a less efficient use of protein for growth. In another study (Bolliet *et al.* 2000), the effect of feeding time in rainbow trout fed different dietary levels of fat on apparent digestibility efficiency and post-prandial protein synthesis was studied. Fish were fed either one hour after light on in the morning or one hour after light off in the evening with a low energy diet (LE, 6% lipid) or a high energy diet (HE, 23% lipid). Regardless of the diet, apparent digestibility and post-prandial protein synthesis were higher in fish fed in the morning than in those fed at the beginning of the night. In fish fed the LE diet in the morning, growth performance and nutrient retention efficiency tended to be higher than in those fed at the beginning of the night. In contrast, fish fed the HE diet in the morning had lower protein growth rate, protein content and protein retention efficiency than those fed in the evening. These results suggest that protein metabolism might be involved in the effect of feeding time on growth and that there is an interaction between the time of feeding and dietary level of fat on growth.

In their study on the combined effect of feeding time and ration on nitrogen metabolism in greenback flounder *Rhombosolea tapirina*, Verbeeten *et al.* (1999) and Chen & Purser (2001) have also shown the effect of feeding time on ammonia and urea excretion, a confirmation that protein metabolism is affected by the time of feeding and contributes to differences in growth efficiency.

CONCLUSION

Feeding rhythms and the cycle of hunger/satiety are controlled by independent mechanisms. Some

Table IV. – Summary of the studied variables affected by the time of feeding and the method used in rainbow trout.

	Method	Reference
Apparent digestibility	digestibility trial	Bolliet et al., 2000
Ammonia excretion	indirect calorimetry measurements	Gélineau et al., 1998
Capacity for protein synthesis	RNA/DNA ratio	Gélineau et al., 1996
Post-prandial protein synthesis	^{3}H-phenylalanine injections	Bolliet et al., 2000
Protein retention efficiency and protein growth rate	growth trials and carcass analysis	Boujard et al., 1995 Bolliet et al., 2000

metabolites, peptides and hormones appear to be directly involved in the hourglass mechanisms related to the cycle of hunger/satiety (CCK, Insulin, plasma levels of glucose, etc...). Other hormones are involved in feeding, growth and energy partitioning and they show significant daily fluctuations. But it remains very difficult to stress a clear and simple picture of the metabolic and physiological rhythms in fish. Indeed, the daily patterns of the studied parameters appears not to be consistent between the different studies. These differences may be related to the techniques used by the authors, the period of the year the experiment was performed, the age of the animal and its sexual state, etc... Further, no studies have been made in free-running conditions, so whether or not the studied parameters have endogenous rhythmicity and would continue to show diel variations in fish without food or under constant lighting conditions is not known so far.

Despite certain discrepancies, it seems that at least 2 synchronizers can be involved in the control of these fluctuations. Depending of the parameters studied, some are affected by the light/dark cycle, others by feeding-time and several appear to be under the control of both. These findings imply, as in mammals (Moore-Ede *et al.* 1976), a multi-oscillatory system in the temporal integration of fish with their environment.

If fish are in different physiological states at different times of the day, they should respond differently to food depending on the time of feeding (Spieler 1979). Accordingly, feeding fish in phase with their internal rhythms might provide the best conditions for nutrient utilisation (Bolliet *et al.* 2001 b).

Although it is believed that feeding is required for the maintenance of a rhythmic pattern of circulating hormones and metabolites (MacKenzie *et al.* 1998), the evidence for an effect of feeding time on the plasma profiles of hormones and metabolites involved in somatic growth is limited. Comparisons among studies are hampered by differences in experimental conditions; sampling interval, season, temperature, fish age and sex, reproductive stage and nutritional state. All are potentially confounding factors that may influence endocrine cycles (Perez-Sanchez *et al.* 1994).

This suggests that the examination of plasma hormone profiles may not be particularly suitable for gaining information about the mechanisms involved in the effect of feeding time on growth of fish. Investigation of hormonal receptors, gene expression or enzymatic activity might provide more pertinent information to elucidate how feeding time affects metabolism and nutrient utilisation.

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PHOTOPERIOD AND GROWTH IN FISH

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POISSONS
CROISSANCE
PHOTOPÉRIODE
GLANDE PINÉALE
MÉLATONINE

RESUMÉ. – La lumière représente un facteur écologique externe fort complexe incluant en fait divers aspects: le spectre de couleurs (qualité de la lumière), l'intensité (quantité) et la photopériode (périodicité). L'environnement aquatique est très particulier à ce sujet et les fluctuations de ces facteurs y sont extrêmement variables. En outre, la réceptivité à la lumière chez les Poissons varie considérablement d'une espèce à l'autre et, au sein de la même espèce, également au cours de la saison et en fonction du stade de développement. La revue proposée ici relate des données et discute sur l'aspect périodique de l'exposition à la lumière et les conséquences sur le développement et la croissance des Poissons. Non seulement la croissance, mais aussi tous les processus biochimiques, les fonctions physiologiques et les comportements sont rythmiques dans la nature et synchronisés par l'alternance jour/nuit. Les données de la littérature démontrent que le développement et la croissance des Poissons suivent des modalités saisonnières, liées aux fluctuations de la photophase. Généralement, les larves ont besoin d'un minimum d'intensité lumineuse pour pouvoir se développer et grandir, ceci en relation avec leur aptitude à détecter leurs proies et à s'alimenter correctement. Les longues photopériodes favorisent un développement harmonieux. Le rôle synergique longueur du jour/disponibilité en nourriture est l'un des points-clés. Chez les juvéniles et animaux plus âgés (Poissons marins et Salmonidés), la réaction à un allongement de la photophase est quasi-unanime, elle stimule la croissance. Seuls certains Poissons plats semblent moins réactifs, peut-être en relation avec leur comportement benthique. Le Saumon atlantique est tout particulièrement sensible, en eau douce et en eau de mer, et durant la smoltification. La question que nous posons est « Comment les effets de la photopériode sont-ils médiés » ? Les travaux menés en physiologie et endocrinologie suggèrent une influence de l'épiphyse. La glande pinéale chez les Poissons est directement photosensible. Chacune de ses cellules photoréceptrices de type cônes contient une horloge circadienne synchronisée par le rythme jour/nuit déclenchant la production de messages rythmiques. En fait, la pinéale ressemble à une rétine très simple sans réseau inter-neuronal organisé. Mais les photorécepteurs de l'épiphyse et de la rétine produisent le messager donneur de temps universel, la mélatonine. Celle-ci est synthétisée et utilisée « sur place » dans la rétine alors qu'elle est libérée dans la circulation par la pinéale. Notre revue synthétise également les informations disponibles sur le rôle de la mélatonine et de sa réceptivité chez les Poissons. Des données très récentes permettent d'imaginer une influence, soit directe via l'hypophyse, soit indirecte via l'hypothalamus, sur la synthèse et la libération d'hormone de croissance. La mélatonine peut aussi jouer un rôle sur le métabolisme thyroïdien et sur la prise alimentaire, deux aspects conditionnant la croissance. Les travaux futurs, combinant approches physiologiques « classiques » *in vitro* et *in vivo* et d'autres, pharmacologiques et moléculaires, permettront très probablement de mieux aborder ces questions du rôle de la photopériode sur la croissance. Tous ces travaux peuvent amener des applications fort prometteuses vers l'aquaculture.

FISH
GROWTH
PHOTOPERIOD
PINEAL GLAND
MELATONIN

ABSTRACT. – Light is a complex external and ecological factor whose components include color spectrum (quality), intensity (quantity) and photoperiod (periodicity). An aquatic environment has peculiar and extremely variable characteristics. Moreover, "receptivity" of fish to light changes profoundly from one species to another and, within the same species, from one developmental stage to another. The present review focuses on the periodic aspect of light supply and its consequences on fish growth. Not only growth, but virtually all biochemical processes, physiological functions and behaviors are rhythmic in nature and synchronized by the 24 h

light/dark (L:D) cycle. Available data indicate that fish growth follows a seasonal pattern which varies as a function of variations in daylength. Generally, larvae need a minimal threshold intensity to be able to develop normally and grow. This is probably related to the aptitude to localize, catch and ingest prey, and long daylength improves larval rearing quality. The synergistic effect of "food availability and daylength" appears to be determining at this stage. Older fish (marine and salmonid species), also react to photoperiod treatments and long daylength stimulates growth. The most studied species is the Atlantic salmon, which is very sensitive to changes in photoperiod, in fresh and seawater, and particularly during the parr-smolt transformation. Flatfish appear as a noticeable exception because growth was not affected by photoperiod in several cases in the species investigated. The question raises to know how are the effects of photoperiod mediated? Early physiological studies suggested a role for the pineal gland. The fish pineal organ is a light sensor. Each of its cone-type photoreceptor cells contains a circadian clock, synchronized by the 24 h light: dark cycle which, in turn, produces rhythmic messages. Actually, the pineal gland resembles a very simple retina with only very few interneurons. Pineal and retinal photoreceptors produce the "time-keeping molecule" melatonin. Retinal melatonin is used and metabolized *in situ*, whereas pineal melatonin has neurohormonal properties. The present paper reviews the information regarding the fish melatonin generating system and melatonin receptors. We also discuss recent evidence indicating the hormone might affect fish growth hormone secretion either directly (on the pituitary) or indirectly (hypothalamus). Melatonin may also influence thyroid hormone metabolism as well as food ingestion, two other factors that affect growth. Future studies combining "classical" physiological approaches (*in vitro* and *in vivo*) together with pharmacological and molecular approaches should provide insights into the mechanisms underlying the control of fish growth by photoperiod. This studies have great potential interest for aquaculture because they should provide indication on the best photoperiod conditions for optimizing fish "natural" growth all year long.

1. INTRODUCTION

From unicellular to vertebrates, environmental factors influence the activity of cells, organisms or populations. In fish, behavioral processes such as locomotor activity, skin pigmentation, thermoregulation, shoaling behavior, etc..., are under the influence of environmental factors. The same holds true for major physiological functions such as growth and reproduction.

Growth implies an increase in size or number of cells over time, with the important connotation of a positive energy balance (Mommsen 1998). Development and growth of teleost fish follows a pattern specific to each species. It differs from growth of birds and mammals in that it is a continuous process so that the older the fish the bigger (Boeuf *et al.* 1999). In fish the energy otherwise used to overcome the effects of gravity (reduced in an aquatic environment) and the energy cost of thermoregulation (required for endotherms) are available for growth. At hatching, fish are the smallest known vertebrates; therefore they will experience quite different metabolic scaling effects compared to other vertebrates. Another aspect contributing to the indeterminate growth pattern is the absence of calcified components with a definitive stop in growth layer increments (bone, otolith). The continuous growth of fish is mostly a consequence of cellular hyperplasia. Growth rates are lower in adults for many reasons including the potential limitations of the intestinal system that scales differently than muscle to animal mass and diffusion rates in the white muscle itself (Mommsen & Moon 2001).

Growth is rather flexible because it depends on a number of factors. As ectotherms, fish are highly dependent on temperature, but many other factors are also known to play a major role on the capacity to develop and grow. These include salinity, pH, oxygen availability, and eventual presence of "natural toxicants" (Boeuf & Le Bail 1999), as well as food availability and developmental stage (Sumpter 1992). Finally another, but nonetheless important, factor controlling growth is light. The main natural source of light is the sun, but other secondary sources are also available originating from the moon, stars, and luminescent organisms. When talking about light, one must consider several aspects including quality (wavelength), quantity (intensity-irradiance) and duration (periodicity) (Boeuf & Le Bail 1999). This review focuses on the latter aspect, *i.e.*, in the effects of the alternating phases of light (L) and dark (D) during the 24 h cycle.

Most of the fundamental rhythms in nature (diurnal or seasonal) are related to the periodicity of light (Edmunds 1988). They results from the rotations of the earth on its axis and around the sun. Many animals, including fish, exhibit a 24-hour cy-

cle in their activities (diel rhythm) which may be a matter of simple photokinesis (Clarke 1965). Fish are more active either during day or at night. For example, light-to-dark transitions are very important to synchronizing locomotor activity rhythms in the Atlantic salmon *Salmo salar* (Richardson & McCleave 1974), and fish feed actively during the day but not at night (Thorpe *et al.* 1988). Hence daylength may indirectly modify growth by increasing food intake or muscle mass by exercise. Most often, daily rhythms are driven by endogenous biological clocks, synchronized by the 24 h L:D cycle, and which would free-run under constant conditions (with a period approximating 24 h). Similarly, circannual rhythms are endogenous rhythms which fluctuate on an annual basis. Organisms with circadian clocks are able to predict and anticipate daily changes, so that the right event will occur at the right time. How do fish integrate the photoperiod information? Where are the biological clocks located? Does the photoperiod system modulates animal growth and how? These questions are also addressed in the present review. More precisely, we will focus attention on the melatonin generating system and its targets, because this molecule is now considered as the "hormonal messenger of photoperiod" in all vertebrates so far investigated (Zachmann *et al.* 1992a).

2. PHOTOPERIOD AND GROWTH

2.1. Larvae

Many studies have been carried out on cultured marine fish larvae, supplying light either continuously or over very long periods, compared to the natural conditions (rabbitfish (Duray & Kohno 1988); halibut (Hallaråker *et al.* 1995a), sole (Fuchs 1978); sea bass (Barahona-Fernandes 1979, Ronzani Cerqueira *et al.* 1991), green back flounder (Hart *et al.* 1996), gilthead sea bream (Tandler & Helps 1985, Ounais-Guschemann 1989), turbot (Person-Le Ruyet *et al.* 1991). Solberg & Tilseth (1987) demonstrated that yolk absorption was independent of the light regime in the cod *Gadus morhua,* except for larvae reared in the dark. In the sea bream *Archosargus rhomboidalis* (Sparidae), high levels of prey promoted good larvae growth under natural lighting conditions, but at low levels of prey growth increased with longer photoperiod (Dowd & Houde 1980). A "daylength-prey abundance" association is usable for the optimization of production cycles. For example, it was possible to produce juvenile halibuts from larvae, using a 6-month delayed photoperiod and ensure year-round production of juveniles (Naess *et al.* 1996). Hence, the "synergistic effect of food availability and light" is the most important factor acting on larval growth; it allows the optimal exploitation of the trophic level. However, a high growth rate may not be good for a normal development as suggested from studies in the sea bass (Ronzani Cerqueira *et al.* 1991).

2.2. Juveniles

A few studies have concluded to a lack of effect of photoperiod on flatfish growth. For example, juvenile yellow tail flounder *Pleuronectes ferrugineus* had similar growth and survival rates under 24L:0D, 18L:6D and 12L:12D conditions (Purchase *et al.* 2000). Also, growth rate of halibuts reared from 5 to 20 g was not affected by light regimes changing from 7 to 12 h L and from 12 to 18 h L (Hallaråker *et al.* 1995b). However, with halibuts of 30 g maintained for 5 months under different photoperiod conditions, a high specific growth and survival rate was observed under a 24L:0D cycle, whereas a 8L:16D cycle gave the poorest results; intermediate values were obtained under natural conditions (Simensen *et al.* 2000). Moreover, fish first maintained under short daylength exhibited an increased growth rate 21 days after being transferred to continuous light (Simensen *et al.* 2000). Turbots, reared for at least 3 months (at 10 and 16°C) under continuous light had slightly higher growth rates than those maintained under natural or constant 16L:8D conditions; however, no difference was seen after 6 –months (Imsland *et al.* 1995). In another series of experiments it was shown that feeding was not affected in turbots maintained for 60 days under six different photoperiods (constant 8L:16D, 16L:8D, 12L:12D, 24L:0D; increasing 12-16L and decreasing 12-8L) (Pichavant *et al.* 1998). However, Imsland *et al.* (1997) observed a better long-term growth (18 months) in turbots exposed to extended daylength during the first winter.

Positive effects of photoperiod on growth have been recorded in other species. A constant 16L:8D cycle enhanced growth in *Sebastes diploproa* compared to a 12L:12D cycle, and this can probably be related to a greater scope for growth due to their lower standard metabolic rate (Boehlert 1981). In the gilthead seabream and sea bass, long photoperiod delayed spawning and increased somatic growth (Silva-Garcia 1996, Kissil *et al.* 2001, Rodriguez *et al.* 2001). The differences appeared after a long exposure time in the seabream (45-145 days depending on the light regime) (Silva-Garcia 1996), and were maintained up to 11 months (Kissil *et al.* 2001). Daily feed consumption was affected by the onset of spawning, and the efficiency of feed utilization and energy retention was also positively correlated with the long photoperiod (Kissil *et al.* 2001). A similar situation had been described in the green sunfish, *Lepomis*

cyanellus maintained for 6 weeks at four photoperiods (constant 8L:16D, 16L:8D, increasing 8-16L and decreasing 16-8L). In this species, food intake is directly correlated to the amount of light to which the fish were exposed (Gross *et al.* 1965). Fish growth and food conversion efficiency were closely correlated and were generally highest in the increasing photoperiod, even when temperature was the same in spring and autumn.

In salmonids, for which there is not a true larval stage, Brännäs (1987) failed to demonstrate an influence of photoperiod during the yolk sac phase or on behavior at emergence in the Atlantic salmon. In the same species, Berg *et al.* (1992) obtained a good relationship between the duration of lighting and growth after first feeding: growth decreased on reduced daylength. This species is particularly receptive to extended daylength and grows very well, even under continuous light, eating continuously during the photophase. In an experiment lasting 192 days after the first feeding, where both temperature and photoperiod were changed, Thorpe *et al* (1989) found that in late summer the greater the growth opportunity (°C X daylength hours), the greater the proportion of young salmon maintaining good growth and within the upper mode of the population (see also below).

In rainbow trout *Oncorhynchus mykiss,* maintained under a natural photoperiodic cycle a reduced rate of decreased daylength favored growth and food conversion efficiency (Mäkinen & Ruohonen 1992). A longer light phase favors food intake and also possibly food conversion (Mason *et al.* 1992). Better growth and food conversion efficiency rate have been observed under continuous illumination during the first year (Maisse & Le Bail unpubl results). In Arctic charr, Mortensen & Damsgård (1993) found that a long photoperiod increased the compensatory growth observed after a previous "warm" (11 °C) temperature and short days pre-treatment. Hence, it appears that growth of non-migrating salmonid species is sensitive to increasing daylength under artificial conditions. However, these results do not take into account any of the other endogenous growth cycles which have been described in these species (Jobling 1987, Saether *et al.* 1996, Noël & Le Bail 1997), and which could also be influenced by light.

