Universiteit Gent
Faculteit der Wetenschappen
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F.A.M.E.

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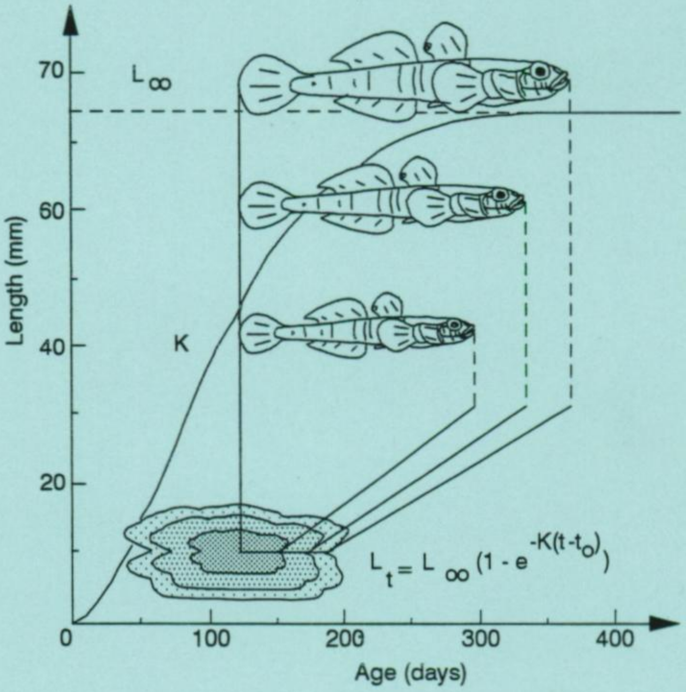

Renato V. Arellano

Promotor: Prof. Dr. M. Vincx
Co-promotor: Dr. J. Mees

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Sciences, Group Biology

Jan Mees

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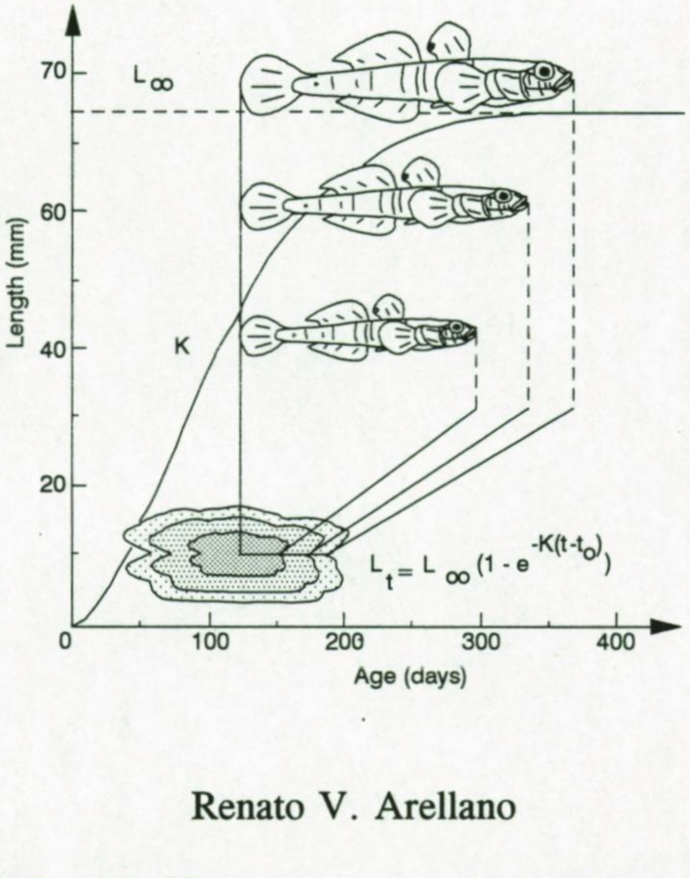

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He who thinks of the consequences
cannot be brave.

Ingush proverb

Who gathers knowledge
gathers pain.

Ecclesiastes 1:18

To Norma, Divina Gracia, Maria Magnolia
and Maria Elena.

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Dr. Maria Karakiri, for sharing her expertise in the preparation and etching of the otoliths;

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My late parents, for their great love.

And, above all, Our Almighty Lord for His bountiful graces.

Renato V. Arellano
University of Gent
15 August 1995

Foreword

This work started in September 1991 after the jury of the thesis committee of the 'International Postgraduate Training course in Fundamental and Applied Marine Ecology (FAME)', Laboratorium voor Ecologie en Systematiek, Vrije Universiteit Brussels (Free University of Brussels), proclaimed me as a recipient of the 'Algemeen Bestuur van de Ontwikkelingssamenwerking (ABOS)' scholarship grant to pursue doctoral studies.

Taking this rare opportunity, I informed my family that I would continue my studies abroad, despite the difficulty of being very far from loved ones. I also informed Dr. Armando B. Cortes, the President of the Cagayan State University at Tuguegarao, Cagayan, Philippines of my intention to pursue doctoral studies, requested an extension of my study leave on official time and was granted his approval without any hesitation.

My initial plan was to continue working on the community ecology and productivity of epibenthic communities of the shallow coastal areas of the Voordelta, The Netherlands. However, Dr. Olivier Hamerlynck, the promotor of my M.Sc. thesis, recommended me to do allied research in the assessment of aquatic resources, rather concentrating on population dynamical studies as this is one of the main problems facing fisheries and ecosystem management in Philippine waters. Thus, a research proposal on otolith microstructure analysis was conceptualised; a proposal that embraced a wide range of techniques, useful for the study of fish ecology and population dynamics.

At the very start, I was a little bit apprehensive to accept this type of research because of my limited knowledge: my background about otolith research dated back from January 13, 1986 when I was attending a training course in fish stock assessment organized at the University of the Philippines, Diliman, Quezon City, Philippines by the 'Food Agriculture Organization of the United Nations and Denmark (FAO/DANIDA)', in collaboration with the 'International Center of Living Aquatic Resource Management (ICLARM)'. The main objective of this course was to train scientists on how to conduct fish stock assessments using length-frequency data. The use of age-length data was not given emphasis and it was only briefly touched upon when comparing its accuracy to the results of the analysis of length-frequency data.

My first time to see the internal structure of otoliths was when I was attending a fisheries management course, one of the subjects of my Postgraduate Diploma in Fishing Gear Technology, which was held from September 1981 to September 1982 at the Grimsby

Institute of Technology, United Kingdom. Preparation of the otoliths was done with the conventional 'burning method'. The duration of this subject was only a couple of days and, despite of my limited knowledge and the obviously very time-consuming nature of otolith microstructure work, the vital role of otolith research in the management of fishery resources and in the study of fish ecology convinced me to accept the work.

In the early stages of the research timetable, I devoted time to a thorough review of the literature on otolith microstructure methodology and the current trends and problems in this field. To augment my practical and theoretical knowledge in this new endeavour, I decided to participate in training courses on these aspects of the research. Thanks to the initiative of Dr. Olivier Hamerlynck and the financial assistance of Prof. Dr. Magda Vincx, I was able to attend a special training at the Elbelabor of the Institut für Hydrobiologie und Fischereiwissenschaft der Universität Hamburg, Germany, from 22 to 31 July 1992. The training focused on the preparation of otoliths for microstructure analysis using light microscope and scanning electron microscope.

With the expertise of Dr. Rüdiger Berghahn, Dr. Maria Karakiri and Aquiles Sepúlveda in otolith microstructure examination and analysis, I was able to gain the basic knowledge necessary for the preparation and interpretation of the otoliths of *Pomatoschistus minutus* and *P. lozanoi*. I also acquired knowledge on the use of a grinding apparatus (a converted record player) to facilitate otolith preparation (a very simple and promising technique which I adopted once I was back in Gent).

Dr. Berghahn suggested me to participate in the 'International Symposium on Otolith Research and Application' which was held at the University of South Carolina, Hilton Head, South Carolina, United States of America, from 23 to 27 January 1993 to further enrich my capabilities in microstructure research. With his brochure about the training and a recommendation to Dr. Hamerlynck and to Prof. Dr. John Mark Dean, Director of the Centre for Environmental Policy, University of South Carolina, I was able to attend the training with the financial help of Prof. Dr. Magda Vincx for my air plane round trip ticket, Prof. Dr. Carlo Heip for my accommodation expense, and Prof. Dr. Jean Mark Dean for my pocket money and free registration fee for the symposium.

The symposium brought together scientists involved in fundamental research on otoliths, fisheries researchers that use them as management tools and students that want to explore the opportunities in this evolving field. Emphasis was on the innovative approaches to otolith research, and on their potential application in the study of fish biology and population dynamics. Scientific sessions and programmes were divided into four sessions: (1) otolith growth and morphology, (2) estimation of fish growth, (3) otoliths in the studies of population biology and (4) chemical tags and otolith composition. Lectures were supported by poster sessions. All day walk-in workshops were conducted on two specific fields: otolith microstructure preparation and interpretation, and the use of image analysis systems for otolith examination.

The symposium was attended by well known scientists from all over the world who presented the new trends in microstructure examination and analysis and 17 students preparing a thesis on otolith research. In addition, the organisers of the symposium requested all participants to bring their own otolith samples for practical exercises on microstructure analysis. As a newcomer in this field of research, I took all possible opportunities to get the most out of this symposium. After the symposium, I was more confident to start up my research at the Marine Biology Section, Zoology Institute, University of Gent.

Introduction and summary

Pomatoschistus minutus Pallas 1770 and *P. lozanoi* de Buen 1923 are sympatric goby species that live demersally in marine and estuarine waters. Due to their high abundance throughout the year and their importance in the food webs of marine ecosystems, they are extensively used in many aspects of ecological studies. This thesis presents a study on age and growth of the *P. minutus* and *P. lozanoi* populations in the ebb-tidal delta of the former Grevelingen estuary (Voordelta, Delta area, south-west Netherlands). Four stations were sampled monthly from September 1992 to July 1994 at two different depths (5 and 10 m). The thesis focuses on the study of (1) the shrinkage and weight loss through preservation, (2) the morphology and function of the otoliths, and, most importantly, (3) the life history and population dynamics of the 2 species using length-frequency data and daily growth increments in their sagittae.

In chapter 1, brief reviews are presented about the life histories of *Pomatoschistus minutus* and *P. lozanoi* in the North Sea and the recent developments in otolith microstructure examination and analysis. Chapter 2 describes the effect of two preservatives on length, weight and otolith structure of the two species; a shrinkage correction factor is established for both species. In chapter 3, the external morphology and function of the sagittae, lapilli, and asterisci are described. Chapter 4 explains the use of the sulcus acusticus and sagitta areas to infer the hearing sensitivity of the two species. Chapter 5 describes the internal structures of the sagittae and the validation of the growth increment deposition in the sagittae. Chapter 6 deals with the age, growth and mortality of *P. minutus* and *P. lozanoi* based on the otolith microstructure analyses. Chapter 7 describes the population dynamics of the two goby species using the analysis of length-frequency data.

The different chapters of this thesis have been submitted to or published in international journals. Chapters 2, 3, 5, 6 and 7 have been submitted with the following titles:

Arellano, R.V., Hamerlynck, O., Vincx, M., Mees, J., Hostens, K., Gijselinck, W. Changes of length, weight, and otolith appearances of *Pomatoschistus minutus* (Pallas) and *P. lozanoi* (de Buen) (Pisces, Gobiidae) after formaldehyde and ethanol fixation.

Arellano, R.V., Mees, J., Hostens, K. Morphology of the otoliths of *Pomatoschistus* *minutus* and *P. lozanoi* (Pisces, Gobiidae).

Arellano, R.V., Mees, J., Hostens, K. Validation of the daily growth increments in the sagittae of *Pomatoschistus minutus* and *P. lozanoi* (Pisces, Gobiidae).

Arellano, R.V., Mees, J., Hostens, K. Age, growth and mortality of two sympatric goby species *Pomatoschistus minutus* and *P. lozanoi* (Pisces, Gobiidae).

Arellano, R.V., Mees, J., Hostens, K. Population dynamics of two sympatric gobies in a shallow coastal area. *Pomatoschistus minutus* and *P. lozanoi* (Pisces, Gobiidae).

Chapter 4 has recently been published as:

Arellano, R.V., Hamerlynck, O., Vincx, M., Mees, J., Hostens, K., Gijselinck, W. (1995). Changes in the ratio of the sulcus acusticus area to the sagitta area of *Pomatoschistus minutus* and *P. lozanoi* (Pisces, Gobiidae). Mar. Biol. 122: 355-360.

Chapter 1 presents a brief review of the literature on the seasonality, spawning behaviour, feeding habits, morphology and ecology of *Pomatoschistus minutus* and *P. lozanoi*. Recent developments in the otolith microstructure research since Pannella (1971) discovered daily growth rings are also reviewed.

Chapter 2 describes the changes of length, weight and otolith appearance of *Pomatoschistus minutus* and *P. lozanoi* after formaldehyde and ethanol fixation. Samples were collected monthly in the ebb-tidal delta of the Grevelingen area (Delta area, south-west Netherlands) from September 1992 to February 1993 by means of a 3 m beam trawl with a codend mesh size of 10 mm. Samples were either stored immediately in 96% ethanol (direct method) or were transferred to 96% ethanol after 24 hours preservation in 7% formaldehyde (indirect method). An insignificant reduction in length and a significant loss in weight were found for both species. The patterns of shrinkage and weight loss with the two preservation methods only differed for the first 24 hours of storage. After an additional day of storage in 96% ethanol, the patterns became analogous. The effects of the direct method on shrinkage and weight loss were more eminent than those of the indirect method during the first week of storage. The effects were found to be smaller in *P. lozanoi* than in *P. minutus* of the same size. For both species, conversion factors from preserved length/weight (PL and PW) to fresh length/weight (FL and FW) were established by simple linear regression. The estimated conversion factors for *P. minutus* are $FL = 2.713 + 0.997 * PL$ and $FW = 0.633 + 2.30 * PW$. For *P. lozanoi*, $FL = 0.589 + 1.024 * PL$ and $FW = 0.185 + 1.302 * PW$. The otoliths of neither goby species showed signs of deterioration or decalcification after one year of storage with both methods of preservation.

Chapter 3 describes the external morphology of the otoliths of *Pomatoschistus minutus* and *P. lozanoi* and assesses possible implication for population dynamics and validation studies. It also establishes relationships between fish length and otolith characteristics on the one hand, and between otolith length and other otolith measures on the other hand, this in order to determine allometric growth and to establish regressions for the back-calculation of fish length. Samples were collected monthly from September 1992 to October 1993 with a beam trawl in the 5 m and 10 m depth strata of the ebb-tidal delta of the Grevelingen area and were preserved by the indirect method (as described earlier). Otoliths (sagittae, lapilli and asterisci) were extracted from the auditory labyrinths by making a frontal plane incision at the cranial part of the head above the eyes. The contours of the otoliths were drawn with the aid of a drawing tube attached to a binocular microscope at 50 x magnification. For each otolith, eight (sagitta) or six (lapillus and asteriscus) characters were measured by a digitizer. The orientation of the otoliths in the skull was similar in both species and otolith shapes and weights did not differ significantly between sexes. Sagittae and lapilli of *P. minutus* were larger and heavier than those of *P. lozanoi*, while the size and weight of the asterisci of both species were not significantly different. The sagittae of *P. minutus* were more corrugated and crenulated than those of *P. lozanoi* after sexual maturity, whereas the lapilli and asterisci of both species never showed corrugations and crenulations. Allometry of the left and right otoliths of both species was isometric, suggesting function for balancing and equilibrium. The somatic growth rates of both species influenced the relationship between fish length and several otolith characters. Juveniles were characterised by faster otolith growth rates, relative to fish length, than adults. It is concluded that the external morphology of the sagittae can be used as an indicator of fish maturity and that sagitta characters can be used for species identification.

Chapter 4 explains the use of the ratio of the sulcus acusticus area to the sagitta area to infer the hearing sensitivity of *Pomatoschistus minutus* and *P. lozanoi*. The goby stock of the ebb-tidal delta of the former Grevelingen estuary was sampled monthly from September 1992 to October 1993. Samples were preserved by the indirect method (as describe earlier). Sagittae were extracted and the areas of the sagitta and sulcus acusticus were measured with a digitizer. The allometry of these two areas were described. The frequency response and auditory threshold of these two sympatric goby species were determined from the ratio of the sulcus acusticus area to the sagitta area (S:O ratio). The S:O ratio of *P. minutus* increased while in *P. lozanoi* the S:O ratio was constant with increasing standard length. The average S:O ratio of *P. lozanoi* was significantly higher than that of *P. minutus* of the same mean length, which makes the former more sensitive to sound frequency. The S:O ratios of these two sympatric fishes were lower than the S:O ratios of demersal and pelagic fishes available from the literature. An interspecific growth difference of sagitta and sulcus acusticus was observed. The sagitta and sulcus acusticus of *P. minutus* are larger than those of *P. lozanoi* of the same length. Differences in the hearing sensitivity are related to differences in S:O ratio, which is most probably due to the differences in the food and spatial niche of these two species.

Chapter 5 explains the validation of the growth increment deposition in the sagittae of *Pomatoschistus minutus* and *P. lozanoi*. Samples were collected during daytime with a beam trawl from the ebb-tidal delta of the Grevelingen from September 1992 to October 1993 and were preserved by the indirect method (as describe earlier). The use of the sagittae of these two sympatric gobies for the determination of their age was validated following the 3 criteria of Fowler (1990): (1) the otoliths display growth increments, (2) the deposition rates of the growth increments correspond to regular time scales, and (3) otoliths continue to grow throughout the species' life. The deposition rate of the growth increments in the sagittae of both species was determined with statistical inference techniques. The sagittae of both species displayed periodic thick opaque zones (checks) with alternating fine striations of translucent and opaque zones (growth increments). When viewed under the scanning electron microscope, they showed distinct deep grooves (checks) with alternating slightly etched wide zones and deeply etched narrow zones (growth increments). The number of growth increments in the first check at the sagitta nucleus (12 to 22) corresponded to the period of hatching of both species under laboratory conditions (Fonds 1973). The second check corresponded to the time of transition from a pelagic to a demersal life style (settlement check). The number of growth increments in the periodic checks (12 to 15, mode of 14) was correlated either to the number of days between full moon and new moon or between the first and last quarters of the moon cycle. The statistical inference technique revealed that the growth increments in the sagittae of both species were deposited at a rate of 1 per day. The sagittae of both species grew throughout the species' life span, though the growth rate changed abruptly when at the time of sexual maturity (30 mm standard length). Thus, the 3 criteria for validation are fulfilled and it was concluded that the sagittae of both species could be used for age and growth studies.

Chapter 6 describes the age, growth and mortality of *Pomatoschistus minutus* and *P. lozanoi* using otolith microstructure examination and analysis. Samples were collected monthly from September 1992 to October 1993 in the ebb-tidal delta of the former Grevelingen estuary (south-west Netherlands) and were preserved by the indirect method. In each month, the otoliths of a representative size range of both sexes of each species were extracted and the microstructure of the sagittae was examined in detail. Daily growth rings were counted using light microscope and scanning electron microscopic techniques. Both techniques yielded highly similar results. Four models were used to back-calculate fish length from the widths of the growth increments. All models yielded very similar results for fish >30 mm standard length. For fish <30 mm standard length, the models of Dahl-Lea and Whitney-Carlander were to be preferred, while the models of Fraser-Lea and Hile tended to overestimate length. The age of both species never exceeded 2 years. Maximum recorded age was 655 (males) and 608 (females) days for *P. minutus* and 496 (males) and 474 (females) days for *P. lozanoi*. Fish growth was found to be directly proportional to the growth of the sagitta radius. In *P. minutus*, the estimated somatic growth rates were 110±36 μm d^{-1} for males and 108 ± 34 μm d^{-1} for females. In *P. lozanoi*, the estimated growth rates were 122 ± 31 μm d^{-1} for males and 123 ± 30 μm d^{-1} for females. In *P. minutus* the growth parameters (L_∞ and K) of the von Bertalanffy growth model were 76.28 mm and 1.06 y^{-1} for males and 75.86 mm and 1.32 y^{-1} for females. In *P. lozanoi*, L_∞ and K were 67.07 mm and 1.31 y^{-1} for males and 65.01 mm and 1.37 y^{-1} for females. Both sexes of *P. lozanoi* had significantly higher mortalities than *P. minutus*. Male *P. minutus* had a significantly lower mortality than females, while no significant differences were observed between the sexes in *P. lozanoi*. Peaks of high mortalities were observed during the spawning season (spring-early summer for *P. minutus*; late summer-early autumn for *P. lozanoi*).

Chapter 7 describes the population dynamics of *Pomatoschistus minutus* and *P. lozanoi* using length-frequency data. The *Pomatoschistus minutus* and *P. lozanoi* populations of the ebb-tidal delta of the Grevelingen area (Delta area, SW Netherlands) were sampled monthly from September 1992 to July 1994 in 2 depth strata (-5 and -10 m). Densities, biomass and length-frequency distributions of males and females of both species were recorded separately and their longevity, growth, population structure and secondary production were studied. Cohorts were identified and separated with the Bhattacharya method and modal progression analyses. Age and growth were described with a seasonally oscillating version of the von Bertalanffy growth equation. Allometry was assessed from the slopes of the length-weight regressions. Production was estimated with the size-frequency method. Since both species are morphologically very similar, the effects of different levels of pooling (sexes and species) on the estimates of growth parameters and secondary production were also assessed.

The restructured length-frequency data displayed 3 cohorts for *Pomatoschistus lozanoi* and 4 for *P. minutus* and the monthly pooled data revealed that both species had a life span of less than 2 years. Only one cohort was produced per year which emerged in June for *P. minutus* and in September for *P. lozanoi*. In *P. minutus*, males attained a theoretical maximum longevity (TML) of 620 days for a theoretical maximum size (TMS) of 79 mm standard length (SL), while females attained a TML of 650 days for a TMS of 70 mm SL. In *P. lozanoi*, the TMS of males of 59 mm SL corresponded to a TML of 600 days, while for females the TMS of 49 mm SL corresponded to a TML of 650 days. Pooling data of both sexes yielded a TMS of 63 mm SL with a corresponding TML of 650 days. Pooling data of both species yielded a TMS of 71 mm SL with a corresponding TML of 720 days.

All growth curves showed a pronounced oscillation and growth stopped once a year. Growth was fastest from July to October and slowest from January to March. Allometry was positive in both sexes of both species, and the growth of males was faster than that of the females. In *Pomatoschistus minutus*, the estimated growth parameters (L_∞, K, C and WP) were 79.4 mm, 1.9 y^{-1}, 1 and 0 for males, and 73.9 mm, 1.65 y^{-1}, 1, and 0.12 for females. When both sexes were pooled, the analyses yielded values of 79.20 mm, 1.70 y^{-1}, 1 and 0.03. In *P. lozanoi*, the growth parameters were 65.30 mm, 1.5 y^{-1}, 1 and 0.13 for males, 54.85 mm, 1.35 y^{-1}, 1, and 0.27 for females, and 64.90 mm, 1.90 y^{-1}, 1 and 0.12 for both sexes pooled. When the data of both species were lumped, the K values were underestimated and L_∞'s were overestimated: the L_∞, K, C and WP became 83.50 mm, 0.94 y^{-1}, 1 and 0.15, respectively.

The production of *Pomatoschistus minutus* (0.427 g AFDW m^{-2} yr^{-1}) was higher than that of *P. lozanoi* (0.046 and 0.091 g AFDW m^{-2} yr^{1} for the 1992 and 1993 cohorts, respectively). Lumping the length-frequency data of different strata, sexes and species seriously overestimated production.

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CHAPTER 1.

Otolith microstructure and the life history of the sympatric gobies *Pomatoschistus minutus* and *P. lozanoi* (Pisces, Gobiidae): a brief review of literature.

1.1. Introduction

The study of the biology of teleost fish species through otolith microstructure examination and analysis requires background information on their ecology and population dynamics. This information is necessary because the otoliths record the past life history of the fish from before the time of spawning up to the time of capture: it will allow for counter-checking and validating whether the information contained in the otoliths corresponds to events in the fish's life or to specific aspects of the ecology of the fish.

This chapter contains two main parts. Firstly, a brief literature review is presented on the life histories of *Pomatoschistus minutus* and *P. lozanoi*, the two commonest goby species inhabiting the coastal waters of north-western Europe, with special attention for our knowledge regarding populations from the Netherlands and Belgium. Recently, an extensive study of the feeding ecology of both species in the shallow coastal waters and estuaries of the Southern Bight of the North Sea was reported (Hamerlynck 1993). Other studies dealing with different aspects of the biology of these two species have also been published (e.g. Fonds 1970, 1971, 1973, Healey 1971, Fonds & Van Buurt 1974, Webb 1980, Miller 1984, Claridge *et al.* 1985, Del Norte-Campos & Temming 1994). Most of these studies only consider *P. minutus*; the morphologically very similar *P. lozanoi* has rarely been studied or even identified as such. Secondly, a brief review on recent developments in the field of otolith microstructure examination and analysis is presented.

1.2. Life history and population dynamics of *Pomatoschistus minutus* and *P. lozanoi*

1.2.1. Geographical distribution

Although over 20 species of gobies have been recorded from European Atlantic coastal waters (Fonds 1973, Miller 1986), only 6 are found over the sandy bottoms of the Dutch and Belgian shallow coastal areas. These are the transparent goby *Aphia minuta* Risso

1810, the black goby *Gobius niger* Linnaeus 1758, the painted goby *Pomatoschistus pictus* Malm 1865, the common goby *P. microps* Krøyer 1838, the sand goby *P. minutus* Pallas 1769 and lozano's goby *P. lozanoi* de Buen 1923 (Fonds 1973, Nijssen & de Groot 1983, Hamerlynck *et al.* 1990, Arellano 1991). The largest species is *G. niger* (maximal length of 170 mm); the smallest species are the *P. pictus*, *P. microps* and *A. minuta* (60 mm) (Nijssen & de Groot 1983).

Gobies of the *Pomatoschistus minutus* complex are the most abundant demersal fish in the coastal waters of the North Sea (Fonds 1973, Miller 1973, Webb 1980, Hamerlynck *et al.* 1990, Hostens & Hamerlynck 1994) and in the inshore waters of England and Wales (Claridge *et al.* 1985, Henderson 1989). In the Voordelta area, *P. minutus* and *P. lozanoi* are very abundant in the ebb-tidal delta of the former Grevelingen estuary, while lower densities have been observed in the more seaward Banjaard area and in the ebb-tidal delta of the Oosterschelde (Arellano 1991, Hostens & Hamerlynck 1994). Both species have a great overlap in geographical distribution and they co-occur in most shallow marine soft bottom coasts and in estuaries from Scandinavia to the Iberian peninsula (Fonds 1971, Claridge *et al.* 1985, Hamerlynck 1990, Petersen 1992).

1.2.2. Seasonal abundance

The abundance of *Pomatoschistus minutus* and *P. lozanoi* in the coastal waters of the Netherlands and Belgium has been reported to fluctuate seasonally. The temporal pattern of abundance is quite similar in both species, although the young cohorts of *P. minutus* generally emerge earlier (June-July) than in *P. lozanoi* (August-September) (Fonds 1973, Hamerlynck 1990). After recruitment of the young cohorts, two clear peaks can be observed in the length-frequency distributions: a high peak representing the abundant small size classes, and a smaller peak representing the fewer survivors of the old cohort. Shortly afterwards, the peak of the old cohort disappears: this is due to either natural mortality or a blending of the two peaks as the younger cohort grows. Highest abundance of gobies has generally been observed from late summer (September) up to early autumn. The abundance of gobies then gradually declines from late November up to the spawning season, lowest densities being recorded in winter and early spring. The monthly densities of *P. minutus* are generally higher than those of *P. lozanoi*, except during the spawning season of *P. minutus*, at which time adult *P. lozanoi* are more abundant (Hamerlynck 1993). During spawning, females have been reported to be more abundant than males.

1.2.3. Reproduction

The reproductive behaviour of all *Pomatoschistus* species is similar (Fonds 1971, Miller 1984). The different aspects of reproduction involve the establishment of a territory, nest-building, courtship, mating, spawning and parental care (Reese 1964). Males build nest under empty bivalve shells (Fonds 1973, Nellbring 1993), lure females to spawn in the nest by means of specific courting behaviour and defend the nest as their territory. The female leaves the nest after spawning, and the male guards the eggs until they hatch. The newly hatched larvae live pelagically for about one month and then settle to the bottom (Fonds 1971, 1973). Females are repeat spawners, producing several batches of eggs per spawning season (Miller 1984) and males can guard batches of eggs from different females at the same time (Fonds 1971).

There is a clear difference in spawning period between the two species in the coastal waters of the North Sea: March to June for *Pomatoschistus minutus* and June to August for *P. lozanoi* (Fonds 1973, Hamerlynck 1990).

1.2.4. Morphology and feeding ecology

Pomatoschistus minutus and *P. lozanoi* differ in size, body pigmentation pattern and the arrangement of dermal papillae (Hamerlynck 1990). *P. minutus* attains a maximal length of around 90 mm total length versus 80 mm for *P. lozanoi* (Nijssen & de Groot 1983). *P. lozanoi* is a more slender and less pigmented species than *P. minutus*. *P. minutus* larger than 15-30 mm standard length are characterized by double spots on the lateral line and have a pigmented dorsal half, while in *P. lozanoi* the spots stay single throughout its life and smaller size classes (15-30 mm) are unpigmented. Subadult *P. lozanoi* (>30 mm) have no black spot on the first dorsal fin and little or no pigmentation on the dorsal half, while in *P. minutus* of the same length the black spot is present and the dorsal half becomes reticulated. For adult individuals, the most important criterium for identification is the papillary pattern of the head lateral-line system (for details see Hamerlynck 1990). Further, male spawners of *P. minutus* have a few double pigment bands on the flank, while male spawners of *P. lozanoi* have seven to nine single bands. Female spawners of *P. lozanoi* have a moustache and a dark blotch on the chin, while female spawners of *P. minutus* have no moustache and only slight pigmentation on the chin (Hamerlynck 1990). The genital papillae of the males of both species are pointed, while those of females are dull (Guitel 1892).

Pomatoschistus minutus is better adapted to live in the extreme conditions of estuaries, while *P. lozanoi* is better adapted to live in the neritic environment (Fonds 1973). These two sympatric goby species have a distinct partitioning of food resources: *P. minutus* is a generalist which feeds mostly on endo- and epibenthic animals, while *P. lozanoi* is a specialist which feeds almost exclusively on hyperbenthic organisms (Hamerlynck *et al.* 1986, 1990, Hamerlynck & Cattrijsse 1944). *P. minutus* is better adapted for biting, while *P. lozanoi* is better adapted for suction feeding (Hamerlynck 1993). Both species are very important in the energy budget of O-group gadoids (Costa 1988, Hamerlynck & Hostens 1993).

1.3. Otolith microstructure

1.3.1. Introduction

Growth is one of the fundamental parameters that has to be assessed in the study of fish population dynamics, secondary production and energy fluxes through the ecosystem. Growth - or changes in the size of fish - can best be evaluated on a rate basis. Therefore, a temporal measurement such as age is essential in growth studies. If the age of a fish is unknown, as is often the case in studies of fish taken from the natural environment, then age determination becomes an important and necessary prerequisite in such studies (Weatherly & Gill 1987).

Age and growth of teleost fishes can be interpreted from the analysis of (1) length-frequency data (Gjøsaeter & Sousa 1983, Pauly 1984, Pauly 1987, Sparre *et al.* 1989), (2) mark-recapture data (Pauly 1983, McCaughran 1987) and (3) the morphology and internal structure of hard structures, like otoliths (Pannella 1971, 1980, Campana & Neilson 1985, Karakiri *et al.* 1989, Alemany & Alvarez 1994, Hood *et al.* 1994, McCormick 1994, Oxenford *et al.* 1994, Panfili *et al.* 1994, Sepúlveda 1994, Gauldie *et al.* 1995), scales (Beamish & McFarlane 1987, Carlander 1987, Miller & Kapuscinski 1994, Ogle *et al.* 1994), fin rays and vertebrae (Clement *et al.* 1992, Conand *et al.* 1995). All these 'hard parts' are mineralised structures that display more or less regular growth rings, making them potentially useful for age determination (Simkiss 1974).

The presence of annual rings in otoliths has been well established for a long time (they have first been used for ageing eels in 1759, Jones 1992). The discovery by Pannella (1971) of daily growth rings in otoliths opened new perspectives for the development and application of otolith increments in growth studies. Daily growth rings have been shown to occur in a wide range of teleosts, in marine and freshwater taxa, from the poles to the equator (Pannella 1971, Barkman 1978, Campana & Neilson 1985). To date, they have especially been valuable as a tool for the study of age and growth in O-group fishes (Campana & Neilson 1985, Brothers 1987, Karakiri *et al.* 1989, Alemany & Alvarez 1994, Hare & Cowen 1994, McCormick 1994, McDowall *et al.* 1994). The application of the otolith increment technique has increased exponentially since Pannella's discovery in 1971 (Campana & Neilson 1985, Jones 1992). It is now in common use for the study of early life history, age, growth, recruitment, migration, mortality, stock assessment and fisheries management (Campana & Neilson 1985, Jones 1992, Stevenson & Campana 1992).