A considerable amount of literature is dedicated to the effects of photoperiod on Atlantic salmon juveniles. Not all of these studies can be referred here, but the effects of photoperiod are so clear for this species that they merit special attention. The major difficulty in extrapolating results is the existence of the major developmental transformation from parr to smolt (see reviews in Fontaine 1975, Hoar 1988, Boeuf 1993). Photoperiod exerts an important role in salmon smoltification (Hoar 1988, Boeuf 1993, Saunders *et al.* 1994, Solbakken *et al.* 1994, Sigholt *et al.* 1995), and growth cannot be dissociated from smoltification. At the end of the freshwater residence phase and just before migration, fish are euryhaline, and they grow very fast. During the first year, before completion of parr-smolt transformation, light stimulates growth. Lundqvist (1980) showed that a longer photoperiod (20L: 4D opposed to natural light or 6L:18D stimulated Baltic salmon growth during autumn. However, the "size-structure" of the experimental population was not considered in this study. The Atlantic salmon has a specific developmental strategy with two modes, weight and size, appearing in the population during the first year, 7 to 9 months before the completion of smolting. Thorpe (1987) proposed that photoperiod synchronises an endogenous rhythm, genetically determined, and regulates the time of the "switch" of the differentiation is made into two growth modes. Decreasing daylength may cause the appearance of bimodality: transfers of fish from continuous light to natural photoperiod (range 12-15 hours) are followed by a segregation in growth rates into lower and upper modes fish (Skilbrei 1991, Skilbrei *et al.* 1997). Under continuous light, bimodality is low or absent and the individual decision to enter the upper mode with fast growth is strongly dependent on the fish size at the time of winter light stimulus. Seven weeks of short-day treatment reduced growth in comparison with the continuous light exposed salmon (Sigholt *et al.* 1998). It is essential for completion of smolting to expose fish to an increasing photoperiod after short-day conditions (Kristinsson *et al.* 1985, Gaignon & Quemener 1992, Björnsson *et al.* 1995). In some cases, one was able to dissociate a pure growth effect of light from those linked to smolting: long term (a few months) constant long daylength stimulates growth, but is increasing daylength necessary for parr-smolt transformation? (Saunders *et al* 1985, Duston & Saunders 1992).

Feeding activity is fundamental, as salmon do not eat at all or at least very little during night time (Thorpe *et al.* 1988), even if they can do during very short photoperiods. Maybe, they can be looking for food at the bottom of the tank (olfactory sense?) during the night (Jorgensen & Jobling 1992). Villarreal *et al.* (1988) suggested that the delays observed in growth, after daylength reduction, reflected a synchronizing effect of photoperiod on an endogenous rhythm of appetite and growth. At present, it seems that growth, linked to daylength, is related to food intake.

All these data lead to the possibility of producing 0^+-age smolts, and at present, an important part of smolt production makes use of light manipulations. One can produce 7-8 month old smolts, with a good growth, and ability to adapt to seawater (Saunders & Duston 1992, Thrush *et al.* 1994, Duncan & Bromage 1998). In the Ifremer laboratory of Brest, 0^+-age smolts of different sizes have been produced using three photoperiod regimes

(16L:8D; 12L:12D and 8L:16D) for 5 months (following three months at 12L:12D; *in* Boeuf & Le Bail, 1999). Fish were reared in indoor 1 m^2 Swedish type tanks in constant light (L:L) and temperature conditions at densities of 15 $kg.m^{-2}$. They were fed dry commercial pellet (Aqualim) daily by an automatic feeder. Growth appeared related to both temperature and lighting conditions.

After seawater transfer, Atlantic salmon growth may also be influenced by daylength. Presently, many farmers in Norway and Scotland use continuous lighting during the autumn or winter (October-April in the North hemisphere) to improve growth: growth in fish subjected to natural daylight is depressed during the autumn and winter, while, conversely, no such growth depression in winter is observed under a continuous light regime (Forsberg 1995). Several authors, using photoperiod treatments, have experimentally demonstrated a substantial improvement of postsmolt growth in sea water (Saunders & Harmon 1988, Kråkenes *et al.* 1991, Hansen *et al.* 1992). However, in these experiments, such treatments not only stimulated growth, but also triggered earlier sexual maturation. It is known that somatic growth is accelerated during the first steps of the gametogenesis, an effect mediated by steroids (Le Bail 1988). Hence, it is possible that under these conditions, a great part of the light-promoted stimulation of growth is related to reproduction. However, in a recent study (Oppedal *et al.* 1997), it has been demonstrated that, if light intensity was sufficient, abrupt changes from natural short photoperiod to continuous additional light (January-June) promoted growth without triggering maturation.

Other studies have been carried out in Pacific salmon species, mainly coho *Oncorhynchus kisutch* and chinook *O. tshawytscha*. In 1978, Clarke *et al.* showed that the sensitivity of young fry to photoperiod varied seasonally. In 1986, Clarke & Shelbourn concluded that bimodal growth in juvenile salmon was a function of a photoperiod phase at the time of first feeding and it was possible to produce underyearling coho smolts. Extended daylength also stimulates growth for Pacific species (Thorarensen & Clarke 1989), as it does for Atlantic salmon. In fact, it is not the accumulation of light exposure that initiates smolting, but rather the time during the day when light is experienced. Moreover, responsiveness to inductive photoperiods depends on the initial photoperiod treatment (Thorarensen & Clarke 1989). Thorarensen *et al.* (1989) exposed young coho salmon to different levels of night illumination ranging from 0.0001 to 0.05 lux, after a first period at short-day (10L: 14D, during 12 weeks) and a second period under inductive lighting (9L: 9D: 1L: 5D or 24L: 0D): they observed slower growth rates for the fish exposed to nocturnal illumination. It seems that a period of total darkness is needed to obtain maximum growth.

In conclusion, increasing daylength exerts a greater influence on salmon smoltification than constant daylength. It seems important for freshwater fish to experience a few weeks of short-day conditions prior to transfer in increasing daylength conditions. Even if in nature this smolting phenomenon cannot be dissociated from somatic growth, the preceding data show that a long daylength (changing or constant) stimulates growth specifically. The observed great dependence of Atlantic salmon on photoperiod might be due to the strains used and high latitude conditions. It would prove interesting to compare the photoperiod responsiveness of northern and Southern strain.

3. THE ENDOLYMPH/OTOLITH SYSTEM

It is of relevant interest to discuss the possible role of the inner ear of teleost because fish otolith exhibits daily and annual rhythmic depositions in relation to photoperiod and light sensitivity. Furthermore, otolith increments have been used for a long time as indicators of life history, aging and somatic growth. They are composed of calcium carbonate crystals in the aragonite form, enmeshed in an inorganic matrix composed largely of a keratin-like protein (Wright *et al.* 1992). Accretion occurs through the alternate deposition of a mineral/matrix-rich layer with a mineral deficient layer. This is done on a daily basis in many species, so that a recognizable daily increment is produced (Pannella 1980).

A few scientists have questioned the role of photoperiod on otolith growth. In Atlantic salmon, deposition is regulated by an endogenous circadian rhythm synchronized to the 24 h L:D cycles (Wright *et al.* 1991). Otolith calcification declines at night and resumes lower levels at dawn: a diel fluctuation in net calcium accretion, linked to plasma calcium concentration, appears (Wright *et al.* 1992). A similar phenomenon is recorded in rainbow trout (Mugiya 1987), Arctic charr (Adams *et al.* 1992) and pike *Esox lucius* (Wang & Eckmann 1992). In embryonic and larval rainbow trout, photoperiod is a potent synchronizer of the daily rhythm of deposition, whatever the photoperiod conditions (6L:6D; 12L:12D; 24L:24D; 24L:0D and 0L:24D; Mugiya 1987). It is not so easy to correlate somatic growth and otolith growth, probably because numerous factors are involved in the control of each of them. Actually, incremental increases in otolith width appear to be linked to photoperiod, whereas increases in the number of rings appear to be related to feeding activity (Neilson & Geen 1982). Other external fac-

tors, such as temperature, may also modify the ratio between somatic and otolith growth, as shown in young turbot (Kossmann, Leroux & Boeuf unpublished observations).

Very little information is available concerning the physiology of the endolymph-otolith complex. The saccule has specialized small and big cells which display all the characteristics of gill ionocytes (Mayer-Gostan *et al.* 1997). The presence of a pH gradient in the inner ear of teleost is a unique common pattern among vertebrates. This is probably related to bio-calcification of otoliths. pH variations could be the major factor affecting the rate of the daily calcium deposition (Payan *et al.* 1997). The lack of spatial uniformity in both the otolith and the saccular endolymph must be taken into account when studying otolith and fish growth (Payan *et al.* 1999). It is not known whether the fine control of photoperiod on otolith growth involves a nervous and/or an endocrine signal. Interestingly, somatic and scale growths were totally inhibited in hypophysectomized goldfish *Carassius auratus* while otolith growth was only slightly reduced (Mugiya 1990); injections of pituitary extracts (GH) restored the normal conditions. On the other hand, starvation resulted in both somatic and otolith growth depression in rainbow trout (Mugiya & Oka 1991).

4. HORMONAL CONTROL OF GROWTH

4.1. Somatotropin

Somatotropin (or growth hormone) originates from the anterior pituitary gland and plays a major role in fish growth and adaptation (Le Bail *et al.* 1993, Sakamoto *et al.* 1993). As early as 1976, Komourdjian *et al.* suggested that somatotropin could play a role as a part of a "light-pituitary axis" in the growth of Atlantic salmon during smoltification. In fact, during this process, plasma GH levels increase "naturally" after the spring equinox, when photoperiod rapidly increases (Boeuf *et al.* 1989, Prunet *et al.* 1989). Generally, increased daylength accelerated the parr-smolt transformation and associated growth, and increased blood GH levels (Björnsson *et al.* 1989, 1995, Stefansson *et al.* 1991, McCormick *et al.* 1995). Exposure to continuous light in autumn and winter causes a "free-running" of an endogenous rhythm governing smolting and a subsequent phase-delay of the smoltification-related increase in circulating GH levels (Björnsson *et al.* 1995, 1998, Björnsson 1997). Similar results of somatotropin increase during smoltification completion have been obtained for masu salmon *Oncorhynchus masou* (Okumoto *et al.* 1989). However, outside of the smolting completion period, increasing light does not necessarily increase GH levels, even if somatic growth is increased, as shown in three Pacific salmon species (Clarke *et al.* 1989). It is interesting to note that in the seabream, the seasonal increase of plasma growth hormone seems more related to daylength than temperature (Perez-Sanchez *et al.* 1994).

In mammals, circulating somatotropin is higher at night than during the day (Harvey & Daughaday 1995). In fish, the daily rhythms in GH content are related to feeding activity (Reddy & Leatherland 1994, Holloway *et al.* 1994) as well as to the L:D cycles (Bates *et al.* 1989, Boujard & Leatherland 1992). In a study of cannulated rainbow trout, Gomez *et al.* (1996) noted peaks in GH values, but they were irregular and asynchronous in individual fish; there was no clear-cut rhythm, but a trend to higher values at night. However, none of these studies provide a link between the daily rhythm and somatic growth capabilities.

It should also be mentioned that generally plasma GH levels were inversely correlated to growth performance in fish (Le Bail *et al.* 1993). GH receptivity studies should be useful to better understand how daylength may influence growth. Adelman (1977) did not observe growth differences between carp *Cyprinus carpio* reared at 9L: 15D and 16L:8D, after treatment with mammalian GH. IGFs are probably very important in the mediation of light influences on growth. Studies of IGFs and insulin have only been possible in fish for the last few years and further experiments will be needed to evaluate a possible direct action of GH and the role of IGFs in these pathways. Recently, Elies *et al.* (1996) cloned and sequenced an IGF1 receptor in two teleost species, turbot and trout. Insulin and IGF1 receptors have been cloned and sequenced and mRNA expression studied in the turbot (Elies *et al.* 1999) and GH receptor has been molecularly characterized (Calduch-Giner *et al.* 2000).

4.2. Thyroid hormones (TH)

The thyroid gland of fish produces high amounts of thyroxine (T_4) which is then transformed into tri-iodothyronine (T_3) in peripheral tissues. The same receptor binds the T_3 and T_4 molecules, but with much higher affinity for the former than for the latter (Eales 1985). T_3 and T_4 levels vary on a seasonal basis in fish sampled in their natural habitat (Osborn & Simpson 1978, Eales & Fletcher 1982); two optima were reached in winter and in summer.

Daily variations in T_4, and less pronounced or even undetectable variations in T_3 are usually described in laboratory fish (Cook & Eales 1987, Gomez *et al.* 1997, Noeske & Spieler 1983). In

rainbow trout, the T_4 rhythm resulted from an interaction between feeding and photoperiod regimes (Boujard & Leatherland 1992). It is noteworthy than in trout and other salmonids, growth rate is significantly correlated to the daily average T_3 value although a marked diurnal rhythm is observed only with T_4 (Eales & Shostak 1985, Boeuf & Gaignon 1989, McCormick & Saunders 1990, Gomez *et al.* 1997). Hence, T_3 levels appear to provide a good estimation of growth responsiveness to light. However, it cannot explain all of the effects of light on fish growth. Indeed, Okumoto *et al.* (1989) found that plasma TH were not affected by changing daylength in masu salmon, although growth was stimulated. In the killifish, *Fundulus heteroclitus* Brown & Stetson (1985) showed that long days (14L:10D) increased, and short days (8L:16D) diminished, the negative feedback sensitivity of the hypothalamus-pituitary axis to TH. They proposed that such a photoperiodically-induced change could aid in the year-round maintenance of thyroxine levels necessary for seasonal adaptation and survival.

The roles of TH during parr-smolt transformation have been reviewed by Boeuf (1993). In the Atlantic salmon, increasing daylength stimulated growth and plasma thyroxin levels, without affecting T_3 (Mc Cormick *et al.* 1987). Under continuous light, T_4 levels remained low, but only true smolts grew "normally" after transfer to seawater. It is hypothesized that under normal photoperiod, the high T_4 levels could act as a growth stimulator at the end of the fresh water stage, in spite of the fact that T_4 has lower affinity than T_3 for the nuclear receptor. However, it should also be noted that T_4 plays many other roles during this period. For example, Iwata *et al.* (1989) discovered that coho and chum salmon *Oncorhynchus keta*, treated with thyroxine changed their phototaxis. Finally, the changes in visual pigments composition observed in the retina during smoltification might reflect modifications in the thyroid function. Indeed, the visual response of the retina to TH is altered after treatment with TH blockers (Alexander *et al.* 1998).

A few studies have noted a relationship between growth, TH levels and the phases of the moon (Grau *et al.* 1981, Farbridge & Leatherland 1987a b, Nishioka *et al.* 1989, Hopkins 1992). However, the effects of the moon would possibly be mediated by the lunar attraction rather than by the direct incident light (Noël & Le Bail 1997).

4.3. Other hormones

Other hormones such as insulin and steroids also have an effect on fish growth; however, information lacks in terms of their relation to the influences of light. Regarding sex steroids, the available information is only related to reproduction and gonadal development. However, puberty is strongly dependent on photoperiod during this phase: the puberty dependent-androgen secretion increase has an influence on somatic growth (Le Bail *et al.* 1988, Le Gac *et al.* 1993). Somatostatin (SRIF) is also known for strongly inhibiting GH secretion in all vertebrates, including fish. McCormick *et al.* (1995) found higher levels of plasma somatostatin-25 in salmon reared under a 9L:15D cycle, but no variation after exposure to longer daylength. Plasma levels of both somatostatins 25 and 14 are higher in stunting coho than in smolts (Sheridan *et al.* 1998). One study, published in 1996 by Zhu & Thomas, demonstrated an influence of different backgrounds and altered illumination on red drum *Sciaenops ocellatus* plasma and pituitary somatolactin (SL, which is a member of the prolactin/GH family of proteins): they found that both plasma and pituitary SL levels were higher in fish exposed for one week to black background and that circulating SL was maximal one day after transfer to a black background tank without illumination. SL may be involved in the adaptation to colored surroundings. However, at present, little is known about a possible involvement of SL in growth regulation.

5. THE MELATONIN GENERATING SYSTEM AND MELATONIN RECEPTORS IN FISH

To synchronize rhythmic functions and behaviors to the daily and annual cycles, an organism needs photoreceptive organs which will transduce the photoperiod information and produce output messages to convey this information to target centers. Fish possess two such photoreceptive organs, the retina of the lateral eyes and the pineal gland. Melatonin is one of the different messages they elaborate in response to the alternation of light and dark (Falcón 1999). As reviewed below, melatonin is considered a "time-keeping" molecule. We will focus attention on the pineal gland because retinal melatonin is unlikely to be involved in the synchronization of rhythmic events outside the retina. Indeed, retinal melatonin is produced, used and metabolized *in situ* where it has autocrine or paracrine effects: it modulates the sensitivity to light, the release of neurotransmitters; also it coordinates retino-motor movements (of cones, rods and retinal pigment epithelium), and outer segment disk shedding (Wiechmann & Smith 2001). Conversely pineal melatonin is released into the blood and cerebro-spinal fluid, and acts through specific receptors on target sites. It is not unreasonable to believe that many of the effects of photoperiod on

physiological and behavioral processes, including growth, are mediated through melatonin.

5.1. The fish pineal gland resembles a simplified retina

The pineal is an evagination from the roof of the diencephalon located just below the skull and connected to the diencephalon by the pineal stalk (Collin 1971, McNulty 1984). In most cases, a lumen filled with cerebrospinal fluid is opened to the 3rd ventricle (Omura & Oguri 1969, McNulty 1984, Falcón *et al.* 1992). Three main cell types make up the pineal parenchyma: photoreceptor cells, neurons and glial (interstitial) cells. Glial cells occupy the full height of the pineal parenchyma and isolate the other cell types from the blood vessels surrounding the organ. The pineal photoreceptor cells resemble to the retinal cones of vertebrates. At one end of the cell, the photoreceptive pole (outer segment) protrudes in the pineal lumen. At the other end, one or several pedicles establish synaptic contacts with dendrites of the 2nd order neurons (Ekström & Meissl 1997, Falcón 1999). This organization much resembles the organization of the retina, however with a much lesser degree of complexity. For example, although few inter-neurons have been described, no rod photoreceptors, bipolar, amacrine and horizontal cells *per se* are seen in the pineal gland.

The 2nd order neurons send their axons to the brain via the pineal tract which runs dorsally to the pineal stalk. It is noteworthy that the central projections from the pineal organ and retina of fish partly overlap (*e.g.*, pretectum, dorsal thalamus and preoptic area) (Ekström 1984, Ekström & Meissl 1997).

5.2. The fish pineal gland elaborates rhythmic messages regulated by light

The fish pineal gland elaborates at least two important messages, in a rhythmic manner, a nervous and a neurohormonal message.

5.2.1. The nervous message, an excitatory neurotransmitter

In response to light, a pineal photoreceptor behaves like a retinal photoreceptor, and the mechanisms of phototransduction are similar in both cell types, actually the two cell types are anatomically and functionally analogous (refs in Falcón 1999). The pineal photoreceptor is depolarized in the dark and hyperpolarized during day (Meissl & Ekström 1988a b, Ekström & Meissl 1997). A major difference between the retinal and pineal photoreceptor is that under prolonged illumination the latter maintains an intensity related membrane potential so that it acts as an indicator of gradual light intensity changes (Ekström & Meissl 1997). It cannot discriminate between rapid light changes as the retinal photoreceptor does. This is consistent with the idea that the pineal gland functions as a luminance detector (Meissl & Dodt 1981). Information is transmitted to the second order neurons *via* an excitatory neurotransmitter, the release of which is inhibited upon photoreceptor hyperpolarization (Ekström & Meissl 1997). As a result, the spike discharges by the axons of the pineal tract are inhibited by light and increased in the dark. Inhibition is directly related to the intensity of the stimulus. The organ can integrate variations in intensity up to a 9 log units range in the pike (Falcón 1999). It is noteworthy that, when studied in parallel, the pineal and the retina of the same species exhibit similar spectral sensitivity curves (Falcón & Meissl 1981). The two organ express similar but different photopigment molecules, most probably as a result of gene duplication (Mano *et al.* 1999). In the pineal gland, spectral sensitivity curves may be recorded at the level of the photoreceptor cells or second order neurons; they are identical in both cases. The responses are usually sensitive in the green range or less often in the green and red range of wavelengths (Meissl & Dodt 1981, Ekström & Meissl 1997, Falcón 1999). In pike, dark adaptation curves show a shift of sensitivity indicating there is a photopic and a scotopic range of sensitivity (Falcón & Meissl 1981). This provides the animal with a greater adaptive advantage compare to those with either one of the sensitivity types.

5.2.2. The neurohormonal message, melatonin

Melatonin is synthesized from tryptophane which is taken up from the circulation. Tryptophane is converted to 5-hydroxytryptophane, by means of the tryptophane hydroxylase (TPOH), and 5-hydroxytryptophane is decarboxylated by the aromatic amino-acid decarboxylase to produce serotonin. Melatonin is synthesized from serotonin by the action of two enzymes: the first one, the arylalkylamine *N*-acetyltransferase (AANAT), converts serotonin to *N*-acetylserotonin; the second one, the hydroxyindole-O-methyltransferase (HIOMT), methylates *N*-acetylserotonin to produce melatonin (Klein *et al.* 1981, 1997). A combination of methods (histochemistry and immunocytochemistry, radio-autography, etc.) allowed to demonstrate that this pathway is active in the photoreceptor cells (refs in Falcón 1999). Melatonin may be either released or deacetylated *in situ* to produce 5-methoxytryptamine and 5-methoxytryptophol (Falcón *et al.* 1985, Yañez & Meissl 1996). Because of its highly lipophylic character, the molecule crosses easily the cell membrane. Other serotonin derivatives,

such as 5-methotrytryptophol, are produced from serotonin by the photoreceptor cells. However, unlike melatonin, their physiological role has yet to be assessed.

The production and release of melatonin by the pineal gland is rhythmic and synchronized to the 24 h L:D cycle. In fish as in all vertebrates classes so far investigated, production is higher during night-time than during daytime (Bolliet *et al.* 1995, 1996a, Falcón *et al.* 1987, 1989, Porter *et al.* 2001). AANAT is the key enzyme which expression and activity are regulated by the L:D cycle. AANAT activity increases after lights off and decreases late at night and early in the morning, as melatonin secretion does (Falcón *et al.* 1987, 1989, Morton & Forbes, 1988, Zachmann *et al.* 1992b). The increase in AANAT activity results, in pike and zebrafish *Danio rerio*, from an increase in AANAT gene expression which starts in the afternoon and decreases after midnight (Bégay *et al.* 1998, Coon *et al.* 1999, Klein *et al.* 1997). The decrease in AANAT activity results from both a decrease in AANAT expression and the light-dependent activation of enzyme proteolysis, as shown in seabream and pike (Falcón *et al.* 2001). In trout, AANAT gene expression is constitutive; variations in AANAT activity lie only upon AANAT protein proteolysis which is high during day and low at night (Bégay *et al.* 1998, Falcón *et al.* 2001). Unexpected illumination at night decreases AANAT activity as well as melatonin release, *in vitro* or *in vivo* (Falcón *et al.* 1989, Max & Menaker 1992, Bolliet *et al.* 1995). In the trout, the spectral sensitivity curves indicate a rhodopsin-like sensitivity (Max & Menaker 1992), as is the case for the release of the excitatory neurotransmitter in this species (Ekström & Meissl 1997).

Like the nervous message, the melatonin message also provides information on daylength, and this is achieved through its release in the blood (and may be in the cerebrospinal fluid). Variations in blood melatonin content are higher during night than during day *i.e.*, they mirror the variations in pineal melatonin production (Gern *et al.* 1978a, Falcón *et al.* 1987, Kezuka *et al.* 1988 1992, Zachmann *et al.* 1992b c, Iigo & Aida 1995, Randall *et al.* 1995, Pavlidis *et al.* 1999, Rebollar *et al.* 1999). As a consequence of the seasonal changes in daylength, the duration and amplitude of the plasma melatonin rhythm varies along with seasons in temperate regions (Kezuka *et al.* 1988, Randall *et al.* 1995), thus providing an accurate information on calendar time. Usually, the melatonin signal is of short duration and high amplitude in summer, and of long duration and short amplitude in winter (Kezuka *et al.* 1988). It has been suggested that duration is controlled by photoperiod, whereas amplitude is controlled by temperature (Garcia-Allegue *et al.* 2001, Samejima *et al.* 2000). Results from *in vitro* studies support these conclusions.

Melatonin is also produced by the retina (see introduction). However, retinal melatonin does not contribute to the circulating levels for the following reasons: (1) only the pineal and plasma melatonin rhythms are in phase (Falcón & Collin 1991, Falcón *et al.* 1987, Zachmann *et al.* 1992b), and in many species (sea bass, pike, brook trout and rainbow trout) ocular melatonin levels are higher during day than during night, *i.e.*, in a 180° anti-phase with the blood rhythm (Falcón & Collin 1991, Zachmann *et al.* 1992b, Zaunreiter *et al.* 1998a b, Garcia-Allegue *et al.* 2001); (2) cultured sea bream, pike and white sucker retinas do not release melatonin into the culture medium (Molina-Borja *et al.* 1996, and unpublished observations); (3) pinealectomy, but not eyectomy, suppresses plasma melatonin in the goldfish and salmon (Kezuka *et al.* 1992, Iigo *et al.* 1997, Mayer 2000). The trout is the only fish species known where pinealectomy diminishes the nocturnal plasma melatonin surge without completely suppressing the L:D variations (Gern *et al.* 1978b).

5.3. The melatonin rhythm is driven by circadian clocks located within the pineal photoreceptors

Experiments conducted *in vivo* and *in vitro* provided indication, in most but not all (see below) of the species investigated, that the rhythm in melatonin secretion was not a simple passive response to the alternation of light and darkness. For example, whether light at night causes a rapid decline in AANAT activity and melatonin secretion, night during day does not necessarily induces an increase in pike (Falcón *et al.* 1987). Under experimentally manipulated photoperiods the melatonin rhythm follows the imposed L:D cycles, but this is achieved progressively (Bolliet *et al.* 1995, Molina-Borja *et al.* 1996). Moreover, the melatonin rhythms have been shown to persist in the pineal gland and blood of animals maintained under constant darkness (D:D), whereas a low-amplitude rhythm may be detected under L:L (Falcón *et al.* 1987, 1989, Bolliet *et al.* 1995, Porter *et al.* 2000). In culture, entire organs, pieces of glands, or dissociated cells maintain a rhythmic release of melatonin under D:D (Falcón *et al.* 1989, Ligo *et al.* 1991, Bolliet *et al.* 1994, 1996a b, Cahill *et al.* 1996, Okimoto & Stetson 1999a b). Altogether this indicates that multiple circadian oscillators drive the melatonin rhythm. Bolliet *et al.* 1996b provided definitive proof that these oscillators are located within the photoreceptor cells. The authors were able to monitor melatonin secretion from individual photoreceptors (using the reverse hemolytic plaque assay) or cultures made exclusively of

photoreceptors (using radioimunoassay); under L:D or D:D, both the amount of melatonin released and the number of melatonin producing photoreceptors, were higher during night (or subjective night) than during day (or subjective day).

Taken together, these findings indicate that single photoreceptor cells contain a circadian clock, a photoreceptive capacity and the ability to secrete melatonin. The L:D cycle synchronizes the clocks which in turn drive the melatonin rhythm. This is achieved through control of AANAT gene expression which is maintained rhythmic under L:L or D:D (Bégay *et al.* 1998, Coon *et al.* 1999).

Interestingly enough is the observation that the pineal gland of salmonids exhibits no rhythm under constant conditions (Gern & Greenhouse 1988, Iigo *et al.* 1997, Thibault *et al.* 1993). Under these conditions, AANAT activity and melatonin levels remain high in the dark and low in the light, independent on the duration of the light and dark phases (above refs). There is, to date, no explanation for these species-dependent variations.

5.4. Temperature modulates melatonin secretion by the fish pineal gland

In cultured pineal glands of pike, temperature cycles superimposed to photoperiod cycles enhance the amplitude of the rhythm when the cryophase coincides with the scotophase, and reduces the amplitude when the cryophase coincides with the photophase (Falcón *et al.* 1994). The opposite holds true in the white sucker (Zachmann *et al.* 1991, 1992c). Temperature cycles are able to synchronize the clocks that drive the rhythm in melatonin secretion. In D:D, melatonin peaks with the cold phase in the white sucker, and with the warm phase in the pike. However, temperature cycles are unable to entrain the circadian clocks, and temperature pulses cannot shift the phase of the clocks as light does (Falcón *et al.* 1994). Moreover, temperature does not affect the period of the free running rhythm under D:D in the sailfin molly and pike (temperature-compensation), but the oscillations are no more seen below a threshold level (Bolliet *et al.* 1994, Okimoto & Stetson 1999a b), consistent with the observation that blood melatonin rhythm is of higher amplitude at 12 °C than at 4 °C in juvenile salmon (Porter *et al.* 2001).

In brief, photo- and thermo-period interact in fish to determine the amplitude and duration of the melatonin rhythm. The effects of temperature cycles are complex, and vary from a species to another. Differences result probably from variations in the metabolisms proper to each species. In cultured pineal glands, cyclic AMP accumulation AANAT activity peak at 12-15 °C in trout, and 18-25 °C in the pike (Thibault *et al.* 1993). AANAT activity from recombinant proteins as well as from gland homogenates exhibit the same maximum, indicating that this is a property of the AANAT protein. Under a 12 °C/20 °C temperature cycle melatonin would peak with cold temperature in the trout, and warm temperature in the pike.

5.5. Melatonin receptors in fish

Melatonin acts through specific membrane bound receptors which belong to the seven transmembrane domain G-protein coupled receptors (Reppert *et al.* 1995). The receptors are usually coupled negatively to the cAMP pathway, but effects on other second messengers have also been reported (Vanecek 1998). Three receptor subtypes have been identified to date in vertebrates: MT1 (Mel1a), MT2 (Mel1b) and Mel1c (Dubocovich *et al.* 2000, Reppert *et al.* 1995, Shiu & Pang 1998). In fish, the full length cloning of a melatonin receptor has been obtained only in pike (Gaildrat *et al.* 2001); partial cloning has been achieved in zebra fish (the three subtypes), trout (Mel1a, Mel1b), and pike (Mel1a) (Gaildrat & Falcón 2000, Mazurais *et al.* 1999, Reppert *et al.* 1995).

Distribution of melatonin receptors has been investigated using *in situ* hybridization in trout (Mazurais *et al.* 1999), RT-PCR in pike (Gaildrat & Falcón 1999, Gaildrat *et al.* 2001), and binding of 2-[^{125}I]-iodomelatonin (^{125}I-Mel) on tissue sections and membrane preparations from goldfish, pike, skate, seabream, salmon and trout (Martinoli *et al.* 1991, Ekström & Vanecek 1992, Davis *et al.* 1994, Iigo *et al.* 1994, Pang *et al.* 1994a, Falcón *et al.* 1996, Gaildrat *et al.* 1998, Mazurais *et al.* 1999). In the brain, the receptors exhibit a widespread distribution which differs, in terms of intensity, between the Mel1a and Mel1b receptors. The highest expression is consistently found in the optic tectum of all fish studied including deep sea gadiform fish (above refs and Priede *et al.*, 2000). Other areas include the olfactive bulbs, telencephalon, preoptic area, thalamus, pretectal area, and cerebellum. Actually, brain melatonin receptors are seen in areas involved in sensory (visual, olfactive, auditive) integration. Melatonin receptors are also expressed in the retina (Gaildrat & Falcón 1999, 2000, Gaildrat *et al.* 2001, Iigo *et al.* 1997). This is consistent with the observation that retinal melatonin has auto/paracrine effects (see above). Studies in the *Xenopus* have indicated the receptors are expressed in photoreceptor and ganglion cells, as well as some unidentified cells of the inner nuclear layer (Wiechmann & Smith 2001). In peripheral tissues, melatonin receptors have been evidenced in fish heart (Pang *et al.* 1994b).

Of great interest is the observation that melatonin receptors are also found in the pituitary gland of pike (Mel1b>Mel1a) and trout (Gaildrat & Falcón 1999, 2000). The number of sites is less

than in the brain, but the affinity for melatonin is the same. As evidenced by *in vitro* autoradiography on tissue sections, the binding of ^{125}I-Mel is located in the antero-ventral part of the organ, an area known to contain gonadotropines (GtHs), prolactin (PRL) and growth hormone (GH) producing cells. Furthermore, melatonin modulates cAMP levels by cultured pike and trout pituitary organs indicating these receptors are functional (above refs).

The binding of ^{125}I-Mel to brain sections or membrane preparations exhibits daily changes in pike, seabream and goldfish (Gaildrat *et al.* 1999, Falcón *et al.* 1996, Iigo *et al.* 1994), but not in salmonids (Ekström & Vanacek 1992, Pang *et al.* 1994a). However, further studies are necessary in salmonids because (1) there were only two sampling times a day, and (2) experiments on whole brain homogenates may obscure variations in restricted areas. It is noteworthy that in the pike, the rhythm in the number of binding sites (high during daytime and low during night-time) is 12 h out of phase when compared with the rhythm in plasma melatonin content (Gaildrat *et al.* 1999). This might reflect a down-regulation of melatonin receptors at night, induced by melatonin itself, as suggested from preliminary unpublished experiments. Interestingly, the variations reported above are maintained in pike under constant conditions. The chronograms obtained under L:L or D:D displayed a slight phase advance when compared to the chronograms obtained under L:D. Altogether, these results support the idea that in fish, photoperiod mediates part of its effects through both the rhythmic production of melatonin by the pineal and the rhythmic expression of the melatonin binding sites in the brain.

6. HOW DOES THE PINEAL GLAND MEDIATE THE EFFECTS OF PHOTOPERIOD ON FISH GROWTH?

As the transducer of the photoperiod information in the organisms, there is indication that the pineal gland, through its output melatonin, might mediate the effects of photoperiod on fish growth. However, investigations on this matter are more than scarce, probably because physiological studies dealing with the effects of pinealectomy on physiological functions often ended with contradictory results. This is reviewed and discussed in the excellent review of Ekström & Meissl (1997). An early physiological study in the goldfish had shown photoperiod-dependent effects of pinealectomy on growth (De Vlaming 1980). Removal of the pineal gland resulted in a reduced growth rate under short, but not long, photoperiod; and melatonin administration reversed the effect under short photoperiod only. A similar experiment was conducted 20 years later in the Atlantic salmon parr (Mayer 2000). In this case, pinealectomy resulted in lower specific growth rates during the period of lengthening photoperiod until summer solstice; but thereafter, *i.e.*, during the decreasing photoperiod, pinealectomized fish exhibited higher growth rates. This indicates that the mechanisms by which the pineal gland may modulate growth are complex. Such a complexity is emphasized by the observation that pinealectomy also affected, in a photo-dependent manner, body lipid content in the golden shiner *Notemigonus crysoleucas* and longnose killifish *Fundulus similis* (De Vlaming 1975, De Vlaming *et al.* 1974). However, melatonin injections exerted almost identical effects than pinealectomy, when one would expect opposite effects.

Although the effects of the pineal gland and melatonin are not yet elucidated, there is evidence suggesting that it participates in the control of fish growth. There are several direct and indirect ways through which melatonin could act. These include a control at the level of the hypothalamus-pituitary axis and/or of peripheral tissues involved in energy supply and food intake. Also, melatonin may be acting either on growth itself or on food intake and food conversion.

6.1. Melatonin and food intake

A recent study by Pinillos *et al.* (2001) has shown that melatonin administration inhibited food intake in the goldfish. Interestingly, the effects were observed after peritoneal injection, not after intra-cerebral injection, precluding a centrally-mediated action. Melatonin effects were antagonized by luzindole, a specific melatonin receptor antagonist in homeotherms. This is consistent with the idea that melatonin and melatonin receptors are found in the gastro-intestinal tract of birds and mammals (refs *in* Pinillos *et al.* 2001). However, although melatonin was found in the gastro-intestinal tissues of sturgeon, trout and carp (Bubenik & Pang 1997), melatonin binding sites could not be clearly identified in the gut of seabream and pike (Falcón *et al.* 1996 & unpubl results) and the MT1 and MT2 melatonin receptor subtypes could not be evidenced in the pike intestine using a PCR approach (Gaildrat & Falcón 1999, 2000, Gaildrat *et al.* 2001). The possibility remains that a melatonin metabolite was acting instead of melatonin. Indeed, in the goldfish serotonin (a melatonin precursor) had similar anorectic effects as melatonin (Pinillos *et al.* 2001) which can be de-acetylated in the liver and pineal to give, among other products, 5-methoxytryptamine a compound closely related to serotonin (Falcón *et al.* 1985, Yañez & Meissl 1996).

It is well known that the fish pineal organ mediates locomotor activity rhythms (refs in Zachmann *et al.* 1992a, Ekström & Meissl 1997), sleep like states (Zhdanova *et al.*,2001) and thermal preference (refs *in* Underwood 1989, Zachmann *et al.* 1992a, Ekström & Meissl 1997). All three behaviors may affect food intake. It was recently shown that trout and catfish display a rhythm in demand-feeding when maintained under normal L:D cycles (Bolliet *et al.* 2001). Under L:L, food availability by itself was able to synchronized rapidly the demand-feeding rhythm to the period of food availability. However, the L:D cycle was a master synchronizer of the demand-feeding rhythm compared to food availability in trout. Finally, as reviewed elsewhere (Spieler 2001), fish grow differently depending on the circadian time feeding. This may have potential implications for aquaculture, but obviously our knowledge on the relationships between behavioral rhythms and food intake and digestion is still at its beginnings.