The otolith microstructure technique has a wider application than the conventional age determination methods, especially in cases where the annual rings are not present, e.g. early life stages, short-lived species and tropical species (Campana & Neilson 1985). Otoliths are more commonly used than the other hard parts mentioned above because they show no resorption under severe stress conditions, such as exposure to low pH (Geen *et al.* 1985), exertion (Campana 1983a) or food deprivation (Marshall & Parker 1982, Campana 1983b). Furthermore, otoliths are usually the first calcified structures formed during the early development of teleosts (Campana & Neilson 1985, Jones 1992). Thus, they consistently record the daily events from the earliest life stages up to the death of the fish. The disadvantage of the otolith increment technique is that the fish must be killed to extract the otoliths. Also, because of their small size, otolith examination and reading can be quite difficult and very time consuming, especially in smaller stages: correct identification of the location of the nucleus, the interpretation of checks or stress marks and the counting of the rings at the outer margin require a lot of practise and expertise.

Important events in the life history of a fish, like hatching (Brothers & McFarland 1981, Hare & Cowen 1994, Sepúlveda 1994), metamorphosis (Bailey *et al.* 1977, Victor 1986), yolk sack resorption (Wilson & Larkin 1980), settlement (Victor 1982, Karakiri *et al.* 1991) and egg deposition (Pannella 1974) can be read from the sagittae as so called 'check marks'. For most tropical teleosts accurate data on the duration of the separate life history stages are lacking, and, as the year class strength of demersal fish species is mainly determined during the 'early life history', i.e. between the end of the larval stage and recruitment (Scott *et al.* 1993), micro-analysis of otoliths can be a very apt tool to reconstruct the life history. Detailed investigation of the early life history stages by daily growth ring examination is a major step forward in the understanding of the processes involved in the determination of exploitable biomass.

1.3.2. Terminology and definitions

'Otoliths' are peculiarly shaped calcium carbonate bodies in the auditory system of fishes (Adams 1940, Carlstrom 1963, Secor *et al.* 1991, 1992). The term actually refers to three pairs of mineralized structures, namely the 'sagittae', the 'lapilli' and the 'asterisci', which are located symmetrically on each side of the head cavity in the semi-circular canals called the 'sacculi', the 'utriculi' and the 'lagenae', respectively (Pannella 1980, Secor *et al.* 1991, 1992).

Prior to the discovery of the daily growth increments in the otoliths of teleost fish, a standard terminology and notation for otolith readers was proposed by Jensen (1965). This report was conceptualised to resolve disagreements between biologists engaged in age determination of fish. Later, there were further attempts to standardise terminology (International Symposium of Otolith Research and Application, January 1992, Hilton Head, USA). Still, many published papers on otolith studies use different terminologies. To avoid confusion, operational definitions of terms should be carefully understood when comparing results of different studies. For example, the term 'otoliths' is sometime used for designating either 'sagittae', 'lapilli' or 'asterisci'.

Age can be read from internal structure of the otoliths. The most important of these are the 'annuli', which are formed on a yearly basis, (Campana & Zwanenburg 1990) and the 'daily growth increments' (Pannella 1971, Otake *et al.* 1994, Ré & Narciso 1994). The characteristics and the optical appearance of the annuli and growth increments differ between species and will depend on the methodologies used for preparation and examination. For example, the annuli of sagittae can be observed as either continuous translucent (or rarely opaque) zones or as grooves (Summerfelt & Hall 1987).

The term 'daily growth increment' is synonymous to 'daily growth ring', 'ring', 'lamella', 'microincrement', 'microband', and 'growth unit' (e.g. Campana & Neilson 1985, Ntiba & Jaccarini 1988, Peñaflor 1988, Ré & Narciso 1994, Sepúlveda 1994, Gauldie *et al.* 1995). Each growth increment is composed of two structures: an 'incremental zone' and a 'discontinuous zone' (Mugiya *et al.* 1981). When examined under a light microscope, the incremental zone appears as a broad, translucent band, while the discontinuous zone is relatively narrow and opaque. When the otolith is etched with a weak acid and viewed under a scanning electron microscope, the incremental zone is observed as a wide, lightly-etched area, while the discontinuous zone appears as a narrow and deeply-etched zone (Campana & Neilson 1985).

The 'incremental zone' and 'discontinuous zone' as defined above for the use in microstructure analyses, are designated as 'translucent zones' and 'opaque zones' in studies reporting on conventional ageing methods. In the conventional method, the term 'opaque zone' is synonymous to 'summer zone', 'fast-growth zone' or 'dense zones', while the 'translucent zone' is synonymous to 'winter zone', 'slow-growth zone' or 'hyaline zone' (Jensen 1965). However, the optical appearance of the different zones is similarly defined in both techniques. Translucent zones allow the passage of light, while opaque zones inhibit the passage of light. Thus, under transmitted light the opaque zones appear dark and the translucent zones appear bright; under reflected light the opaque zones appear bright and the translucent zones appear dark (Summerfelt & Hall 1987).

The term 'nucleus' is synonymous to 'centre', 'core', 'focus', 'primordium', 'kernel' and 'origin' (Jensen, 1965, Campana & Neilson 1985, Summerfelt & Hall 1987, Campana & Zwanenburg 1990). The term 'zone' has been used synonymously to 'annulus', 'year ring', 'year mark', 'band', 'winter ring', 'summer ring' and 'growth zone'. The term 'check' is synonymous to 'check mark', 'check ring', 'false ring', 'secondary ring', and 'split'. Checks are composed of hyaline matter and they are not counted for age determination (Jensen 1965). They appear as discontinuities which are evidence of periods of extreme stress (Campana & Neilson 1985). They may appear either periodically or non-periodically (Pannella 1971, 1980, Campana 1984) and are observed as the deepest grooves on the otoliths when viewed under scanning electron microscopy. Under light microscope, they have the appearance of very thick opaque zones.

1.3.3. Methodology

A successful otolith microstructure examination depends on the adequacy of the methods used for storage, preparation, mounting and observation. For a manual on otolith removal and preparation for microstructural examination, and a review of the existing methods for dissection, cleaning, storage, sectioning, polishing, and etching we refer to Secor *et al.* (1991, 1992).

Methods of preservation can influence the quality of the otoliths. Both frozen specimens and fish that are preserved either in formaldehyde or in ethanol (Brothers *et al.* 1976, Methot & Kramer 1979, Ré 1983, Berghahn *et al.* 1993), will shrink before otolith removal and the degree of shrinkage depends on the species, the type and strength of preservative, the time between death and preservation, and the size of the fish (Theilacker 1980, Butler 1992). Samples preserved in 95% ethanol tend to shrink most (Butler 1992), while preservation with <70% ethanol or with formaldehyde solutions will result in otolith decalcification (Radtke & Waiwood 1980, Butler 1992). This is caused by the rapid lowering of the pH as the water from the tissues dilute the preservative. The ethanol should thus be changed within 24 hours, and the pH of the preservative should be monitored regularly (Butler 1992).

Extracted otoliths of adults and juveniles are either stored dry, in ethanol or mounted on microscope slides. Otoliths can be fixed with different mounting media: e.g. epoxy (e.g. Nishimura & Yamada 1984, Castonguay 1987, Alhossaini & Pitcher 1988, Currens *et al.* 1988, Eckmann & Pusch 1989), Protex (e.g. Bailey 1982, Laroche *et al.* 1982, Moksness *et al.* 1987), Euparal (Struhsaker & Uchiyama 1976, Fowler 1989, Karakiri *et al.* 1989, Uchida *et al.* 1989, Berghahn & Karakiri 1990), Spurr (Haake *et al.* 1982, Dean *et al.* 1983, Beckman & Dean 1984, McMichael & Peters 1989), crystal bond (Bradford & Geen 1987, Post & Prankevicius 1987), polyester resin (Brothers *et al.* 1976, Keener *et al.* 1988, Heath 1989, Thresher *et al.* 1989), Eukitt (Butler 1989), instant glue (Campana & Neilson 1982, Campana *et al.* 1987, Nyman & Conover 1988), Krazy glue (Campana & Hurley 1989), thermoplastic cement (Davis *et al.* 1985, Vero *et al.* 1986, Graham & Orth 1987, Mosegaard *et al.* 1988), Permount (Lough *et al.* 1982, Bolz & Lough 1983, Lagardere 1989, Stevenson, *et al.* 1989), Epon (Pannella 1971, Penney & Evans 1985, Eckmann & Rey 1987), Canada balsam (Taubert & Coble 1977, Marshall & Parker 1982, Essig & Cole 1986, Vero *et al.* 1986), DPX (Fagade 1980), Flo-Texx (Fives *et al.* 1986, Warlen 1988), fingernail polish medium (Haldorson *et al.* 1989, Hovenkamp 1989), shellac (Karakiri & Hammer 1989, Karakiri *et al.* 1989, 1991, Berghahn & Karakiri 1990), Gurr's neutral mounting medium (Jenkins 1987), histological mounting medium (Kendall *et al.* 1987, Yoklavich & Boehlert

1987), immersion oil (McFarland *et al.* 1985, Messieh *et al.* 1987, Thorrold 1989), cyanoacrylate glue (McGurk 1984), glycerine (Mugiya 1987, Ntiba & Jaccarini 1988), acrylic adhesive (Simoneaux & Warlen 1987) and epoxide resin (Walline 1985, Wilson 1988). The choice of the mounting medium depends on several criteria: (1) they must be hard and flexible to maintain the position of the otolith in the media during grinding and polishing, (2) they may not cause the otolith to crack during grinding and polishing, (3) they must be transparent to allow easy viewing of otolith (Secor *et al.* 1992).

For light microscopy examination, preparation of otoliths is designed to enhance light transmission differences between incremental and discontinuous zones (Campana & Neilson 1985). Good sectioning and polishing are prerequisites for otolith microstructure examination: their purpose is to remove excess mounting media (Secor *et al.* 1991, 1992) and to enhance the clarity of the growth increments (Campana & Neilson 1985). Clearing compounds such as immersion oil, Euparal, Canada Balsam, glycerine and xylene are used to increase the transparency of the mounting media and the light transmission through the otolith, as well as to enhance the distinction between the incremental and discontinuous zones (Isely & Noble 1987, Mugiya 1987, Tsukamoto & Kajihara 1987, Heath 1989, Karakiri *et al.* 1989, Mugiya & Uchimura 1989). Otoliths can be sectioned and polished manually on wet sandpaper (Castonguay 1987, Secor *et al.* 1991, 1992), with rotating polishing wheels (Morales-Nin 1987, Yoklavich & Boehlert 1987) and with converted record players (Karakiri & von Westernhagen 1988). Sectioning and polishing must be done with caution because the process is irreversible and leads to ruined preparations when carried out past the otolith midplane (Campana & Neilson 1985).

For scanning electron microscopy examination, preparation of the otolith relies on the differences in chemical composition of the incremental and discontinuous zones: differential etching produces contoured otolith surfaces (Campana & Neilson 1985, Secor *et al.* 1991). Weak acids, such as 1-5% HCl, or calcium chelators such as ethylene-dinitrilo tetra-acetic acid (EDTA) are to be preferred as etching agents (Secor *et al.* 1991). Etching time must be regulated in such a way that otoliths are not over-etched and that no microstructure features are lost.

The advantages and disadvantages of the light microscopy and scanning electron microscopy techniques are linked to the size, morphology, and features of the otolith to be examined (Campana & Neilson 1985). Light microscopy is a fast and flexible means of observation: otolith preparation and equipment needs are minimal, and focal lengths can be adjusted to follow the contours of nonplanar increments. Limitations include the need for otoliths translucent enough to allow light transmission, potential confusion of increments with visual artifacts, and a resolution limit that may not be sufficient to differentiate increments of narrow width. Using scanning electron microscopy, the resolution exceeds that needed for observation of the most narrow increments, and visual artifacts do not occur. Counts and measurements can be made from photographs which reduces observational errors. Limitations of the SEM technique include the need for expensive specialised equipment and more elaborate sample preparation. Grinding and polishing of otoliths are mandatory procedures and they must be carried out precisely to the midplane to ensure adequate etching. SEM is not appropriate for the observation of otoliths with nonplanar increments. Age-dependent etching times can complicate preparations of extended growth sequences.

1.3.4. External and internal morphology of the otoliths

The external morphology of the otoliths of teleost fish is complex and species-specific (Nolf 1985). Growth increments consist of incremental and discontinuous zones (Pannella 1974, Blacker 1975, Mugiya *et al.* 1981, Campana & Neilson 1985) which occur through differential deposition of calcium carbonate and protein over a 24 hours period (Mugiya *et al.* 1981, Campana & Neilson 1985). Incremental zones are well calcified with aggregates of needle-like crystals of calcium carbonate, mainly in the aragonite form (Mugiya *et al.* 1981), and, occasionally, vaterite configurations (Campana 1983b) which are oriented with the long axis perpendicular to the otolith margin. Discontinuous zones are mainly composed of the protein otolin (Dunkelberger *et al.* 1980, Ross & Pote 1984). Mugiya *et al.* (1981) reported that the calcium uptake by goldfish otoliths slowed down or stopped at sunrise and resumed after 3 hours. During this period, calcification stopped (possibly as a result of reduced mascular cell secretion) and the discontinuous zone was deposited. Interaction between the incremental zones and the discontinuous zones determines the shape of the otoliths (Gauldie & Nelson 1990).

Growth increment formation is linked to an endocrine driven, endogenous circadian rhythm (Simkiss 1974, Campana & Neilson 1985). Endocrine secretion shows a circadian periodicity (Simpson 1978, Menaker & Binkley 1981) which controls most physiological processes, including skeletal deposition (Simpson 1978). Endogenous circadian rhythm in fishes include free-run capability (continuation of a circadian rhythm in the absence of periodic stimuli) and entrainment to daily environmental cues ('zeitgeber') such as the photoperiod, temperature and feeding periodicity (Campana & Neilson 1985). Exogenous factors such as food availability (Campana 1983a, Neilson & Geen 1985, Berghahn & Karakiri 1990, Jenkins *et al.* 1993), changes in temperature (Brothers 1978, Barkman & Bengtson 1987, Berghahn & Karakiri 1990, Jenkins *et al.* 1993), stress (Campana 1983b, Berghahn 1987), temporary hypoxia (Berghahn 1989, Berghahn & Karakiri 1990), tidal rhythms (Campana 1984, Eckmann & Rey 1987) and light regime (Marshall & Parker 1982) influence both fish growth and otolith growth. The width of the daily growth rings is in all cases proportional to the daily growth of the fish (Campana & Neilson 1985).

Influence of temperature, photoperiod and food on the formation of daily growth increments is well documented in several studies, but the results are conflicting (e.g. Taubert & Coble 1977, Tanaka *et al.* 1981, Campana & Neilson 1982, Radtke & Dean 1982, Mugiya 1987, Berghahn 1989, Karakiri & von Westernhagen 1989). The growth increments of the otoliths of *Tilapia mossambica* were formed daily under a 24 hours light-dark cycle, but not in fish exposed to constant light (Taubert & Coble 1977). The growth increment counts on the otolith of embryonic and larval *Salmo gairdneri* were highly correlated with light-dark cycle and increments were also found to be formed under constant light and constant darkness (Mugiya 1987). Reversal of the light-dark cycle reversed the order of formation of the incremental and discontinuous zones in the otoliths of juvenile *Tilapia nilotica* (Tanaka *et al.* 1981). Campana & Neilson (1982) stated that the production of daily growth increments in *Platichthys stellatus* was unaffected by the photoperiod. The photoperiod has been reported to have only limited effects on growth increment width (Karakiri & von Westernhagen 1989). The formation of hyaline zones is also enhanced by high water temperature and starvation (Berghahn 1989).

Daily growth increment formation is not inhibited under constant temperature, provided it is high enough for growth (Taubert & Coble 1977, Campana & Neilson 1982, Radtke & Dean 1982, Mugiya 1987): within a wide range of environmental temperatures, increment counts do not vary, except at very low temperatures (Taubert & Coble 1977, Campana & Neilson 1982, Mugiya 1987). Still, Karakiri & von Westerhagen (1989) observed, under laboratory conditions, that wider growth increments were deposited in the otoliths of O-group plaice *Pleuronectes platessa* at higher temperature (10°C) than at lower temperature (5°C). Interaction of temperature with ration level affected the otolith increment width of *Oncorhynchus tshawytscha* (Neilson & Geen 1982, 1985). However, exposure of young plaice to extremely high temperatures (27°C) for a short time resulted in thinner increment widths than those observed in starved fishes or in fishes exposed to UV-B radiation (Berghahn & Karakiri 1990).

Shift of feeding time doesn't affect the time of formation of incremental and discontinuous zones (Tanaka *et al.* 1981). Still, feeding frequency has been shown to be correlated to the frequency of growth increment formation (Neilson & Geen 1982, 1985). Multiple feeding periods induce the formation of new growth increments in the otolith (Campana & Neilson 1985). Also, Campana (1983a) reported that feeding frequency affected increment appearance and the incidence of subdaily increments in *Salmo gairdneri*, but not in *Platichthys stellatus*. He assumed that this difference in effect was probably due to the higher metabolic rate of *Salmo gairdneri*.

Checks are records of periods of heavy perturbation or stress (Campana & Neilson 1985) which can occur periodically and non-periodically (Pannella 1971, 1980, Campana 1984). Periodic checks are linked to the lunar cycle (Pannella 1980, Campana 1984), while the non-periodic checks are associated with such events as hatching, sexual maturity or environmental stress (Pannella 1980, Campana 1983b, Berghahn & Karakiri 1990). Campana (1983a) reported that the sagittae of stressed young coho salmon *Oncorhynchus kisutch* showed checks and reduced calcium deposition, although simultaneous protein deposition was not monitored. He hypothesised that check prominence and the reduction in the otolith calcium:protein ratio are proportional to the level of stress. The presence of nonperiodic checks can lead to a misleading interpretation of the growth sequence. On the other hand, periodic checks can be used to determine the age and size at which important life history transitions occur, and to validate daily growth increments if the checks are formed through large-scale stress phenomena (Campana & Neilson 1985, Geffen 1992). Periodic checks (lunar bands) in the otoliths of marine fish have been reported in several studies (e.g. Pannella 1971, 1974, 1980, Brothers *et al.* 1976, Rosenberg 1982, Campana 1984).

1.3.5. Validation

Growth increments in the otoliths are useful in age and growth studies if they are validated to be deposited daily (Geffen 1992). The validation process requires good procedures: capture-recapture experiments with fish of known age released to the wild or experiments with fish maintained in the laboratory are to be preferred for validation studies.

One of the disadvantages of laboratory conditions is that the natural behaviour of fish, and thus its growth and the optical appearance of the growth increments, is affected (Campana & Neilson 1985, Rice *et al.* 1985, Hovenkamp 1990). However, manipulation of environmental variables such as light, temperature, feeding, etc. can reflect the sensitivity of otolith growth to environmental change (Karakiri & von Westernhagen 1989, Berghahn & Karakiri 1990).

Several techniques are used for validating the growth increments in otoliths. The deposition rates of the growth increments can be validated by: (1) marking the otoliths with oxytetracycline, tetracycline, acetazolimide etc. (Mugiya & Muramatsu 1982, McMichael & Peters 1989, Molony & Choat 1990); (2) marking the otoliths with stress, e.g. capture stress (Boehlert & Yoklavich 1985), handling stress (Campana 1983b) or temperature stress (Berghahn & Karakiri 1990); (3) monitoring known age larvae (Bergstad 1984, Rosa & Ré 1985, Leak & Houde 1987, Lagardere 1989); (4) statistical inferences (Post & Prankevicius 1987, De Vries *et al.* 1990, Sogard 1991, Ré & Narciso 1994, Arellano *et al.* submitted c); (5) inferences from older fish (Taubert & Tranquilli 1982, Radtke & Targett 1984, Hill & Radtke 1988); (6) marginal increment analysis (Tanaka *et al.* 1981, Ré *et al.* 1985, Sepúlveda 1994); (7) timing of the initial increment formation (Dabrowski & Tsukamoto 1986, Maillet & Chekley 1990, Karakiri *et al.* 1991); (8) relating otolith growth to somatic growth and manipulation of ring deposition (Moksness & Wespestad 1989, Wright *et al.* 1990) and (9) assessing the physiological and biochemical mechanisms of increment deposition (Mugiya & Oka 1991).

1.3.6. Use and interpretation of otolith microstructure data

Validated growth increment counts can be used to infer age (Geffen 1992). Increment widths, the corresponding otolith radii, and the fish growth-otolith growth relationship are the basic data needed for the different back-calculation models used to establish age-length keys (Francis 1990, Arellano *et al.* submitted d). These data can be used to estimate the daily growth rate (Jenkins *et al.* 1993, Panfili *et al.* 1994) or the growth parameters of the von Bertalanffy growth model (Hood *et al.* 1994), and to infer the age and length of fish at the time of spawning, settlement, hatching and other transitory events in its life (Victor 1982, 1986, Campana & Neilson 1985, Karakiri *et al.* 1991). Non periodic and periodic checks can be used to infer periods of perturbation or stress (Campana & Neilson 1985).

The slope of the fish growth-otolith growth relationship is used to infer the allometric growth of the otoliths. The external morphology of the otoliths can be used for identification of species and stocks. The ratio between the areas of the sulcus acusticus and the sagitta can be used for the determination of the sound frequency sensitivity and auditory threshold of teleosts (Gauldie & Nelson 1990, Lombarte 1992, Arellano *et al.* 1995).

The ratio of $^{210}Pb/^{226}Ra$ in the otoliths has been used to estimate age (Bennet *et al.* 1982, Campana & Zwanenburg 1990, Fenton *et al.* 1990, 1991), and the concentrations of Sr and Ca in fish otoliths can be indicators of the ambient temperature to which fish have been exposed (Gauldie *et al.* 1992, 1994, 1995, Gunn *et al.* 1992, Sadovy & Severin 1992). Additionally, otolith elemental composition has been reported to be a potential measure of pollution (Papadopoulu *et al.* 1978, 1980) and as an indicator of stock identity (Calaprice 1971, Edmonds *et al.* 1989). To date, the application of otolith microstructure examination and analysis has focused mainly on the determination of age and growth and factors affecting them. One of the promising fields to be given emphasis is that of otolith elemental analysis.

CHAPTER 2.

Changes of length, weight and otolith appearance of *Pomatoschistus minutus* (Pallas) and *P. lozanoi* (de Buen) (Pisces, Gobiidae) after formaldehyde and ethanol fixation.

Abstract

Two methods of preservation were used to determine shrinkage and weight loss for *Pomatoschistus minutus* and *P. lozanoi*. Samples were either stored immediately in 96% ethanol (direct method) or were transferred to 96% ethanol after 24 hours preservation in 7% formaldehyde (indirect method). An insignificant reduction in length and a significant loss in weight were found for both species. The patterns of shrinkage and weight loss with the two preservation methods only differed for the first 24 hours of storage. After an additional day of storage in 96% ethanol, the patterns became analogous. The effects of the direct method on shrinkage and weight loss were more eminent than those of the indirect method during the first week of storage. The effects were found to be smaller in *P. lozanoi* than in *P. minutus* of the same size. For both species, conversion factors from preserved length/weight (PL and PW) to fresh length/weight (FL and FW) were established by simple linear regression. The estimated conversion factors for *P. minutus* are FL = 2.713 + 0.997 * PL and FW = 0.633 + 2.30 * PW. For *P. lozanoi*, FL = 0.589 + 1.024 * PL and FW = 0.185 + 1.302 * PW. The otoliths of neither goby species showed signs of deterioration or decalcification after one year of storage with both methods of preservation.

2.1. Introduction

The relevance of accurate length and weight measurements are emphasized in many studies on age and growth (e.g. Bolz & Lough 1983, Crisp 1984, Pauly 1984, Sparre *et al.* 1989, Woodbury & Ralston 1991), population dynamics and stock assessment (e.g. Pauly 1984, Arellano 1988, 1989, Sparre *et al.* 1989, Mees *et al.* 1994) and ecological studies (e.g. Arellano 1991, Hamerlynck *et al.* 1992, 1993b, Hostens & Hamerlynck 1994). They are the basic data for the estimation of biomass, growth and secondary production, and thus for the quantification of energy flows in aquatic ecosystems (Crisp 1984, Pauly 1984, Giguere *et al.* 1989, Hostens 1989, Sparre *et al.* 1989).

Length based methods are frequently used for the assessment and management of fish stocks. The basic length or weight frequency data are used to estimate age structure, growth parameters and mortality in populations of fish (Pauly 1984, Sparre *et al.* 1989) and invertebrates (Pauly 1984, Arellano 1988, 1989, Mees *et al.* 1994). Length-frequency data are usually preferred over age structured data because they are easy to collect, which contrasts sharply with the difficulties involved in the ageing of aquatic species.

It is routine practice to preserve and store fish samples before processing. The samples are either preserved chemically in formaldehyde or ethanol (Mckern *et al.* 1974, Messieh 1975, Methot & Kramer 1979, Wilson & Larkin 1980, Radtke *et al.* 1988) or by deep freezing or icing (Lockwood & Daly 1975, Bailey *et al.* 1977, Winters 1981, Radtke *et al.* 1988). The choice between these methods depends on the specific aims of the research. In age and growth research using otoliths, the fish samples are often preserved indirectly: they are stored in formaldehyde for a short period of time (usually less than 24 hours) after which they are transferred to ethanol for longer storage (Mckern *et al.* 1974, Essig & Cole 1986). Samples preserved in formaldehyde for a longer time will show decalcification of the otoliths (Butler 1992). Alternatively, the samples are preserved directly in ethanol (Messieh 1975, Methot & Kramer 1979, Wilson & Larkin 1980, Hettler 1984, Victor 1986, Comyns *et al.* 1989, Kramer 1990, Laidig *et al.* 1991, Szedlmayer *et al.* 1991, Crabtree *et al.* 1992) or frozen (Deegan & Thompson 1987, Kramer 1990, Woodbury & Ralston 1991). The use of low ethanol concentrations (less than 70%) will also cause deterioration and decalcification of the otoliths (Methot & Kramer 1979). Higher ethanol concentrations combined with small sample volumes will buffer the pH of the preservative, which is otherwise lowered due to water extraction during storage (Berghahn, pers. comm.). Thus, samples for otolith researches are usually preserved by either indirect or direct methods of preservation.

It has been demonstrated before that chemically preserved animals loose weight and shrink (Amosov 1960, Parker 1963, Fudge 1968, Hopkins 1968, Halliday & Roscoe 1969, Omori 1970, Howmiller 1972, Lockwood 1973, Lockwood & Daly 1975, Durbin & Durbin 1978, Landry 1978, Omori 1978, Champalbert & Kerambrun 1979, Pace & Orcutt 1981, Williams & Robins 1982, Crisp 1984, Hay 1984, Bottger & Schnack 1986). In general, reduction of length has been shown to be insignificant, while weight loss often exceeds 20% of the initial weight (Lockwood 1973). These changes in length and weight can result in serious underestimates of age and growth (Butler 1992). To circumvent this bias, preserved lengths and weights should be back-transformed to fresh lengths and weights prior to analysis. Species-specific regressions should be derived from several samples measured fresh and after fixation with the preservatives used (Winters 1981).

The aim of the present study is to investigate the combined effects of 7% formaldehyde and 96% ethanol preservation on length, weight and otolith appearance for the gobies *Pomatoschistus minutus* and *P. lozanoi*. Regressions for the preserved length and weight measurements to fresh length and weight are presented for both species.

2.2. Materials and methods

2.2.1. Data collection and shrinkage experiment

The gobies *Pomatoschistus minutus* and *P. lozanoi* were collected monthly in the ebb-tidal delta of the Grevelingen area (South-west Netherlands) from September 1992 to February 1993 by means of a 3 m beam trawl with a codend mesh size of 10 mm. On each occasion, individuals belonging to representative length classes were selected and transported fresh to the laboratory for shrinkage experiments.

The fresh length and weight of each fish was measured to the nearest mm (standard length (SL) from the tip of the lower jaw to the end of the notochord) and weighed to the nearest mg. The samples were then equally divided in two parts which were preserved with two different methods. The first subsample was preserved in 7 % neutral formaldehyde for 24 hours and then transferred to 96% ethanol for long storage (indirect method). The second subsample was immediately preserved in 96% ethanol (direct method). The fish samples were washed with running tap water and air dried for 5 to 10 minutes before they were preserved in 96% ethanol.

The SL and weights of the fish were measured after 1 day and after 1, 2, 3, 4, 6, 9 and 26 weeks of storage. In order to compare the effects of the 7% formaldehyde and the 96% ethanol preservation, the fish preserved with the indirect method were also measured after the second day (i.e. after their first day in ethanol).

The fish samples were always air dried at room temperature prior to length and weight measurements. They were considered to be air dried when weight in the balance was stable.

At the end of the shrinkage experiment, the species and sex of the gobies were identified with the keys of Guitel (1892) and Hamerlynck (1990).

2.2.2. Observations on otolith appearance

The three pairs of otoliths, the sagittae, the lapilli and the asterisci, were extracted after one year of storage. Otoliths with milky, etched and/or discoloured appearances were considered to show signs of deterioration and decalcification (Butler 1992, Berghahn, pers. comm.).

2.2.3. Data analysis

Estimation of shrinkage and weight loss

The percentage shrinkage in length (%L) and the percentage weight loss (%W) were calculated with the following equations:

$$\%L = \frac{FL - PL}{FL} \times 100$$

$$\%W = \frac{FW - PW}{FW} \times 100$$

where FL and PL are the fresh and preserved length and FW and PW are the fresh and preserved weight of the fish.

Statistical analysis

The homogeneity of variances was tested with a Bartlett's test to determine whether parametric statistics could be applied. If not, the data were analyzed with the non-parametric Kruskal-Wallis test (Sokal & Rohlf 1981).

To asses the possible differential effect of the preservatives on male and female *Pomatoschistus minutus* and *P. lozanoi* and of different methods of preservation, the fresh mean SL and the fresh mean weights were statistically tested with analysis of variance (ANOVA) at the $p<0.05$ level. Results were only compared when the fresh mean SL or the fresh mean weight between sexes and between methods of preservation were not significantly different.

To establish the conversion factors for *Pomatoschistus minutus* and *P. lozanoi*, linear regression equations of the preserved lengths/weights vs. the fresh lengths/weights were estimated. To determine whether the intercepts passed through zero and whether the slopes equalled one, a t-test (Walpole 1982) was applied at the $p<0.01$ level.

2.3. Results

A total of 267 *Pomatoschistus minutus* and 147 *P. lozanoi* were used for shrinkage experiments. At the end of the experiment, 69 males (size range from 36 to 75 mm SL) and 28 females (size range from 30 to 74 mm SL) from the indirect method and 106 males (size range from 37 to 78 mm SL) and 64 females (size range from 31 to 71) from the direct method were identified for *P. minutus*. For *P. lozanoi*, 47 males (size range from 25 to 50 mm SL) and 39 females (size range from 24 to 56 mm SL) from the indirect method and 36 males (size range from 29 to 45 mm SL) and 25 females (size range from 33 to 40 mm SL) from the direct method were identified.

2.3.1. *Pomatoschistus minutus*

Shrinkage

For both sexes, there were no significant differences in mean fresh SL between the subsamples used for the 2 preservation methods (Table 2.1). In the subsample used for the indirect method, the fresh SL of males and females were not significantly different, while in the subsample used for the direct method they were.

The shrinkage patterns in both preservation methods differed during the first 24 hours of storage (Figures 2.1a, b, e & f). Thereafter, a similar shrinkage pattern was observed in both sexes and in both methods of preservation. The greatest reduction in length occurred after one day of preservation in 96% ethanol. After 24 hours of storage with the indirect method (the one day in formalin) some of the fish showed an increase in length and no overall shrinkage was observed in the sample. In the direct method, shrinkage was more eminent and it stabilised earlier.

Table 2.1. Analysis of variance on the fresh mean standard lengths and the fresh mean weights of *Pomatoschistus minutus* (PMF: female; PMM: male) and *P. lozanoi* (PLF: female; PLM: male) used in the shrinkage experiment. ID = Indirect method; DI = Direct method. * Kruskal-Wallis Test.

	Fresh length			**Fresh weight**		
	ID	DI	$p<0.05$	ID	DI	$p<0.05$
PMF	49.63	45.28	0.129	1.764	1.365	0.239
PMM	54.46	56.55	0.444	2.607	2.717	0.802
$p<0.05$	0.115	0.001	-	0.066	0.000*	-
PLF	37.21	35.40	0.171	0.557	0.479	0.364
PLM	38.70	37.69	0.118	0.647	0.596	0.163
$p<0.05$	0.428	0.118	-	0.293	0.163	

Analysis of variance (Table 2.2) showed that the reduction in length in both sexes and in both methods of preservation (6% for both females and males in the indirect method, 5% for both females and males in the direct method) was insignificant after 26 weeks of preservation. Thus, the data from both sexes were pooled (indirect method). To back-calculate the fresh length, a linear regression line was fitted to a plot of preserved length (PL) against fresh length (FL). The regression equation was $FL = 2.713 + 0.997 * PL$, $r^2 = 0.99$, $N = 97$. The slope did not differ significantly from one ($t = 0.365$; $p>0.05$); the intercept was significantly different from zero ($t = 6.210$; $p<0.01$).