6.2. Melatonin and the control of hormones involved in fish growth

The evidence that melatonin receptors are present in the antero-ventral part of the pike and trout pituitary glands, and that melatonin modulates cAMP levels in cultured organs indicates that some hormonal output(s) is (are) under melatoninergic control. Preliminary investigations in trout indicate that GH is one possible candidate. Indeed, the release of GH by dissociated and cultured trout pituitary cells was increased in the presence of different melatonin concentrations. As observed for cAMP, the adenyl cyclase stimulator forskoline induced increases in GH release; under these pharmacological conditions melatonin effects are rather inhibitory. These results, although preliminary, are a good indication that melatonin may affect fish growth by a direct action on the GH producing cells of the pituitary. There is to date not enough data to explain why melatonin exerted two opposite effects. The presence of two melatonin receptor subtypes within the somatotrophs might be one requisite (Gaildrat & Falcón 1999, 2000).

The control of GH secretion by the fish somatotrophs is a process that involves both stimulatory (*e.g.*, dopamine, thyrotropin releasing hormone [TRH], GH-releasing factor) and inhibitory (*e.g.*, norepinephrine, serotonin, somatostatin [SRIF], GH) agents (Peng & Peter 1997, Agustsson & Bjornsson 2000, Agustsson *et al.* 2000). It is not unreasonable to believe that melatonin may affect GH secretion indirectly through controlling upstream regulatory factors. Dopamine and serotonin are two good candidates. Indeed, recent studies demonstrated that melatonin injections reduced dopamine content in trout hypothalamus and pituitary (Hernandez-Rauda *et al.* 2000). Previous investigations had shown that melatonin was able to modulate serotonin metabolism in the fish hypothalamus (refs in Zachmann *et al.* 1992, Ekström & Meissl 1997). Future studies should aim to investigate whether these hypothalamic effects of melatonin are directed on dopaminergic and serotoninergic neurons that innervate the GH secreting cells.

Another possible way through which melatonin could influence fish growth is the pituitary/thyroid axis. In mammals, a type II iodothyronine deiodinase expression and activity has been evidenced in the pineal gland (Smith *et al.* 2001 and refs). In frogs and tadpoles melatonin is a potent inhibitor of T_4 secretion by the thyroid (Wright *et al.* 2000). In fish, little information is available regarding the interactions between melatonin and the thyroid. In catfish, pinealectomy reduces ^{131}I uptake by the thyroid, it increases plasma levels of T_3 and decreases those of T_4, but increases both T_3 and T_4 levels in the thyroid gland (refs in Ekström & Meissl 1997). The effects depend on the reproductive status.

6.3. What role for the nervous message?

When considering the effects of the pineal gland of fish, one must consider not only melatonin, but also the nervous message which is conveyed through the pinealofugal innervation to the brain centers. Unfortunately, virtually nothing is known on the role the nervous innervation plays. The only available information was obtained from neural tract tracing methods which made possible to delineate the brain areas innervated by the pinealofugal ganglion cells (for refs see Ekström & Meissl 1997). The most striking observation lies in the fact that many of these areas are also innervate by retinofugal projections. The pre-optic nuclei of the hypothalamus seem to occupy a key position for what concerns pituitary function (Holmqvist *et al.* 1994). Indeed, it receives projections from both the retina and pineal gland, it expresses melatonin receptors, and contains dopaminergic neurons that innervate the pituitary gland. This emphasizes that the hypothalamic optic nucleus constitutes a photoneuroendocrine control center, activated by light, which probably plays an important role during growth and parr-smolt transformation by modulating the release of pituitary hormones. The lateral habenular *nuclei* are other putative dopaminergic nuclei that also receive innervation from the pineal gland and retina. The pretectal area, like the optic nuclei, also receive inputs from both the retina and pineal gland and posses melatonin receptors. Other areas of interest include the dorsal and ventral thalamus and the periventricular hypothalamus.

7. CONCLUSIONS

Many living species depend on the diurnal and annual lighting cycles for normal development, growth and reproduction. Daylength appears to be an important "*zeitgeber*" in fish. Many studies have demonstrated the positive influence of long daylength on growth and a few species, such as the Atlantic salmon, are extremely sensitive to it. Today, all this knowledge is used in salmoniculture, photoperiod manipulations being easily applied and not overly expensive. Long photoperiods or continuous daylight appear as a palliative for the compensation of low winter temperatures in highest latitude countries. This approach, however, may not be applicable to all species. Some fish do not respond and others need a (very) long time before expressing better growth. Research will have to be pursued in this area in the future to obtain more determining responses. Physiological mechanisms are not yet elucidated. How does photoperiod directly affect fish growth through a putative role of melatonin? During the last two decades, there has been a lack of interest, due to the difficulty to obtain clear-cut effects of pinealectomy or/and melatonin administration. Among different reasons, the experimental paradigms did not considered the respective roles of both the pineal and retina, the multiplicity of the messengers elaborated and released by these two organs and the fish seasonal physiological status. An issue to the elucidation of light receptivity and subsequent physiological responsiveness will come from studies combining "classical" physiological approaches (*in vitro* and *in vivo*) together with pharmacological and molecular approaches.

List of abbreviations: AANAT: arylalkylamine (serotonin) N-acetyltransferase; cyclic AMP: adenosine cyclic 3': 5'-monophosphate; ^{125}I-Mel: 2-[^{125}I]-iodomelatonin; HIOMT: hydroxyindole-O-methyltransferase; L:D: light/dark; L:L: constant light; RT-PCR: reverse transcription-polymerase chain reaction; SL: somatolactine; SRIF: somatostatin; T_3: tri-iodothyronine; T_4: thyroxine; TRH: thyroid hormones; TRH: thyrotropin releasing hormone.

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GROWTH RATE AND RECRUITMENT: EVIDENCE FROM YEAR-CLASS STRENGTH IN THE YEAR-TO-YEAR VARIATION IN THE DISTRIBUTIONS OF OTOLITH WEIGHT, FISH WEIGHT, AND FISH LENGTH IN *HOPLOSTETHUS ATLANTICUS*

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LENGTH
WEIGHT
OTOLITH WEIGHT
HOPLOSTETHUS ATLANTICUS
FISHING-DOWN
YEAR-CLASS
GROWTH CURVE

ABSTRACT – An innovative method of differences was developed to recover year class structure from a series (1984 to 1992) of random samples of fish length, fish weight and otolith weight taken from random stratified trawl surveys of Orange Roughy, *Hoplostethus atlanticus* (Trachichthyidae: Teleostei). The apparent stability of the sample distributions over the period 1984 to 1992 was likely to be caused by stratified recruitment, not a consequence of old age. Both strong year class effects, and an estimate of the growth curve, of *H. atlanticus* were recovered using the method of differences. Strong year classes were correlated with periods of low ocean turbulence. The growth curve developed from the year class structure was highly correlated with growth curves derived from daily microincrements.

LONGUEUR
POIDS
POIDS DE L'OTOLITHE
HOPLOSTETHUS ATLANTICUS
SUREXPLOITATION
CLASSE ANNUELLE
COURBE DE CROISSANCE

RÉSUME – Une méthode innovante des différences est développée pour retrouver la structure de classes par année à partir d'une série d'échantillonnages au hasard (1984 à 1992) de la longueur, du poids des Poissons, et du poids des otolithes provenant de campagnes de chalutage (random stratified samples) recueillant l'Hoplostèthe, *Hoplostethus atlanticus* (Trachichthyidae : Téléostéen). La stabilité apparente des distributions dans les prises entre 1984 et 1992 est probablement due à un recrutement stratifié, plutôt que la conséquence d'une abondance d'individus âgés. Les effets importants des classes annuelles et une estimation de la courbe de croissance de *H. Atlanticus* sont retrouvés en utilisant la méthode des différences. Les fortes classes sont corrélées aux périodes de basse turbulence de l'Océan. La courbe de croissance obtenue à partir de la structure des classes annuelles est hautement corrélée aux courbes de croissance basées sur l'analyse des stries journalières.

INTRODUCTION

The Orange Roughy, *Hoplostethus atlanticus* (Trachichthyidae: Teleostei), is a pandemic deep-water fish species occurring at depths 600 to 1200 m in many parts of the world's oceans (Paulin 1979, Merrett & Wheeler 1983, Gordon & Duncan 1987). Since the late 1970's, *H. atlanticus* has been the basis of a commercial fishery in New Zealand and Australian waters (Wilson 1982, Robertson & Grimes 1983), and recent exploration for commercial quantities of *H. atlanticus* has taken place in the North Atlantic (Anon 1994, Dubuit 1995). There are commercial fisheries for *H. atlanticus* in Namibia (Anon 1998), and off the coasts of Madagascar and Chile. In less than twenty years *H. atlanticus* has gone from an obscure fish in museum collections to a popular culinary item, particularly in the United States of America. Over this period growing knowledge of the biology of *H. atlanticus* has lead to the revision of several of the earlier paradigms applied to the management of the fishery in New Zealand waters. Exploration of the sea-bed with new acoustic technologies has greatly extended the geographical range over which seamounts (the favored habitat of *H. atlanticus*) are known to occur. It is now evident that there are many spawning grounds not a single spawning ground to which all fish migrate, confirming earlier observations by Pankhurst *et al.* (1987).

Continuing exploitation of the resource has lead to a revision of the early estimates of stock size

that were made in the late 1970's and early 1980's. Apparent decline in stocks of *Hoplostethus atlanticus* has occurred in the face of what were believed to be conservative yield estimates (Robertson & Grimes 1983, Smith *et al.* 1991). Downward estimates of the virgin stock size by hind-casting techniques have also occurred over this period (Francis *et al.* 1993). Unlike most species of fish, the decline in apparent stock size was thought to have occurred without a fishing-down effect. Size distributions of *H. atlanticus* apparently remained stable without the expected preferential loss of the largest size classes (Gauldie *et al.* 1989).

The apparent stability of size classes in the face of declining numbers has had considerable impact on age estimates of *Hoplostethus atlanticus.* Age estimates based on morphological and chemical properties of the otolith have been through a progressive inflation of maximum ages from 20^+ years (van den Broek 1983a b, Kotlyar 1980), through 50 years (Mace *et al.* 1990), to 150-200 years (Fenton *et al.* 1991, Smith *et al.* 1995), to extreme ages of 500^+ years. It was believed that growth rates were very low because of the extraordinary antiquity of the fish, and that the consequent low recruitment and extreme age at first spawning of 30^+ years resulted in sensitivity to over-fishing. Taken in isolation, ages of greater than 20^+ years for a fish rarely heavier than 3 kg may seem extreme, but the paradigm of extreme old age in fishes has been used to explain declining stocks of many other species of fish. The apparent stability of size frequencies in the face of fishing pressure was widely perceived as an indicator of very old, slow growing fish, although no formal explanation of how this might occur has been published. There has also been a continuing parallel effort to estimate age in *H. atlanticus* using more conventional methods that have lead to estimates of maximum ages in the range 14 to 22 years (van den Broek 1983 a b, Gauldie *et al.* 1989); still a great age for a small fish with a maximum size of about 3 kg. Although extreme ages have been discredited (West & Gauldie 1995, Romanek & Gauldie 1996, Gauldie 1998), the problem of no apparent fishing-down effect remains. The apparent stability of the size distribution in the catch curves typical of the fishery is one of the many puzzling features of the biology of *H. atlanticus.* The estimated biomass of *H. atlanticus* in New Zealand waters was thought to have declined by 70% or more over the period 1984 to 1992 covered in this study (Smith *et al.* 1991), yet the length and weight distributions in the catch have remained superficially similar and more or less stationary, as will be shown below.

We will show that the argument for slow growth based on the apparent stability of the size distribution data for *H. atlanticus* is unlikely to be correct.

Closer examination of the size distribution data for *H. atlanticus* that was made available for this study (fish length, fish weight and otolith weight), showed evidence of year class structure in what had previously been assumed to be a more or less stationary distribution over time. We describe a variation on the method of differences that is commonly used to establish year class progressions that reveals a significant amount of year class structure embedded in the *H. atlanticus* data.

Because the samples were taken at yearly intervals, the progression of year classes allows a direct estimate of the growth curve of *H. atlanticus*. We compare this direct estimate of the growth curve with growth curves derived from other direct and indirect methods. In addition, year class structure allows us to explore possible oceanographic causes that may have influenced recruitment in *H. atlanticus.*

MATERIALS AND METHODS

1. Fish length and weight, and otolith weight data : A set of 7149 observations of fish length, weight and otolith weight of *Hoplostethus atlanticus* was prepared in 1994 for the New Zealand Exploratory Fishing Company using data and specimens held by the New Zealand Ministry of Fisheries. The data and specimens were derived from the random stratified trawl surveys of the fishery for *H. atlanticus* on the Chatham Rise, east of New Zealand, conducted by the Ministry of Fisheries. A random set of 20 samples was taken from each of a number of trawl surveys amounting to a total sample size of between 842 to 933 for each successive year from 1984 to 1990, and including 1992. There were no trawl surveys commissioned by the Ministry of Fisheries in 1991. This data is as close to a random sample of the true population characteristics of *H. atlanticus* that it is possible to obtain in the context of sampling by trawl surveys.

In the following text "otolith" refers to the sagitta. Otoliths were collected at sea and stored dry in envelopes after determination of fish lengths (standard length), sex and fish weight. Otoliths were weighed to 10 mg precision, fish weight measured to a tenth of gm, and lengths (standard length) measured to a tenth of cm. The data set consisted of 8 year class (sample sizes in parentheses), 1984 (883), 1985 (842), 1986 (894), 1987 (915), 1988 (927), 1989 (933), 1990 (894), and 1992 (860).

2. Statistical analysis of the data: This study used smoothing techniques and linear regressions in addition to the standard tests for the significance of the differences between means and medians available in the Data Desk software package. The statistical nomenclature in Data Desk is followed here.

Linear regressions were fitted using Data Desk. Recovering the underlying trend in a data of two variables y, x is usually achieved by fitting the data to a function that can range from a straight line to a complex polynomial. The appropriateness of the fit in the absence of a prior model is determined by the r^2 value of the regression which provides an estimate of the proportion of the variation in y that is explained by the variation in x. Alter-

natively, in the absence of a prior model to which the data can be regressed, one can substitute an arbitrary smoothing function that follows the trend in the data. Two smoothing techniques that are available for this purpose are the lowess and the median smooth.

The lowess technique adjusts the fit to the trend in the data by calculating a running mean value based on an adjustable window of sample points (Cleveland 1979). The more points in the window, the less sensitive the lowess smooth is to short term trends. Consequently, there is an arbitrary window size that determines the cut-off point between too sensitive to short term trends, and too insensitive to short term trends. A more arbitrary, but less adjustable, method is available in median smoothing which has the least subjective control, and from a common sense point of view describes where most of the y data points may lie over the most of the range in x. Median smoothers have the advantage of behaving as low-pass filters on equally spaced data that avoid the biasing effects of brief spikes in the data sequence (Tukey 1977). This study used the lowess and the median smoothing programs available in the Data Desk statistical package.

3. Wind-driven turbulence data: Wind-driven upper ocean mixing is estimated from observations of either anemometers or ocean wave topology (Beaufort scale), following Bakun (1990), where the upwelling (mixing downward, turbulence, etc.) is related to wind speed cubed. Derived wind velocity data for the geographical area bounded by longitudes 170°E and 170°W and latitudes 40°S to 45°S are taken from the CEOS (Climate in Eastern Ocean Systems) CD-ROM containing the COADS data set (available through NOAA, ORSTOM and FAO). This area covers the historical boundaries of the eastern Pacific Ocean stocks of New Zealand *H. atlanticus* to which the size frequency data refer.

4. The method of differences for establishing year class progressions: Many species of fish have clearly separated length modes evident in their catch curves, each length mode corresponding to a successive year class. Catch curves for such species readily show strong and weak year classes in which the area under the curve of a particular length mode may be much greater, or much lesser, than the other length modes in the catch curve. For example, Fig. 1 a-c show the kind of catch curves that might be sampled at three successive time intervals from a fishery based on a species with well-separated length modes, each corresponding to a different year class. Because such length modes are clearly separated, it is customary in examining such data to simply decide by eye on year class structure, and strong (or weak) year class progressions. But, in effect, the examination by eye is no more than a way of looking for differences in the location of length mode peaks between the sample at one time interval, and the sample at the next time interval. If there was no difference in the location of length modes between sampling intervals, then one would consider that no growth had occurred in the particular cohort of fish that comprise that length mode. But if there was a visible difference in location, then one would consider that growth equivalent to the separation between peaks had occurred in that particular cohort over the time between sampling. This kind of analysis is usually done intuitively, but it amounts to a subtraction from the catch curve at one time interval, of that of the previous time

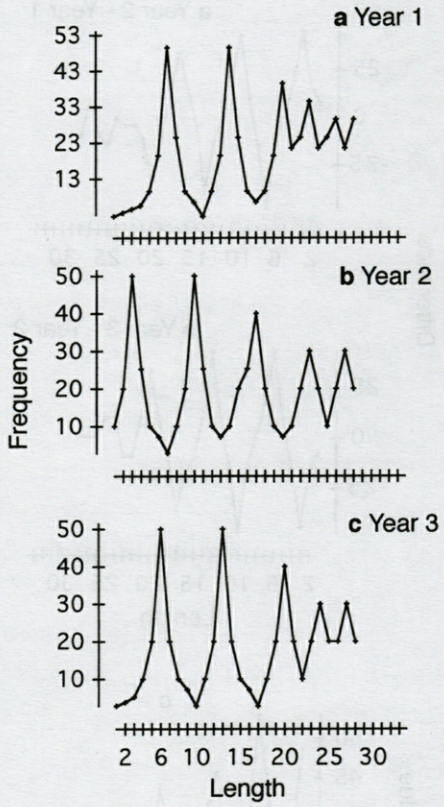

Fig. 1. – Changes in length modes over time for an idealized fishery are shown as length frequencies at time 1, time 2 and time 3.

interval. Thus, "no difference in location" by subtraction would lead to low value; and "a visible difference in location" would lead to a high value. Following the example in Fig. 1 a-c, the graphs in Fig. 2 a and b show, respectively, the differences obtained from subtracting time 1 from time 2, and time 2 from time 3 ("time" as indicated in Fig. 1 a to c). The difference graphs in Fig. 2 a and b contain further information about year class progression. If the difference between time 2 and time 3 (Fig. 2 b) is regressed against the difference between time 1 and time 2 (Fig. 2 a) at progressively lagged intervals, then the plot of the r^2 of the regression reveals the phase relations (i.e., the pattern of the change in frequency over time), between the underlying length mode structure measured at different time intervals; as is shown in the plot of r^2 against lag Fig. 2 c.