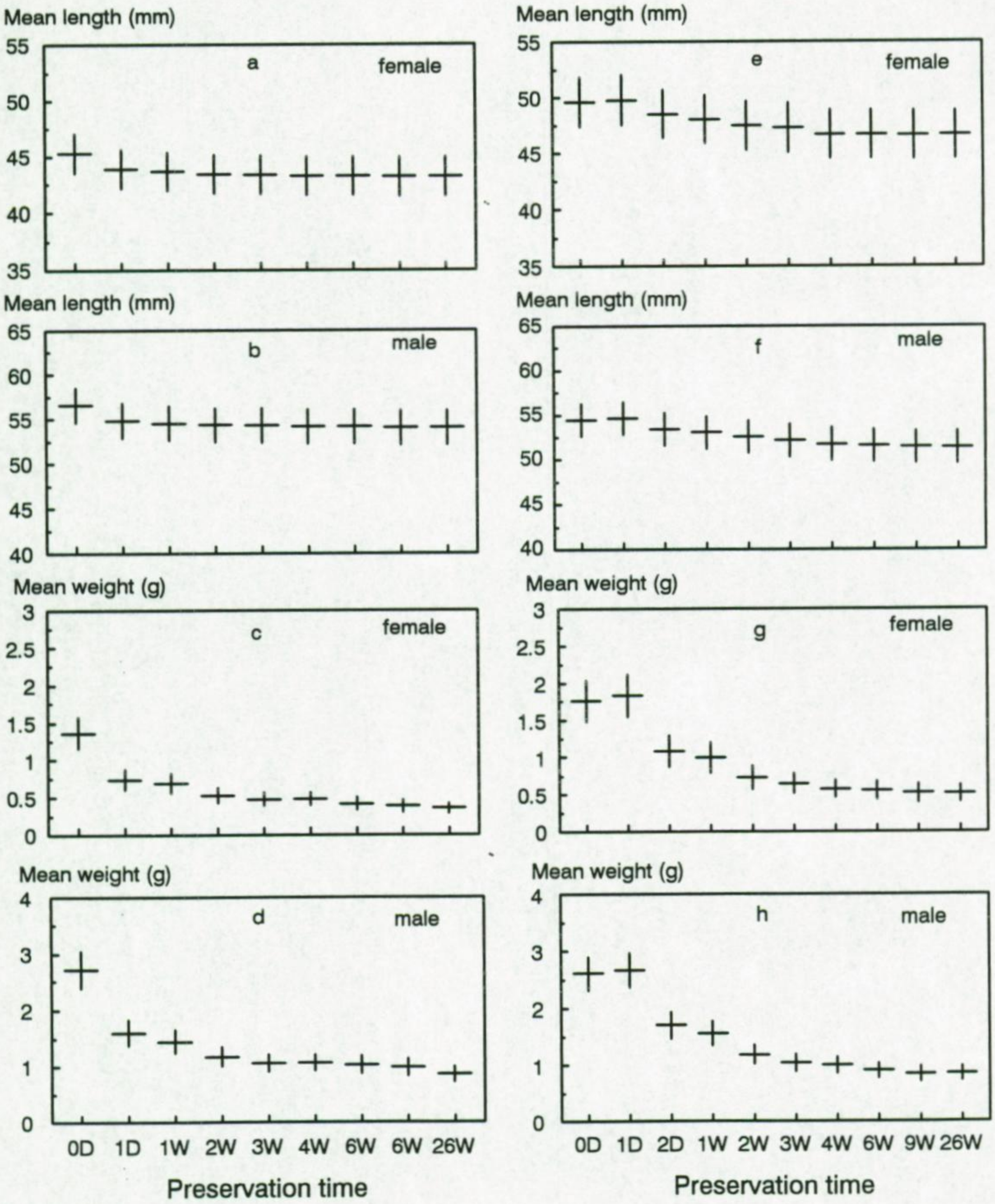


Figure 2.1. Shrinkage in length (a,b,e & f) and weight loss (c,d,g & h) of female (a,c,e & g) and male (b,f,d & h) *Pomatoschistus minutus* in the direct (a-d) and indirect (e-g) methods of preservation. Vertical bars represent the standard errors.

Table 2.2. Analysis of variance on the effect of the two methods of preservation on the lengths of *Pomatoschistus minutus* (PMF: female; PMM: male) and *P. lozanoi* (PLF: female; PLM: male). FL = fresh length; PL = preserved length.

	Indirect			**Direct**		
	FL	PL	$p<0.05$	FL	PL	$p<0.05$
PMF	49.63	46.68	0.348	45.28	43.20	0.404
PMM	54.46	51.38	0.236	56.55	54.00	0.358
$p<0.05$	-	0.12	-	-	-	-
PLF	37.21	35.90	0.652	35.40	33.40	0.353
PLM	38.70	37.13	0.982	37.69	35.56	0.156
$p<0.05$	-	0.549	-	-	0.179	-

Weight loss

The fresh mean weights of both sexes were not significantly different in the subsample used for the indirect method, while in the subsample used for the direct method the difference was significant (Table 2.1).

After one day of storage in 96% ethanol, the patterns of weight loss were similar for both sexes in both methods of preservation (Figures 2.1c, d, g & h). The effects of the two methods of preservation only differed during the first 24 hours of storage: in the indirect method the samples gained weight, while in the direct method they lost weight. Most of the weight loss occurred after one day storage.

In both methods of preservation, the loss in weight of both sexes (71% for females and 67% for males in the indirect method, 74% for females and 68% for males in the direct method) was highly significant after 26 weeks of storage (Table 2.3). The data from both sexes were subsequently pooled (indirect method).

A linear regression line was fitted to a plot of preserved weight (PW) against fresh weight (FW). The regression equation was $FW = 0.633 + 2.30 * PW$, $r^2 = 0.97$, $N = 97$. The slope differed significantly from one ($t = 30.24$; $p<0.01$); the intercept was significantly different from zero ($t = 11.33$; $p<0.01$).

Table 2.3. Analysis of variance on the effect of the two methods of preservation on the weights (grams) of *Pomatoschistus minutus* (PMF: female; PMM: male) and *P. lozanoi* (PLF: female; PLM: male). FW = fresh weight; PW = preserved weight. * Kruskal-Wallis Test.

	Indirect			**Direct**		
	FW	PW	$p<0.05$	FW	PW	$p<0.05$
PMF	1.764	0.514	0.000	1.365	0.352	0.000*
PMM	2.607	0.861	0.000*	2.717	0.867	0.000*
$p<0.05$	-	0.188*	-	-	-	-
PLF	0.557	0.339	0.008	0.479	0.112	0.001
PLM	0.647	0.336	0.000*	0.596	0.146	0.000*
$p<0.05$	-	0.951	-	-	0.193	-

2.3.2. *Pomatoschistus lozanoi*

Shrinkage

The fresh mean SL of female and male *Pomatoschistus lozanoi* were not significantly different in both methods of preservation (Table 2.1). Also, the fresh mean SL were not significantly different between sexes.

The shrinkage patterns of both sexes were analogous to the shrinkage patterns of *Pomatoschistus minutus* (Figures 2.2a, b, e & f) in both preservation methods.

Analysis of variance (Table 2.2) showed that the reduction in length was insignificant between both sexes (4% for both females and males in the indirect method, 6% for both females and males in the direct method) after 26 weeks of storage. Thus, the data from both sexes in both methods could be pooled. To back-calculate the fresh length, a linear regression line was fitted to a plot of preserved length (PL) against fresh length (FL). The regression equation was $FL = 0.589 + 1.024 * PL$, $r^2 = 0.98$, $N = 86$. The slope did not differ significantly from one ($t = 1.83$; $p>0.05$); the intercept did not differ significantly from zero ($t = 1.22$; $p>0.05$).

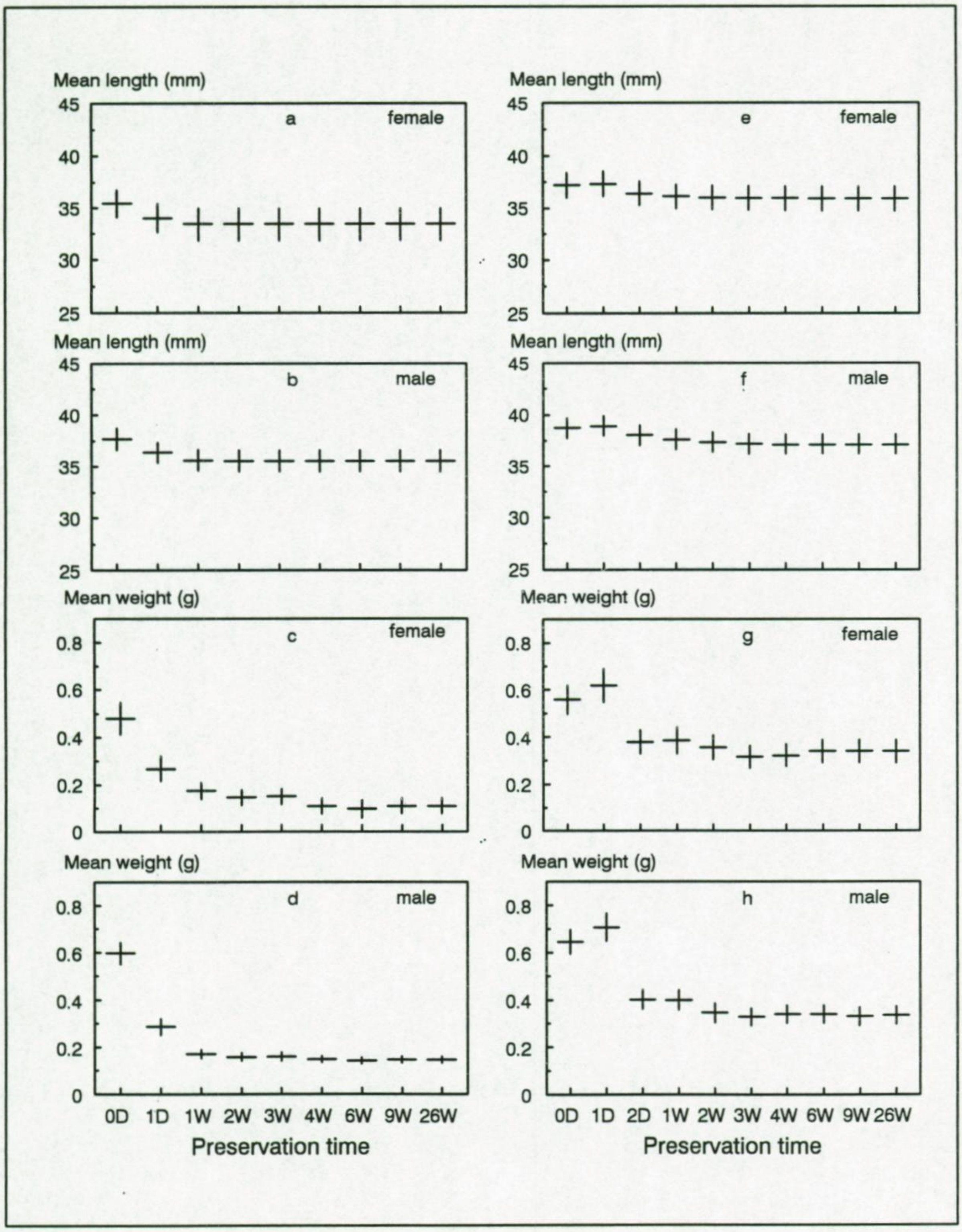


Figure 2.2. Shrinkage in length (a,b,e & f) and weight loss (c,d,g & h) of female (a,c,e & g) and male (b,f,d & h) *Pomatoschistus lozanoi* in the direct (a-d) and indirect (e-g) methods of preservation. Vertical bars represent the standard errors.

Weight loss

The fresh mean weights of male and female *Pomatoschistus lozanoi* were not significantly different in both preservation methods (Table 2.1).

The patterns of weight loss were analogous to the patterns observed for *Pomatoschistus minutus* (Figures 2.2c, d, g & h).

After 26 weeks of preservation, the weight loss of both sexes differed significantly between preservation methods (39% for females and 48% for males in the indirect method, 77% for females and 76% for males in the direct method) (Table 2.3). The weight loss between sexes was not significantly different for both preservation methods. The data from both sexes were subsequently pooled (indirect method).

To derive the fresh weight, a linear regression line was fitted to a plot of preserved weight (PW) against fresh weight (FW). The regression equation was $FW = 0.185 + 1.302 * PW$, $r^2 = 0.98$, $N = 64$. The slope differed significantly from one ($t = 13.96$; $p<0.01$); the intercept was significantly different from zero ($t = 19.05$; $p<0.01$).

2.3.3. Observations on otolith appearance

After more than one year of preservation by the direct and indirect method, the appearance of the otoliths of *Pomatoschistus minutus* and *P. lozanoi* showed no signs of deterioration and decalcification.

2.4. Discussion and conclusions

Marine species begin to shrink after death because osmoregulation stops. The degree of shrinkage varies with the time between death and preservation (Butler 1992). The present shrinkage experiment only dealt with the effect of the chemicals used as preservatives. The shrinkage and weight loss occurring between death and preservation were not taken into account. It is assumed that shrinkage after death up to the time of preservation was minimal or at least equal for the different subsamples.

The experiment showed that the patterns of shrinkage and weight loss in both methods of preservation were analogous after one day of preservation in ethanol. They only differed in the first 24 hours of storage. The differential effects of the two methods of preservation for the first 24 hours of storage are due to differences in the chemical properties of the two preservatives used. The elongation of length and gain in weight of the fish in formaldehyde are due to the absorption of water. The faster reduction in length in ethanol is due to dehydration.

The final effect of the preservatives on length in *Pomatoschistus lozanoi* was not significant. This was indicated by the intercept which passed through zero and the 1:1 ratio of preserved length to fresh length, which was still maintained after the experiment. For *P. minutus*, though the 1:1 ratio of preserved length to fresh length was maintained, the intercept was different from zero. This means that the conversion factor of +2.713 mm is significant. This conversion factor is much higher than the conversion factor for 0-group *Pleuronectes platessa* (0.85 mm) estimated by Lockwood & Daly (1975). One possible explanation, besides the fact that both species have a different morphology, is the difference in preservatives concentration and method used. Lockwood & Daly (1975) used 4 % neutral formaldehyde.

Weight losses are clearly very significant in both sexes of both species. These were manifested in the intercepts and slopes which were highly significant from zero and one, respectively. The weight is probably lost as water and fat. Therefore, it is assumed that *Pomatoschistus minutus* had a higher water and fat content than *P. lozanoi*. Still, since no chemical analysis was done, the nature and causes of the reduction in length and weight cannot be explained with the results of the experiments. Differences in the reduction of length and weight between both species may also be due to body size differences. It is known that individuals of *P. minutus* are more robust than *P. lozanoi* of the same length (Hamerlynck 1990).

In conclusion, preserved samples cannot be compared with fresh samples unless conversion factors for length/weight are established. The only exception is the length conversion for *Pomatoschistus lozanoi*.

Proper techniques in the preservation of otoliths are very important in microstructure examination. Decalcification of otoliths due to inappropriate preservation techniques is an important source of error (Neilson 1992), which may lead to the underestimation of fish age. Decalcified otoliths will cause a loss of some very important ecological information concerning the target species. The two preservation methods did not have deteriorating effects on otolith appearance after the period of the experiment and are recommended for further use.

CHAPTER 3.

Morphology of the otoliths of *Pomatoschistus minutus* and *P. lozanoi* (Pisces, Gobiidae).

Abstract

The external morphology of the otoliths of two sympatric goby species, *Pomatoschistus minutus* (Pallas) and *P. lozanoi* (de Buen), was studied. The orientation of the otoliths in the skull was similar in both species and otolith shapes and weights did not differ significantly between sexes. Sagittae and lapilli of *P. minutus* were larger and heavier than those of *P. lozanoi*, while the size and weight of the asterisci of both species were not significantly different. The sagittae of *P. minutus* were more corrugated and crenulated than those of *P. lozanoi* after sexual maturity was reached, whereas the lapilli and asterisci of both species never showed corrugations and crenulations. Allometry of the left and right otoliths of both species was isometric, suggesting that they function for balancing and equilibrium. The somatic growth rates of both species influenced the relationship between fish length and several otolith characters. Juveniles were characterised by faster otolith growth rates, relative to fish length, than adults. It is concluded that the external morphology of the sagittae can be used as an indicator of fish maturity and that sagitta characters can be used for species identification.

3.1. Introduction

Otoliths are crystalline $CaCO_3$ structures in the endolymphatic sac of teleost fish (Secor & Dean 1989, Gauldie & Nelson 1990). They function for balancing and as sound transducers (Parker 1908, Schuijf 1981, Fay 1980, Arellano *et al.* 1995).

Since the discovery of growth increments in the otoliths (Pannella 1971), their use in age and growth studies has increased exponentially (Jones 1992). The occurrence of internal structures, such as translucent and opaque zones, checks and daily growth increments are widespread phenomena in the otoliths of fish living in freshwater and marine habitats from temperate to tropical areas (Campana & Neilson 1985, Gauldie & Nelson 1990). Otolith growth increments are very precise indicators of fish age (Reznick *et al.* 1989, Pannella 1971, 1974, Campana & Neilson 1985) and they have been shown to contain valuable information on the past life of fish. Otoliths have been used to study somatic growth rates (Campana & Neilson 1985, Secor & Dean 1989, Wright *et al.* 1990, Mugiya & Tanaka 1992), seasonal, fortnightly, weekly, and daily growth checks (Pannella 1971, 1974, Taubert & Coble 1977, Campana 1984), past changes in environmental conditions (Taubert & Coble 1977, Campana & Neilson 1982, Radtke & Dean 1982, Radtke *et al.* 1987, 1988, Berghahn 1989, Karakiri & von Westernhagen 1989, Berghahn & Karakiri 1990), feeding rhythms and changes in the ration level (Struhsaker & Uchiyama 1976, Pannella 1980, Tanaka *et al.* 1981, Campana 1983a, Neilson & Geen 1982, 1985, Karakiri & Hammer 1989, Karakiri *et al.* 1989, Berghahn & Karakiri 1990) and planktonic larval duration and settlement (Victor 1986, 1991, Karakiri & Hammer 1989, Karakiri *et al.* 1989, 1991). Otolith shapes have further been used as taxonomic tools in fossil fish species (Nolf 1974, 1982, 1985, Gaemers 1984) and for stock identification (Parrish & Sharman 1959, Kotthaus 1961, Messieh 1972).

A detailed description of the morphology of otoliths is one of the basic requirements before they can be used in age and growth studies. The morphology of otoliths is complex and species-specific (Nolf 1974, 1982, 1985). The otolith shape is determined by the interaction of the protein matrix with the calcium carbonate crystals in the growth increments, and growth has been shown to vary in different parts of the otoliths (Gauldie & Nelson 1990). Thus, selection of the appropriate measure for age and growth rate estimates is important. Campana (1992) recommended axis length and increment clarity as criteria because otolith radii do not always show a complete growth increment sequence due to either inconsistencies in the preparation or to uncontrolled factors associated with otolith growth.

Growth rates can be deduced from daily growth increment width and from the relationship between otolith length and fish length (Campana & Neilson 1985, Francis 1990). The accuracy of growth estimates will depend upon the characteristics of the otolith-fish length relationship. Uncoupling of otolith-fish length relationships has been reported in several studies (e.g. Brothers 1981, Marshall & Parker 1982, Mosegaard *et al.* 1988, Reznick *et al.* 1989, Secor & Dean 1989, Mugiya & Tanaka 1992), and this circumstance must be determined prior to the back-calculation of size.

The aims of this study are: (1) to describe the external structures of the otoliths of *Pomatoschistus minutus* and *P. lozanoi* and to assess possible implications for age, growth and validation studies; (2) to establish relationships between fish length and otolith characteristics on the one hand, and between otolith length and other otolith measures on the other hand in order to determine allometric growth and to establish regressions for the back-calculation of fish length. Data on the internal structure of the otoliths will be reported elsewhere (Arellano *et al.* submitted c).

3.2. Materials and methods

3.2.1. Field and laboratory works

Pomatoschistus minutus and *P. lozanoi* were collected monthly from September 1992 to October 1993 with a beam trawl (opening of 3 m headrope and 50 cm height, codend mesh size of 10 mm) in the 5 m and 10 m depth strata of the ebb-tidal delta of the Grevelingen area (Delta area, south-west Netherlands). The fish were immediately preserved in 7% seawater-buffered formaldehyde.

In the laboratory, the samples were transferred to 96% ethanol for longer storage. After 3 months, the standard lengths (SL) were measured to the nearest mm and all individuals were identified to species and sexed according to Guitel (1892) and Hamerlynck (1990). The preserved lengths were converted to fresh lengths using regression equations (Arellano *et al.* submitted a). Each month, length-frequency distributions were established per sex and per species (size classes of 1 mm SL). From each size class 1 or 2 individuals were then selected for further analysis.

Otoliths (sagittae, lapilli and asterisci) were extracted from the auditory labyrinths by making a frontal plane incision at the cranial part of the head above the eyes. The cranium was opened at the incision to expose the brain and the auditory labyrinth, and the otoliths were taken out with forceps and were cleaned from adherent tissues with dissecting needles. The contours of the otoliths were drawn with the aid of a drawing tube attached to a binocular microscope at 50 x magnification. The otoliths were air dried and weighed with a microbalance with a precision of 0.001 μg and were kept in small vials with 96% ethanol. Some otoliths of large individuals ($>$ 60 mm SL) were gold plated for 3 minutes for scanning electron microscopy (SEM).

For each otolith, eight (sagitta) or six (lapillus and asteriscus) characters were measured (Figure 3.1). Axes and planes of the otoliths were described following the criteria and terminology of Summerfelt & Hall (1987). Nomenclature of the sagitta characters was based on the terminology introduced for Baltic sprat (Aps *et al.* unknown).

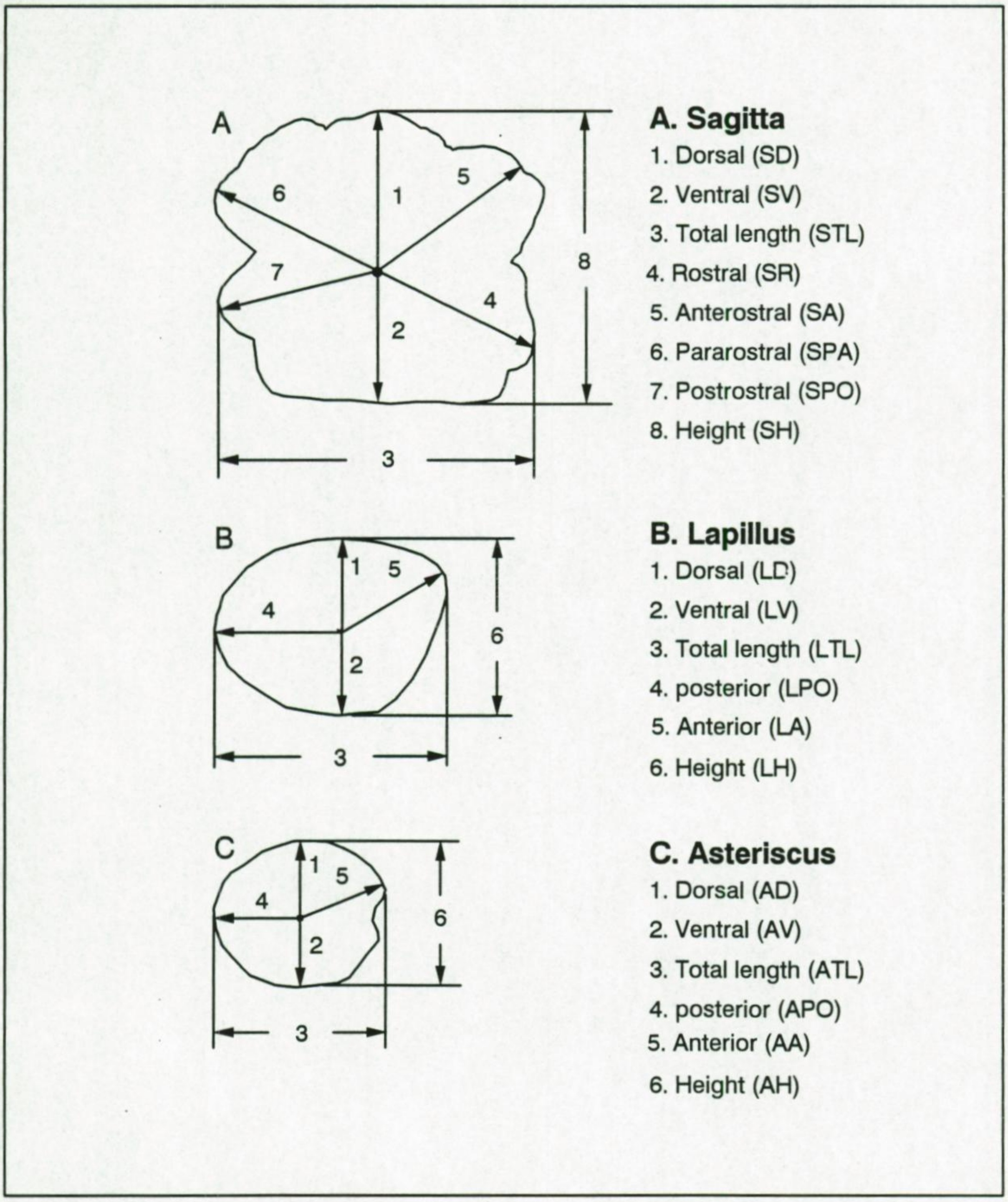


Figure 3.1. Schematic drawings of the right sagitta (A), lapillus (B), and asteriscus (C) indicating the measured characters and their abbreviations.

3.2.2. Data analysis

Data for the comparison of the mean surface areas and weights of the otoliths of male and female *Pomatoschistus minutus* and *P. lozanoi* were submitted to Bartlett's test of homogeneity of variances to determine if parametric statistics could be used (Sokal & Rohlf 1981). Exploratory analyses of the data for relationships between different otolith characters and SL or otolith lengths were done with scatterplots and normal plots of residuals. Outliers were detected with the use of plots of the raw residuals and were deleted when they were outside the range of ± 3SD. The significance of the regression equations was tested with analysis of variance (ANOVA); the slopes were compared with analysis of covariance (ANCOVA).

Growth in weight and surface area of left and right otoliths were compared using allometry (Gould 1966). ANOVA was used to compare the surface areas and weights of the otoliths between sexes, between species, and between otoliths of individuals of the same mean SL.

Allometry of otolith characters relative to standard length or otolith length was determined from the slopes of the regressions. When the x and y axes are both in mm, slopes equal to 1 represent isometry, while slopes < 1 or > 1 represent negative or positive allometry, respectively. If the units of measurements are not the same, i.e. when the x axis is in mm and the y axis is in mm^2, 2 represents isometry (Gould 1966); for the allometry of the SL-otolith weight relationship, 3 represents isometry (Pauly 1984).

3.3. Results

A total of 6648 otoliths of *Pomatoschistus minutus* (598 pairs of male and 510 pairs of female sagittae, lapilli and asterisci) and a total of 4164 otoliths of *P. lozanoi* (366 pairs of male and 328 pairs of female sagittae, lapilli and asterisci) were extracted.

3.3.1. Position and location of sagitta, lapillus and asteriscus

The position and location of the otoliths in the skull was similar in both species (Figure 3.2). The three pairs of otoliths were located symmetrically on both lateral walls of the brain.

The anterostral and rostral planes of the sagitta displayed an angle of about 45° relative to the fish's central axis. The pararostral and postrostral planes were oriented toward the caudal end of the fish. The ventral plane was resting on the otic cleft of the skull base, and the dorsal plane was adjacent to the top of the skull.

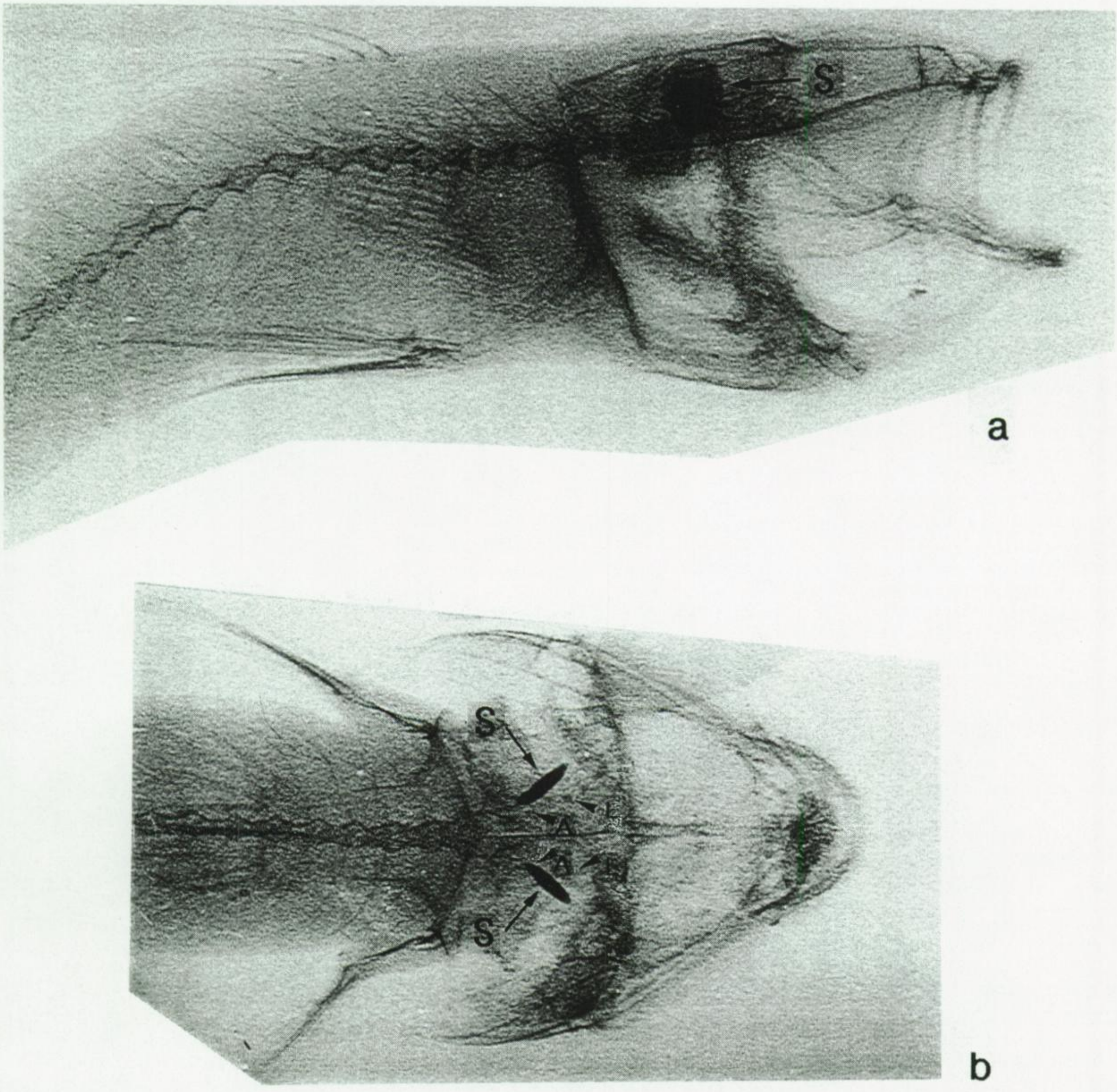


Figure 3.2. *Pomatoschistus minutus*. X-ray pictures of the otoliths in the skull. a: lateral view, b: top view, S: sagitta, L: lapillus, A: asteriscus.

The lapillus was located on the ventral-anterior proximal side of the sagitta. Orientations of the lapillus characters were similar to the orientations of the sagitta.

The asteriscus was located on the ventral-posterior proximal side of the sagitta. Orientations of the asteriscus characters were also similar to the orientations of the sagitta. However, the ventral plane of the asteriscus was not resting on the otic cleft of the skull base.

3.3.2. External morphology

Allometry between the left and right otoliths was isometric for the sagittae, lapilli and asterisci of both *Pomatoschistus minutus* and *P. lozanoi*. Otolith surface areas and weights of males and females of the same mean SL were not significantly different. Thus, means of the left and right otoliths were calculated and the data of males and females were pooled per size class. In both species, the sagittae were significantly larger and heavier than the lapilli and asterisci, and the lapilli were larger and heavier than the asterisci (ANOVA, $p<0.005$). Sagittae and lapilli of *P. minutus* were larger and heavier than those of *P. lozanoi* of the same mean SL (ANOVA, $p<0.005$), while size and weight of the asterisci of both species were not significantly different ($p>0.05$).

In both species the otoliths differed in colour: the sagittae were opaque and whitish and the asterisci were completely transparent. The lapilli had intermediate characters. The sagittae and lapilli were slightly darker near the centre.

The proximal and distal sides, and the sulci acustici, of the sagittae, lapilli and asterisci of *Pomatoschistus minutus* and *P. lozanoi* are shown in Figures 3.3, 3.4, 3.5 & 3.6. Otolith morphology of the two species was similar unless otherwise specified. The proximal sides (adjacent to the brain's lateral wall) of the sagittae and asterisci were flat to concave, while those of the lapilli were convex. The distal sides (opposite to the brain's lateral wall) of all otoliths were convex and the sulcus acusticus was always adjacent to the brain's lateral wall.

The sulci acustici of the three pairs of otoliths had a quite different shape. The sulcus acusticus of the sagitta resembled a 'footprint' and was located at the centre of the otolith (Figures 3.3d, 3.4d, 3.5b & 3.6b). In the lapillus, the sulcus acusticus rather looked like a letter "C" and it was located more anteriorly (Figures 3.3e, 3.4e, 3.5d & 3.6d). In the asteriscus, the sulcus acusticus had the shape of a 'crooked stick' oriented

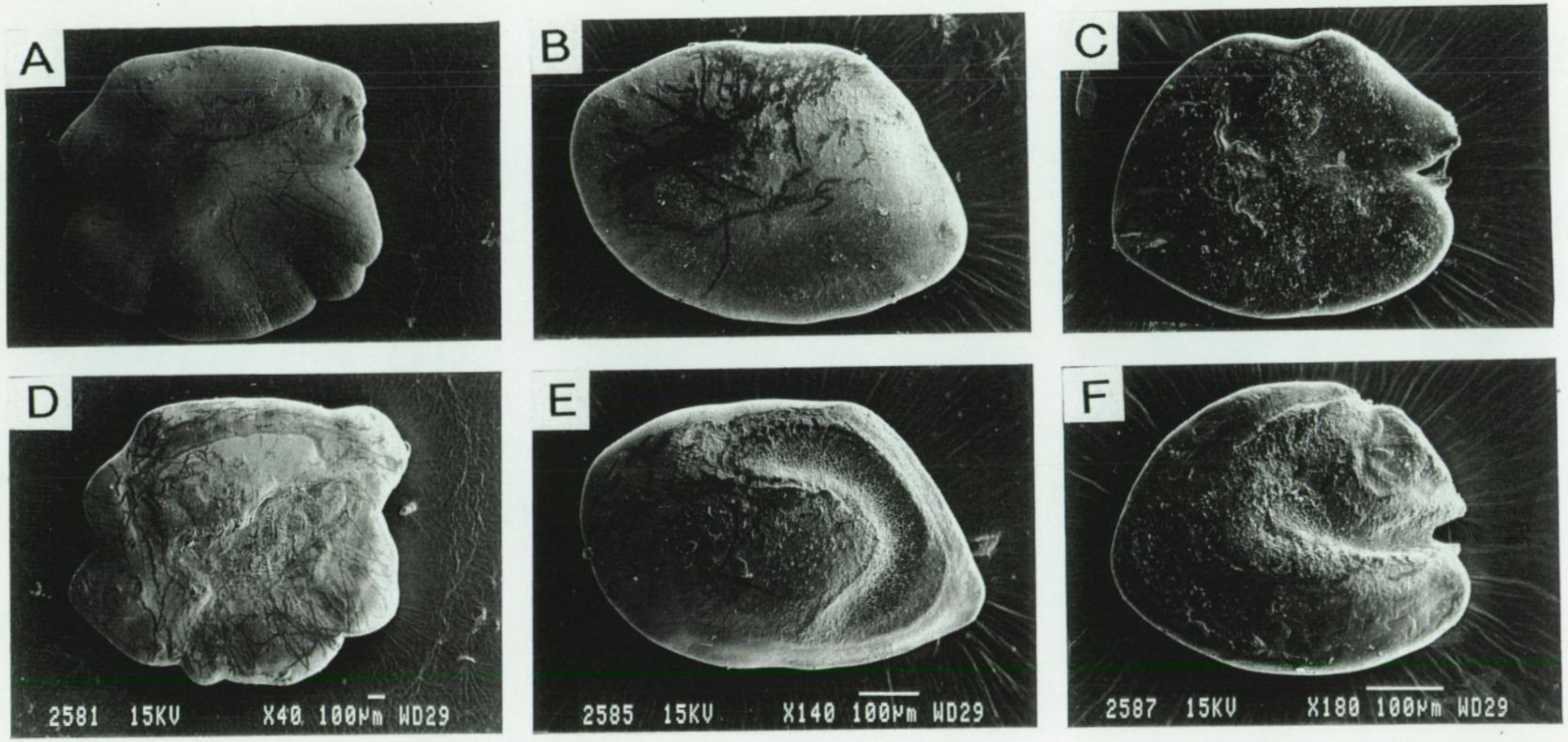


Figure 3.3. *Pomatoschistus minutus*. Distal (A, B & C) and proximal (D, E & F) sides of the sagittae (A & D), lapilli (B & E) and asterisci (C & F).

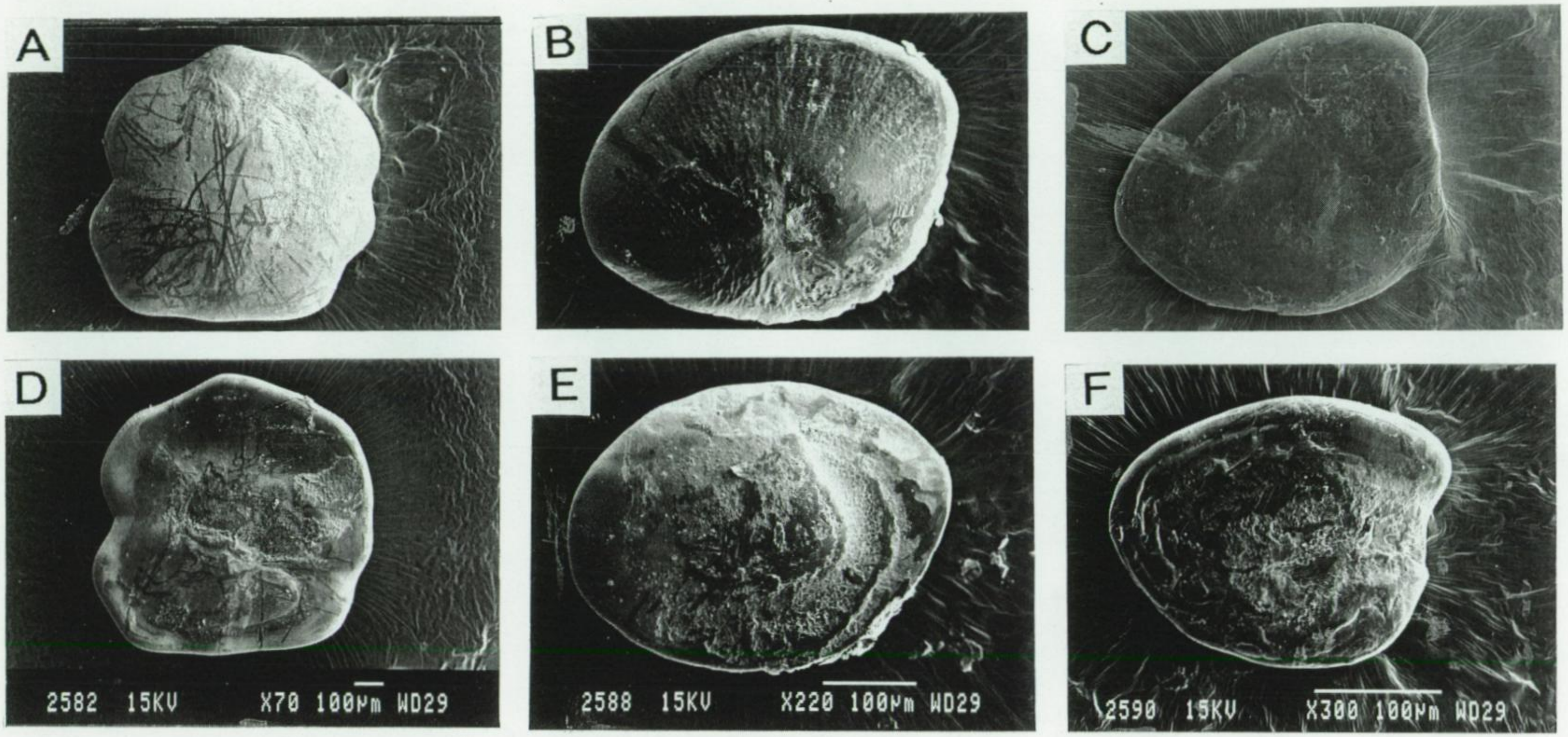


Figure 3.4. *Pomatoschistus lozanoi*. Distal (A, B & C) and proximal (D, E & F) sides of the sagittae (A & D), lapilli (B & E) and asterisci (C & F).

from the anterior edge to the centre (Figures 3.3f, 3.4f, 3.5f & 3.6f). The general shape of the sulcus acusticus showed no clear changes as the fish grew. The average ratio of the sulcus acusticus area to the sagitta area was significantly higher in *Pomatoschistus lozanoi* than in *P. minutus* of the same SL (Arellano *et al.* 1995). No attempt was made to calculate the ratios of the sulcus acusticus area to the lapillus and asteriscus areas.

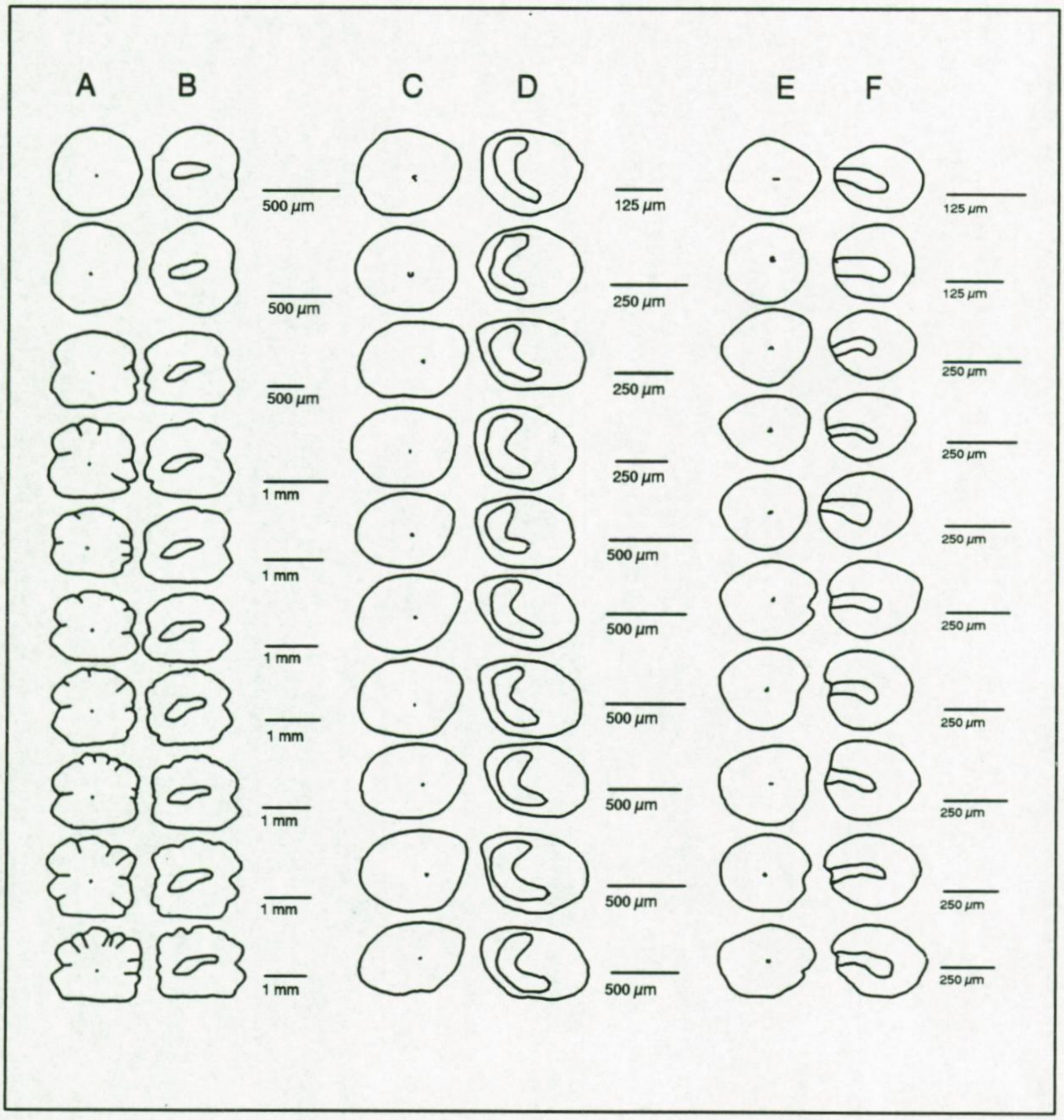


Figure 3.5. *Pomatoschistus minutus*. Morphological development of sagittae (A & B), lapilli (C & D) and asterisci (E & F) as the fish grew.

The distal sides of the sagittae showed vague corrugations and crenulations when the fish reached about 30 mm SL, and these features became more distinct as the fish grew. The sagittae of *Pomatoschistus minutus* were much more corrugated and crenulated (Figure 3.5a) than the sagittae of *P. lozanoi* (Figure 3.6a). Generally, in fish > 30 mm SL, the ventral planes of the sagittae were flat, while the dorsal planes were rounded,

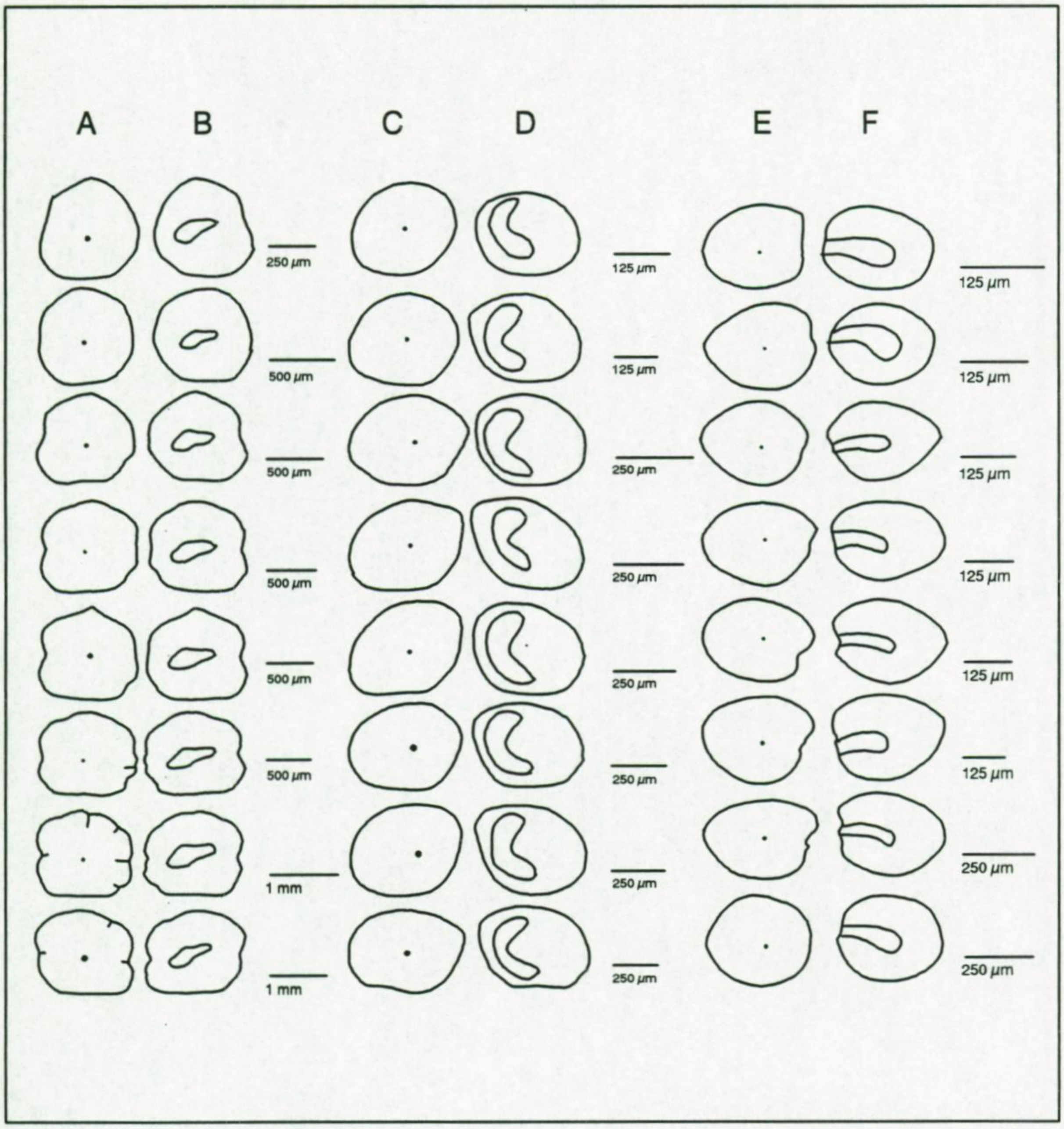


Figure 3.6. *Pomatoschistus lozanoi*. Morphological development of sagittae (A & B), lapilli (C & D) and asterisci (E & F) as the fish grew.

corrugated and crenulated. Distinct depressions separated the rostral from the anterostral and the pararostral from the postrostral parts in larger individuals (> 50 mm SL) of both species. The depressions between pararostral and postrostral were more distinct in *P. minutus* than in *P. lozanoi*.

The distal sides of the lapilli and the asterisci of both species showed smooth surfaces (Figures 3.3b & c, 3.4b & c, 3.5c & e, 3.6c & e). The asteriscus shape did not change as the fish grew. The ventral and anterior planes of the lapilli were flat, while the dorsal planes were rounded. Both the ventral and dorsal planes of the asterisci were rounded. In both species, a distinct depression separated the anterior from the lower part of the asteriscus.

3.3.3. Allometric growth

Pomatoschistus minutus

The relationships between standard length and sagitta characters are summarised in Table 3.1. All regressions were linear and significant except for the SL-sagitta surface area regression which showed a significant power function (Arellano *et al.* 1995). Most scatterplots show an inflection point (all otolith characters except for surface area) at 32 mm SL (see Figures 3.7a-c for the relationship between SL and sagitta total length STL, lapillus total length LTL and asteriscus total length ATL). Therefore, regressions were estimated separately for the size ranges from 18 to 32 mm SL and from 33 to 78 mm SL. Allometry of all sagitta characters was negative with respect to SL. The slopes estimated for the 18 to 32 mm SL size class were significantly higher than those estimated for the 33 to 78 mm SL size class. Only the slope of the sagitta width regression did not differ significantly between size classes.

The relationships between sagitta total length and the other sagitta characters are summarised in Table 3.2. All regressions were linear and significant except for the STL - sagitta surface regression which showed a significant power function. Only the 'STL-height', 'STL-dorsal' and 'STL-ventral' regressions had inflection points at 32 mm SL. The slopes estimated for the 18 to 32 mm SL size class were significantly higher than those estimated for the 33 to 78 mm SL size class, except for the 'STL-ventral' slope which did not differ significantly between size classes.

Table 3.1. Pomatoschistus minutus. Relationship between fish length (SL) and sagitta, lapillus and asteriscus characters (see abbreviations of otolith characters in Figure 3.1) and comparison of slopes. SC: sagitta circumference, SW: sagitta width, LC: lapillus circumference, LW: lapillus width, LS: lapillus surface, AC: asteriscus circumference, AW: asteriscus width, AS: asteriscus surface, a: intercept, b_1: slope for size range 18 to 32 mm SL, b_2: slope for size range 33 to 78 mm SL, r^2: coefficient of determination, N: number of samples, (*** = $p<0.005$; ** = $p<0.01$; * = $p<0.05$; NS = $p>0.05$).

Relationship	**a**	**b_1/b_2**	**r^2**	**N**	**Sig. of regr.**	**Sig. of b_1**
Size range 18 to 32 mm SL		**SAGITTA**				
SL-STL	-0.149	0.031	0.72	69	***	
SL-SC	-0.410	0.104	0.78	69	***	
SL-SH	-0.062	0.030	0.84	69	***	
SL-SW	0.061	0.008	0.63	68	***	
SL-SD	-0.072	0.018	0.80	69	***	
SL-SV	-0.001	0.013	0.78	69	***	
SL-SR	-0.085	0.017	0.74	69	***	
SL-SPO	-0.085	0.017	0.71	69	***	
SL-SPA	-0.084	0.017	0.75	69	***	
SL-SA	-0.082	0.017	0.79	69	***	
Size range 33 to 78 mm SL						
SL-STL	0.278	0.026	0.88	1034	***	*
SL-SC	0.836	0.088	0.89	1032	***	*
SL-SH	0.402	0.021	0.89	1036	***	***
SL-SW	0.160	0.007	0.60	781	***	NS
SL-SD	0.173	0.013	0.87	1030	***	***
SL-SV	0.224	0.009	0.77	1032	***	***
SL-SR	0.218	0.013	0.77	1034	***	***
SL-SPO	0.113	0.013	0.87	1029	***	**
SL-SPA	0.194	0.013	0.81	1034	***	***
SL-SA	0.138	0.014	0.84	1034	***	**
Size range 18 to 32 mm SL		**LAPILLUS**				
SL-LTL	-0.030	0.012	0.67	70	***	
SL-LC	0.007	0.034	0.72	69	***	
SL-LH	0.012	0.009	0.74	69	***	
SL-LW	0.014	0.004	0.68	66	***	
SL-LD	-0.009	0.005	0.63	70	***	
SL-LV	0.021	0.004	0.67	69	***	
SL-LA	-0.026	0.006	0.63	69	***	
SL-LPO	-0.018	0.007	0.63	70	***	
SL-LS	$6.57*10^{-4}$	1.472	0.84	1042	***	
Size range 33 to 78 mm SL						
SL-LTL	0.254	0.007	0.74	971	***	***
SL-LC	0.805	0.018	0.73	977	***	***
SL-LH	0.218	0.005	0.70	969	***	***
SL-LW	0.124	0.002	0.69	782	***	***
SL-LD	0.117	0.002	0.65	978	***	***
SL-LV	0.102	0.002	0.61	981	***	***
SL-LA	0.114	0.003	0.71	975	***	***
SL-LPO	0.154	0.004	0.66	975	***	***
Size range 18 to 32 mm SL		**ASTERISCUS**				
SL-ATL	-0.016	0.008	0.64	69	***	
SL-AC	-0.010	0.023	0.77	69	***	
SL-AH	-0.013	0.007	0.79	69	***	
SL-AW	-0.029	0.003	0.52	69	***	
SL-AD	-0.002	0.004	0.70	69	***	
SL-AV	-0.010	0.004	0.73	69	***	
SL-AA	-0.005	0.004	0.62	69	***	
SL-APO	-0.019	0.005	0.65	69	***	
SL-AS	$1.41*10^{-4}$	1.657	0.89	999	***	
Size range 33 to 78 mm SL						
SL-ATL	0.145	0.005	0.74	925	***	***
SL-AC	0.400	0.015	0.79	937	***	***
SL-AH	0.115	0.004	0.74	931	***	***
SL-AW	0.036	0.001	0.57	782	***	***
SL-AD	0.055	0.002	0.75	938	***	***
SL-AV	0.058	0.002	0.66	946	***	***
SL-AA	0.061	0.002	0.71	943	***	***
SL-APO	0.093	0.002	0.63	942	***	***

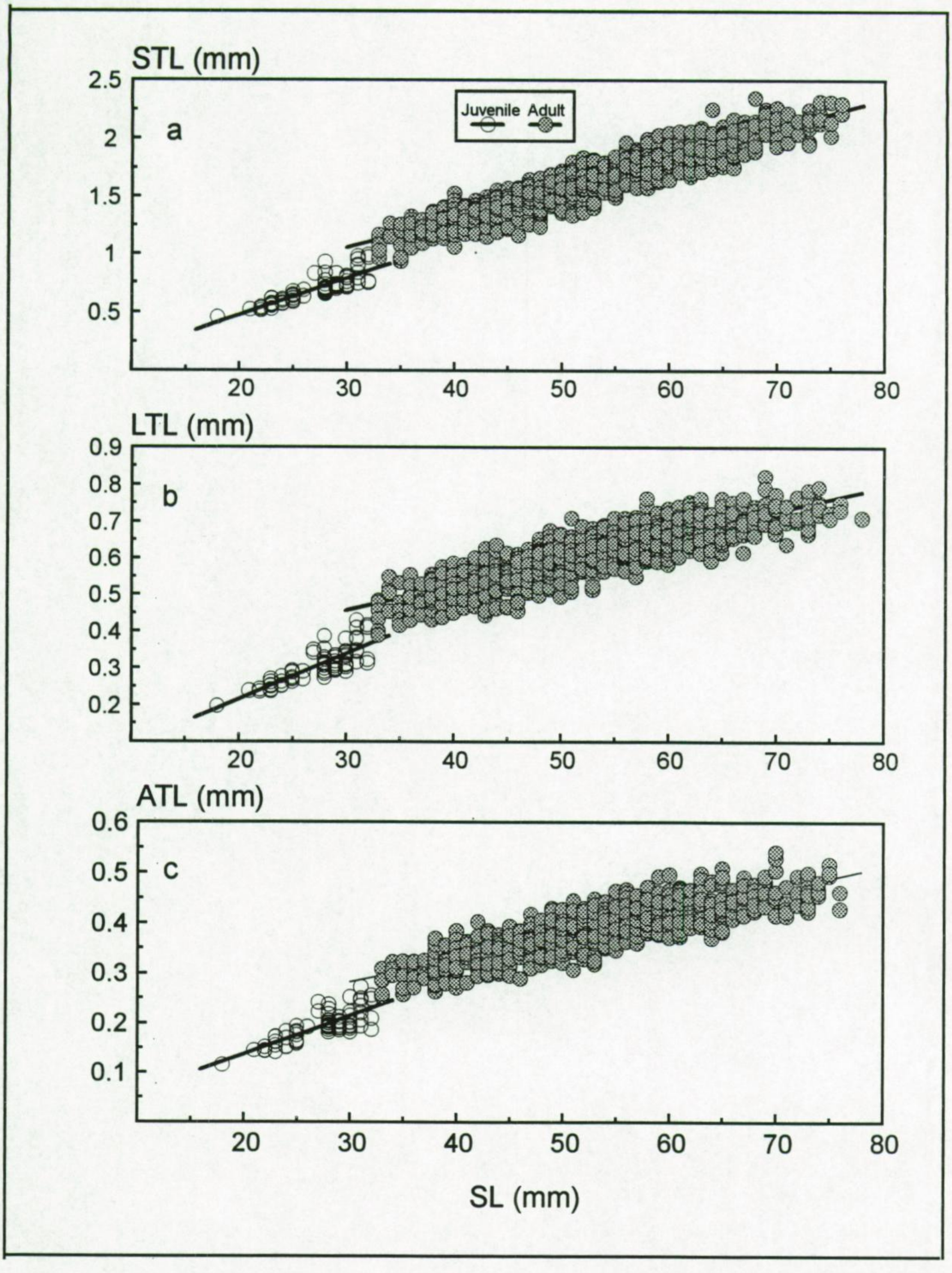


Figure 3.7. *Pomatoschistus minutus*. Relationship between fish length (SL) and (a) sagitta length (STL), (b) lapillus length (LTL) and (c) asteriscus length (ATL).

Table 3.2. Pomatoschistus minutus. Relationship between sagitta length (STL), lapillus length (LTL) and asteriscus length (ATL) and the other sagitta, lapillus and asteriscus characters (see abbreviations of otolith characters in Figure 3.1) and comparison of slopes. SC: sagitta circumference, SW: sagitta width, SS: sagitta surface, LC: lapillus circumference, LW: lapillus width, LS: lapillus surface, AC: asteriscus circumference, AW: asteriscus width, AS: asteriscus surface, a: intercept, b: slope, r^2: coefficient of determination, N: number of samples, (*** = $p<0.005$; ** = $p<0.025$; NS = $p>0.05$).

Relationship	**a**	**b**	**r^2**	**N**	
Size range 18 to 78 mm SL		**SAGITTA**			
STL-SC	0.082	3.273	0.98	1083	***
STL-SW	0.112	0.244	0.79	824	***
STL-SR	0.015	0.532	0.96	1093	***
STL-SPO	0.017	0.489	0.95	1086	***
STL-SPA	0.033	0.507	0.96	1090	***
STL-SA	0.008	0.540	0.95	1093	***
STL-SS	0.823	1.828	0.99	1098	***
Size range 18 to 32 mm SL					
STL-SH	0.157	0.859	0.93	69	***
STL-SD	0.051	0.512	0.91	69	***
STL-SV	0.094	0.363	0.85	69	***
Size range 33 to 78 mm SL					
STL-SH	0.230	0.780	0.94	1022	***
STL-SD	0.088	0.454	0.89	1013	***
STL-SV	0.133	0.336	0.86	1011	***
Comparing the slopes (18 to 32 and 33 to 78 mm SL)					
STL-SH					**
STL-SD					**
STL-SV					NS
		LAPILLUS			
LTL-LC	0.137	2.631	0.98	1062	***
LTL-LH	0.067	0.644	0.93	1064	***
LTL-LW	0.050	0.283	0.90	865	***
LTL-LD	0.034	0.343	0.89	1069	***
LTL-LV	0.033	0.304	0.87	1069	***
LTL-LA	-0.009	0.503	0.94	1070	***
LTL-LPO	0.012	0.534	0.94	1056	***
LTL-LS	0.544	1.790	0.98	1067	***
		ASTERISCUS			
ATL-AC	0.045	2.919	0.96	1055	***
ATL-AH	0.030	0.798	0.88	1059	***
ATL-AW	-0.009	0.290	0.79	860	***
ATL-AD	0.016	0.400	0.84	1058	***
ATL-AV	0.015	0.397	0.83	1062	***
ATL-AA	-0.002	0.500	0.88	1059	***
ATL-APO	0.011	0.530	0.94	1053	***
ATL-AS	0.596	1.876	0.97	1059	***

Allometry of all sagitta characters was negative with respect to STL, except for sagitta circumference (positive allometry).

The relationships between SL and lapillus and asteriscus characters are summarised in Table 3.1. The relationships between LTL and ATL and the other lapillus and asteriscus characters are summarised in Table 3.2. The results were identical to those obtained for the sagitta (see higher). However, no inflection points were observed in the regressions of the lapillus and asteriscus characters on LTL and ATL (Figures 3.8a & 3.10a).

The relationships between SL and otolith weights are summarised in Table 3.3. All regressions showed power functions and were significant. Allometry was always negative. The sagitta regression had the highest slope value and was closest to isometry. Negative allometry was highest for the asteriscus.

Table 3.3. *Pomatoschistus minutus* and *P. lozanoi*. Relationship between fish length (SL) and otolith weight. SOW: sagitta weight, LOW: lapillus weight, AOW: asteriscus weight, a: intercept, b: slope, r^2: coefficient of determination, N: number of samples, (*** = $p<0.005$).

Relationship	**a**	**b**	**r^2**	**N**	
P. minutus					
SL-SOW	$5.91*10^{-5}$	2.572	0.87	1075	***
SL-LOW	$4.22*10^{-5}$	1.875	0.81	973	***
SL-AOW	$3.83*10^{-5}$	1.521	0.51	900	***
P. lozanoi					
SL-SOW	$1.14*10^{-4}$	2.384	0.87	583	***
SL-LOW	$8.47*10^{-5}$	1.670	0.75	534	***
SL-AOW	$6.35*10^{-5}$	1.387	0.53	510	***

Pomatoschistus lozanoi

The relationships between SL, STL, LTL and ATL and the different sagitta, lapillus and asteriscus characters are summarised in Tables 3.4 & 3.5. The results are analogous to the results obtained for *P. minutus* (Figures 3.8b, 3.9a-c, 3.10b). However, the inflection points in the regressions of SL and otolith characters was now located at 30 mm SL (Figures 3.9a-c). Inflection points were further only observed in the STL-postrostral and in the STL-pararostral regressions (Table 3.5). The relationships between SL and otolith weights are summarised in Table 3.3. Results of these analyses were similar to the results for *P. minutus*.

Table 3.4. Pomatoschistus lozanoi. Relationship between fish length (SL) and sagitta, lapillus and asteriscus characters (see abbreviations of otolith characters in Figure 3.1) and comparison of slopes. SC: sagitta circumference, SW: sagitta width, LC: lapillus circumference, LW: lapillus width, LS: lapillus surface, AC: asteriscus circumference, AW: asteriscus width, AS: asteriscus surface, a: intercept, b: slope, b_1: slope for size range 19 to 30 mm SL, b_2: slope for size range 31 to 57 mm SL, r^2: coefficient of determination, N: number of samples, (*** = $p<0.005$; ** = $p<0.025$; NS = $p>0.05$).