While the difference graphs are unnecessary in the case of the kind of length mode progression shown in Fig.1 a to c, they provide a useful tool for analyses of length mode progressions for data that have less obvious length modalities. For example, Fig. 3 a to c show the same length modes as in Fig. 1 a to c, but with a normally distributed overlay of non-modally distributed underlying lengths. Visual inspection of the three size distributions in Fig. 3 a to c would not indicate any underlying modality in length distribution. But, by subtracting a from b, and b from c in Fig. 3, one would recover exactly the same difference graphs as in Fig. 2 a and b, from which the same lagged regression as in Fig. 2 c

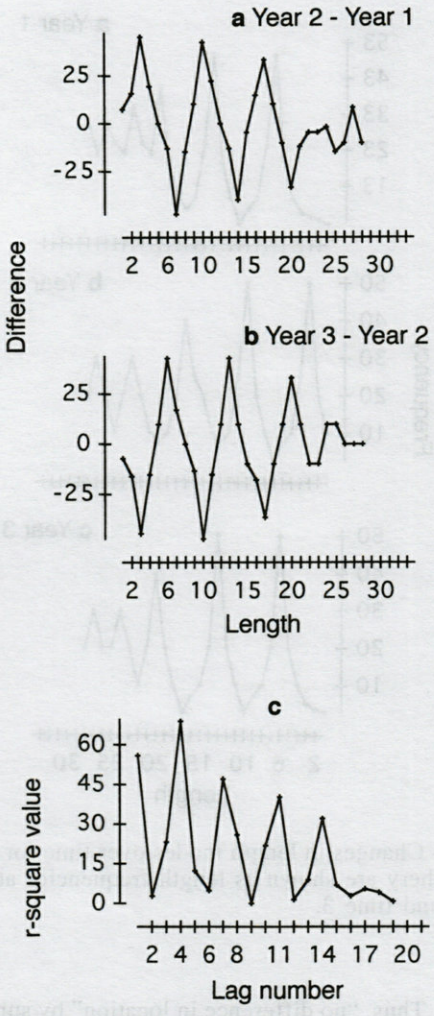

Fig. 2. – Frequencies from the method of differences resulting from (a) subtraction of time 1 from time 2 in Fig. 1; and (b) subtraction of time 2 from time 3 in Fig.1. c. The plot of r^2 of the regression of the frequencies from the method of differences (a) from Fig. 2, lagged against (b) from Fig. 2.

would show the strong length modality that was otherwise hidden in the data.

We believe that the novel approach of difference graphs to length mode analysis can be applied to the size data in the catch curves of *H. atlanticus* (fish length, fish weight and otolith weight), that have a similar appearance (see Fig 6, 9 and 11 below) to the size distributions in Fig. 3 a to c. The phase relations between different years of samples can also be established using the plots of the r^2 of the regressions of the data obtained from the method of differences.

RESULTS

General relationships in the data

Otolith weight is plotted against fish weight in Fig. 4. The graph in Fig. 4 has been fitted with a

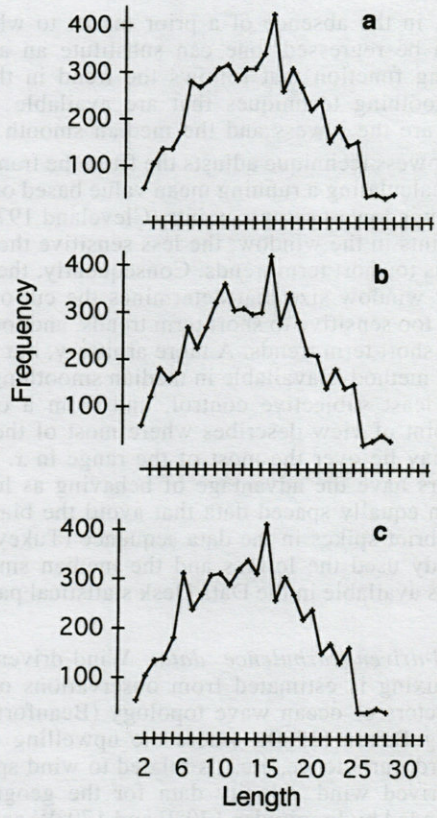

Fig. 3 a-c. – Length mode data from Fig. 1 each with the same overlay of normally distributed length frequencies.

linear regression (r^2 = 41.4%). Although the plot in Fig. 4 indicates a non-linear relationship between otolith weight and fish weight, transformation of the data to provide a more linear relationship does not markedly improve the fit to the linear regression. The maximum value of r^2 = 48.4% was obtained with the transformation formula: $(\text{otolith weight})^{0.1} = (\text{fish weight})^{1.76}$. Thus, the relationship between otolith weight and fish weight is non linear, but not markedly so. The scatter in otolith weight increased markedly with fish weight, but less than half of the variation in otolith weight is explained by the variation in fish weight in *Hoplostethus atlanticus* reflecting the de-coupling of otolith growth rate and fish growth rate seen in many species (Mosegaard *et al.* 1988, Wright *et al.* 1990).

Otolith weight is plotted against fish length in Fig. 5a, in which the graph has been fitted with a linear regression (r^2 = 45.3%). Transforming the data using the formula $(\text{otolith weight})^{0.1} = (\text{fish length})^{1.76}$, increases the value of r^2 to 58.6%. Thus, about 60% of the variation in otolith weight was explained by the variation in fish length, reflecting the de-coupling of otolith growth rate and fish growth rate. The scatter in the data increases with increasing fish length indicating a weakening of the correlation between otolith weight and fish length with increasing size.

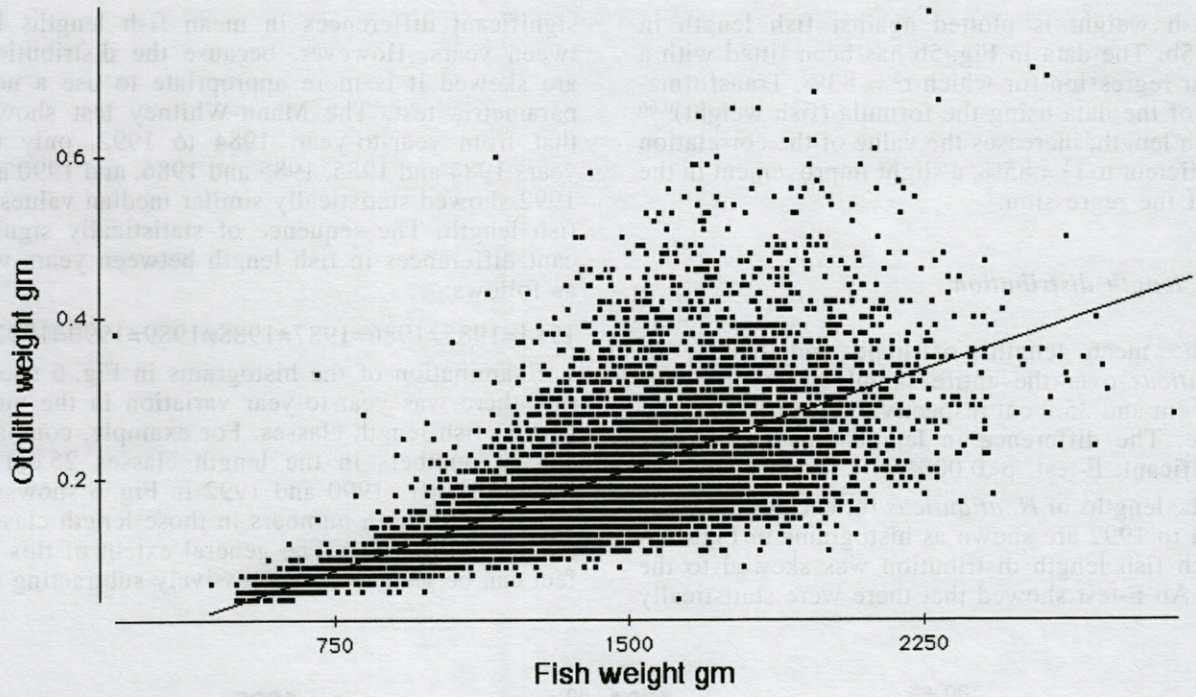

Fig. 4. – Otolith weight plotted against fish weight fitted with linear regression.

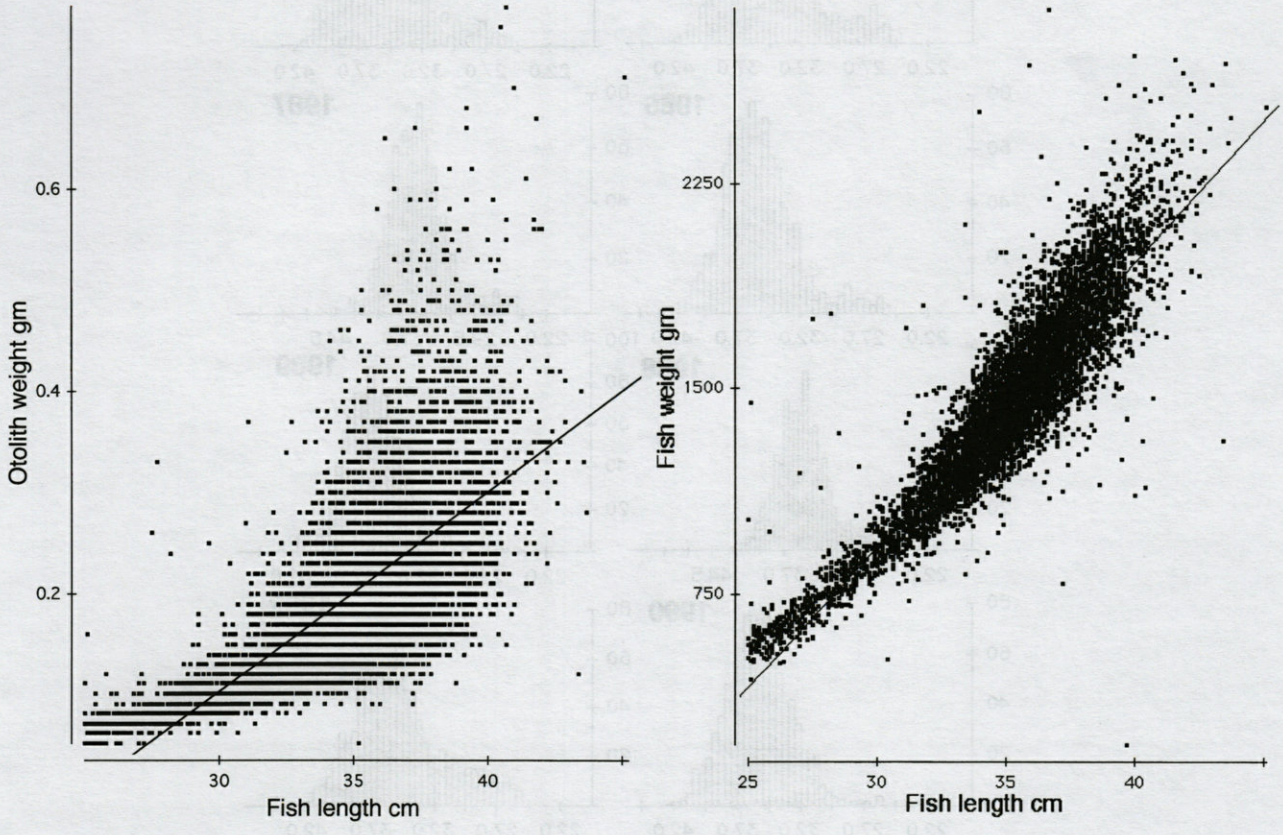

Fig. 5. – a. Otolith weight plotted against fish length fitted with linear regression; b. Fish weight plotted against fish length fitted with linear regression.

Fish weight is plotted against fish length in Fig. 5b. The data in Fig. 5b has been fitted with a linear regression for which $r^2 = 83\%$. Transformation of the data using the formula $(\text{fish weight})^{0.66} = \text{fish length}$, increases the value of the correlation coefficient to $r^2 = 85\%$, a slight improvement in the fit of the regression.

Fish length distribution

The mean lengths of male and female *H. atlanticus* over the entire sample were different, 34.3 cm and 35.7 cm respectively; about 4% difference. The difference in length was statistically significant, F-test, $p<0.0001$.

The lengths of *H. atlanticus* for each of the years 1984 to 1992 are shown as histograms in Fig. 6 in which fish length distribution was skewed to the left. An F-test showed that there were statistically significant differences in mean fish lengths between years. However, because the distributions are skewed it is more appropriate to use a non-parametric test. The Mann-Whitney test showed that from year-to-year, 1984 to 1992, only the years 1984 and 1985, 1985 and 1986, and 1990 and 1992 showed statistically similar median values in fish length. The sequence of statistically significant differences in fish length between years was as follows:

1984=1985≠1986≠1987≠1988≠1989≠1990=1992.

Examination of the histograms in Fig. 6 shows that there was year-to-year variation in the numbers in fish length classes. For example, comparison of numbers in the length classes 25 cm to 30 cm in years 1990 and 1992 in Fig. 6 shows an almost trebling in numbers in those length classes from 1990 to 1992. The general extent of this effect can be gauged by successively subtracting the

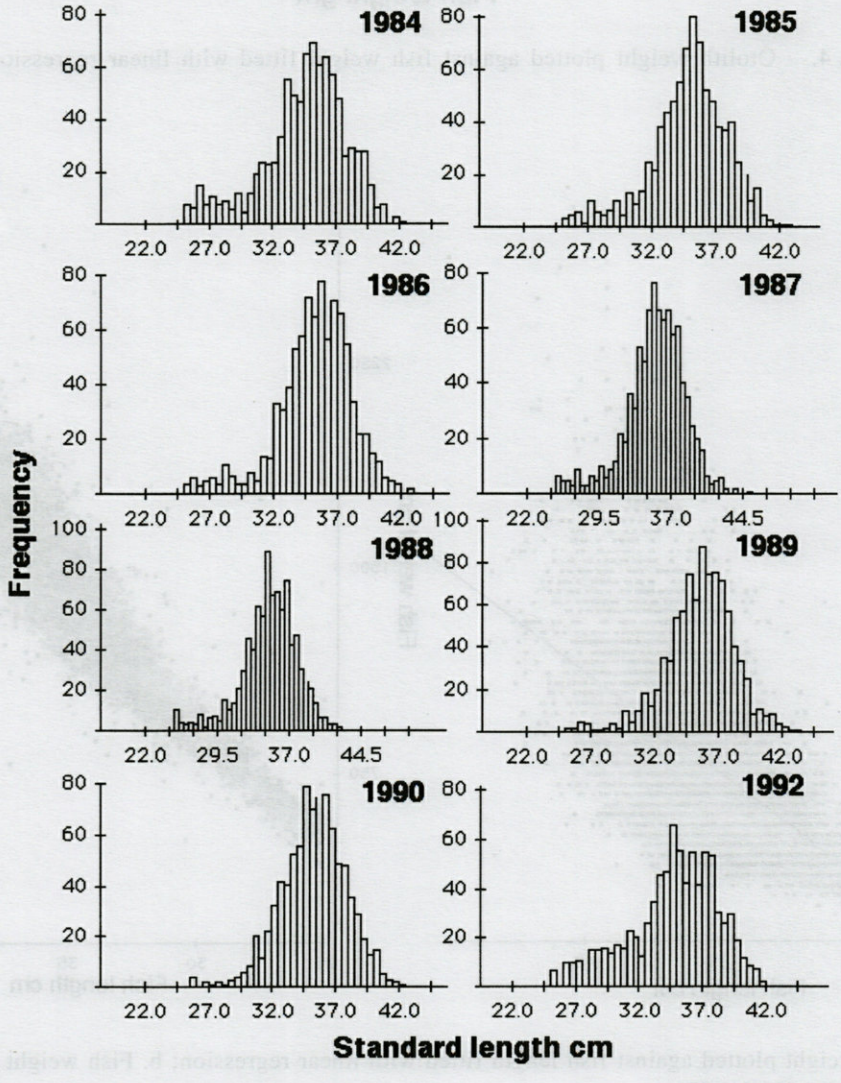

Fig. 6. – Histograms of fish length plotted by years 1984 to 1992.

numbers in each annual length class from the numbers in the same length class in the year ahead. For example, 85-84 in Fig. 7 is the difference in length classes resulting from 1984 value being subtracted from 1985 values. The seven sets of differences for the years 1992-1990, 1990-1989, 1989-1988, 1988-1987, 1987-1986, 1986-1985, and 1985-1984 are shown in Fig. 7.

The subtraction means that negative values in the difference distributions indicate a decrease in that size class with respect to the previous year, and positive differences indicate an increase in that size class with respect to the previous year. When each of the difference graphs in Fig. 7 was fitted with median smoothed curves it was evident that there was a year-to-year oscillation in year-to-year differences around zero. However, the oscillations were out of phase from sample to sample. The sets of arrows identified from *A* to *J* show an apparently positive phase cycle, i.e. year-classes, moving through successive years before finally damping out to zero.

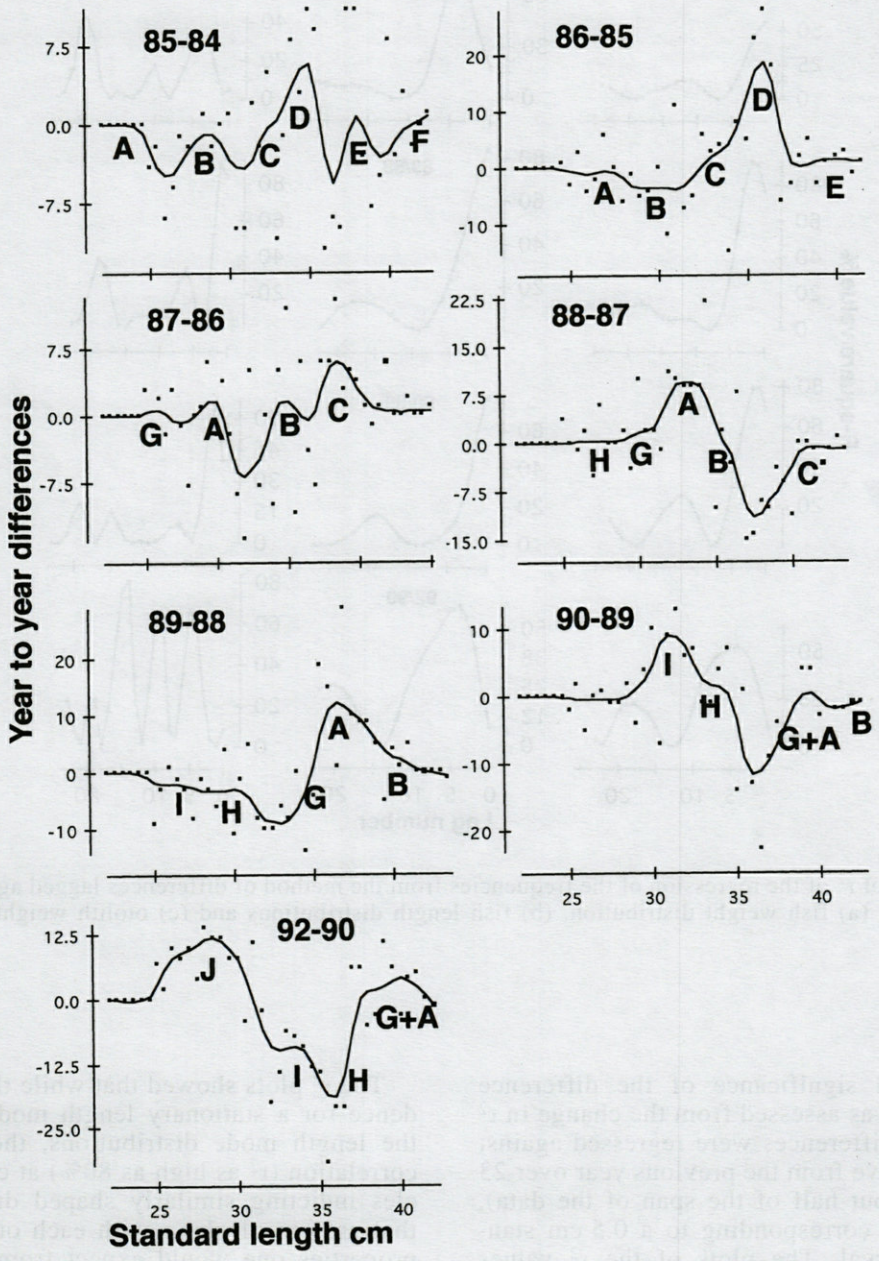

Fig. 7. – Differences from year to year in fish length plotted against fish length class. The fish length divisions on the abscissa in 85-84 have the same values in all graphs. Successive year classes values are marked alphabetically from the left.