Relationship	a	b_1/b_2	r^2	N	Sig. of reg	Sig. of b_1
Size range 19 to 30 mm SL		**SAGITTA**				
SL-STL	-0.158	0.036	0.86	62	***	
SL-SC	-0.343	0.113	0.87	60	***	
SL-SH	0.035	0.030	0.85	61	***	
SL-SW	0.094	0.008	0.45	54	***	
SL-SD	0.014	0.017	0.75	62	***	
SL-SV	0.011	0.014	0.77	60	***	
SL-SR	-0.106	0.019	0.80	62	***	
SL-SPO	-0.072	0.018	0.84	62	***	
SL-SPA	-0.078	0.019	0.82	62	***	
SL-SA	-0.060	0.018	0.79	62	***	
Size range 31 to 57 mm SL						
SL-STL	0.160	0.025	0.84	622	***	***
SL-SC	0.608	0.081	0.84	621	***	***
SL-SH	0.280	0.022	0.84	627	***	***
SL-SW	0.127	0.007	0.68	504	***	NS
SL-SD	0.152	0.012	0.79	628	***	***
SL-SV	0.121	0.010	0.76	627	***	***
SL-SR	0.024	0.015	0.79	629	***	***
SL-SPO	0.183	0.010	0.75	628	***	***
SL-SPA	0.093	0.013	0.79	627	***	***
SL-SA	0.076	0.014	0.79	630	***	***
Size range 19 to 30 mm SL		**LAPILLUS**				
SL-LTL	0.022	0.012	0.77	60	***	
SL-LC	0.096	0.035	0.80	59	***	
SL-LH	0.032	0.010	0.83	59	***	
SL-LW	0.043	0.004	0.71	56	***	
SL-LD	0.004	0.006	0.75	60	***	
SL-LV	0.030	0.004	0.67	60	***	
SL-LA	-0.004	0.006	0.65	60	***	
SL-LPO	-0.004	0.007	0.76	60	***	
SL-LS	$6.06*10^{-4}$	1.486	0.87	668	***	
Size range 31 to 57 mm SL						
SL-LTL	0.132	0.009	0.75	602	***	***
SL-LC	0.401	0.025	0.80	602	***	***
SL-LH	0.127	0.006	0.79	606	***	***
SL-LW	0.084	0.002	0.60	509	***	***
SL-LD	0.059	0.004	0.76	609	***	***
SL-LV	0.068	0.003	0.67	606	***	***
SL-LA	0.061	0.004	0.70	600	***	***
SL-LPO	0.073	0.005	0.71	610	***	***
Size range 19 to 30 mm SL		**ASTERISCUS**				
SL-ATL	0.008	0.008	0.75	56	***	
SL-AC	0.048	0.024	0.75	56	***	
SL-AH	0.004	0.007	0.74	56	***	
SL-AW	0.029	0.001	0.03	52	NS	
SL-AD	-0.004	0.004	0.71	56	***	
SL-AV	0.006	0.003	0.62	56	***	
SL-AA	-0.010	0.004	0.64	56	***	
SL-APO	0.013	0.005	0.64	56	***	
SL-AS	$12.6*10^{-4}$	1.697	0.87	634	***	
Size range 31 to 57 mm SL						
SL-ATL	0.064	0.006	0.76	571	***	**
SL-AC	0.174	0.020	0.79	578	***	**
SL-AH	0.042	0.006	0.76	571	***	**
SL-AW	0.024	0.001	0.40	500	***	
SL-AD	0.023	0.003	0.73	578	***	**
SL-AV	0.020	0.003	0.68	578	***	NS
SL-AA	0.031	0.003	0.68	577	***	**
SL-APO	0.047	0.003	0.67	577	***	**

Table 3.5. Pomatoschistus lozanoi. Relationship between sagitta length (STL), lapillus length (LTL) and asteriscus length (ATL) and the other sagitta, lapillus and asteriscus characters (see abbreviations of otolith sagitta characters in Figure 3.1) and comparison of slopes. SC: sagitta circumference, SW: sagitta width, SS: sagitta surface, LC: lapillus circumference, LW: lapillus width, LS: lapillus surface, AC: asteriscus circumference, AW: asteriscus width, AS: asteriscus surface, a: intercept, b: slope, r^2: coefficient of determination, N: number of samples, (*** = $p<0.005$; NS = $p>0.05$).

Relationship	**a**	**b**	**r^2**	**N**	
Size range 19 to 57 mm SL		**SAGITTA**			
STL-SC	0.152	3.198	0.98	692	***
STL-SW	0.106	0.264	0.78	561	***
STL-SR	-0.062	0.594	0.96	689	***
STL-SH	0.166	0.849	0.95	691	***
STL-SD	0.092	0.464	0.91	691	***
STL-SV	0.068	0.395	0.88	690	***
STL-SA	-0.002	0.546	0.94	692	***
STL-SS	0.834	1.878	0.98	692	***
Size range 19 to 30 mm SL					
STL-SPO	0.009	0.515	0.96	61	***
STL-SPA	0.009	0.531	0.95	62	***
Size range 31 to 57 mm SL					
STL-SPO	0.121	0.401	0.89	624	***
STL-SPA	0.022	0.517	0.91	627	***
Comparing slopes (19 to 30 and 31 to 57 mm SL)					
SPO					***
SPA					NS
		LAPILLUS			
LTL-LC	0.086	2.765	0.97	672	***
LTL-LH	0.055	0.693	0.91	674	***
LTL-LW	0.052	0.264	0.81	568	***
LTL-LD	0.020	0.385	0.87	672	***
LTL-LV	0.033	0.314	0.82	676	***
LTL-LA	-0.005	0.496	0.91	673	***
LTL-APO	0.013	0.523	0.91	675	***
LTL-LS	0.581	1.856	0.96	676	***
		ASTERISCUS			
ATL-AC	0.026	2.897	0.96	666	***
ATL-AH	0.006	0.802	0.88	664	***
ATL-AW	0.010	0.217	0.56	566	***
ATL-AD	0.008	0.386	0.83	663	***
ATL-AV	0.003	0.403	0.82	669	***
ATL-AA	0.005	0.460	0.85	662	***
ATL-APO	0.012	0.534	0.93	655	***
ATL-AS	0.600	1.947	0.95	664	***

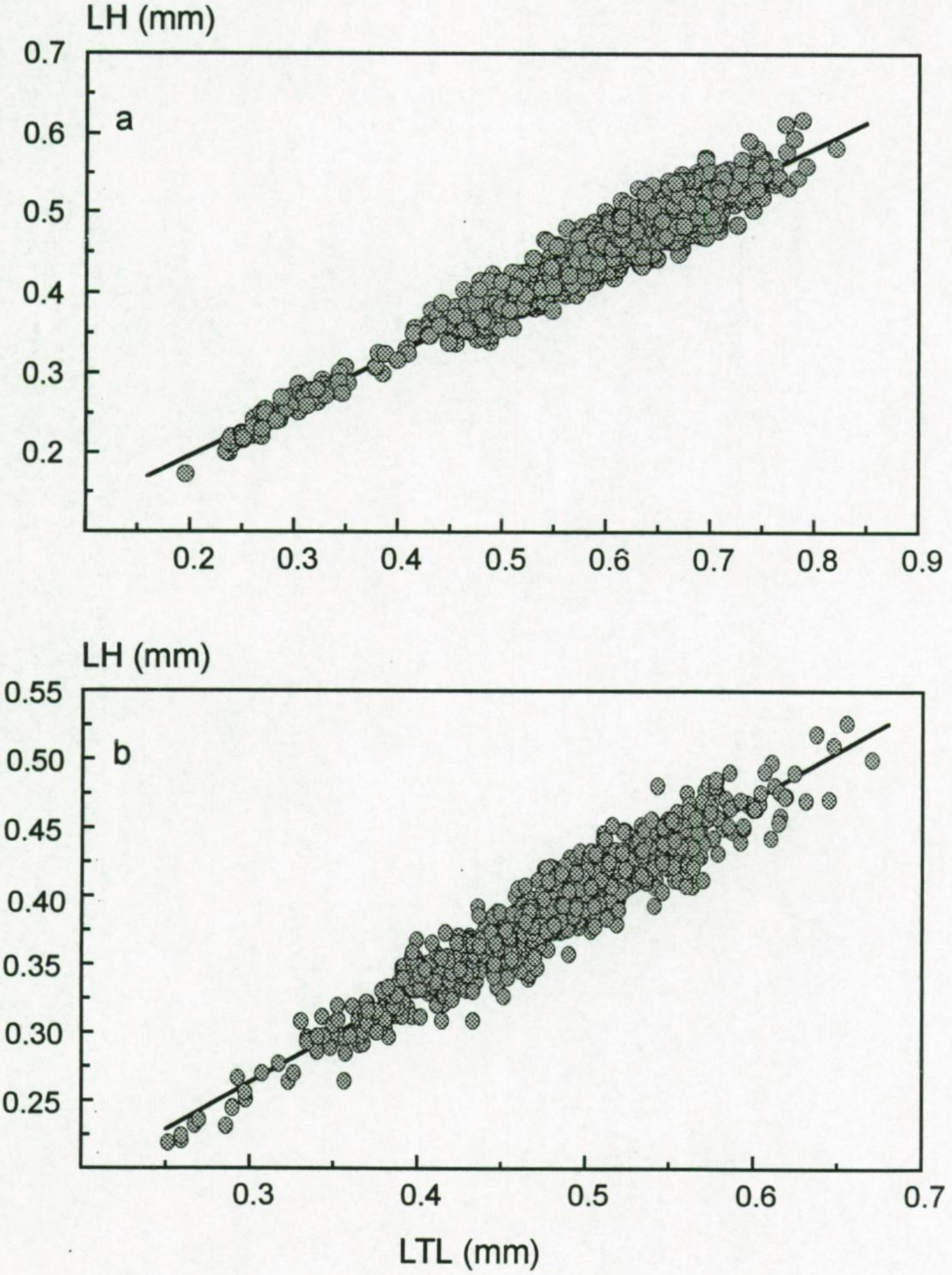


Figure 3.8. *Pomatoschistus minutus* (a) and *P. lozanoi* (b). Relationship between lapillus length (LTL) and lapillus height (LH).

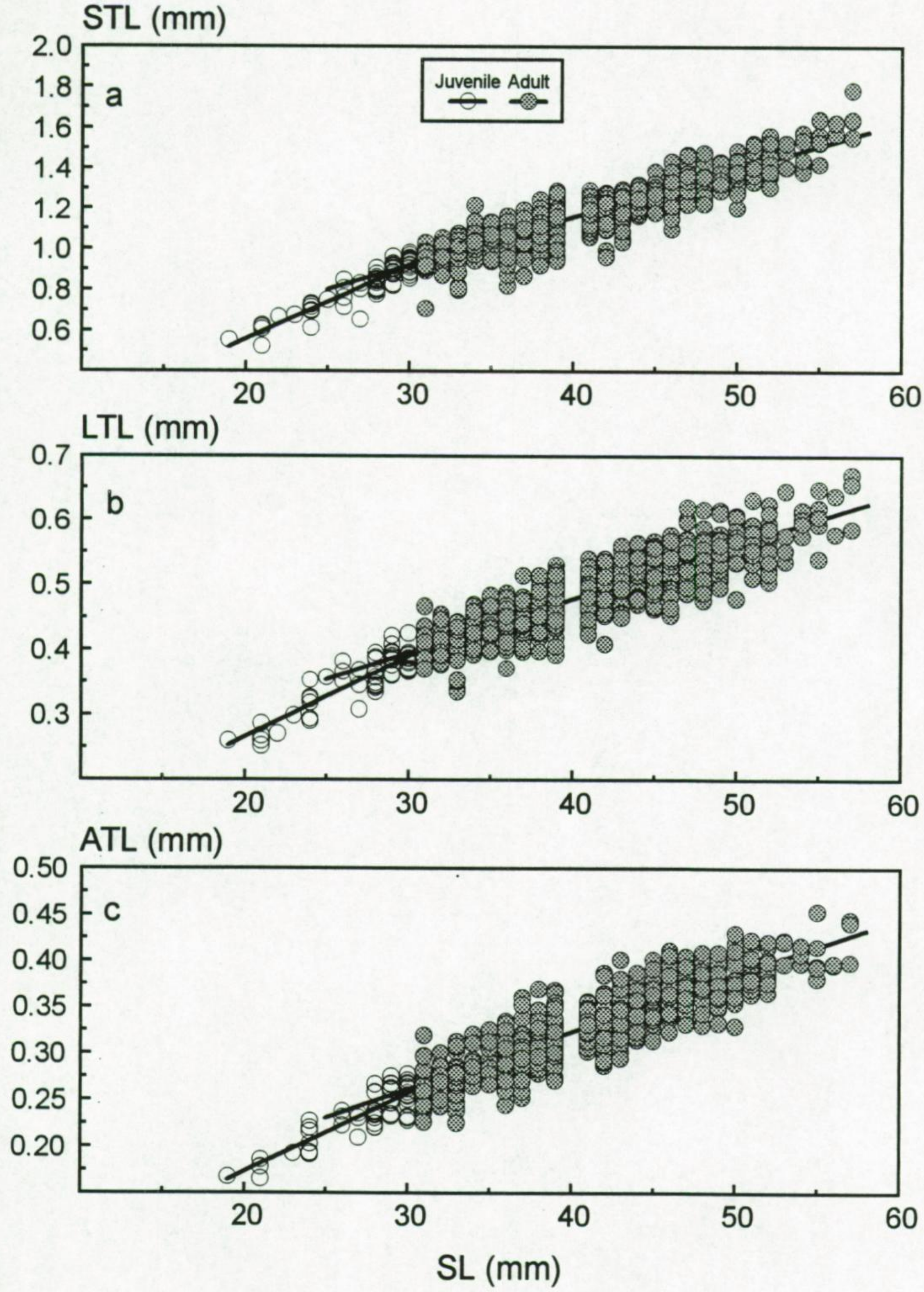


Figure 3.9. *Pomatoschistus lozanoi*. Relationship between fish length (SL) and sagitta length (STL), (b) lapillus length (LTL) and asteriscus length (ATL).

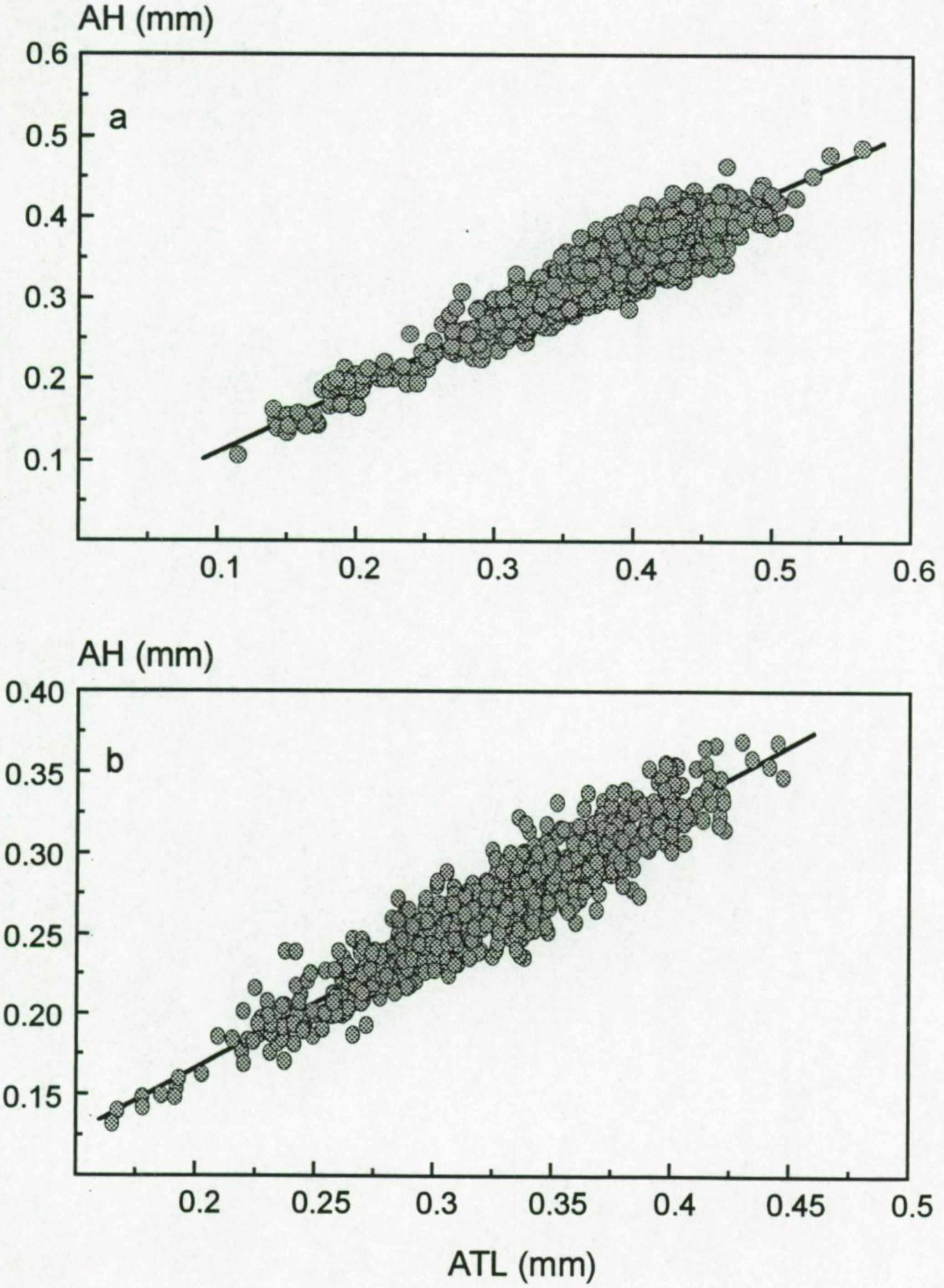


Figure 3.10. *Pomatoschistus minutus* (a) and *P. lozanoi* (b). Relationship between asteriscus length (ATL) and asteriscus height (AH).

3.4. Discussion

3.4.1. External morphology

Precise knowledge of the otolith's orientation in the auditory labyrinth is a prerequisite in extracting otoliths efficiently. The orientation in the auditory labyrinth and the relative sizes of the sagittae, lapilli and asterisci are similar to those observed in other teleost fish (Nolf 1974, Secor *et al.* 1991, Victor 1991), although Pannella (1980) states that the asterisci are generally slightly larger than the lapilli.

The observed isometry of the left and right otoliths, and the fact that they are located symmetrically on the lateral walls of the brain, suggests that the sagittae, lapilli and asterisci of *Pomatoschistus lozanoi* and *P. minutus* function primarily for balancing and equilibrium. This function of otoliths is typical for all teleost fishes (e.g. Secor *et al.* 1991). The ratio of the sulcus acusticus area to the sagitta area was used to hypothesize on the hearing sensitivity of both species (Arellano *et al.* 1995). A consistent isometric growth was observed for the surface areas and circumferences of the left and right sagittae, lapilli, and asterisci. All other otolith characters also displayed isometry, or, rarely, weak positive or negative allometry. Because otoliths function for balancing and equilibrium, it is expected that the growth of otolith characters is always isometric. The exceptions may be linked to the difficulty of identifying the exact centres (origin of measurement) of the otoliths: especially in small gobies (< 35 mm SL) the centres and some of the characters of the otoliths are not yet distinct. Also, the non significance of the regression of SL to asteriscus width for *P. lozanoi* in the size class of 19 to 30 mm SL, and the low r^2's of all regressions involving otolith width, are probably due to measurement errors.

Changes in the external morphology of the sagittae are correlated with the life history of the gobies. In *Pomatoschistus minutus*, the sagittae of juveniles had more or less round and smooth distal sides. As the fish reached sexual maturity at a SL of 30 (Hamerlynck 1990) or 32 (this study) mm, the sagitta shape changed and corrugations and crenulations started to appear. Adults had distinctly corrugated and crenulated sagittae. This suggests that the calcium carbonate deposition rate in the ventral and dorsal parts was reduced after sexual maturity (particularly on the sagitta dorsal edge). Thus, the dorsal side of the sagitta will become flattened as the fish grow older. The higher slope values for the 'STL-dorsal' regression as compared to that of the 'STL-ventral' regression, and the fact that the slopes of the 'STL-ventral' regressions of both size classes were not significantly different (Table 3.2), suggests that the dorsal side of the sagitta is a 'free growth zone' while the ventral side is a 'restricted growth zone'. Ventral growth is probably controlled by the skull structure: the rounded shape of the ventral side in small gobies suggests that the ventral edge is not yet in contact with the otic cleft of the skull and that growth is not yet restricted; the flattened shape after sexual maturity is indicative of physical contact with the otic cleft resulting in restricted growth. Changes in the external morphology of the sagittae are also reflected in the internal structure of the otoliths: concentric, uninterrupted and wide growth increments at the centre are evidences of free growth, while interrupted and narrow growth increments near the margins are evidences of restricted growth (Arellano *et al.* submitted c).

Physical contact between the otoliths and the skull always results in restricted otolith growth (Gauldie & Nelson 1990) and also provides a fulcrum for the angular rotation of the otolith that generates the shearing forces to activate the kinocilia of the macula (Schuijf 1981). The fact that the ventro-lateral sides of the sagittae (up to the lower edge of the sulcus acusticus) are thicker than the dorso-lateral sides, suggests that, when the otolith stops growing ventrally, $CaCO_3$ continues to be deposited in the former area. Thus, the ventral growth increments become congested and interrupted and can not be used for age and growth estimation. Otolith growth towards the sulcus face has also been observed in other species (e.g. Gauldie & Nelson 1990).

Deposition of calcium carbonate in the sagittae of *Pomatoschistus lozanoi* was also reduced after sexual maturity. However, restriction of growth mainly occurred in the postrostral and - to a lesser extent- pararostral parts.

The morphological changes in the sagittae of *Pomatoschistus minutus* and *P. lozanoi* thus suggest that the sagittae of larvae and juveniles are light enough to be floating in the endolymphatic fluid, and that the heavier sagittae of sexually mature individuals will sink to the otic cleft of the skull.

Growth of the sagittae of *Pomatoschistus minutus* was fastest in frontal and caudal direction and was slower dorsally, while in *P. lozanoi* the opposite was observed. This observation is supported by the decreasing ratio of sagitta height to sagitta total length as *P. minutus* grew (Figure 3.11). The fact that the sagittae of *P. lozanoi* grew slower in the caudal direction than dorsally may also be correlated with its maximum size. The maximum size of *P. lozanoi* is smaller (65 mm SL) than that of *P. minutus* (78 mm SL), which possibly makes posterior growth more restricted in the former species. However, this hypothesis still remains speculative and needs further investigation.

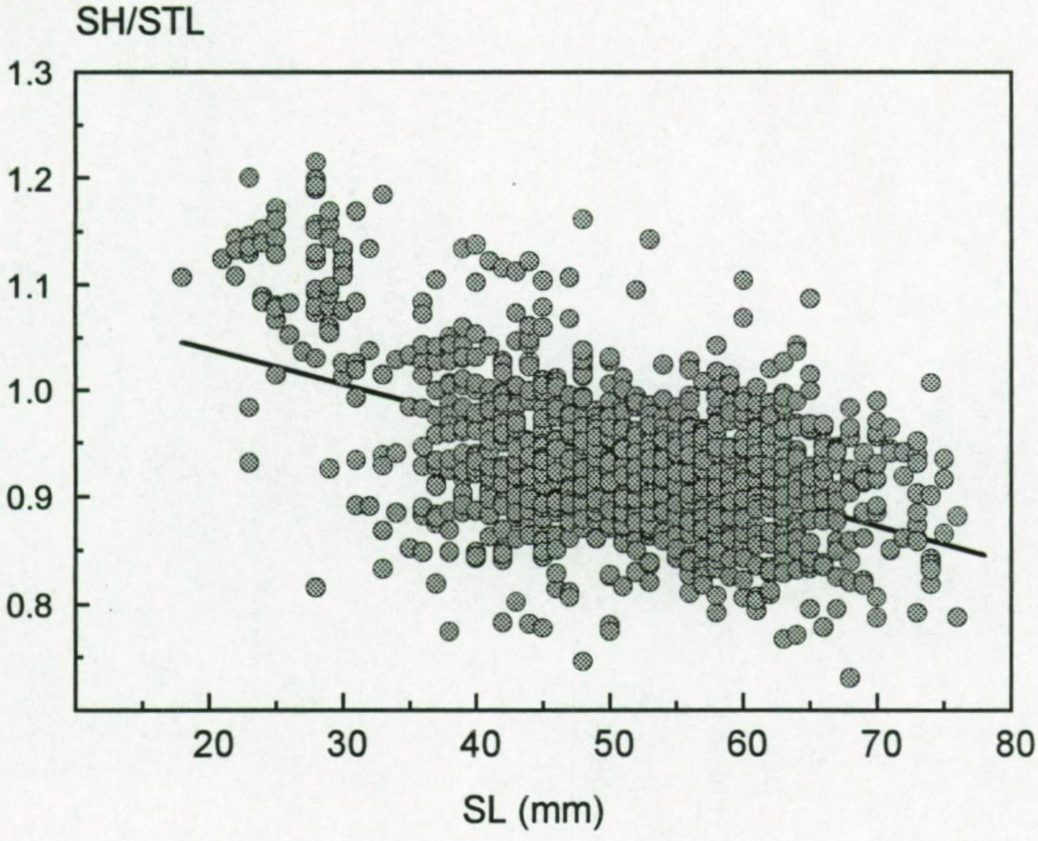


Figure 3.11. *Pomatoschistus minutus*. Relationship between fish length (SL) and the ratio of the sagitta height (SH) to the sagitta length (STL).

Growth of the asterisci and the lapilli of *Pomatoschistus minutus* and *P. lozanoi* also decreased significantly as the fish reached sexual maturity. Maintenance of shape and surface smoothness of the asterisci in all fish life stages implies that their growth is never restricted by the skull, which is probably correlated with their small sizes and light weights.

Isometry between the surface area and the weight of the asterisci of *Pomatoschistus minutus* and *P. lozanoi* indicates that the calcium carbonate deposition in the lagena is more or less equal in both species; the larger and heavier sagittae and lapilli in *P. minutus* suggests that the calcium carbonate deposition rates in the sacculus and utriculus of *P. minutus* are faster than in *P. lozanoi*.

In both species, the shape of the sulcus acusticus did not change with growth. This implies that the physical contact between the sensory epithelium of the macula and the sulcus acusticus (Fay & Popper 1985) does not deform the sulcus acusticus. However, the increasing ratio of the sulcus acusticus area to the sagitta area (Arellano *et al.* 1995) and the more distinct outline of the sulcus acusticus in adult individuals (personal observation) probably result from increasing macula growth. The sulcus acusticus is the pivot point of otolith movement and the outline of the sulcus acusticus has been observed to be very similar in size and shape to that of the macula (e.g. average ratio of 0.95 in *Hoplostethus atlanticus*) (Gauldie 1988).

3.4.2. Allometric growth

The stronger negative allometries between SL and otolith characters observed after reaching sexual maturity reflect slowing calcium carbonate deposition rates. Thus, there is evidence of uncoupling of otolith and somatic growth. This has also been observed in other species (e.g. Marshall & Parker 1982, Mosegaard *et al.* 1988, Reznick *et al.* 1989, Secor & Dean 1989, Mugiya & Tanaka, 1992). Otolith growth consists of organic matrix formation and subsequent calcification (Mugiya & Tanaka 1992). The organic matrix formation is a biological process and as such proportional to somatic growth: the matrix contains mainly proteins and protein synthesis is controlled by similar hormonal and biochemical processes for all tissues. The matrix functions both as a crystallisation site and as a crystal growth inhibitor (Crenshaw 1982). Calcification is a physico-chemical process and is less affected by biological events than matrix formation. Thus, the slowing down of the somatic growth rate will reduce organic matrix formation in the otolith. Additionally, physiological efficiency declines with age. Mashiko & Jozuka (1962) reported that the accumulation of ^{45}Ca in carp declined with age. Rosenthal (1956) also reported that the uptake rate of ^{45}Ca by guppies is higher in young than in mature fish. From these points of view, the inflection points detected at 30 mm in *Pomatoschistus lozanoi* and at 32 mm SL in *P. minutus* are probably linked with changes in hormonal and biochemical processes as fish reach sexual maturity. This finding further shows that back-calculations using otolith readings will overestimate growth in adults and underestimate growth in juveniles, if the whole data set is treated as one regression.

3.5. Conclusions

The external appearance, position and location of the otoliths in the skull of *Pomatoschistus minutus* and *P. lozanoi* are relatively similar. However, the sagittae and lapilli of *P. minutus* are larger and heavier than the sagittae and lapilli of *P. lozanoi*. The asteriscus surface areas and weights of both species are not significantly different, suggesting that calcium carbonate deposition rates in the lagena are similar. Allometry of the sagittae can be used as a taxonomic tool to distinguish between these closely related and morphologically highly similar species. Both species showed uncoupling of otolith growth and somatic growth at about 30 mm SL. Thus, otoliths can be used as an index of fish maturity.

CHAPTER 4.

Changes in the ratio of the sulcus acusticus area to the sagitta area of *Pomatoschistus minutus* and *P. lozanoi* (Pisces, Gobiidae).

Abstract

The *Pomatoschistus minutus* (Pallas) and *P. lozanoi* (de Buen) stock of the ebb-tidal delta of the former Grevelingen estuary was sampled monthly from September 1992 to October 1993. Sagittae were extracted and the areas of the sagitta and sulcus acusticus were measured with a digitizer. The frequency response and auditory threshold of these two sympatric goby species were determined from the ratio of the sulcus acusticus area to the sagitta area (S:O ratio). The S:O ratio of *P. minutus* increased while in *P. lozanoi* the S:O ratio was constant with increasing standard length. The average S:O ratio of *P. lozanoi* was significantly higher than that of *P. minutus* of the same mean length, which makes the former more sensitive to sound frequency. The S:O ratios of these two sympatric fishes were lower than the S:O ratios of demersal and pelagic fishes available from the literature. An interspecific growth difference of sagitta and sulcus acusticus was observed. The sagitta and sulcus acusticus of *P. minutus* are larger than those of *P. lozanoi* of the same length.

4.1. Introduction

Otoliths are useful sources of information on age (Campana & Neilson 1985, Gauldie & Nelson 1990, Jones 1992), daily growth (Pannella 1980, Campana & Neilson 1985), biological records of environmental changes (Pannella 1980, Radtke 1984a), equilibrium and acceleration (Stevenson & Campana 1992), and sound transduction of teleost fishes (Parker 1908, Morris & Kittleman 1967, Popper 1977, Fay 1980, Schuijf 1981, Fay & Popper 1985, Gauldie 1988). They are also used for stock identification (Parrish & Sharman 1959, Jones 1992), as a taxonomic tool in fossil fish species (Gaemers 1984).

The sagittae, one of the three pairs of otoliths, which are located in the saccular vestibule of the pars inferior of the vestibular apparatus (Fay & Olsho 1979, Secor *et al.* 1991) are peripheral mechanoreceptors (Schuijf 1981, Secor *et al.* 1991). The sagitta acts as a transmitter of mechanical stimuli to the cilia of the macula inserted in the sulcus acusticus

(Morris & Kittleman 1967). Gauldie (1988) postulated that the ratio of the macula area to the otolith area (M:O ratio), and subsequently the ratio of the sulcus acusticus area to the otolith area (S:O ratio), are related to the frequency response and auditory threshold of the sagitta (see Table 4.1).

Table 4.1. Macula:otolith (M:O) ratio for the sagittae ± SD (standard deviation).

Source	**Species**	**M:O ratio (± SD)**	**Sample size**
Gauldie (1988)	*Thunnus albacores*	0.540	2
	Leionura atun	0.426	1
	Gadus morhua [a]	0.362	3
	Arripis trutta	0.233	1
	Cheilodactylus macropterus	0.229	1
	Pseudophycis bacchus	0.222	1
	Cheilodonichthys kumu	0.216	1
	Hoplostethus mediterraneus	0.202 (±0.020)	10
	Trachurus declivis	0.202 (±0.015)	18
	Hoplostethus atlanticus	0.195 (±0.026)	26
	Tautoga onitis [a]	0.195	3
	Perca fluviatilis [a]	0.175	4
	Seriotella brama	0.173	1
	Genypterus blacodes	0.171	1
	Kathestoma giganteum	0.149	1
	Rhombosolea plebeia	0.129	3
Lombarte (1992)	*Merluccius capensis* [a]	0.281 (±0.024)	239
	Merluccius paradoxus [a]	0.312 (±0.028)	225
Present study	*Pomatoschistus minutus* [a]	0.084 (±0.016)	224
	Pomatoschistus lozanoi [a]	0.092 (±0.015)	224

[a] M:O calculated from sulcus:otolith (S:O) ratio by the different authors.

The aim of the present paper is to determine whether there are interspecific and ontogenic differences in the frequency response and auditory threshold of two sympatric gobies *Pomatoschistus minutus* and *P. lozanoi*. The S:O ratios of these species were compared to the S:O ratios of other species available from the literature.

4.2. Materials and methods

The right and left sagittae were extracted from *Pomatoschistus minutus* and *P. lozanoi* collected on a more or less monthly basis from the ebb-tidal delta of the Grevelingen area (southwest Netherlands) from September 1992 to October 1993. The standard length (SL: from the tip of the lower jaw to the end of the notochord) of the preserved fish were measured to the nearest mm and converted to fresh SL length (Arellano *et al.* submitted a).

The areas of the right and left sagittae and the sulcus acusticus were drawn with the aid of a drawing tube and a binocular microscope at 50x magnification. The sagittae were air-dried to facilitate distinguishing the sulcus acusticus area (Figure 4.1). The two areas, expressed in mm^2, were measured with a digitizer (Hipad Plus, Tbasic).