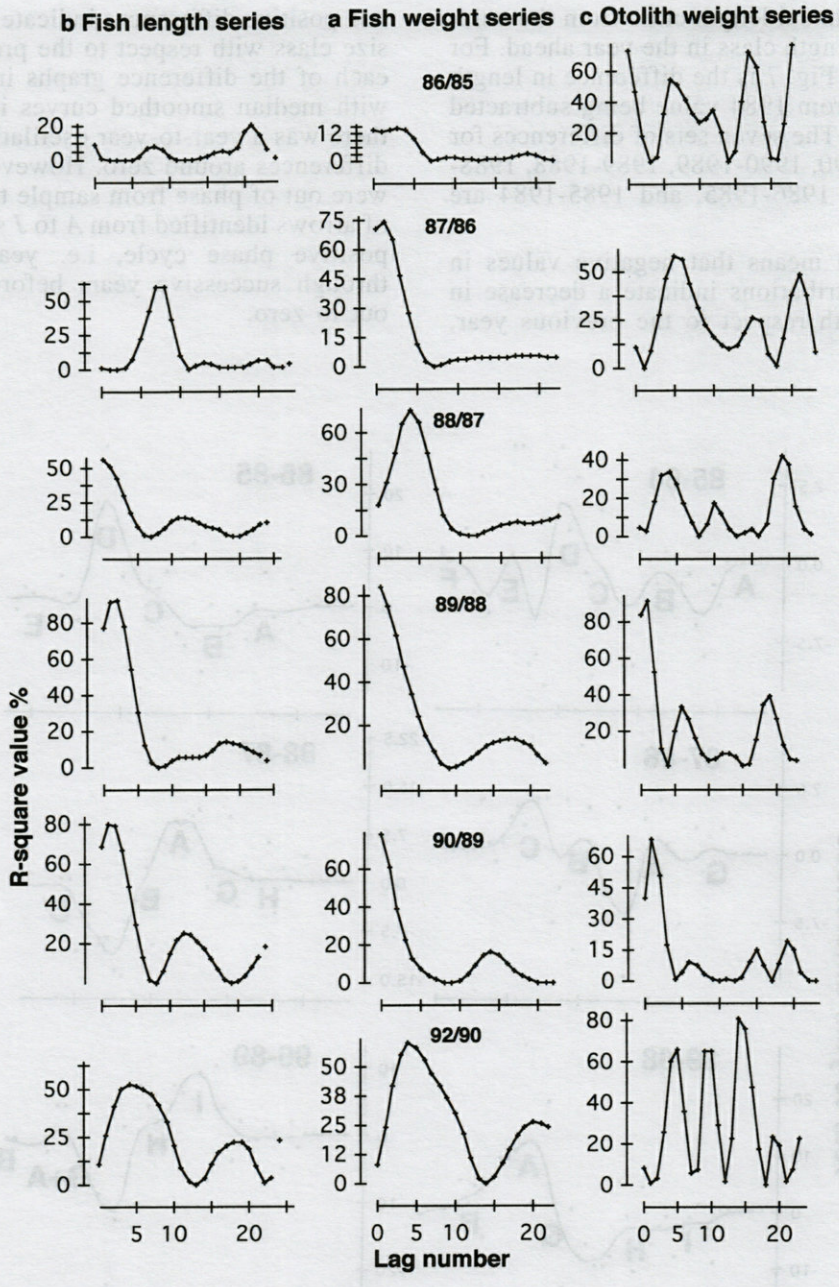

Fig. 8. – The plots of r^2 of the regression of the frequencies from the method of differences lagged against the previous year are shown for (a) fish weight distribution, (b) fish length distributions and (c) otolith weight distributions.

The biological significance of the difference curves in Fig. 7 was assessed from the change in r^2 value when the differences were regressed against the difference curve from the previous year over 23 lag intervals (about half of the span of the data), each lag interval corresponding to a 0.5 cm standard length interval. The plots of the r^2 values against lag interval for regressions between the median smoothed successive annual differences in Fig. 7 are shown in Fig. 8a.

The r^2 plots showed that while there was no evidence for a stationary length modes concealed in the length mode distributions, there were strong correlation (r^2 as high as 80%) at certain phase cycles indicting similarly shaped difference curves that are out of phase with each other; exactly the properties one would expect from a length mode progression representing a strong year class. In addition, the peaks in the r^2 values shift in respect to length in progressive years, thus supporting the in-

terpretation of a cycle of strong year classes moving through the data that was apparent from an inspection the lettered peaks in Fig. 7.

Fish weight distribution

The mean weights of male and female *H. atlanticus* of the total sample were different, 1322.3 gm and 1558.3 gm respectively; about 17.8% difference. The differences in weight was statistically significant, F-test, $p \leqslant 0.0001$.

The weights of individual *H. atlanticus* for each of the years 1984 to 1992 are shown as histograms in Fig. 9 in which it is evident that fish weight distribution was skewed to the left, the opposite direction to the otolith weight histograms below. An F-test showed that there were statistically significant differences in mean fish weights between years; however, because the distributions are skewed it is more appropriate to use a non-parametric test. The Mann-Whitney test showed that from year-to-year, 1984 to 1992, only the years 1984 and 1985, 1985 and 1986, and 1990 and 1992 showed statistically similar median values in fish weight. The sequence of statistically significant differences in fish weight between years was as follows:

1984=1985=1986≠1987≠1988≠1989≠1990=1992.

Examination of the histograms shows that there was year-to-year variation in the numbers in fish weight classes. For example, comparison of numbers in the weight classes 600 gm to 1100 gm in

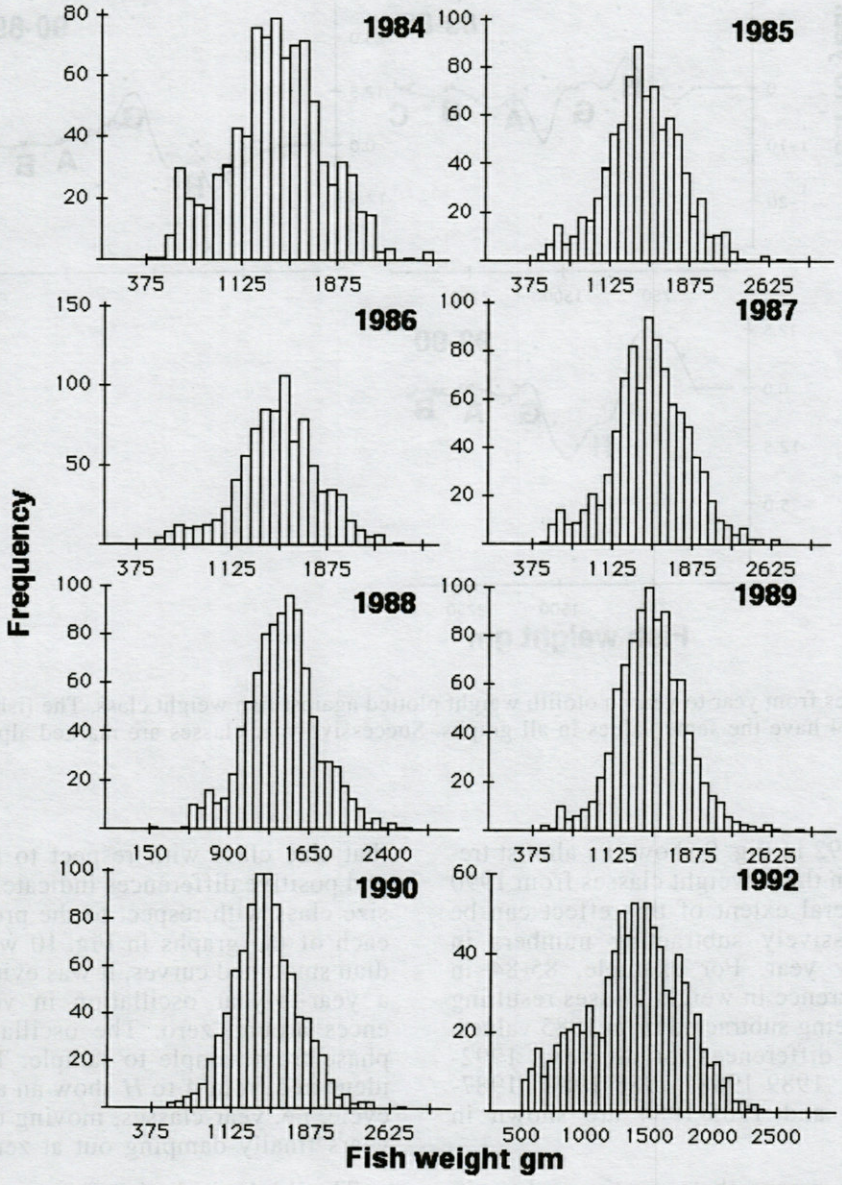

Fig. 9. – Histograms of fish weight plotted by years 1984 to 1992.

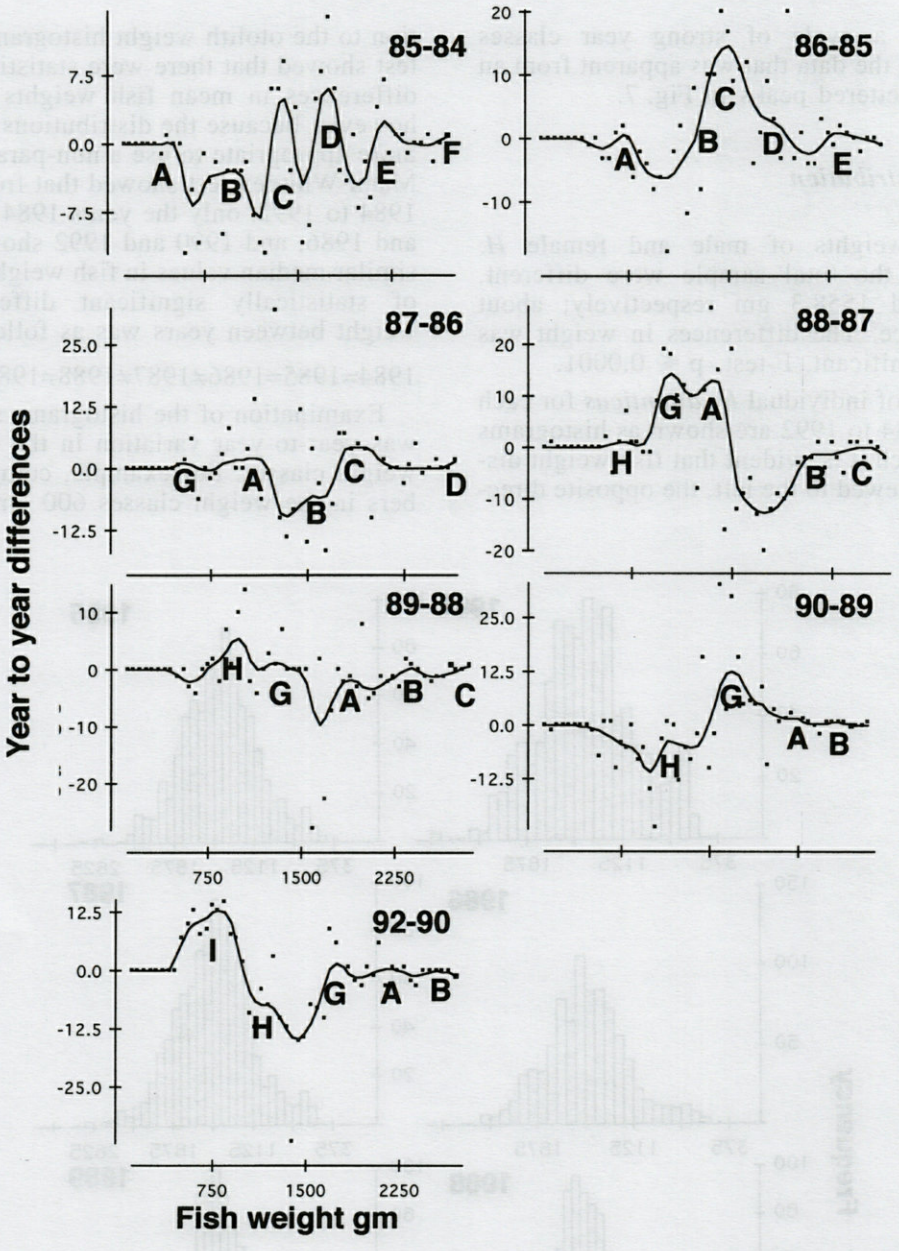

Fig. 10. – Differences from year to year in otolith weight plotted against fish weight class. The fish weight divisions on the abscissa in 85-84 have the same values in all graphs. Successive year classes are marked alphabetically from the left.

years 1990 and 1992 in Fig. 9 shows an almost trebling in numbers in those weight classes from 1990 to 1992. The general extent of this effect can be gauged by successively subtracting numbers in weight classes by year. For example, 85-84 in Fig. 10 is the difference in weight classes resulting from 1984 value being subtracted from 1985 values. The seven sets of differences for the years 1992-1990, 1990-1989, 1989-1988, 1988-1987, 1987-1986, 1986-1985, and 1985-1984 are shown in Fig. 10.

The subtraction means that negative values in the difference distributions indicate a decrease in that size class with respect to the previous year; and positive differences indicate an increase in that size class with respect to the previous year. When each of the graphs in Fig. 10 was fitted with median smoothed curves, it was evident that there was a year-to-year oscillation in year-to-year differences around zero. The oscillations were out of phase from sample to sample. The sets of arrows identified from *A* to *H* show an apparently positive cycle, i.e. year-classes, moving through successive years finally damping out at zero.

The biological significance of the difference curves in Fig. 10 was assessed from the change in

r^2 value when the differences were regressed against the difference curve from the previous year over 23 lag intervals (about half of the span of the data), each lag interval corresponding to a 50 gm standard weight interval. The plots of the r^2 values against lag interval for regressions between the median smoothed successive annual differences in Fig. 10 are shown in Fig. 8b.

The r^2 plots showed that while there was no evidence for a stationary length modes concealed in the weight mode distributions, there were strong correlation (r^2 as high as 80%) at certain phase cycles indicting similarly shaped difference curves that are out of phase with each other; exactly the properties one would expect from a length mode progression representing a strong year class. In addition, the peaks in the r^2 values shift in respect to length in progressive years, thus supporting the interpretation of a cycle of strong year classes moving through the data that was apparent from an inspection the lettered peaks in Fig. 10.

Otolith weight distribution

The mean weights of otoliths of males and females in the total sample were different, 0.199 g and 0.205 g respectively. The difference in weight was statistically significant, F-test, $p < 0.0027$. However, given the variation in otolith weight with fish size, this minor difference (about 3%) is probably not biologically significant.

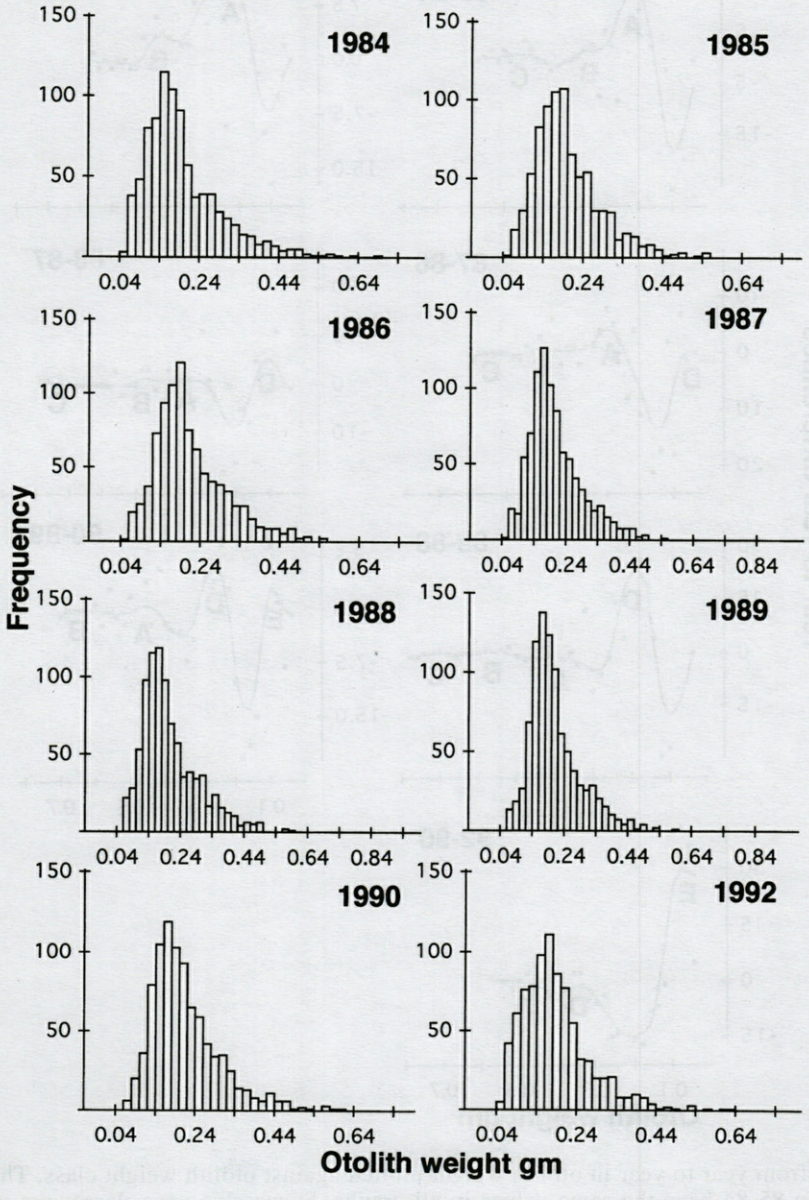

Fig. 11. – Histograms of otolith weight plotted by years 1984 to 1992.