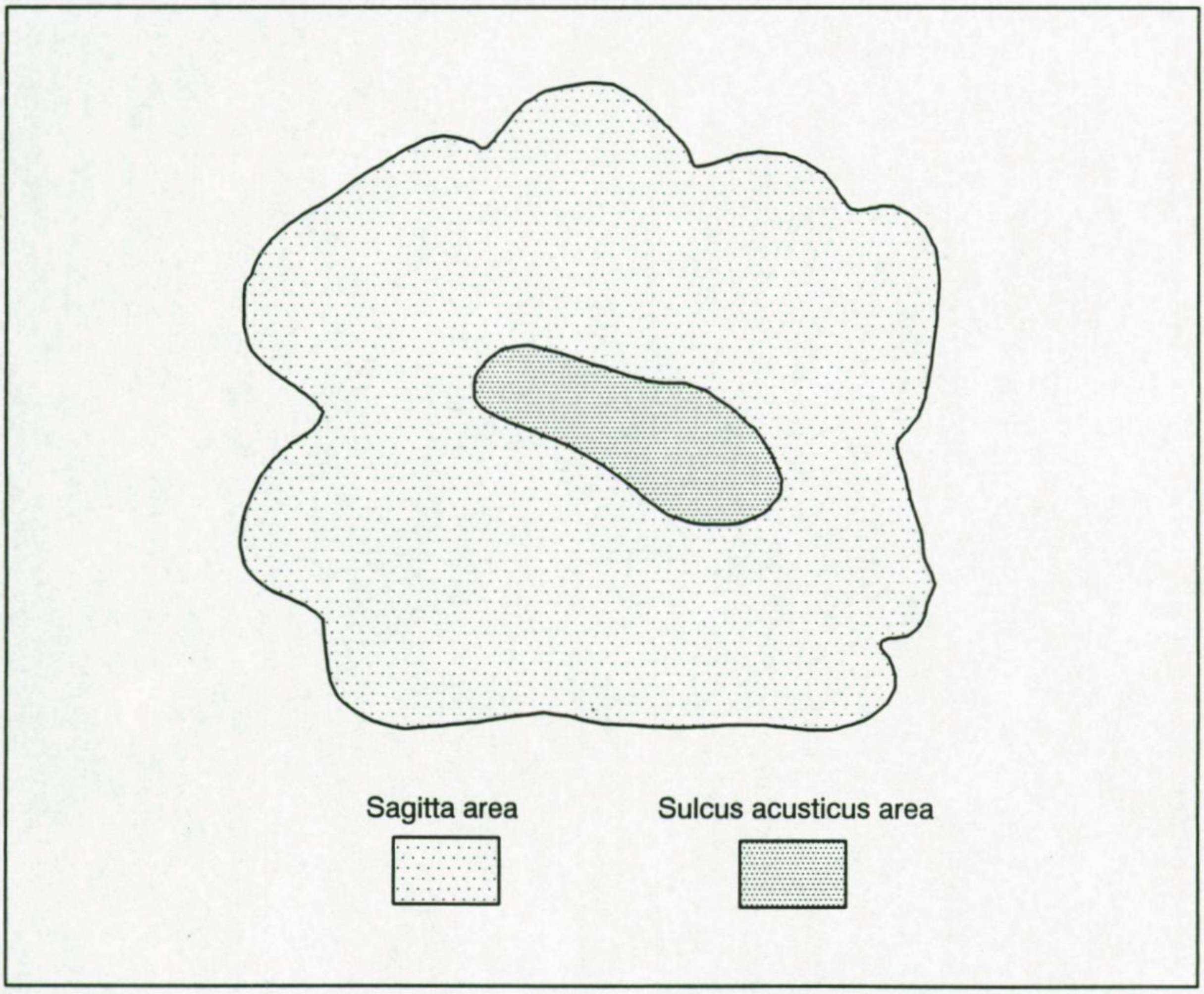


Figure 4.1. *Pomatoschistus minutus*. Proximal side of a left sagitta with the areas measured.

The presence of extreme outliers was detected by means of a casewise plot of residuals. Observations falling outside the mean ± 3 * SD were considered as outliers. Log-transformation of the data, e.g. power function relationship, was applied when the observed residuals did not follow the normal expected line in the normal probability plot. Homogeneity of variances was assessed by Bartlett's Test (Sokal & Rohlf 1981), prior to the analyses of variance (One-Way Between-Group ANOVA) on the areas of sagitta and sulcus acusticus at the 5% level of significance. Tests were done between left and right sagittae and sulcus acusticus and between both species of the same mean length. If differences were not significant, the ratio of the sulcus acusticus to the sagitta was calculated as the mean of the right and left sagittae and sulcus acusticus.

The allometric growth of the sagitta and the sulcus acusticus was determined relative to fish growth in length by the slope of the regression equations and by the distribution of the data points in the scatterplot. A slope equal to 2 represents isometry and slopes significantly different from 2 represent either positive (> 2) or negative allometry (< 2). For the growth of the sulcus acusticus area relative to the sagitta area, a slope equal to 1 represents isometry. These slope values were only applied when the dependent and independent variables showed a power function relationship (Gould 1966). To determine if the S:O ratios of the two species changed with respect to the increase in SL of the fish, the slope values were tested. If the slope value was significantly different from 0 (> 0) the S:O ratio changed as the fish grew. The S:O ratios between species were compared by means of ANOVA. Analysis of covariance (ANCOVA) was used to compare the slopes. The confidence limits of the slope at 95% level were computed.

4.3. Results

A total of 1109 *Pomatoschistus minutus* (599 males with a size range of 18 to 79 mm and 510 females with a size range of 22 to 75 mm) and 696 *P. lozanoi* (367 males with a size range of 18 to 55 mm and 329 females with a size range of 20 to 55 mm) were used in this study.

4.3.1. Comparison of sagitta and sulcus acusticus

All data showed homogenous variances and were normally distributed. The areas of the right and left sagittae and sulcus acusticus showed no significant differences for both *Pomatoschistus minutus* and *P. lozanoi*. The data were subsequently pooled to represent averages of the areas of the right and left sagittae and sulcus acusticus in each species. The areas of both sagittae and sulcus acusticus were larger in *P. minutus* than in *P. lozanoi* (F

= 61.55, $p<0.05$ for the sagitta area; F = 32.16, $p<0.05$ for the sulcus acusticus area).

4.3.2. Growth of the sagittae and sulcus acusticus

The different relations between the sagitta area (SA), sulcus acusticus area (AS) and SL for both *Pomatoschistus minutus* and *P. lozanoi* are given in Table 4.2.

The growth of the sagitta area relative to fish growth was significantly different between species. In *Pomatoschistus minutus*, allometry was positive ($b = 2.08 \pm 0.07$, $t = 2.51$, $p<0.025$) and in *P. lozanoi* allometry was negative ($b = 1.71 \pm 0.05$, $t = 10.60$, $p<0.025$). The rate of otolith growth was significantly higher in *P. minutus* than in *P. lozanoi* ($F = 444.7$, $p<0.001$).

Table 4.2. *Pomatoschistus minutus* and *P. lozanoi*. Relations between sagitta area (SA), sulcus acusticus area (AS) and standard length (SL) for the two gobies.

Species	**n**	**r**	**p**
P. minutus			
$SA = 0.00463\ SL^{2.08}$	214	0.97	<0.05
$AS = 0.0000167\ SL^{2.31}$	214	0.96	<0.05
$AS = 0.082\ SA^{1.13}$	222	0.98	<0.05
P. lozanoi			
$SA = 0.00194\ SL^{1.71}$	220	0.97	<0.05
$AS = 0.000135\ SL^{1.78}$	220	0.93	<0.05
$AS = 0.09\ SA^{1.03}$	220	0.93	<0.05

In both species the growth of the sulcus acusticus area in relation to the fish growth showed a similar pattern as the growth of the sagitta area ($b = 2.31 \pm 0.09$, $t = 6.63$, $p<0.025$ in *Pomatoschistus minutus*; $b = 1.78 \pm 0.10$, $t = 4.59$, $p<0.025$ in *P. lozanoi*). The slopes of the growth curves were significantly higher in *P. minutus* than in *P. lozanoi*

(F = 369.2, $p<0.001$).

The growth of the sulcus acusticus area relative to the sagitta area in *Pomatoschistus minutus* showed positive allometry (b = 1.13 ±0.03, t = 8.55, $p<0.025$) while growth in *P. lozanoi* was isometric (b = 1.03 ±0.05, t = 1.36, $p>0.025$). The growth of the sulcus acusticus area of *P. lozanoi* was geometrically similar to the growth of the sagitta area. The slope of the growth curve was significantly greater in *P. minutus* than in *P. lozanoi* (F = 19.71, $p<0.001$).

4.3.3. S:O ratio in relation to fish length

The relationship of the S:O ratio to fish length was linear (Figure 4.2). The regression for *Pomatoschistus minutus* was significant (F = 60.50, $p<0.05$) while the *P. lozanoi*

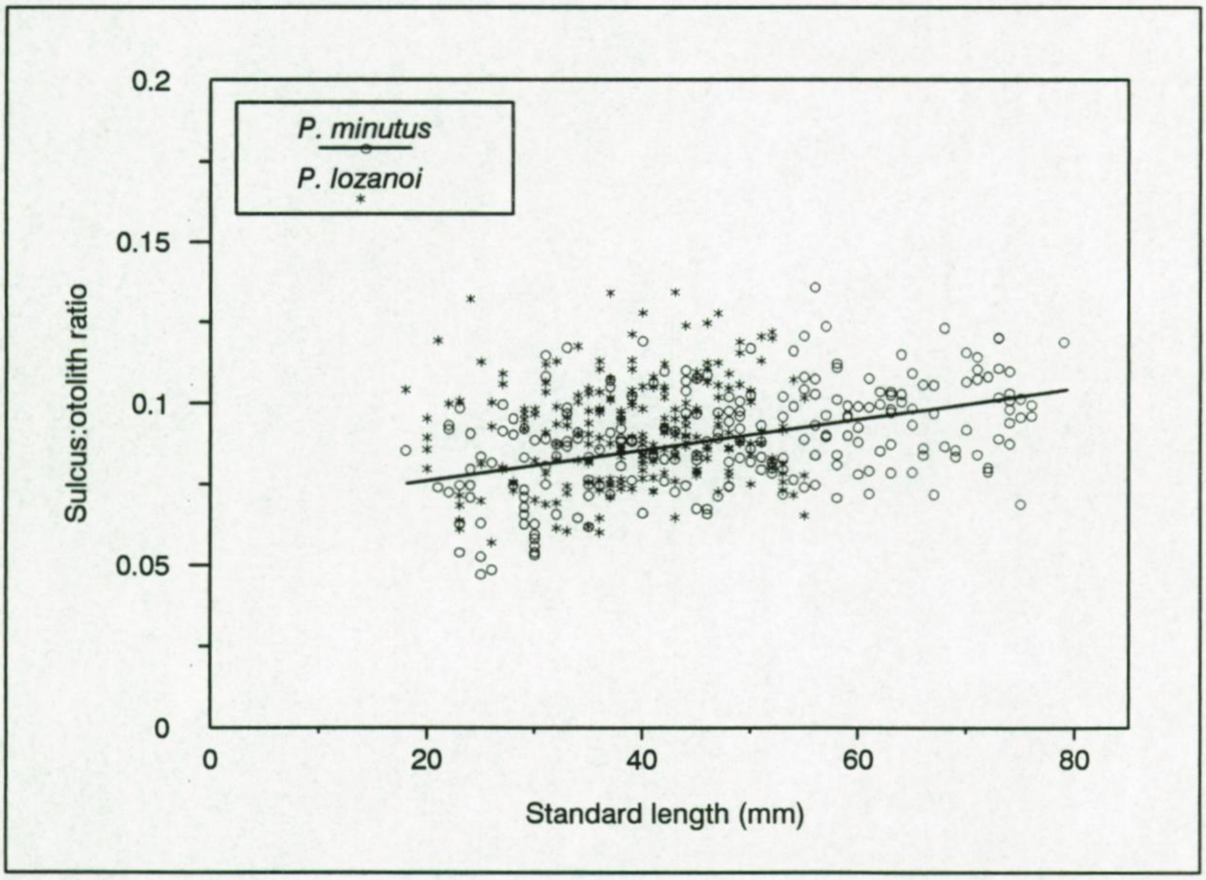


Figure 4.2. *Pomatoschistus minutus* and *P. lozanoi*. Relationship between sulcus:otolith ratio (S:O) and standard length (SL, mm) in *P. minutus* (S:O = 0.066 + 0.000483SL) and *P. lozanoi* (S:O = 0.084 + 0.000193SL).

regression was not ($F = 2.66$, $p > 0.05$). The slope was significantly different from 0 for *P. minutus* ($b = 4.83 \times 10^{-4} \pm 1.2 \times 10^{-4}$, $t = 7.89$, $p < 0.025$). There was a predictable relationship between the S:O ratio and fish length in *P. minutus*, but not in *P. lozanoi*. The average S:O ratio of *P. lozanoi* was significantly higher than that of *P. minutus* ($F = 16.18$, $p < 0.025$) of the same mean length (range 20 to 55 mm).

4.4. Discussion and conclusions

4.4.1. Allometric growth

The shapes of the otoliths of teleost fishes are complex and mostly species-specific (Nolf 1985). In very rare cases (three observations in *Pomatoschistus minutus* and 1 observation in *P. lozanoi*), the shapes of the right and left sagittae were different due to aberrant calcium deposition on one side. In these cases, the sagitta's function for balancing and equilibrium is not met. The detection of homogeneous variances in the data and the overall geometrical similarities between the right and left sagittae indicate that no sex-related intraspecific growth variation occurs.

Still, in these two morphologically very similar sympatric gobies, differences in the allometric growth of the sagittae were detected. The differential increase of sagittal growth (positive allometry) in *Pomatoschistus minutus* and the differential decrease (negative allometry) of sagittal growth in *P. lozanoi* with respect to fish growth indicate that the sagitta attains a larger relative size in *P. minutus*. The interspecific growth differences of the sagittae are related to differences in size of the skull and the endolymphatic sac. Sagittal growth at the ventral side is known to be restricted by the groove in the base of the otic cleft (Gauldie & Nelson 1990). Furthermore, the body structure of *P. minutus* is more robust than *P. lozanoi* of the same length (Hamerlynck 1990, personal observation). Although the relative sizes of the endolymphatic sac and the skull were not measured, it is deduced that *P. minutus* accommodates larger sagittae than *P. lozanoi*. This observation is not common in other congeneric teleost fishes. An example is the growth of the sagittae and the sulcus acusticus of *Merluccius capensis* and *M. paradoxus*, which both showed negative allometric growth with respect to fish length (Lombarte 1992).

The ecological significance and behavioral differences of the two goby species are reported in several studies. *Pomatoschistus minutus* and *P. lozanoi* are demersal marine species which spend part of the year in the estuarine environment as adults (Hamerlynck *et al.* 1993b). They are the most abundant goby species of the *Pomatoschistus* complex along the shallow waters of the North Sea (Hamerlynck 1993). In general, gobies are important links in the trophic chain to fishes, birds, reptiles and mammals (Hamerlynck 1993) and they are the main prey of gadoids (Granitto 1985, Fosså 1991, Salvanes *et al.* 1992, Hamerlynck & Hostens 1993). In the Belgian coastal area, *P. minutus* spawned earlier (March to June) than *P. lozanoi* (June to August) (Hamerlynck 1990). Despite the fact that these two species coexist in shallow water areas, they showed quite different food niches (Hamerlynck *et al.* 1990). Both species feed more intensively during the night (Gibson & Hesthagen 1981, Berge *et al.* 1983) due to predator avoidance (Hamerlynck *et al.* 1993a). *P. lozanoi* is considered a specialist which feeds more on pelagic and hyperbenthic species while *P. minutus* is a generalist which feeds more on benthic species (Hamerlynck *et al.* 1990, Hamerlynck & Cattrijsse 1994).

Based from the ecological significance and behavioral differences between *Pomatoschistus minutus* and *P. lozanoi*, the differences in the allometric growth of the sagittae and sulcus acusticus are most probably related to the different food niches of these two species.

4.4.2. Hearing sensitivity

There are two major pathways for sound to the inner ear: through the swimbladder (indirect path, see below) and through direct stimulation of the ears by impinging particle motion (direct path). In the direct path, Fay and Popper (1985) stated that as the fish's body tissue moves at almost the same amplitude as the surrounding water particles, the otoliths move at different amplitudes and in different phases due to their greater inertia. With this movement, bending occurs between the sensory hairs of the macula and the sulcus acusticus of the sagitta, which results in stimulation of the sensory hair cells.

The relative morphological designs of the sulcus acusticus and the sagittae have been used to determine the hearing sensitivity of fish (Gauldie 1988, Lombarte 1992). The relation between the frequency response and the auditory threshold function and the shape of the otolith (M:O ratio) was modelled by Gauldie (1988). The sagitta acts as a long lever pivoted at the sulcus acusticus with a fixed short lever at the pivot point provided by the protein matrix which connects the sagitta to the hair cells of the macula. Any movement of the long lever results in a displacement of the short lever. For the same movement of the two levers less shear displacement is generated by the long lever. Thus, the model emphasizes that a fish with a high M:O ratio (greater shear displacement) is more sensitive to particular sound frequencies than a fish with a low M:O ratio.

The interspecific variation of the frequency response and auditory threshold in the two goby species is due to the different allometric growth of the sulcus acusticus (AS) relative to the growth of the sagittae (SA). *Pomatoschistus lozanoi* showed no significant changes of hearing sensitivity as the fish grows (isometric growth of AS to SA), while *P. minutus* showed a progressive increase (increase in S:O ratio) in hearing sensitivity (positive allometric growth of AS to SA). But the sulcus acusticus of *P. lozanoi* is larger in size relative to the sagitta (higher average S:O ratio). Therefore, *P. lozanoi* is more sensitive to sound than *P. minutus* of the same mean length.

The trend of hearing capabilities for *Pomatoschistus lozanoi* was similar to the findings of Gauldie (1988) for fishes collected from Australian coral reefs. Observations for *P. minutus* were similar to the findings of Lombarte (1992) for fishes collected in the north Atlantic. The S:O ratios of the two gobies are relatively low in comparison with other species (Table 4.1). Generally, small demersal fishes living in shallower waters, like *P. minutus* and *P. lozanoi*, have lower S:O ratios than the larger demersal fishes living in deeper waters like *Gadus morhua, Merluccius capensis* and *M. paradoxus*. Pelagic fishes have still higher M:O ratios than demersal fishes (e.g. *Thunnus albacores* compared to *Gadus morhua*) (Gauldie 1988).

In the indirect pathway of sound to the ear, the expansion and contraction of the air-filled swimbladder according to the local fluctuations in sound pressure cause fluid motion in the ears and motion of the otoliths against the sensory hairs (Von Frisch 1936).

A brief review of the literature on the gobies swimbladder by Hesthagen and Koefoed (1979) revealed that gobies may either lack or have a reduced swimbladder. Different studies revealed that both *Pomatoschistus* species have swimbladders (Fonds 1970, Hesthagen & Koefoed 1979). In addition, the swimbladder volumes (as percentage of body weight) of *P. minutus*, collected in Oslofjord (Norway), were significantly greater in May (4.7%) than of those caught in June (2.5%) (Hesthagen & Koefoed 1979). This temporal variation seems to be correlated with the more benthic life style in June. For *P. lozanoi*, it is not clear if there is a temporal variation of the swimbladder volumes (Fonds 1970).

The more benthic mode of feeding of *Pomatoschistus minutus* is evidence of less movements of their otoliths, which is justified by the lower average S:O ratio. The higher average S:O ratio of *P. lozanoi* is probably linked with vertical migration during the search for food as this species has a more pelagic mode of feeding. Vertical migration would create an intense physical contact between the sensory hairs of the macula and the sulcus acusticus. This physical contact provides a fulcrum for the rotation of the otolith that generates the shearing forces to activate the kinocilia of the macula (Schuijf 1981). Thus, a wider area of sulcus acusticus relative to the area of the sagitta is expected. In addition, during this upward movement the expansion and contraction of swimbladder is more intense than when the fish is more or less stationary at the bottom.

Such speculations are supported by previous studies (e.g. Gauldie 1988, Lombarte 1992) that pelagic fishes are more sensitive to sound frequencies than demersal fishes.

Additionally, *Pomatoschistus minutus* and *P. lozanoi* were collected from eight sampling stations in two depth strata in the ebb-tidal delta of the Grevelingen from September 1992 to July 1994. Analysis of length-frequency data revealed that *P. minutus* were more abundant (72%) in deeper water while *P. lozanoi* were more abundant (55%) in the shallower water (Arellano *et al.* submitted e). This habitat segregation may be a behavioral response to avoid competition. Hamerlynck *et al.* (1990) observed food resource partitioning between these species. In addition, avoidance from visual predators may contribute to the higher abundance of *P. lozanoi* in the shallower waters. This correlates with the lower water transparency recorded in the 5 m depth stratum.

The progressive increase in the S:O ratios in *Pomatoschistus minutus* as it grows indicates that the sulcus acusticus (b = 2.31) grows faster than the sagitta (b = 2.08), despite the fact that they both show positive allometry. This conclusion is similar with the findings for marine gadoids in northern Alaska (Frost & Lawry 1981), for macrourid fishes of the Pacific and Atlantic oceans (Wilson 1985), and for marine gadoids in the southeast Atlantic (Lombarte 1992). They associated this effect with water depth. Other environmental factors, such as lower temperatures (Morales-Nin 1987), can slow otolith growth. These observations may be applied to *P. minutus*, which are more abundant in deeper waters.

In the present study, it is concluded that even two congeneric sympatric species whose sagittae have similar morphological features have different trends in their hearing capabilities. This is related to a differences in the S:O ratio, which is most probably due to the differences in their food and spatial niches.

CHAPTER 5.

Validation of the daily growth increments in the sagittae of *Pomatoschistus minutus* and *P. lozanoi* (Pisces, Gobiidae).

Abstract

The use of the sagittae of two sympatric gobies *Pomatoschistus minutus* (Pallas) and *P. lozanoi* (de Buen), for the determination of their age was validated following the 3 criteria of Fowler (1990). We tested whether (1) the otoliths display growth increments, (2) the deposition rates of the growth increments correspond to regular time scales, and (3) otoliths continue to grow throughout the species' life. The deposition rate of the growth increments in the sagittae of both species was determined with statistical inference techniques. The sagittae of both species displayed periodic thick opaque zones (checks) with alternating fine striations of translucent and opaque zones (growth increments). When viewed under the scanning electron microscope, they showed distinct deep grooves (checks) with alternating slightly etched wide zones and deeply etched narrow zones (growth increments). The number of growth increments in the first check at the sagitta nucleus (12 to 22) corresponded to the period of hatching of both species under laboratory conditions (Fonds 1973). The second check corresponded to the time of transition from a pelagic to a demersal life style (settlement check). The number of growth increments in the periodic checks (12 to 15, mode of 14) was correlated either to the number of days between full moon and new moon or between the first and last quarters of the moon cycle. The statistical inference technique revealed that the growth increments in the sagittae of both species were deposited at a rate of 1 per day. The sagittae of both species grew throughout the species' life span, though the growth rate changed abruptly when at the time of sexual maturity (30 mm standard length). Thus, the 3 criteria for validation are fulfilled and it is concluded that the sagittae of both species can be used for age and growth studies.

5.1. Introduction

Information on both age and growth of teleost fish can be obtained from the analysis of length-frequency data (e.g. Pauly 1984, Sparre *et al.* 1989, Longhurst & Pauly 1987), mark-recapture studies (e.g. Longhurst & Pauly 1987, Wootton 1990) and/or direct ageing from hard parts such as bones, scales, vertebrae and otoliths (Campana & Neilson 1985). Difficulties with sampling gear selection and continuous spawning are often sources of error or uncertainty in the interpretation of the shifts of modes in length-frequency distributions (Longhurst & Pauly 1987). Poor recovery of samples, high mortality and loss of tags can result in imprecise estimates of age and growth in mark-recapture studies (Wootton 1990). Direct ageing techniques are often preferred because they are least prone to subjective interpretation, and only small samples are needed to construct accurate growth curves (Hoedt 1992). In temperate areas, the fisheries management sectors mostly rely on direct ageing, and otoliths are preferred above other hard parts (Bagenal 1974).

Due to the uncertainty in age and growth results based on one method, comparing the results of different methods of ageing will always be required (Longhurst & Pauly 1987). Additionally, the periodicity of the growth increments in the otoliths needs to be validated (Geffen 1992, Hoedt 1992). The deposition rate of the growth increments can be validated using different techniques. Most used are marking of otoliths, monitoring of known-age larvae, statistical inferences, inferences from older fish, and marginal increment analyses (Geffen 1992). The choice between these techniques will depend on the specific purpose and situation of the study.

The present study focuses on the validation of the sagittae of *Pomatoschistus minutus* and *P. lozanoi* based on the 3 criteria of Fowler (1990): (1) the otoliths should display growth increments, (2) the deposition rates of these growth increments should correspond to regular time scales, and (3) the otoliths should continue to grow throughout the species' life. Deposition of the growth increments in the sagittae was validated by correlating the number of growth increments in the hatching marks, settlement marks, and periodic marks (see below) to the number of days before hatching, the duration of the pelagic phase (as observed in laboratory experiments) and the number of days lapsed between the lunar cycles, respectively.

5.2. Materials and methods

5.2.1. Sampling

Pomatoschistus minutus and *P. lozanoi* were collected during daytime with a beam trawl (3 m opening and 10 mm codend mesh size) from the ebb-tidal delta of the Grevelingen (Voordelta area, South-west Netherlands). Fish were collected monthly from September 1992 to October 1993. Samples were immediately preserved in neutralized formaldehyde (7% final concentration). Within 24 hours after sampling, samples were transferred to 96% ethanol for long storage.

5.2.2. Treatment of samples and data analysis

Pomatoschistus minutus and *P. lozanoi* were measured to the nearest mm standard length (SL) and the otoliths were extracted from the auditory labyrinth. Each month, a representative size-class of both sexes of both species was selected (Table 5.1). The preserved lengths were converted to fresh lengths using species-specific regression equations (Arellano *et al.* submitted a).

Table 5.1. *Pomatoschistus minutus* and *P. lozanoi*. Size distribution and numbers of otoliths for ring counts used. SD: standard deviation, N: number of samples.

	P. minutus				P. lozanoi			
Sex/Month	Size range	Mean	SD	N	Size range	Mean	SD	N
Male								
9 Sep 92	30-66	52.87	7.20	39	25-44	35.70	4.83	27
1 Oct 92	37-71	56.19	10.80	26	29-47	38.21	4.38	24
30 Oct 92	49-67	57.00	9.17	3	38-54	44.25	7.14	4
8 Dec 92	30-66	51.75	14.07	8	27-53	39.25	7.85	8
9 Feb 93	35-65	49.67	10.07	9	32-52	40.25	6.80	8
11 Mar 93	36-60	49.50	8.73	6	23-51	37.50	10.13	6
8 Apr 93	33-59	46.67	13.05	3	25-55	40.00	9.85	9
11 May 93	41-62	48.50	9.26	4	27-53	42.88	8.97	8
8 Jun 93	15-46	25.25	9.59	8	37-53	47.00	5.80	7
16 Jul 93	27-52	43.33	9.18	6	-	-	-	-
7 Sep 93	37-68	52.78	11.32	9	18-49	34.40	13.30	5
11 Oct 93	39-71	54.63	11.65	8	29-50	40.71	7.65	7
Female								
9 Sep 92	36-63	48.44	6.93	36	29-40	36.25	4.92	4
1 Oct 92	34-62	48.72	7.87	18	27-48	37.85	6.75	20
30 Oct 92	49-57	53.67	4.16	3	28-50	38.75	9.71	4
8 Dec 92	31-64	48.63	11.29	8	27-48	36.83	8.59	6
9 Feb 93	35-63	48.57	10.18	7	27-50	40.63	8.45	8
11 Mar 93	36-59	47.67	8.45	6	20-40	28.75	8.54	4
8 Apr 93	39-60	49.14	8.03	7	23-53	41.17	10.42	6
11 May 93	39-66	51.17	9.58	6	37-55	45.88	6.81	8
8 Jun 93	19-61	27.50	13.90	8	30-50	42.25	7.69	8
16 Jul 93	24-47	34.83	8.66	6	45	-	-	1
7 Sep 93	37-62	49.13	8.92	8	20-44	32.00	12.25	4
11 Oct 93	49-70	58.63	6.61	8	29-43	36.14	5.98	7

To detect the presence of internal structures such as growth increments, periodic checks and hatching marks (first criterion), the sagittae of both species were examined with a light microscope (LM) and a scanning electron microscope (SEM). For LM examination, the right sagitta was glued on a microscope slide (with EPON A and EPON B compounds) and then ground with moist course and fine sandpapers (grid sizes of 500 and 1000) until the midplane was nearly reached. Fine polishing up to the midplane was done with aluminium powder (0.05 μm grain size) on a suede cloth. For SEM examination, the left sagittae were fixed on SEM stubs with shellac, and were first ground with a silicium carbide water mixture (12 μm grain size), stopping just before the midplane was reached. Then they were polished with a fine silicium carbide water mixture (6 μm grain size) up to midplane. The apparatus used for grinding was a converted record player modified after Karakiri & von Westernhagen (1988) (Figure 5.1). The polished sagittae were washed with tap water, dried (35°C for 30 minutes) and etched with weak acetic acid (5%) for 5 to 7 minutes. The etched sagittae were gold-plated for 3 minutes before SEM viewing.

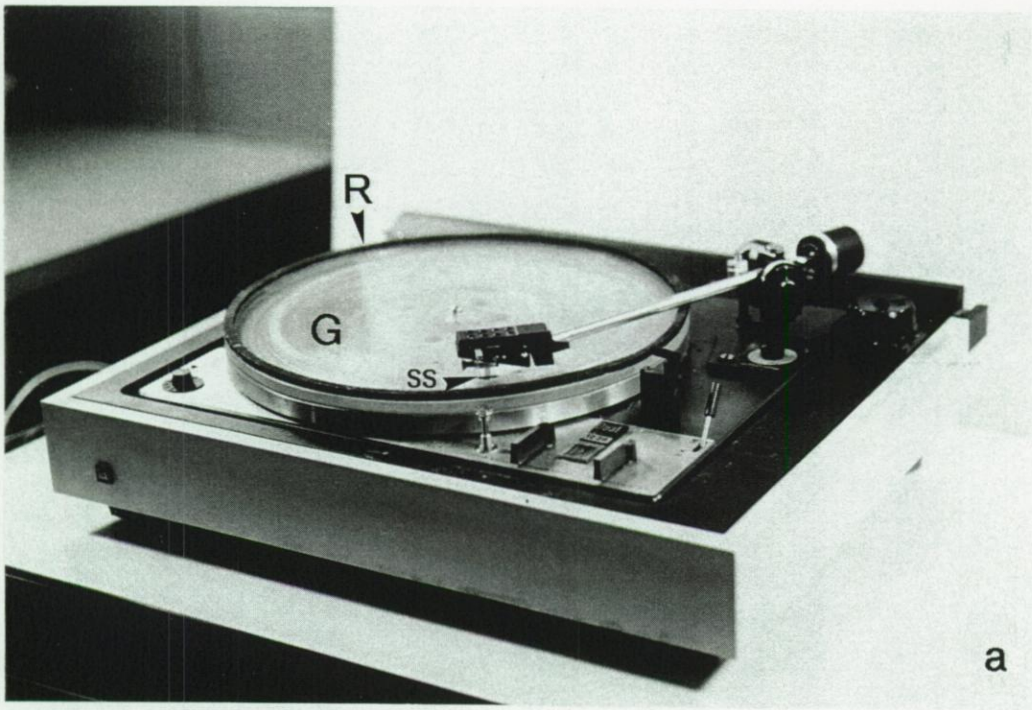
To verify whether the observed growth increments correspond to regular time intervals (second criterion), the relationship between the number of days that had lapsed between different phases of the moon and the sampling dates and the number of growth increments between the last periodic check and the outer margin of the sagittae, was investigated with linear regression analyses. A slope not significantly different from 1 then means that deposition of the growth increments occurred at a rate of 1 per day. Further, the number of growth increments in the first and second checks and the number of rings between 2 periodic checks were regressed on the number of hatching days (as reported in the literature) and the number of days in a lunar cycle, respectively.

To check whether otolith growth continues throughout the species' life span (third criterion), the sagittal lengths of *Pomatoschistus minutus* and *P. lozanoi* were regressed upon their standard lengths. If the sagittae have continuous growth, this regression should show a significant linear relationship (Fowler & Doherty 1992). Student's t-tests were used to assess whether the intercepts and slopes of the regressions were significantly different from 0 and 1, respectively (Walpole 1982).

5.3. Results

The internal structures of the sagittae of *Pomatoschistus minutus* and *P. lozanoi* are shown in Figures 5.2, 5.3, 5.4 & 5.5.

The overall pattern is identical for both species: the etched sagittae show complex



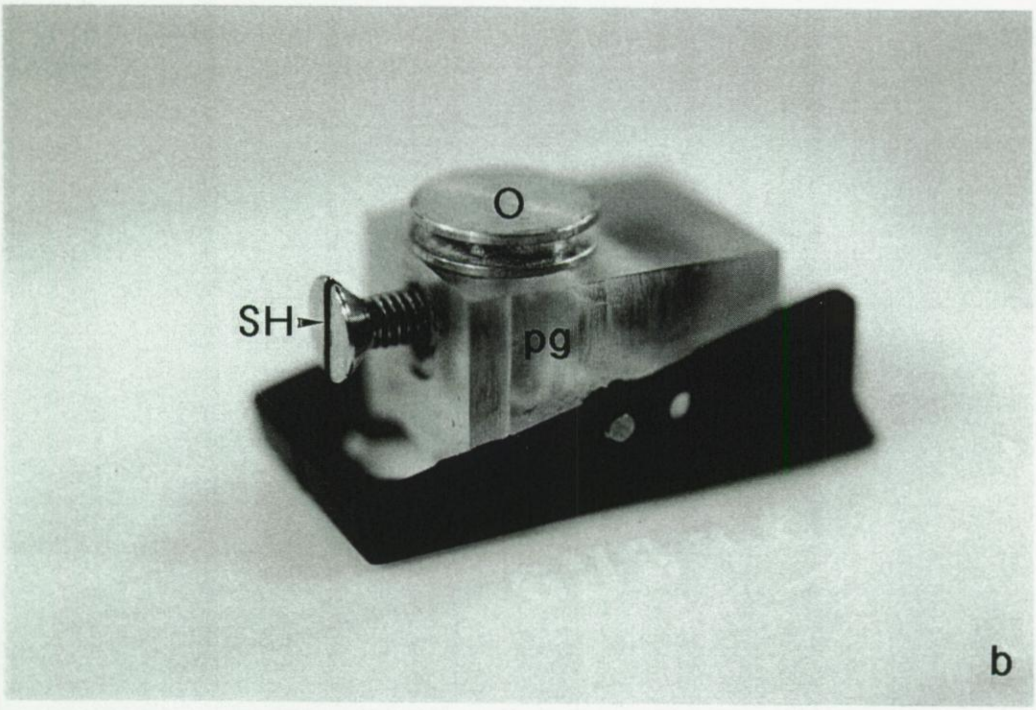


Figure 5.1. (a) Converted head of record-player in operation. SS: SEM stub with attached otolith, G: glass disc, R: rubber. (b) The converted head upside down, pg: plexiglass, o: otolith attachment area, SH: screw head.