The weights of otoliths of *H. atlanticus* for each of the years 1984 to 1992 are shown as histograms in Fig. 11. It is evident from the histograms that otolith weight distribution was skewed to the right. An F-test showed that there were statistically significant differences in mean otolith weights between years; however, because the distributions are skewed it is more appropriate to use a non-parametric test. The Mann-Whitney test showed that from year to year, 1984 to 1992, only the four-year period 1986 to 1990 showed no statistically significant year-to-year change in otolith weight. The sequence of statistically significant differences in otolith weight between years was as follows:

1984≠1985≠1986=1987=1988=1989=1990≠1992.

Examination of the histograms shows that there was year-to-year variation of numbers in otolith weight classes. For example, comparison of the numbers in the weight classes 0.04 to 0.14 gm in years 1990 and 1992 in Fig. 11 shows an almost doubling in numbers in these weight classes form 1990 to 1992. The general extent of this effect can be gauged by successively subtracting numbers in weight classes by year. For example, 85-84 in Fig. 12 is the difference in weight classes resulting from 1984 values being subtracted from 1985 values. The seven sets of differences for the years 1992-1990, 1990-1989, 1989-1988, 1988-1987, 1987-1986, 1986-1985 and 1985-1994 are shown in Fig. 12.

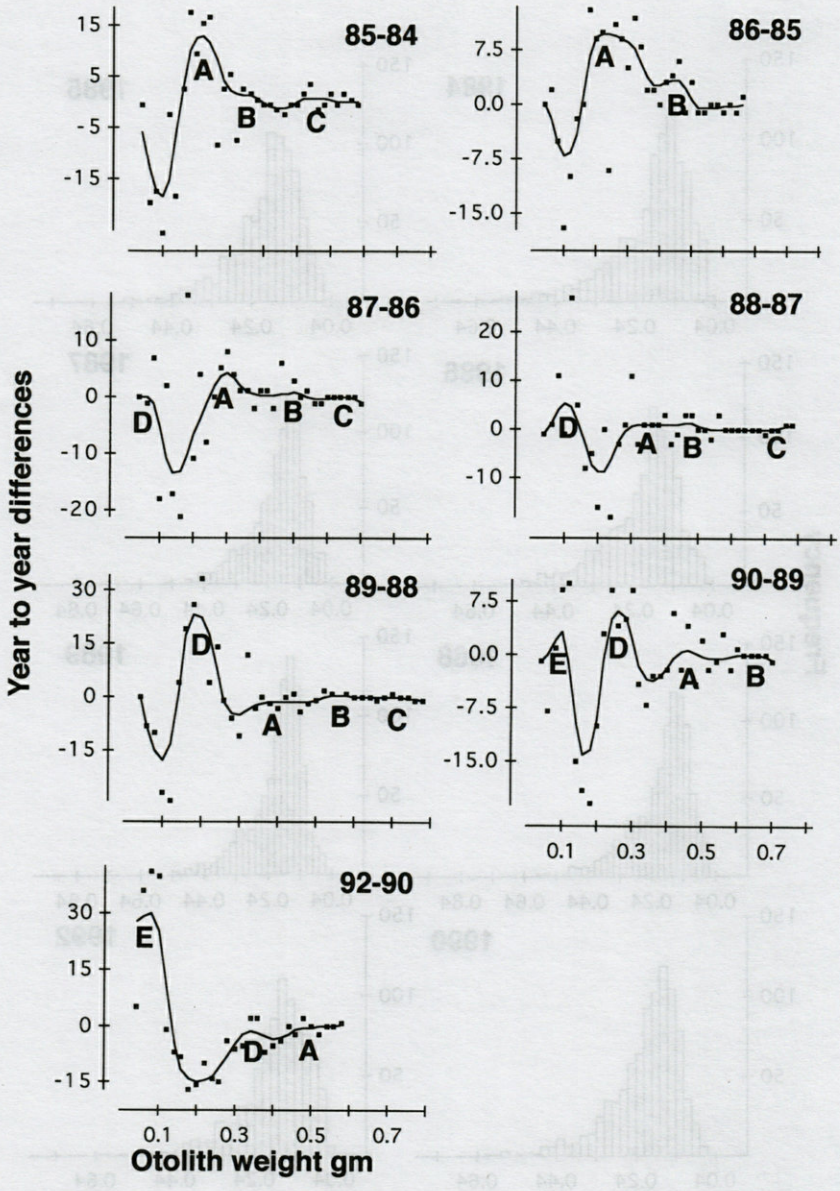

Fig. 12. – Differences from year to year in otolith weight plotted against otolith weight class. The otolith weight divisions on the abscissa in 85-84 have the same values in all graphs. Successive year classes are marked alphabetically from the left.

The subtraction means that negative values in the difference indicate a decrease in that size class with respect to the previous year, and positive differences indicate an increase distribution in that size class with respect to the previous year. When each of the graphs in Fig. 12 was fitted with median smoothed curves, it was evident that there was a year-to-year oscillation in year-to-year differences around zero; however, the oscillations were out of phase from sample to sample. The peaks identified from *A* to *E* showed an apparently positive cycle (i.e. year-classes), moving through successive years finally damping out at zero. Two positive cycles apparently began in 1988 and 1992 respectively.

The biological significance of the difference curves in Fig. 12 was assessed from the change in r^2 value when the differences were regressed against the difference curve from the previous year over 23 lag intervals (about half of the span of the data), each lag interval corresponding to a 15 mg standard otolith weight interval. The plots of the r^2 values against lag interval for regressions between the median smoothed successive annual differences in Fig. 12 are shown in Fig. 8c.

The r^2 plots showed that while there was no evidence for a stationary length modes concealed in the length mode distributions, there were strong correlation (r^2 as high as 60%) at certain phase cycles indicting similarly shaped difference curves that are out of phase with each other; exactly the properties one would expect from a length mode progression representing a strong year class. In addition, the peaks in the r^2 values shift in respect to length in progressive years, thus supporting the interpretation of a cycle of strong year classes moving through the data that was apparent from an inspection the lettered peaks in Fig. 12.

Estimating length at age

The samples from 1984 to 1990 were made at yearly intervals. Therefore, if the same cohort can be recognized as a peak that can be identified in two, or more, successive years, then a length-at-age relationship can be developed in the following way.

Suppose that each of the length modes recognized in each yearly sample represent successive cohorts. Then, the age in years of the first cohort is *a*, the age of the second cohort is *a+1*, the third *a+2*, etc. each peak represents a length mode so that the length corresponding to the first cohort is l_a, the second l_{a+i}, etc. Plotting l_{a+i} against a_i will give a specific length-at-age curve starting at age *a*. If it is assumed that growth from age zero to age a_i is linear, then length from zero age can be extrapolated; thereby giving the full growth curve.

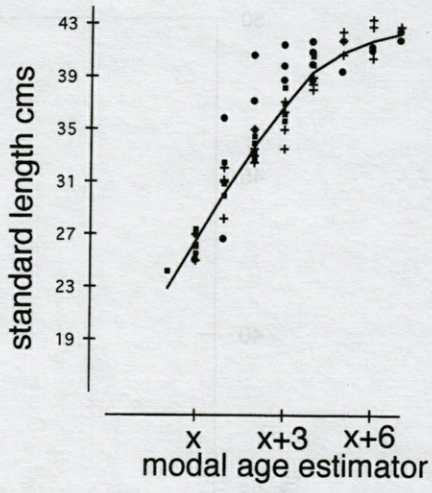

Fig. 13. – Length at age (*x, x+1*...etc) estimated from the subtraction method using otolith weight (filled circles), fish weight (filled squares) and fish length (crosses) fitted with a lowess smoothed curve.

Assuming that the lettered modes in the year to year difference curves for fish length, fish weight and otolith weight (Figs. 7, 10 and 12) are indicators of different cohorts, then the subsequent cohort lengths l_{a+i} are plotted against age class $a+i$ from each successive cohort in Fig. 13. The age and length data from cohorts in the fish length, fish weight and otolith weight data are indicated respectively by a cross, filled square and filled circle in Fig. 13.

Rather then attempt to fit a predetermined type of growth curve to the data points in Fig 13, a lowess smoothing curve was fitted, assuming zero length at zero age. The lowess curve is shown as a curved line in Fig. 13. It is evident from Fig. 13 that *a* has a value of 7 years by extrapolation along the lowess curve. By comparison, a length at age curve for *H. atlanticus* based on daily microincrement age is shown in Fig. 14. Lowess curves for females and males are shown as a dotted lines and a filled line respectively in Fig. 14. The similarity of the two length at age curves is evident. A direct regression of lengths at age over the size range 25 to 45 cm SL from both sets of growth curves in Figs. 13 and 14 resulted in a statistically significant regression ($p \leqslant 0001$)with an r^2 value of 97%.

Year class strength and recruitment

Changes in abundance of fishes are often related to changes in ocean productivity that is influenced by changing oceanographic conditions. Ocean productivity is tied to the degree of mixing of the deeper, nutrient-rich water with the water in the photic layer. One proxy for the degree of mixing between the deeper and the photic layer water is

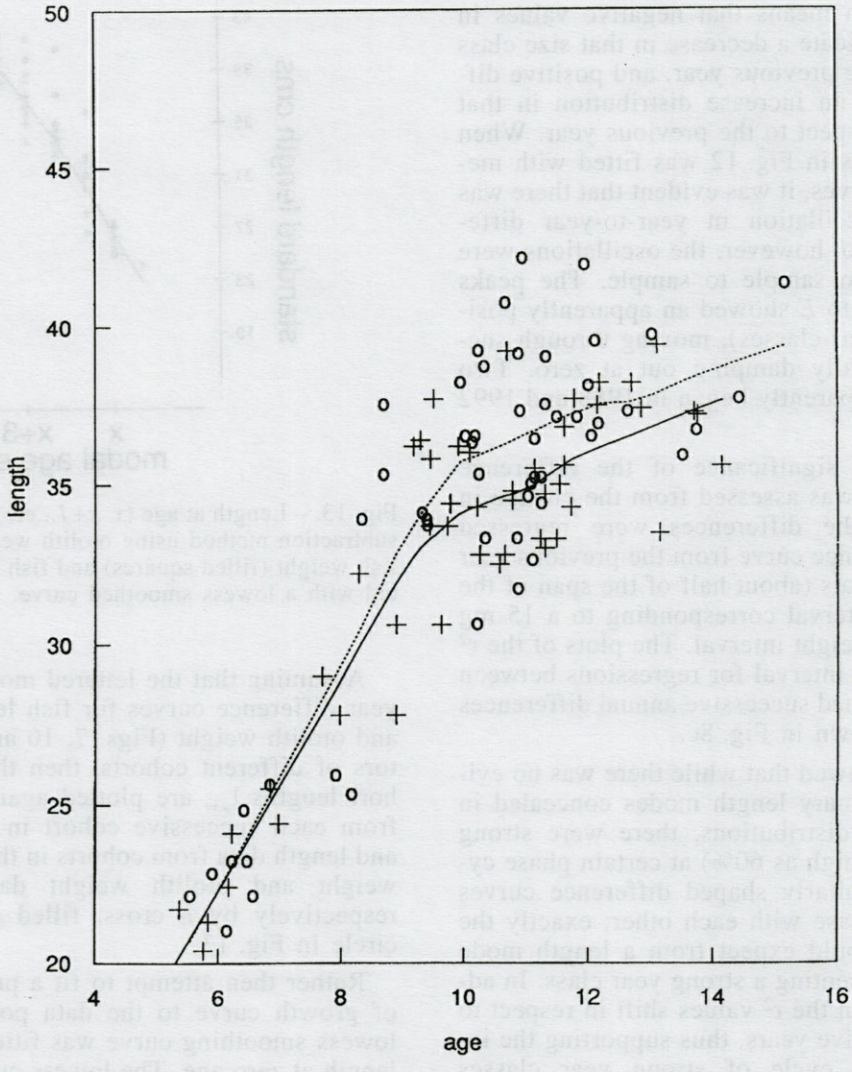

Fig. 14. – Length at age estimated by the daily microincrement method; females (open circles) and males (crosses) fitted separately with a lowess smoothing curve.

the cube of wind velocity (Backun 1990). In this study we have used the wind velocity observations in the CEOS-COADS data set. Annual turbulence effects are taken as the sum of the monthly average cubed wind speed in the region comprising the eastern New Zealand stocks of *H. atlanticus*. The annual turbulence 1970 to 1991 is plotted against year in Fig. 15a.

Following the assumption that the lettered modes in the year to year difference curves represent cohorts whose year of spawning can be identified, then it is possible to use the area under the curve of each lettered length mode as a measure of year class strength for each cohort. The average annual contribution of each cohort as area under the curve is taken as a measure of the year class strength for that cohort. The plot of year class strength is plotted against year of spawning in Fig. 15 b shows two stronger peaks in 1978/1979 and 1986, with a weaker peak in 1981. Examination of the plot of annual turbulence on year of occurrence that is also plotted in Fig. 15 b shows 1978/1979, 1981 and 1986 to be low turbulence years. The years 1973 and 1974 were also low turbulence years but are outside of the range of the cohort year class strength measure available from the 1984-1992 data.

The assumption that ocean productivity in the year of spawning determines year class strength can be tested by plotting year class strength (from area under the difference curves) against annual turbulence (Fig. 15c). The data in Fig. 15c have been fitted with a both linear and non-linear regression. The slope of the linear regression line is significantly different from zero ($p=0.039$) and the r^2 value is 0.36, indicating the 36% of the variation in annual year class strength is explained by the variation in ocean turbulence, which we use as an analog productivity measure. Fitting polynomial equations to year-class strength in Fig. 15c yields a

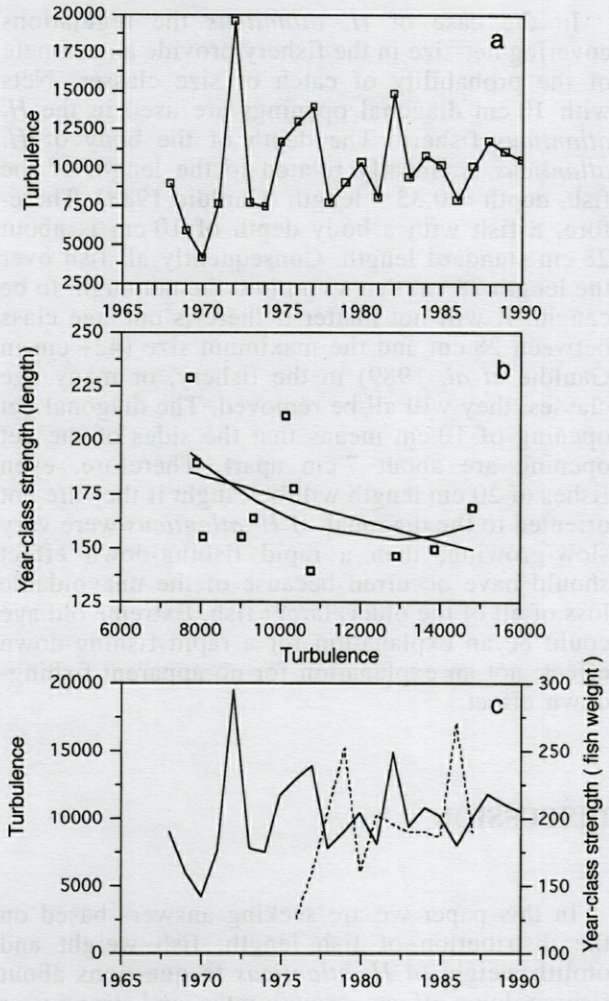

Fig. 15 – a. Annual turbulence plotted against year of spawning; b. Annual turbulence and year class strength both plotted against year of spawning; c. Fish weight year class strength plotted against year of spawning fitted with linear and non-linear regressions.

higher r^2 value, 0.44, but there is no reason to choose a non-linear over a linear regression.

Fishing-down effect

Most fisheries scientists refer to the progressive loss of larger size classes in a new or expanding fishery as a fishing-down effect because of the decrease in numbers involved. However, it is also referred to by the less intuitively obvious term "fishing-up effect" which is in the sense of the Russian equivalent: 'juvenation of the age structure'. A discussion of the origins and applications of the 'fishing-up effect' is in Ricker (1975: 262-264).

The fishing down effect results in the progressive loss of larger, presumably older, size classes. In most fisheries with size limits on net openings (including the *H. atlanticus* fishery) the specific probability that a fish going into the net will be caught is mostly a function of size. The general probability of a fish being available to go into the net is more complex, but the simplified in-net model will suffice for our discussion of the fishing-down effect. Consequently, at any particular size distribution, the larger size classes have a disproportionately higher probability of being caught. The fishing-down effect is similar whether age classes and size classes are congruent or not; congruent meaning each size comprises a discrete age class. The fishing-down effect does not markedly decrease when age classes and size classes are not congruent because of the spread of ages within size classes.

The Congruent Model matrix is shown in Table Ia. The rows are size classes and the columns are age classes. The number of cells is arbitrary and does not have to be symmetrical. Each cell in the matrix can contain two numbers. The lower number is the probability, p_s, that the size class, irrespective of age, will be caught; bearing in mind that the mechanism of the fishing-down effective will be size-selective. The upper number is the proportion of fish in each age class within each size class. This proportion represents the proportion of fish in the *i*th age class that are in the *j*th size class. This proportion, p_{age}, gives the spread of an age class over different size classes. This is the column marked p_{sum} is the size class probability, the horizontal sum of the products $[p_s * p_{age}]$ within each cell. The probability p_{sum} represents the proportion of each size class lost in each fishing pass. A similar treatment is presented in Ricker (1975).

The values for the probability of being caught, p_{sum} for each size class is the same for each of Table Ia b and has been chosen arbitrarily to reflect the increasing probability of catch with size. In the congruent model (Table Ia) age classes are identical with size classes, so that p_{age} has a constant value of one. In the absence of any fishing effect, in the congruent model the size frequency distribution of a thousand fish would comprise a series of non-overlapping, equal-sized length modes in each age class. The matrix in Table Ia can be used to simulate the fishing down effect by removing the numbers of fish in each size class corresponding to the size-class probabilities in column p_{sum}. After each pass, the remaining fish are all moved up one length class, and another 143 fish corresponding to the new cohort are added as size class 1. Constant recruitment is not likely to occur in nature, but simplifies the example. A series of length distributions corresponding to each successful pass for 7 passes using the congruent model are shown in Fig. 16a. After 7 passes the size distribution has stabilized to a new level in which the larger size classes are greatly reduced. The total population has stabilized at 683 out of the original 1000 fish.