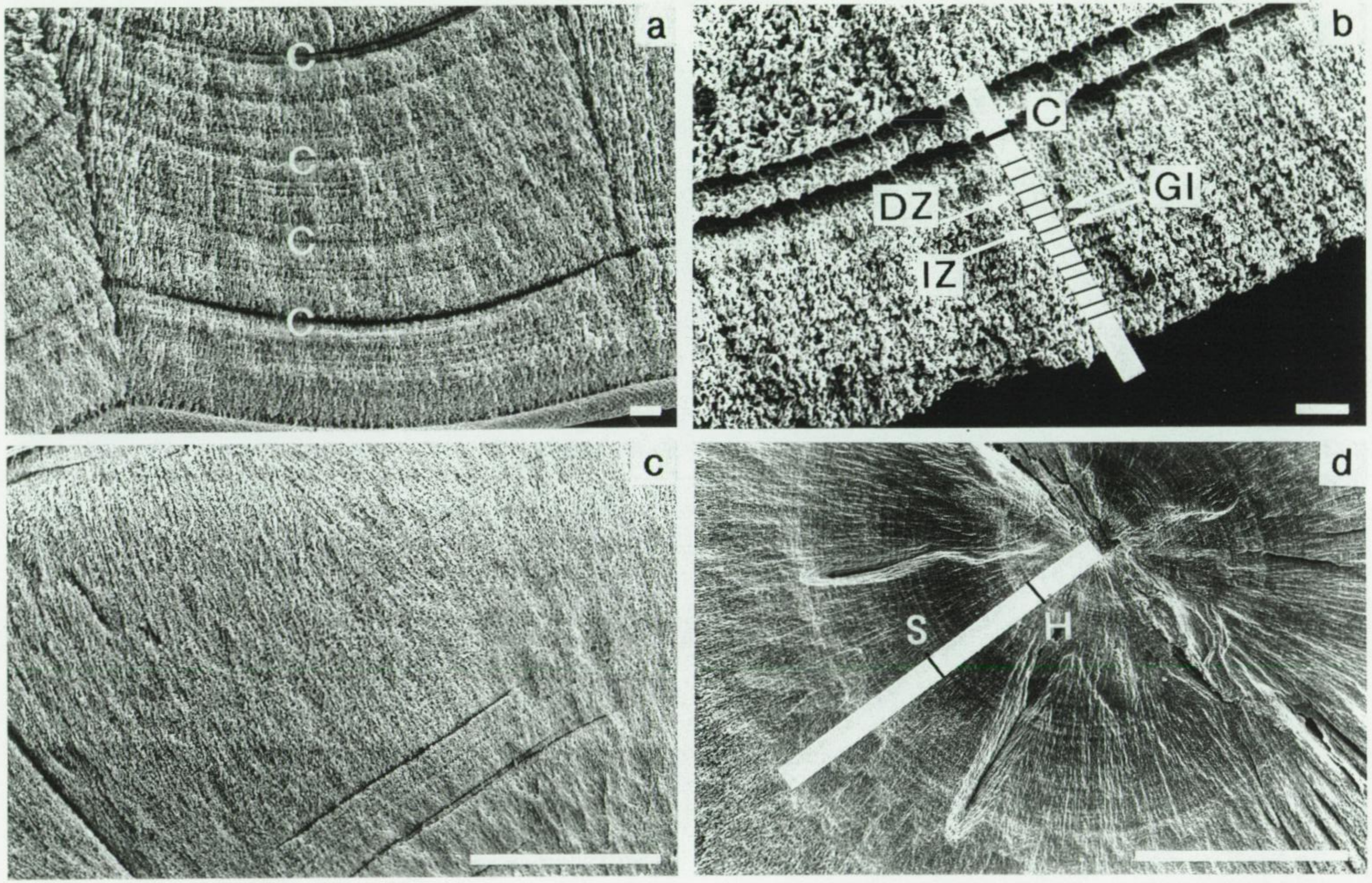


Figure 5.2. *Pomatoschistus minutus*. SEM pictures of the internal structures of the sagittae. a: periodic checks (c), b: growth increments (GI); incremental zone (IZ); discontinuous zone (DZ) in the sagitta margin, c: growth increments in the margin, d: hatching (H) and settlement (S) marks. Scale bars: a,b = 10 μm; c,d = 100μm.

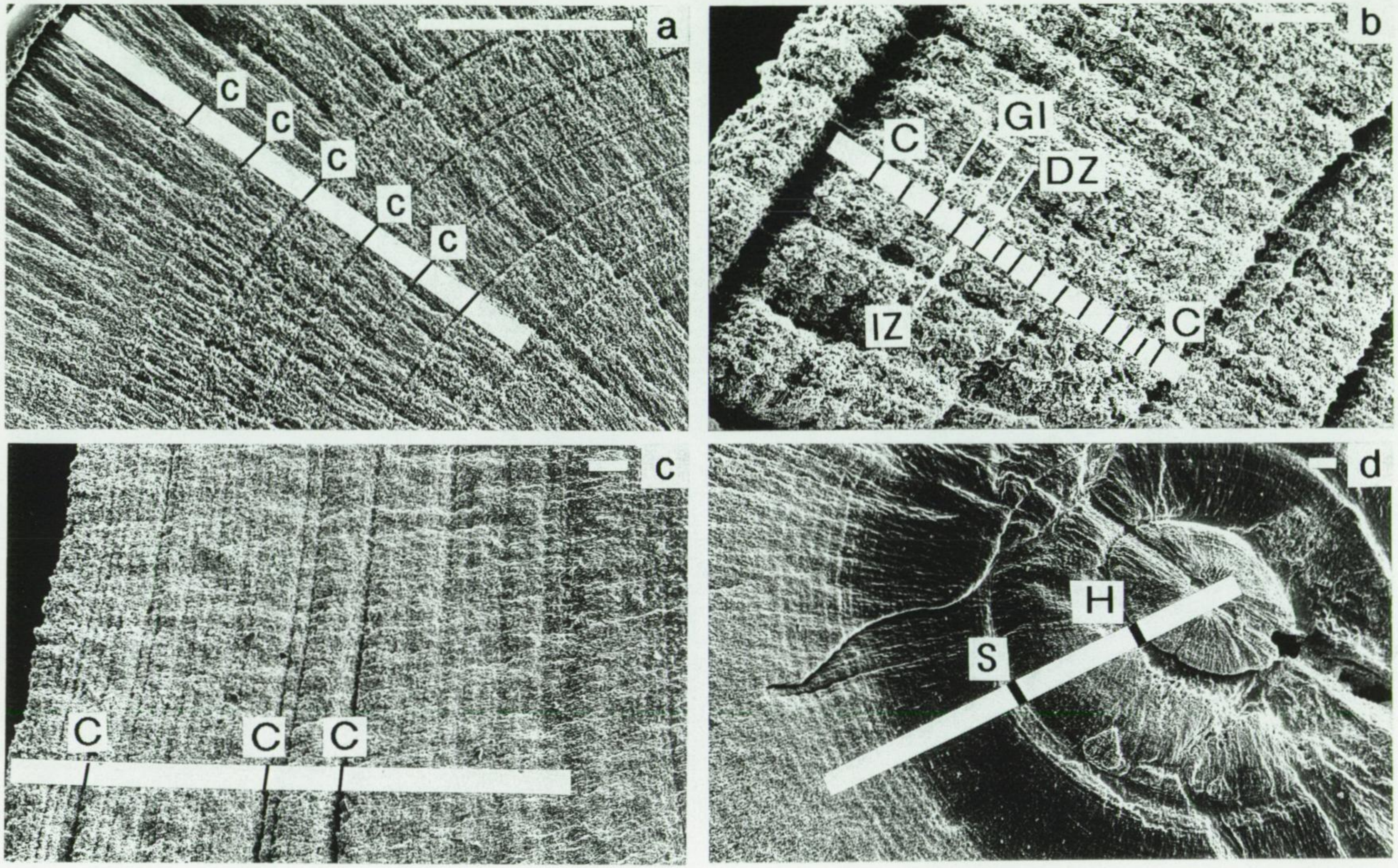


Figure 5.3. *Pomatoschistus lozanoi*. SEM pictures of the internal structures of the sagittae. a: periodic check (c), b: growth increments (GI); incremental zone (IZ); discontinuous zone (DZ) in the periodic checks, c: periodic checks and growth increments in the margin, d: hatching (H) and settlement (S) marks. Scale bars: a = 100 μm; b,c,d = 10 μm.

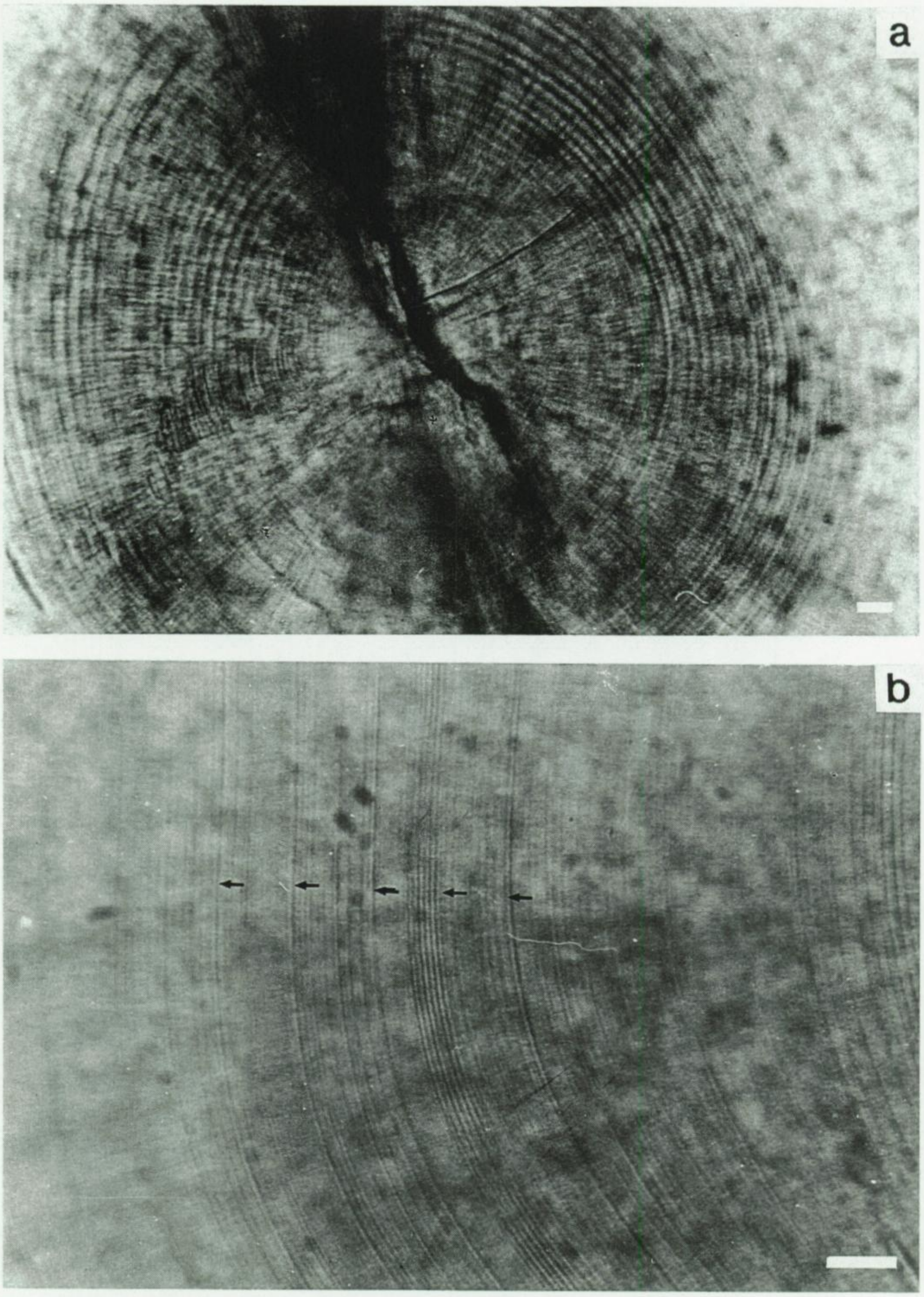


Figure 5.4. *Pomatoschistus minutus*. LM pictures of the internal structure of the sagittae. Scale bars: a & b = 10 μm.

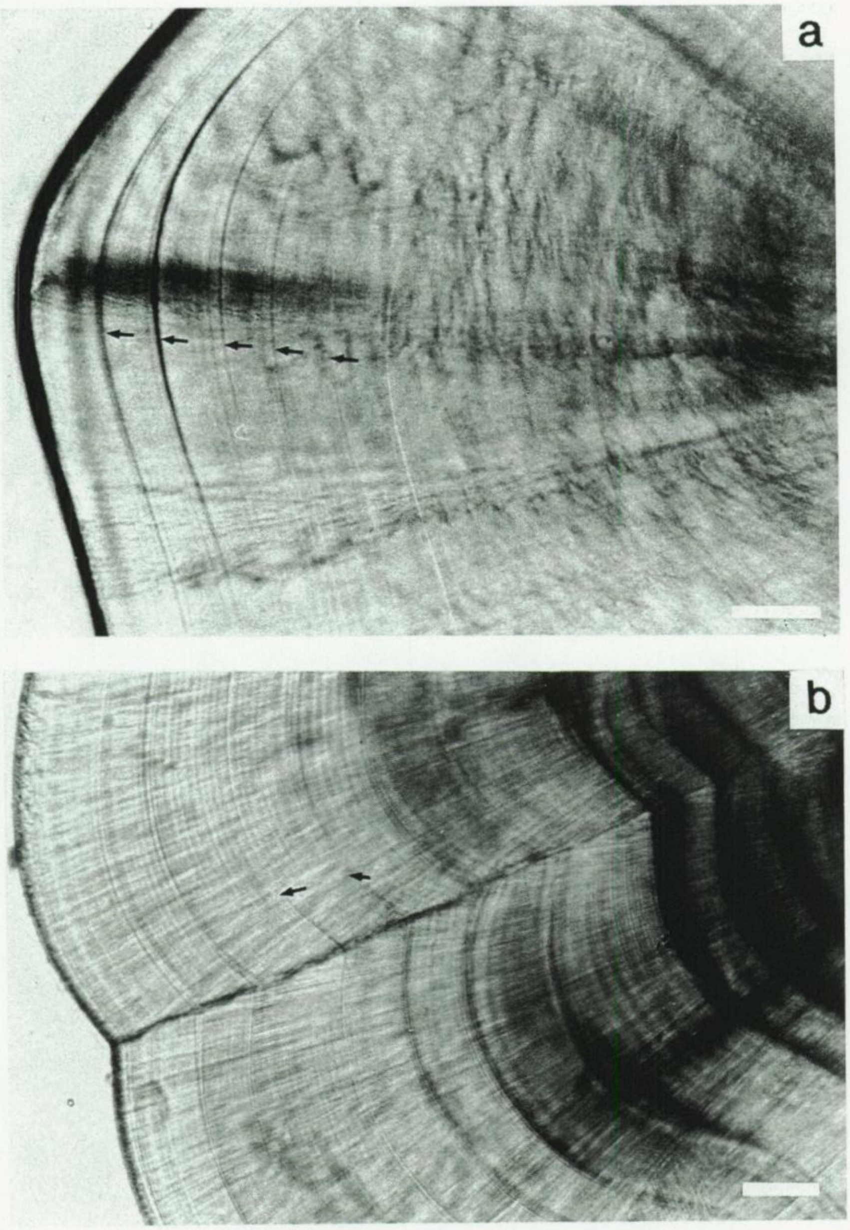


Figure 5.5. *Pomatoschistus lozanoi*. LM pictures of the internal structure of the sagittae. Scale bars: a = 50 μm, b = 30 μm.

internal structures, periodic checks being prominent features (Figures 5.2a, 5.3a & b). These periodic checks contain fine striations of 12 to 15 alternating lightly etched wide zones and deeply etched narrow zones (Figures 5.2b, 5.3b & c). Sagittae viewed under LM show alternating fine translucent and opaque zones within periodic thick opaque zones (Figures 5.4 & 5.5). Distinct checks are also observed near the nucleus of the sagittae (Figures 2d & 3d).

The relationship between the number of days between the last distinct phase of the moon (either full moon, new moon, first quarter or last quarter) and the sampling date, and the number of fine striations between the last periodic check and the outer margin of the sagittae are summarised per sex and species in Table 5.2. All regressions were linear, and only the regression of female *Pomatoschistus minutus* was not significant. None of the slopes was significantly different from 1, suggesting that the deposition of the growth increments occurred daily in the wild population. None of the intercepts of the regressions were significantly different from 0, indicating that the first growth increment was 'theoretically' deposited at birth.

Table 5.2. Regression coefficients of days that lapsed between the last phase of the moon and the day of sampling and the number of growth rings between the outermost periodic check and the margin of the sagitta. a: intercept, b: slope, r^2: coefficient of determination, N: number of samples.

				b = 1	**a = 0**		
Species	**a**	**b**	**$p<0.001$**	**$p>0.001$**	**$p>0.001$**	**r^2**	**N**
P. minutus							
Male	-0.686	0.997	***	NS	NS	0.715	133
Female	-3.960	1.225	NS	NS	NS	0.288	113
P. lozanoi							
Male	-3.255	1.202	***	NS	NS	0.329	111
Female	-3.707	1.249	***	NS	NS	0.332	79

The numbers of growth rings in the first check (hatching mark) of the sagittae of both sexes and both species are shown in Figure 5.6. In *Pomatoschistus minutus*, the number of hatching days ranged from 8 to 41 d for males (peaks of hatching from day 13 to day 22), while that of females ranged from 5 to 32 d (with peaks between day 14 and day 20). In *P. lozanoi*, the number of hatching days ranged from 6 to 32 d for males (peaks of hatching: 10 to 22 d), while that of females ranged from 8 to 24 d (peaks of hatching: 13 to 21 d).

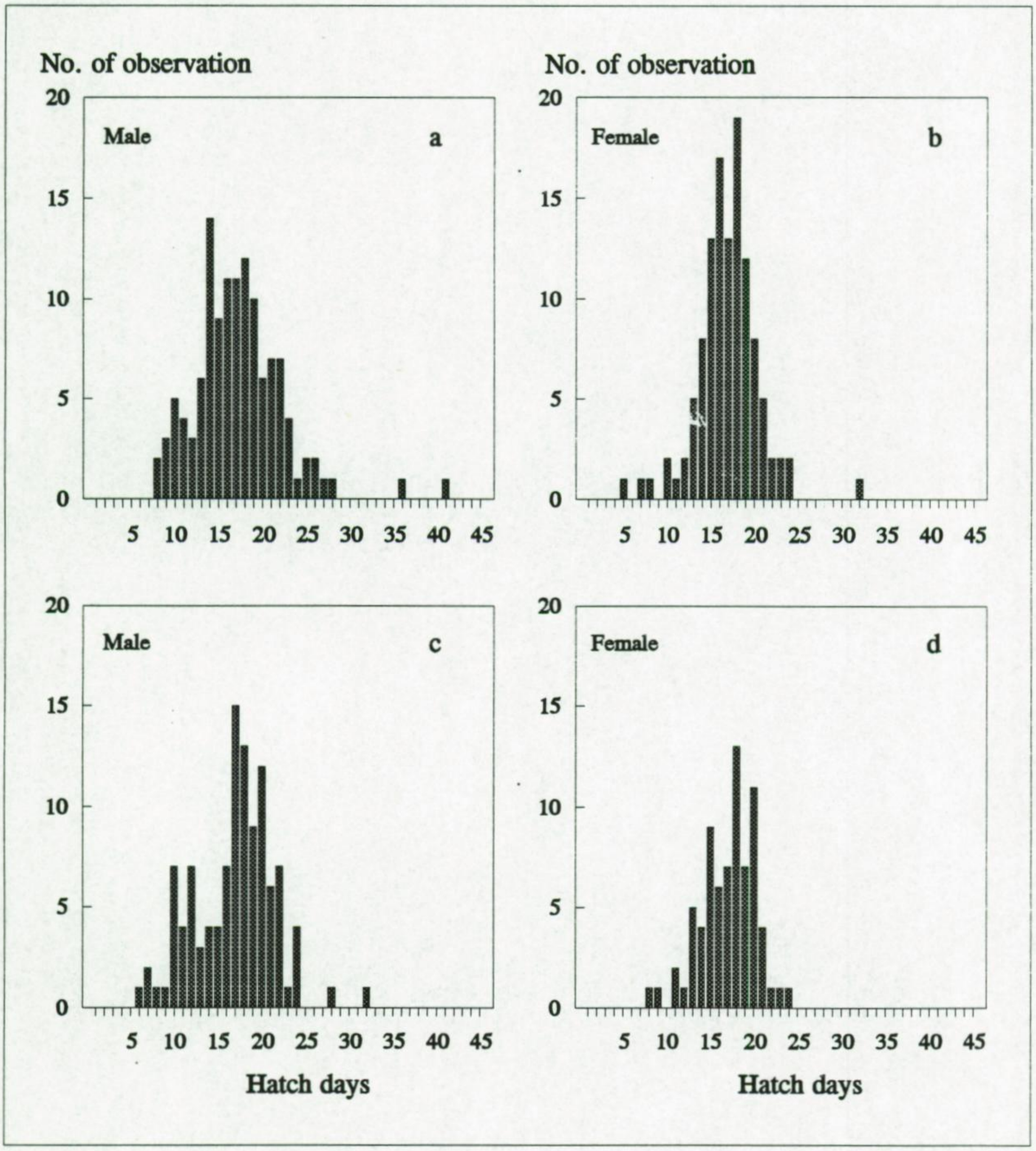


Figure 5.6. *Pomatoschistus minutus* (a,b) and *P. lozanoi* (c,d). Number of growth increments between the sagitta centre and the first check mark.

The number of growth increments between the hatching mark (first check) and settlement mark (second check) of the sagittae of both sexes of both species are shown in Figure 5.7. In *Pomatoschistus minutus*, the number of settlement days ranged from 7 to 86 d for males (peaks of settlement from day 21 to day 40), while that of females ranged from

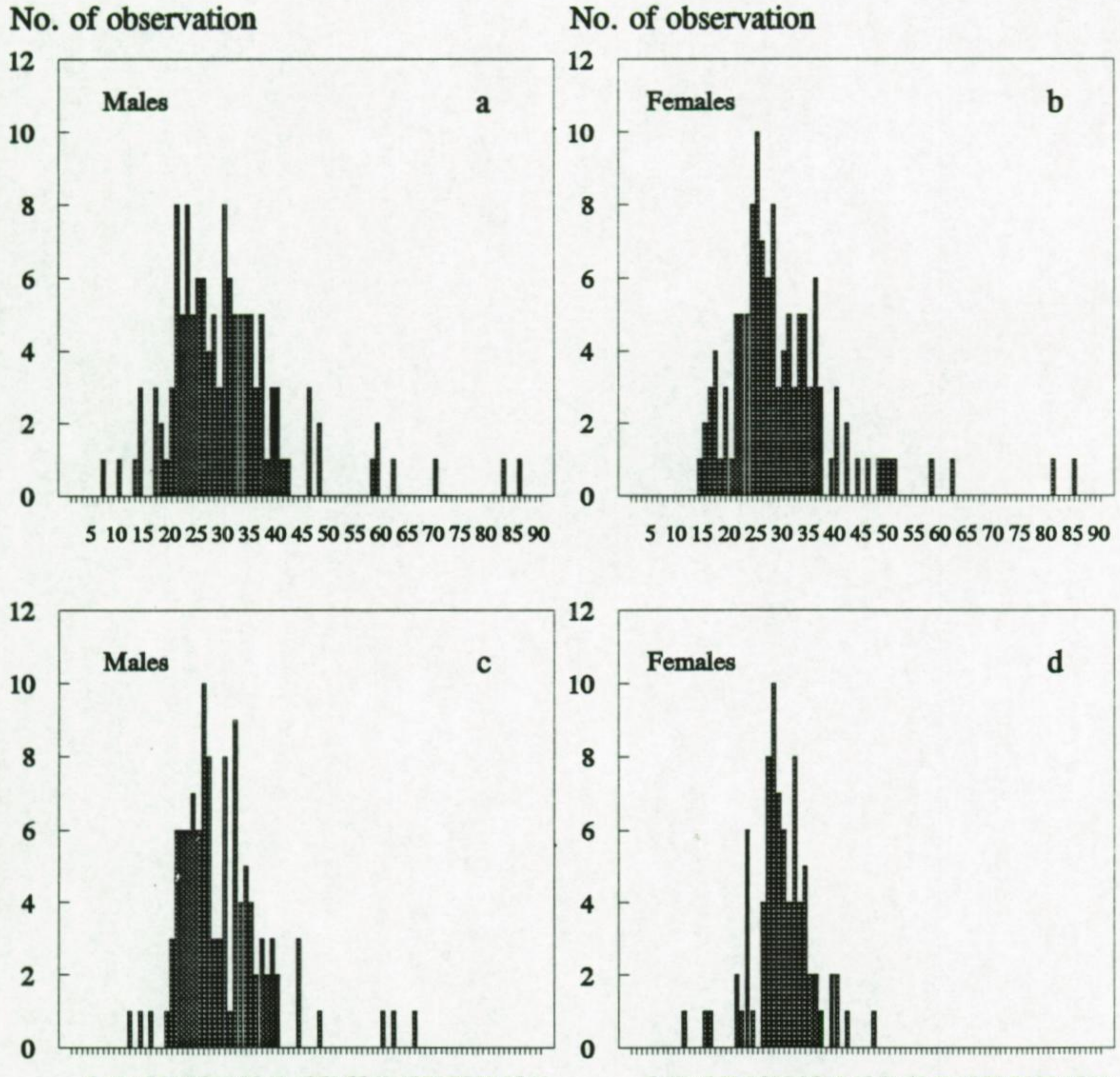


Figure 5.7. *Pomatoschistus minutus* (a,b) and *P. lozanoi* (c,d). Number of growth increments between the hatching mark and the settlement mark.

14 to 85 d (peaks again between day 21 and day 40). In *P. lozanoi*, the number of settlement days ranged from 12 to 66 d for males (peaks of settlement: 20 to 35 d), while that of females ranged from 11 to 47 d (peaks of settlement: 23 to 34 d).

The relative number of growth increments between the periodic checks of both sexes of *Pomatoschistus minutus* and *P. lozanoi* is shown in Figure 5.8. The modes of the distributions were always 14 (range of frequently occurring counts: 12 to 15).

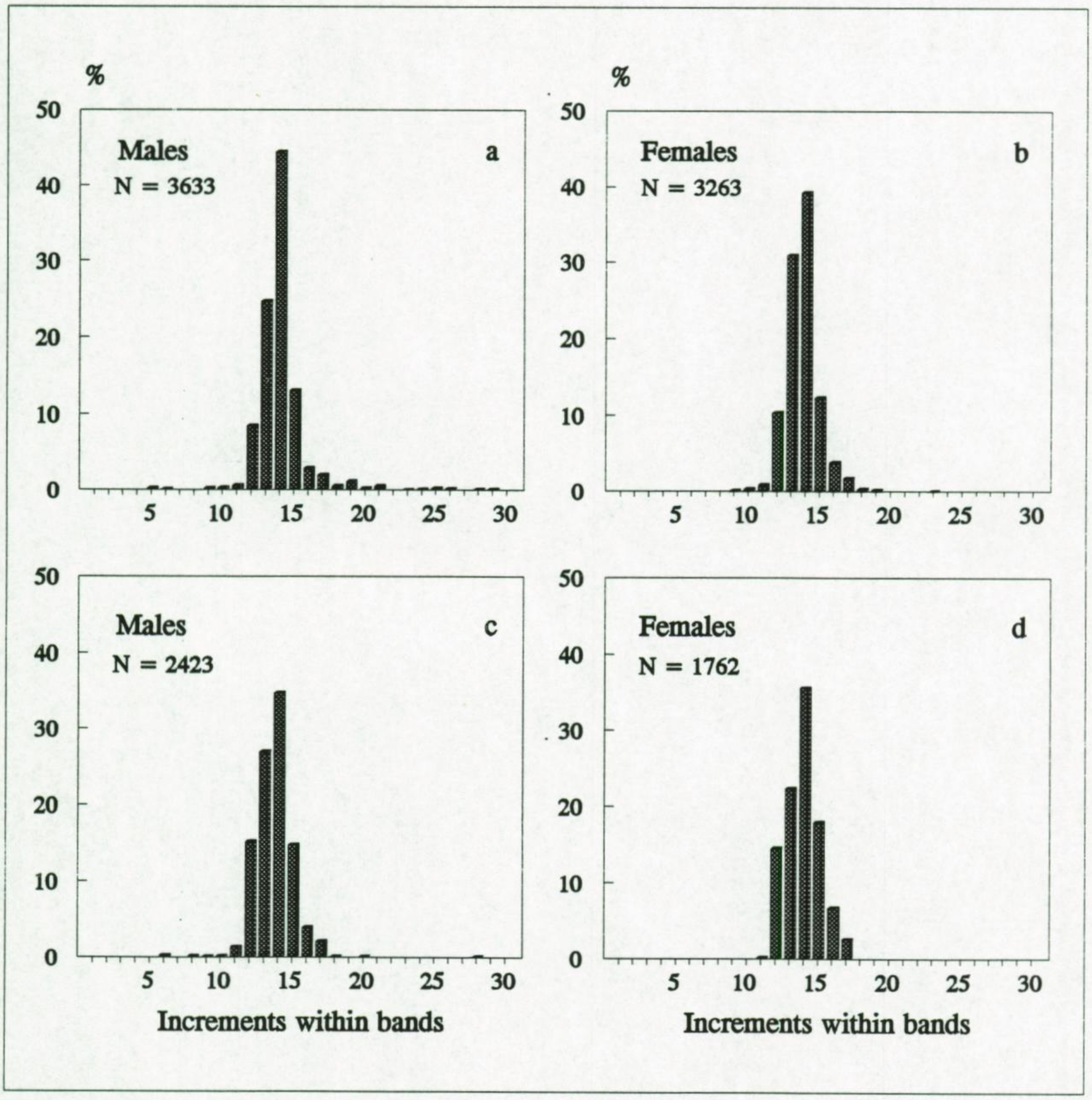


Figure 5.8. *Pomatoschistus minutus* (a,b) and *P. lozanoi* (c,d). Number of growth increments between two periodic checks.