Table I – The rows are size classes, the columns are age classes within each size class. Each cell in the row/column matrix has two numbers. The lower number is the probability that the fish in that size class (irrespective of age) will be caught. This is the probability p_s. The upper number is the proportion of fish the total population of fish that is in each age class within each size class. This proportion represents the probability p_{class} The column marked p_{sum} is the size class probability, the horizontal sum of the product of $p_s.p_{age}$. a. Congruent model matrix: in the congruent model each size class is an age class as well, so that proportion p_{age} is 1, and there is no spread of ages among size classes. b. Non.congruent model matrix: in the non-congruent model age classes are spread over more than one size class and the size class probability p_{sum} is non-linear on size.

a

Age / Size	1	2	3	4	5	6	7	P_{sum}
1	*1*							
	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
2		*1*						
	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
3			*1*					
	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
4				*1*				
	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
5					*1*			
	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
6						*1*		
	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
7							*1*	
	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

b

Age / Size	1	2	3	4	5	6	7	P_{sum}
1	*0.3*	*0.2*						
	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.005
2	*0.3*	*0.3*	*0.2*	*0.15*				
	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.0475
3	*0.3*	*0.3*	*0.3*	*0.25*	*0.25*	*0.2*	*0.1*	
	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.18
4		*0.25*	*0.3*	*0.3*	*0.3*	*0.3*	*0.25*	
	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.34
5		*0.05*	*0.2*	*0.25*	*0.25*	*0.2*	*0.3*	
	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.75
6				*0.5*	*0.2*	*0.2*	*0.25*	
	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.56
7					*0.1*	*0.1*	*0.1*	
	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.3

The Non-congruent Model matrix is shown in Table Ib. The lower numbers in the cells are the same as for Table 1a, but the upper numbers reflect a spread of ages within each size class. Consequently, the relation between p_{sum} the size class catch probability and size is non-linear. However, the effects of a series of fishing passes over a population with a constant annual recruitment of 143 fish into the smallest year class shows a similar fishing-down effect to the congruent model (Fig. 16 b). The only difference is that the non-linear relation between p_{sum} and size that results in a one-pass phase delay in the approach to equilibrium of the largest size class.

It is evident from Fig. 16a b that the probability of a size-class being taken determines the approach to fishing-down equilibrium, not the distribution of age classes within size classes.

In the case of *H. atlanticus* the regulations covering net-size in the fishery provide an estimate of the probability of catch of size classes. Nets with 10 cm diagonal openings are used in the *H. atlanticus* fishery. The depth of the body of *H. atlanticus* is linearly related to the length of the fish, depth = 0.35 x length (Gauldie 1988). Therefore, a fish with a body depth of 10 cm is about 28 cm standard length. Consequently all fish over the length 28 cm that swim into the net ought to be caught. It will not matter if there is one age class between 28 cm and the maximum size (42+ cm in Gauldie *et al.* 1989) in the fishery, or many age classes, they will all be removed. The diagonal net opening of 10 cm means that the sides of the net opening are about 7 cm apart. Therefore, even fishes of 20 cm length will be caught if they are not oriented to the diagonal. If *H. atlanticus* were very slow-growing, then a rapid fishing-down effect should have occurred because of the unavoidable loss of all of the older, larger fish. Extreme old age could be an explanation for a rapid fishing-down effect; not an explanation for no apparent fishing-down effect.

DISCUSSION

In this paper we are seeking answers based on the distribution of fish length, fish weight and otolith weight of *H. atlanticus* to questions about fishing down effects, growth rates, and strong year classes, as well as their cause. Before examining the answers that we have obtained, there is a need to be sure that the materials and methods that we have used are adequate to the task.

The samples of fish length, fish weight and otolith weight were as near to representative of the population of *H. atlanticus* as it is possible to obtain in the context of trawl surveys. It is unlikely that we will ever have access to data of better quality than that used in this study.

The general relationships between fish length, fish weight and otolith weight of *H. atlanticus* have the same basic form as in other species of teleosts, so that there is no reason to expect any unusual biological properties in the catch curves of fish length, fish weight and otolith weight that are unique to *H. atlanticus*. The scatter in the length-weight relationship increases with increasing size which accounts for the over-lap in year class size modes, as well as the increasing scatter in the length-at-age curve.

There have been a number of attempts to associate age with otolith weight (Brander 1974, Fletcher 1991), but they have usually been confounded by the need to assign prior ages to otolith weight size classes from an age estimation procedure not di-

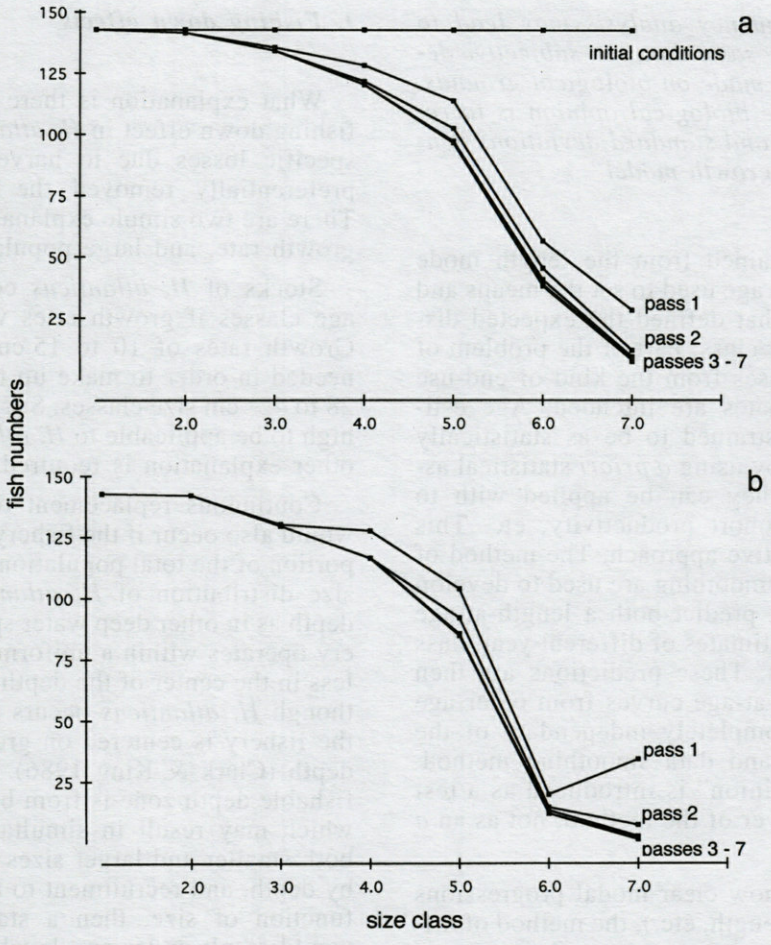

Fig. 16 – The fishing-down effect for the congruent model (a) and non-congruent model (b).

rectly related to weight (e.g., Boehlert 1985). Age estimation methods based on otolith weight generally use weight as a key to ages determined by other methods. This paper describes a new method of year-to-year differences aimed at directly assessing year-class strength, (and by implication age), from weight distributions of otoliths of *H. atlanticus* that does not require prior age estimation.

The statistical methods used in this study, F-test, Mann-Whitney tests and regression are all part of the standard suite of statistical tools used by fish biologists. Smoothing is widely applied to data sets both when the underlying functional relationship in known (Hinton & Campbell 1974, Tessler *et al.* 1993, Riggs & Tessler 1994), and when it is not known (Tukey 1977, Lancaster & Salkauskas 1986). In this study an arbitrary median smooth (Tukey 1977) was applied to the year-to-year subtraction data. This approach avoids the prior biological assumptions required by statistical fitting of modes in size class distribution, and provides a temporal progression of size modes that can be compared with other sources of information about growth in *H. atlanticus*. We have also used standard lowess smoothing (Cleveland 1979) to establish trend lines in growth curves.

The use of length modes, to assign ages to length classes is one of the oldest "common-sense" approaches to fish age estimation (see Ricker 1975: 203-207). Many fishes species show distinct length modes in the early life of the fish that have been used to estimate age. In many species of fish, including *H. atlanticus*, the older year classes do not show such obvious modalities. Nonetheless, the justification for a maximum age of 150+ years for *H. atlanticus* has been based on extrapolation of apparent length modes of the first three age classes (Gauldie 1998).

There have been many attempts to overcome the subjectivity of length mode recognition from size distributions of mixed-mode older year classes by using statistical methods (Cassie 1954, Tanaka 1953, Hasselblad 1966, McNew & Summerfelt 1978, Schnute & Fournier 1980, Macdonald & Green 1988). Such methods are not always as objective as they may seem. In the words of Schnute & Fournier (1980: 1348):

"Because length-frequency analysis may lead to many solutions for the same data set, subjective decisions must often be made on biological grounds. One way to introduce biological opinion is to require that the means and standard deviations conform to an assumed growth model".

Thus, the age obtained from the length mode analysis is always the age used to set the means and standard deviations that defined the expected distribution of each year class. Part of the problem of prior assumptions arises from the kind of end-use for which age estimates are intended. Age estimates are often constrained to be as statistically rigorous as possible by using *a priori* statistical assumptions, so that they can be applied with to length/age curves, cohort productivity, etc. This paper uses an alternative approach. The method of differences and data smoothing are used to develop estimates of age that predict both a length-at-age curve, and provide estimates of different year class recruitment strengths. These predictions are then tested against length-at-age curves from other age estimates that are completely independent of the original subtraction and data smoothing method. Thus, "biological opinion" is introduced as a test of the predictive power of the method, not as an *a priori* definition.

In fisheries that show clear modal progressions in fish size (weight, length, etc.), the method of differences by subtraction between year classes is applied routinely although indirectly, and visually, by the progressive absence of a year class in successive years at a particular size class. The method of differences used here is, in principal, no different to the widely used visual method of identifying modal progression. Instead of the difference between years being the subtraction of a positive mode from what amounts to a "zero" mode, the difference is between a larger mode and a smaller mode. However, the application of the method of subtraction used here is a departure from common fisheries practice. The method of differences was applied directly in this study and median smooth curves fitted to the subtracted data yielded evidence of year class progressions through the fishery from 1984 to 1992 in the three categories otolith weight, fish weight and fish length.

The method of differences is the quantitative application of the qualitative visual method routinely used by fish biologists to distinguish both year classes, and year class progressions. The extent to which the method of differences is robust is determined by the same criterion by which the qualitative visual method is judged: are the result supported by other, entirely independent, observations? Against this background, we can now examine the answers to the question of fishing down effects, year class strength and growth rate.

1. Fishing down effects

What explanation is there for the absence of a fishing down effect in *H. atlanticus* when the size-specific losses due to harvesting ought to have preferentially removed the larger size classes? There are two simple explanations: relatively rapid growth rate, and large population size.

Stocks of *H. atlanticus* could replenish larger age classes if growth rates were relatively rapid. Growth rates of 10 to 15 cm per year would be needed in order to make up the loss of fish in the 28 to 42^{+} cm size classes. Such growth rates are too high to be applicable to *H. atlanticus*, therefore another explanation is required.

Continuous replacement of larger size classes would also occur if the fishery was sampling only a portion of the total population of *H. atlanticus*. The size distribution of *H. atlanticus* is stratified by depth as in other deep water species, while the fishery operates within a uniform depth range more or less in the center of the depth range of the fish. Although *H. atlanticus* occurs from 600 to 1200 m, the fishery is centered on grounds at about 800 m depth (Clark & King 1986). Recruitment into the fishable depth zone is from both above and below, which may result in simultaneous recruitment of both smaller and larger sizes. If size was stratified by depth, and recruitment to the 800 m zone was a function of size, then a stable size distribution would result under any levels of fishing pressure. Thus, the lack of an apparent fishing down effect in the size distribution of *H. atlanticus* can be explained by size partitioning by depth. This would result in only a relatively small proportion of both smaller and larger fish in the population being exposed to capture.

Size partitioning by depth is a simple explanation for stability of size frequencies. However, there still remains the possibility that factors related to the deep-water habitat of *H. atlanticus* contribute to the stability of size classes. Stability of length classes under fishing pressure in Cichlids and Coregonids has been attributed to extreme environments with inherently low energy inputs (Johnson 1983). As such, these fishes fall into the general class of K-selected species. In such species, size is usually poorly correlated with age. In K-selected life history strategies, organisms tend to be promoted from an unstable pool of smaller organisms to a stable pool of larger organisms only when depletions of large organisms occur. K-selection life history strategies appear to be fairly common in deep water fishes (Merrett 1994, Merrett & Haedrich 1997), including *H. atlanticus* (Gauldie *et al.* 1989). Part of the reason may be a uniformly low energy input into the deep ocean.

Size is the causal factor in most fishing down effects, not age. Age can only be a causal factor in

circumstances where growth rates are so high that smaller fish are promoted into larger size classes rapidly enough to match losses due to fishing. An argument that no apparent fishing down effect is related to great age in *H. atlanticus* is unsupportable.

2. Year class strength

Year class strength in most fish species is a function of many variables. Most of the variables that control year class strength cannot be measured over the period for which the commercial fishery for *Hoplostethus atlanticus* has existed. However one variable, upper ocean turbulence, that has a strong impact on ocean productivity (Bakun *et al.* 1982, Bakun 1990, Cury *et al.* 1995), can be recovered from the historical climate and meteorology data.

When the average year class strength based on fish weight and fish length modes is plotted against wind-driven turbulence measurements the coefficients of determination (r^2 values) show that 36% of the variation in year class strength can be explained by the variation in ocean turbulence in the linear model; and a higher proportion of the variation in year class strength, 44%, can be explained by the variation in ocean turbulence in the non-linear (polynomial) model. Year class strength is likely to be affected by many variables. It may be possible in low turbulence conditions that there is lower cloud cover and higher sunshine that compensates for lower nutrients; and, conversely, at high turbulence conditions there are more storm clouds and more nutrients compensating for lower sunshine, resulting in a non-linear relation. Nonetheless, the more conservative linear regression model indicates an increase in year class strength with low turbulence.

The plot of fish-weight year class strength against year of spawning showed stronger year classes in 1978/1979, 1981 and 1986. The effect of the 1986 year class should reach a peak in 1995 as nine-year olds, the dominant age class in the fishery for *Hoplostethus atlanticus* following the microincrement aging method (Gauldie *et al.* 1989). However, the 1986 year class followed three years of weaker recruitment and as far as we know has been followed by nine years of neither high nor low turbulence. It is possible that weak recruitment from 1980 to 1985, and from 1987 to 1994 has contributed to the apparent decline in stocks of *H. atlanticus*. In addition, the low turbulence recorded in 1973 and 1974 may have resulted in a particularly strong recruitment that would have been evident in the fishery when commercial exploitation of stocks of *H. atlanticus* began in 1977. The decline in stocks from 1977 estimated by hind-casting techniques may reflect variability in recruitment strength as much as the effects of fishing pressure.

Examination of modes derived from the method of differences showed that within years and between years over the period of 1984 to 1987 there were seven-year classes in the *H. atlanticus* fishery. Length modes in raw size data from the *H. atlanticus* fishery are usually more-or-less bimodal, with one large adult mode and a much smaller, apparently juvenile, mode (Gauldie *et al.* 1989, Mace *et al.* 1990). The three length modes in small (< 10 cm SL) *H. atlanticus* used by Mace *et al.* (1990) to validate ages up to 56 years may be artifacts of sampling (Gauldie 1998). However, Gordon & Duncan (1987) reported clearly recognizable length modes in *H. atlanticus* sampled from the Porcupine Sea Bight in the North Atlantic at 19, 24, 26, 29, 33 and 36 cm (standard length). The 19 cm mode is missing in the *H. atlanticus* subtraction data reported here, but the remaining length modes (24, 26, 29, 33 and 36 cm) appeared in the subtraction data corresponding to ages 6, 7, 8, 9 and 10 years respectively calculated from the growth curve.

The passage of year classes evident from the subtraction method points to the 1978 spawning as a strong recruitment year that appeared in the 85-94 data as seven-year-old fish and persisted until at least 1992. There was possibly another successful spawning in 1976 that appeared as 9-year-old fish in 85-84, and there was also another possible successful spawning in 1985 that appeared a seven-year-old fish in 92-90.

3. Growth rate

Age estimation for *H. atlanticus* has been difficult to test because of the lack of conventional length modes (or other modalities) in the catch curves from the fishery (as shown in this paper), but comparison of length-at-age curves from length mode progressions, derived from the subtraction method, with length-at-age curves estimated from a daily microincrement method showed similarly shaped curves. Alternative ages of 150^{+} years for *H. atlanticus* have been proposed by Fenton *et al.* (1991); Mace *et al.* (1990) claimed maximum ages of 29 years. The length-at-age curve derived from the method of differences were clearly dissimilar to the length-at-age curves of both Fenton *et al.* (1991) and Mace *et al.* (1990). However, the growth curves obtained from the method of differences are not only similar to those obtained from daily microincrements (verified by the Gauldie-Romanek model, Romanek & Gauldie 1996, Gauldie & Romanek 1998, Payan *et al* 1997, 1998); but are also give similar maximum ages to the original growth curves of *H. atlanticus* obtained from assumed annual marks in otoliths de-

scribed in van den Broek (1983 a, b) and Kotlyar (1980). The microincrement method of aging shows that the fishery for *H. atlanticus* is dominated by the age classes 4-12 years (Gauldie *et al.* 1989). The method of differences indicates 6 year classes in the fishery of 4 to 10 year old fish, with the age groups 11 to 16 represented by a small number of size classes.

Common practice usually requires that an age estimation method be shown a priori to be correct before it is applied to a fisheries problem. The methods used to establish *a priori* correctness inevitably contain assumptions that can lead to circularities. The method of differences is free of any prior biological assumptions, but requires an *a posteriori* test of its potential value. Growth curves obtained by the method of differences matched closely (r^2 = 97%) the shape of growth curves generated by microincrement ageing of the otolith of *H. atlanticus*. It is unlikely that two completely independent estimates of the growth curve could be so similar just by chance.

Conclusions

1. The absence of a fishing down effect in the catch curves of *H. atlanticus* cannot be explained as a consequences of old age. The most likely explanation is that there are considerable reserves of *H. atlanticus* that migrate into the fishable zone. This would account for both the stability of the size distribution and the decline in apparent biomass within the fishable zone.

2. The method of differences provides information about year class structure of *H. atlanticus* that is correlates recruitment with ocean productivity. The correlation of recruitment with productivity provides prediction of future recruitment patterns that can be used as a direct experimental test of the robustness of both the prediction of recruitment and the method of differences itself.

3. The high correlation between the growth curve obtained by the method of differences and the growth curves based on daily microincrements is remarkable. It is even more remarkable when one considers that there is little or no correlation between the published growth curves of *H. atlanticus* that are all supposedly based on the same annual marks from otolith sections.

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Les manuscrits, dactylographiés en double interligne sur le recto seulement des feuilles numérotées (ne pas excéder 20 pages) sont présentés en trois jeux complets, sous leur forme définitive.

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