The regressions of sagittal length (STL) on SL of *Pomatoschistus minutus* and *P. lozanoi* are shown in Figures 5.9a-b. All regressions were linear and significant: STL = -0.149 + 0.031 SL, $r^2 = 0.72$, N = 69, $p<0.005$ for juvenile *P. minutus*; STL = 0.278 + 0.026 SL, $r^2 = 0.88$, N = 1034, $p<0.005$ for adult *P. minutus*; STL = -0.158 + 0.036 SL, $r^2 = 0.86$, N = 62, $p<0.005$ for juvenile *P. lozanoi*; STL = 0.160 + 0.025 SL, $r^2 = 0.84$, N = 622, $p<0.005$ for adult *P. lozanoi*. This indicates that the sagittae of both species

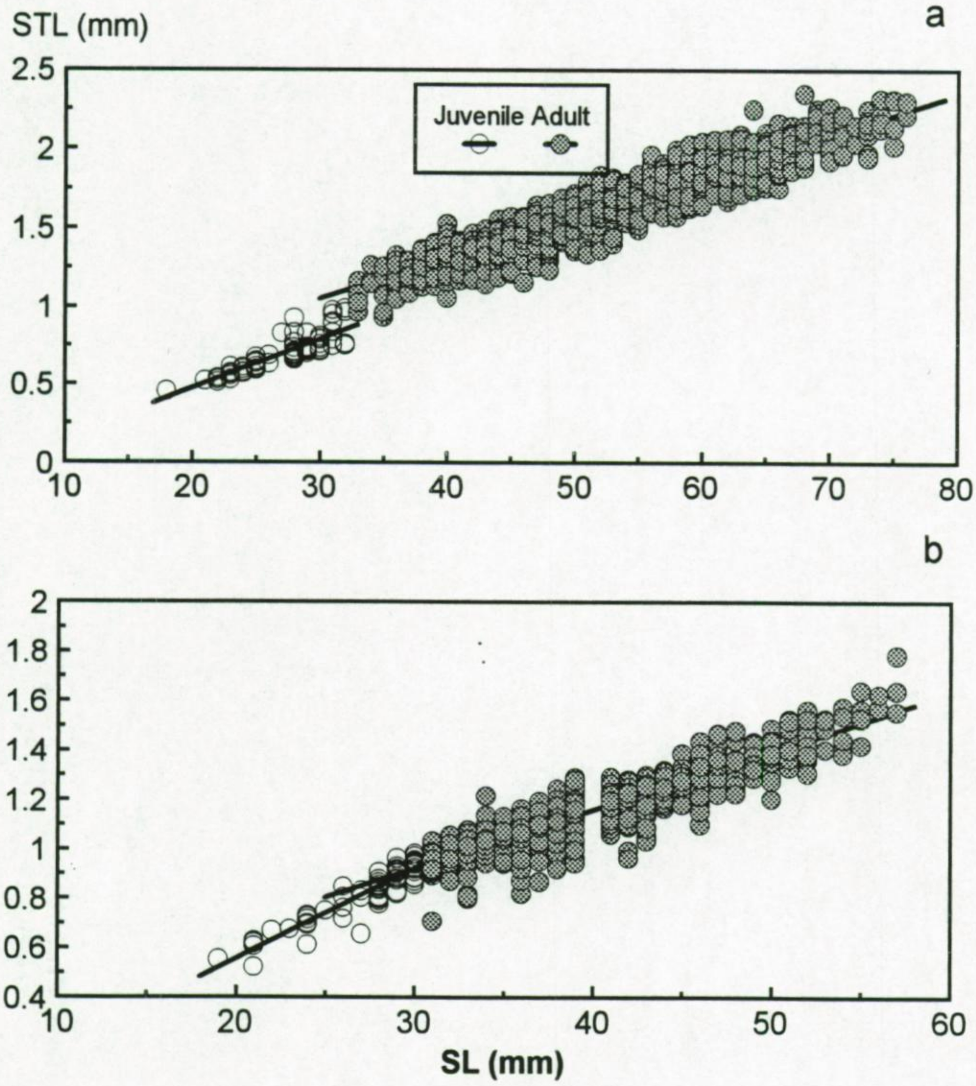


Figure 5.9. *Pomatoschistus minutus* (a) and *P. lozanoi* (b). Relationship between fish length (SL) and sagitta length (STL).

continued to grow as the fish grew in SL. Otolith growth of juveniles was faster than that of adults.

5.4. Discussion

Accurate knowledge of the population dynamics of fish stocks requires estimates of age (Pauly 1984, Sparre *et al.* 1989). Age-length keys established from otoliths are reliable tools for estimating the growth parameters of the von Bertalanffy growth model (Longhurst & Pauly 1987). However, otoliths can only be used in age and growth studies if the growth increments are deposited daily, and if they continue to grow throughout the life of the fish (Fowler 1990, Fowler & Doherty 1992).

The three criteria to validate the sagittae of *Pomatoschistus minutus* and *P. lozanoi* - i.e. they must show internal structures which are deposited at regular time scales and continue to be deposited throughout life, are readily fulfilled. The fine growth increments in the sagittae of both species (daily growth rings) are interrupted by periodic checks at fortnightly intervals. The latter rings are in synchrony with either the intervals between full moon and new moon or between the first and last quarters of the lunar cycle. Probably, the fortnightly periodic checks are deposited at the full and new phases of the moon since tidal currents are strongest during these periods.

Several studies (e.g. Pannella 1971, 1980, Campana 1983b, 1984, Berghahn & Karakiri 1990) have shown that the formation of periodic checks is related to the lunar cycle, and, more specifically, to periods of higher perturbation (activity and stress). Pannella (1980) stated that feeding activity was tidally regulated and that there was a close relationship between the sharpness of the growth increments and the alteration of periods of activity and rest. Active species showed faint separations between growth increments, while more passive species showed marked incremental boundaries. This is because ionic exchanges and respiration are enhanced during activity, whereas during periods of rest all metabolic functions and respiration are minimal.

Several studies have addressed the activity patterns of *Pomatoschistus* species. Peak feeding activities of *P. minutus* were observed during and shortly after the high tide, decreasing with the ebb tide (Gibson & Hesthagen 1981, Del Norte-Campos & Temming 1994). The stomach fullness index of *P. lozanoi* collected during spring tide was highest at the low tide (Hamerlynck *et al.* 1993a). In *P. microps*, the highest feeding activity was observed either at high or low tides (Cattrijsse pers. comm.). Thus, the peaks in activity of both species under consideration seem to occur during those periods in the tidal cycle when weakest currents prevail (at high or low tides), while activity is lowest when currents are strongest (during flood and ebb tides).

Extrapolating these observations to the time scale of the lunar cycle, we hypothesise that the activity of *Pomatoschistus minutus* and *P. lozanoi* is lowest during the periods around full and new moon, i.e. in periods when the strongest tidal currents prevail, and that the periodic checks are formed at these times. The formation of - basically similar - internal structures in the sagittae of *Pomatoschistus minutus* and *P. lozanoi* is assumed to be under genetic and lunar control. Species belonging to the same family have similar patterns of growth increments even if they live in different habitats (Pannella 1980). The occurrence of the periodic checks in the sagittae of both species indicates that all members of the populations were influenced by the same external factors. Thus, the causative factor accounting for the deposition of the periodic checks is easily identified, i.e. the lunar cycle. Despite the overriding similarity in the internal structure of the sagittae, these two sympatric goby species showed clear differences in the external structures of the sagittae (Arellano *et al.* 1995).

Daily growth increments in otoliths can be used for the study of larval duration (e.g. Victor 1982, 1987, Brothers *et al.* 1983, Radtke 1984b, Jones 1986, Mugiya 1987, Karakiri *et al.* 1991, Jenkins & May 1994, Sepúlveda 1994, Sponaugle & Cowen 1994). The number of growth increments in the first check near the nucleus of the sagittae of *Pomatoschistus minutus* and *P. lozanoi* approximately corresponds to the number of hatching days observed in laboratory experiments. Indeed, Fonds (1973) found that embryos of *P. minutus* and *P. lozanoi* reached the 'black eyed' stage after 3 to 10 days and hatched after 6 to 20 days. The newly hatched larvae swam immediately toward the water surface. They lived pelagically for at least one month and then migrated to the demersal habitat at a size of ca. 12 mm SL (Fonds 1973). Thus, the first check is a hatching mark, indicating the transition of the newly hatched larvae from the demersal to the pelagic habitat. The second distinct check can be seen as a settlement mark, indicating the transition from the pelagic to the demersal habitat.

Since the discovery of otolith growth rings (Pannella 1971), the deposition of growth increments on a daily basis is considered to be a widespread phenomenon among teleost fish, both from tropical and temperate areas (Campana & Neilson 1985). Regressions of the number of days that had lapsed between the last distinct phase of the moon and the sampling date and the number of fine striations between the last periodic check and the sagitta margin, revealed that the growth increments in the sagittae of both species were deposited at a rate of 1 per day.

Three additional observations support this conclusion. Firstly, there is the close agreement between the number of growth increments between two periodic checks and the number of days between the full moon and new moon. Secondly, there is the close agreement of the number of growth increments in the first check and number of hatching days as observed from the laboratory experiments (see above). Further, - the analysis of length-frequency data of *Pomatoschistus minutus* and *P. lozanoi* collected in the Dutch Wadden Sea (Fonds 1973) and in the Voordelta area (Arellano *et al.* submitted e) revealed that both species live for less than 2 years. The close agreement between the number of growth rings in the sagittae and the relative ages estimated from the length-frequency data (Arellano *et al.* submitted d & e) again confirmed that the growth increments are deposited daily.

The linear relationship between sagittal length and standard length revealed that the sagittae of both species continue to grow throughout their lives. The greater width of the growth increments near the sagittal nucleus as compared to the growth increments near the sagittal outer margin, suggests that the sagitta growth of young fish is faster than that of older fish. The inflection points in the regressions at about 30 mm SL (Figure 5.9) indicate that the calcium carbonate deposition rates slow down when the species reach sexual maturity (Hamerlynck 1990). The sagittae of both species grow at different rates; possible causes of these differences will be reported elsewhere (Arellano *et al.* submitted b).

5.5. Conclusions

We were able to demonstrate that the deposition of growth rings in the sagittae of *Pomatoschistus minutus* and *P. lozanoi* occurred at a rate of 1 per day. Clear periodic checks (stress marks) were found to be correlated with the lunar cycle. Generally, 14 daily growth increments were deposited between two periodic checks. Hatching and settlement marks were also observed in both species. Growth of the sagittae continued throughout the live span of the two species. Therefore, it is concluded that the sagittae of these two species can be useful tools for the determination of their age.

CHAPTER 6.

Age, growth and mortality of two sympatric goby species *Pomatoschistus minutus* and *P. lozanoi* (Pisces, Gobiidae).

Abstract

The gobies *Pomatoschistus minutus* and *P. lozanoi* were collected monthly from September 1992 to October 1993 in the ebb-tidal delta of the former Grevelingen estuary (south-west Netherlands). In each month, the otoliths of a representative size range of both sexes of each species were extracted and the microstructure of the sagittae was examined in detail. Daily growth rings were counted using light microscope and scanning electron microscopic techniques. Both techniques yielded highly similar results.

Four models were used to back-calculate fish length from the widths of the growth increments. All models yielded very similar results for fish >30 mm standard length. For fish <30 mm standard length, the models of Dahl-Lea and Whitney-Carlander were to be preferred, while the models of Fraser-Lea and Hile tended to overestimate length.

The age of both species never exceeded 2 years. Maximum recorded age was 655 (males) and 608 (females) days for *Pomatoschistus minutus* and 496 (males) and 474 (females) days for *P. lozanoi*. Fish growth was found to be directly proportional to the growth of the sagitta radius. In *P. minutus*, the estimated somatic growth rates were 110 ± 36 μm d^{-1} for males and 108 ± 34 μm d^{-1} for females. In *P. lozanoi*, the estimated growth rates were 122 ± 31 μm d^{-1} for males and 123 ± 30 μm d^{-1} for females.

In *Pomatoschistus minutus* the growth parameters (L_∞ and K) of the von Bertalanffy growth model were 76.28 mm and 1.06 y^{-1} for males and 75.86 mm and 1.32 y^{-1} for females. In *P. lozanoi*, L_∞ and K were 67.07 mm and 1.31 y^{-1} for males and 65.01 mm and 1.37 y^{-1} for females.

Both sexes of *Pomatoschistus lozanoi* had significantly higher mortalities than *P. minutus*. Male *P. minutus* had a significantly lower mortality than females, while no significant differences were observed between the sexes in *P. lozanoi*. Peaks of high mortalities were observed during the spawning season (spring-early summer for *P. minutus*; late summer-early autumn for *P. lozanoi*).

6.1. Introduction

The sand goby *Pomatoschistus minutus* and lozano's goby *P. lozanoi* are the most abundant demersal fish species in the shallow coastal waters of the Southern Bight of the North Sea (Hamerlynck *et al.* 1993b). They constitute a major prey item of a variety of commercially exploited fish, a.o. bib *Trisopterus luscus* and whiting *Merlangius merlangus* (Hamerlynck & Hostens 1993). Owing to their high abundance in the coastal waters of Belgium and the Netherlands, they have been extensively used in ecological research (e.g. Fonds 1971, 1973, Hamerlynck 1990, 1993, Hamerlynck *et al.* 1989, 1992, 1993b, Hamerlynck & Cattrijsse 1994). Despite the exhaustive studies on these two sympatric gobies, some aspects of their population dynamics are poorly described. To date, only little information is available concerning their life span, growth characteristics and total mortality. In the present study age, growth and mortality of *P. minutus* and *P. lozanoi* collected from the ebb-tidal delta of the former Grevelingen estuary (Delta area, south-west Netherlands) are derived from the analyses of otolith microstructure.

6.2. Materials and methods

6.2.1. Field and laboratory works

Pomatoschistus minutus and *P. lozanoi* were collected monthly from September 1992 to October 1993 with a beam trawl (opening of 3 m headrope and 50 cm height, codend mesh size of 10 mm) in the 5 m and 10 m depth strata of the ebb-tidal delta of the Grevelingen area (Delta area, south-west Netherlands). The fish were immediately preserved in 7% seawater-buffered formaldehyde.

In the laboratory, the samples were transferred to 96% ethanol for longer storage. After 3 months, the standard lengths (SL) were measured to the nearest mm and all individuals were identified to species and sexed according to Guitel (1892) and Hamerlynck (1990). Each month, length-frequency distributions were established per sex and per species (size classes of 1 mm SL). From each size class 1 or 2 individuals were then selected for further analysis.

Otoliths were extracted from the auditory labyrinths by making a frontal plane incision at the cranial part of the head above the eyes. The cranium was opened at the incision to expose the brain and the auditory labyrinth, and the sagittae were taken out with forceps and were cleaned from adherent tissues with dissecting needles. The contours of the otoliths were drawn with the aid of a drawing tube attached to a binocular microscope at 50 x magnification. The sagitta radii were measured with a digitiser.

For light microscope (LM) examination, the right sagittae were glued on a microscope slide (with EPON A and EPON B compounds) and then ground with moist course and fine sandpapers (grid sizes of 500 and 1000) until the midplane was nearly reached. Fine polishing up to the midplane was done with aluminium powder (0.05 μm grain size) on a suede cloth. For scanning electron microscope (SEM) examination, the left sagittae were fixed on SEM stubs with shellac, and were first ground with a silicium carbide water mixture (12 μm grain size), stopping just before the midplane was reached. Then they were polished with a fine silicium carbide water mixture (6 μm grain size) up to midplane. The apparatus used for grinding was a converted record player modified after Karakiri & von Westernhagen (1988) (see Figure 5.1). The polished sagittae were washed with tap water, dried (35°C for 30 minutes) and etched with weak acetic acid (5%) for 5 to 7 minutes. The etched sagittae were gold-plated for 3 minutes before SEM viewing.

The growth increments along the pararostral of the sagittae were counted with the aid of a micrometer eyepiece attached to a light microscope. If growth increments were blurred along part of the counting path, counts were extrapolated by dividing the axis length of that counting path by the average of the clear increment widths in the counting path. The growth increment widths were measured from the edge of the incremental zone to the edge of the discontinuous zone. The number of the growth increments and its widths were also counted and measured from the SEM pictures.

6.2.2. Data Analysis

Back-calculation of length

To establish age-length keys for both sexes of *Pomatoschistus minutus* and *P. lozanoi*, standard lengths were back-calculated from the widths of the daily growth increments with the Dahl-Lea (Lea 1910), Fraser-Lee (Lee 1920), Hile (1941) and Whitney-Carlander (Whitney & Carlander 1956) models.

The Dahl-Lea equation is expressed as:

$$L_i = (S_i/S_c)\,L_c$$

where L_i and S_i are the fish length and the otolith radius at the time of formation of the *i*th otolith growth increment, L_c and S_c are the fish length and the otolith radius at the time of capture.

The Fraser-Lee equation is expressed as:

$$L_i = c + (L_c - c)\,(S_i/S_c)$$

where L_i, S_i, L_c and S_c are defined above and *c* is the intercept of the linear regression between the otolith radius and fish length.

The Hile equation is expressed as:

$$L_i = -\,(a/b) + (L_c + a/b)\,(S_i/S_c)$$

where L_i, S_i, L_c and S_c are defined above, and *a* and *b* are the intercept and slope of the linear regression between fish length and otolith radius.

The Whitney-Carlander equation is expressed as:

$$L_i = [(c + dS_i)/(c + dS_c)]\,L_c$$

where L_i, S_i, L_c and S_c are defined above and *c* and *d* are the intercept and slope of the linear regression between otolith radius and fish length.

Growth

The growth of *Pomatoschistus minutus* and *P. lozanoi* was described with a basic von Bertalanffy growth function (VBGF, von Bertalanffy 1938) expressed as:

$$L_t = L_\infty(1 - e^{-K(t - t_0)})$$

where L_t is the length at age t, L_∞ is the asymptotic length, K is a growth constant, t is the age and t_0 is the age at zero length (for the description of the growth of the sagittae L_t and L_∞ are replaced by R_t and R_∞, i.e. the radius of the sagitta).

L_∞ (or R_∞) and K were estimated using a Gulland and Holt Plot (Gulland & Holt 1959):

$$K = - b$$

and

$$L_\infty = - a/b$$

where a and b are the intercept and slope of the linear relationship between mean length $(L_{t+dt}+L_t)/2$ and the growth rate dL_t/dt.

The t_0 was estimated using a von Bertalanffy plot (von Bertalanffy 1934), expressed as:

$$t_0 = -a/b$$

where a and b are the intercept and slope of the linear relationship between age t and $-\ln(1-L_t/L_\infty)$.

Mortality

The mathematical model used to describe the decline in numbers of the

Pomatoschistus minutus and *P. lozanoi* populations (an equivalent of the 'catch equation', Baranov 1918) is:

$$N_t = N_o \ e^{-Zt}$$

where N_t is the number of fish remaining at time t, N_o is the number of fish at time $t=0$ and Z is total mortality.

The Z was calculated with the Beverton & Holt (1956) equation:

$$Z = K \ \frac{L_\infty - \bar{L}}{\bar{L} - L'}$$

where $\bar{L}$ is the mean length of a fish of length L' and longer, and L' is the length for which all fish of that length and longer are under full exploitation.

6.3. Results

6.3.1. Age and longevity

The growth increment counts along the pararostral of the sagittae of both sexes of *Pomatoschistus minutus* and *P. lozanoi* are summarised in Table 6.1. The number of growth rings counted from male *P. minutus* of 15 to 71 mm were 112 to 655. For female *P. minutus* of 19 to 70 mm, the number of growth rings were 119 to 608. In *P. lozanoi*, males of 18 to 55 mm had 150 to 496 growth rings, while females of 20 to 55 mm had 157 to 474 growth rings.

The regressions between the LM and SEM growth ring counts are shown in Figure 6.1. The regression for *P. minutus* was SEM = 5.852 + 0.976 * LM, r^2 = 0.988, N = 71; while the regression for *P. lozanoi* was SEM = -1.809 + 1.029 * LM, r^2 = 0.990, N = 79. All regressions were positive, linear and significant ($p<0.001$). All slopes and intercepts were not significantly different from 1 and 0, respectively (*P. minutus*: t = 1.951 for the slope, t = 1.894 for the intercept, $p>0.025$; *P. lozanoi*: t = 1.524 for the slope, t = 0.759 for the intercept, $p > 0.025$).

Table 6.1. *Pomatoschistus minutus* and *P. lozanoi*. Size and age distribution. $\bar{x}$L: mean length; N: number of samples; $\bar{x}$A: mean age; SD: standard deviation.

Sex/Month	Size range	$\bar{x}$L	SD	N	Age range	$\bar{x}$A	SD
P. minutus							
Male							
9 Sep 92	30-66	52.87	7.20	39	256-655	456.12	80.44
1 Oct 92	37-71	56.19	10.80	26	321-612	475.76	100.50
30 Oct 92	49-67	57.00	9.17	3	459-533	483.67	42.72
8 Dec 92	30-66	51.75	14.07	8	271-537	440.88	100.97
9 Feb 93	35-65	49.67	10.07	9	340-548	451.89	74.85
11 Mar 93	36-60	49.50	8.73	6	328-481	429.50	55.52
8 Apr 93	33-59	46.67	13.05	3	302-522	428.67	113.72
11 May 93	41-62	48.50	9.26	4	389-546	436.25	73.72
8 Jun 93	15-46	25.25	9.59	8	112-455	193.50	110.85
16 Jul 93	27-52	43.33	9.18	6	194-389	308.17	68.31
7 Sep 93	37-68	52.78	11.32	9	323-523	409.00	63.86
11 Oct 93	39-71	54.63	11.65	8	349-579	459.38	80.70
Female							
9 Sep 92	36-63	48.44	6.93	36	203-543	399.08	73.14
1 Oct 92	34-62	48.72	7.87	18	313-587	438.11	72.64
30 Oct 92	49-57	53.67	4.16	3	391-494	444.67	51.64
8 Dec 92	31-64	48.63	11.29	8	281-494	403.00	69.69
9 Feb 93	35-63	48.57	10.18	7	314-501	404.57	73.26
11 Mar 93	36-59	47.67	8.45	6	357-497	411.17	53.80
8 Apr 93	39-60	49.14	8.03	7	368-541	449.43	61.84
11 May 93	39-66	51.17	9.58	6	339-608	446.83	91.88
8 Jun 93	19-61	27.50	13.90	8	119-454	193.63	109.13
16 Jul 93	24-47	34.83	8.66	6	187-343	267.17	63.73
7 Sep 93	37-62	49.13	8.92	8	325-553	428.00	79.74
11 Oct 93	49-70	58.63	6.61	8	435-567	494.75	42.06
P. lozanoi							
Male							
9 Sep 92	25-44	35.70	4.83	27	215-371	294.00	45.13
1 Oct 92	29-47	38.21	4.38	24	269-453	335.21	43.57
30 Oct 92	38-54	44.25	7.14	4	300-435	348.75	60.31
8 Dec 92	27-53	39.25	7.85	8	235-453	326.13	64.06
9 Feb 93	32-52	40.25	6.80	8	209-430	340.00	44.92
11 Mar 93	23-51	37.50	10.13	6	169-403	293.67	81.93
8 Apr 93	25-55	40.00	9.85	9	205-496	327.22	90.47
11 May 93	27-53	42.88	8.97	8	190-426	335.38	73.47
8 Jun 93	37-53	47.00	5.80	7	235-427	370.14	70.56
16 Jul 93	-	-	-	-	-	-	-
7 Sep 93	18-49	34.40	13.30	5	150-355	268.20	98.82
11 Oct 93	29-50	40.71	7.65	7	283-397	330.00	41.46
Female							
9 Sep 92	29-40	36.25	4.92	4	244-360	302.75	57.30
1 Oct 92	27-48	37.85	6.75	20	211-435	339.80	55.95
30 Oct 92	28-50	38.75	9.71	4	225-391	302.50	71.80
8 Dec 92	27-48	36.83	8.59	6	227-421	307.17	78.33
9 Feb 93	27-50	40.63	8.45	8	232-404	343.00	69.54
11 Mar 93	20-40	28.75	8.54	4	157-332	240.25	74.03
8 Apr 93	23-53	41.17	10.42	6	200-418	338.50	77.40
11 May 93	37-55	45.88	6.81	8	289-474	378.75	65.10
8 Jun 93	30-50	42.25	7.69	8	191-439	331.88	93.77
16 Jul 93	45	-	-	1	-	-	-
7 Sep 93	20-44	32.00	12.25	4	161-376	268.25	108.93
11 Oct 93	29-43	36.14	5.98	7	233-353	301.14	55.04

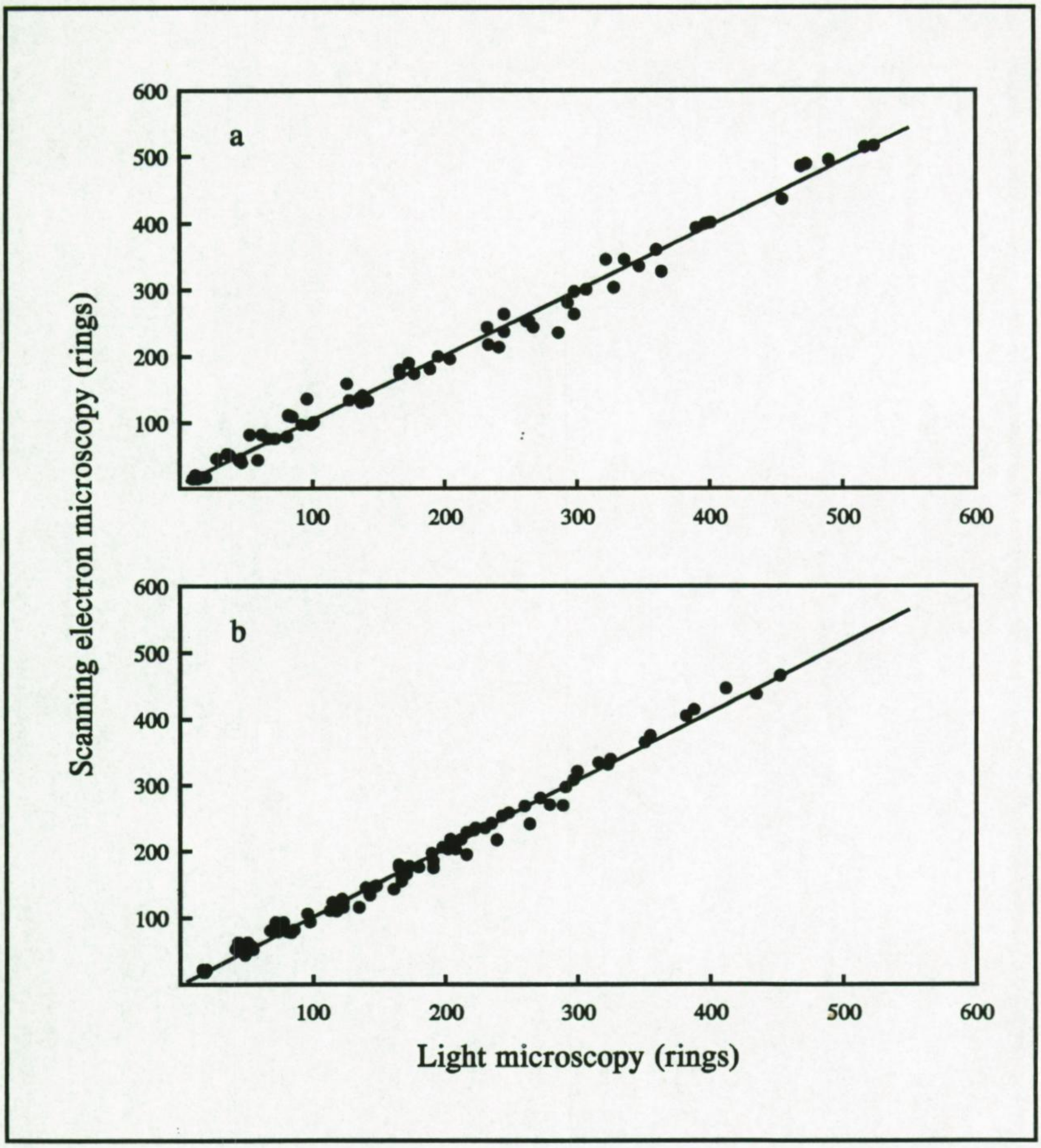


Figure 6.1. *Pomatoschistus minutus* (a) and *P. lozanoi* (b). Relationship between growth increment counts with light microscope and scanning electron microscope.

6.3.2. Growth rates

The growth parameters of the VBGF of both sexes of *Pomatoschistus minutus* and *P. lozanoi* estimated from the age-length data derived from the 4 different back-calculation equations are summarised in Table 6.2. In male *P. minutus*, the L_∞ ranged from 76.05 to 76.50 mm (76.28 ± 0.22), K from 1.03 to 1.13 y^{-1} (1.06 ± 0.05) and t_0 from -0.01 to 0.13 (0.08 ± 0.07). In female *P. minutus*, the L_∞ ranged from 73.48 to 79.58 mm (75.86 ± 2.94), K from 1.23 to 1.41 y^{-1} (1.32 ± 0.10) and t_0 from -0.01 to 0.13 (0.38 ± 0.07). The data points used for estimating the L_∞ and K of both sexes spanned the sizes of about 30 to 55 mm (Figures 6.2a-d). The back-calculated lengths of

Table 6.2. *Pomatoschistus minutus* and *P. lozanoi*. Growth parameters of the von Bertalanffy growth model estimated from the age-length data back-calculated from different equations. L_∞: asymptotic length, K: growth constant, t_o: age at zero length, r^2: coefficient of determination and SD: standard deviation.

Species/Sex/Equation	**L_∞(mm)**	**K(y^{-1})**	**t_o**	**r^2**
P. minutus				
Male				
Dahl-Lea	76.44	1.03	0.13	0.96
Hile	76.14	1.13	0.08	0.96
Whitney-Carlander	76.50	1.03	0.13	0.96
Fraser-Lee	76.05	1.04	-0.01	0.98
Mean	76.28	1.06	0.08	
SD	0.22	0.05	0.07	
Female				
Dahl-Lea	73.48	1.41	0.38	0.97
Hile	79.58	1.23	0.51	0.99
Whitney-Carlander	73.53	1.41	0.38	0.97
Fraser-Lee	76.83	1.23	0.38	0.99
Mean	75.86	1.32	0.41	
SD	2.94	0.10	0.07	
P. lozanoi				
Male				
Dahl-Lea	64.54	1.41	0.18	0.99
Hile	66.79	1.31	0.14	0.99
Whitney-Carlander	64.53	1.41	0.18	0.99
Fraser-Lee	72.43	1.12	-0.02	0.99
Mean	67.07	1.31	0.12	
SD	3.73	0.14	0.10	
Female				
Dahl-Lea	64.03	1.43	0.17	0.98
Hile	68.38	1.24	-0.05	0.92
Whitney-Carlander	64.03	1.43	0.17	0.98
Fraser-Lee	63.59	1.37	0.01	0.97
Mean	65.01	1.37	0.08	
SD	2.26	0.09	0.11	

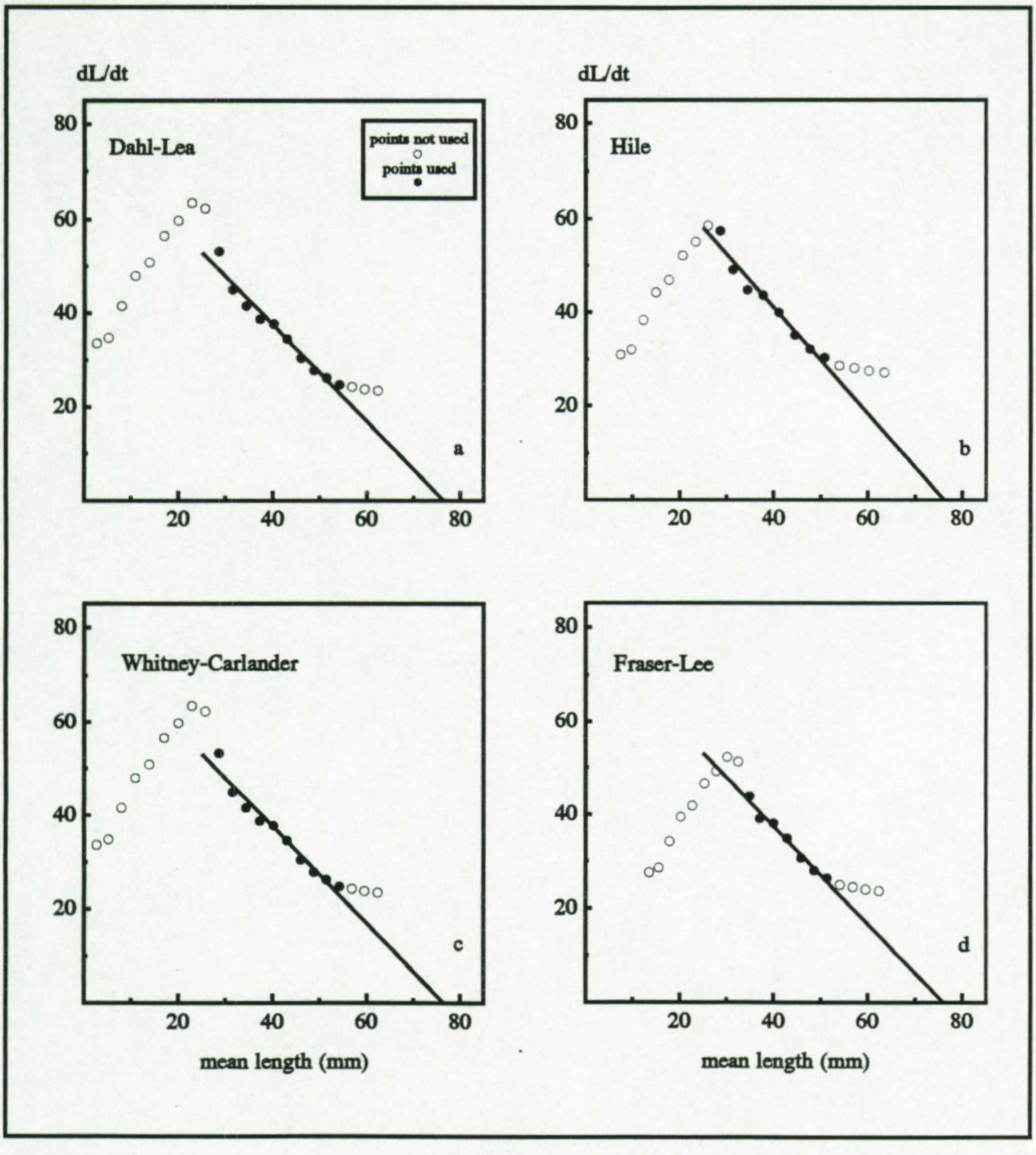


Figure 6.2. *Pomatoschistus minutus*. Gulland and Holt plot of mean length against growth rate for the estimation of the growth parameters of the von Bertalanffy growth model using the age-length data derived from the 4 different back-calculation models.

P. minutus derived from the different equations (with the fitted VBGF) are shown in Figures 6.3a-b.

In male *Pomatoschistus lozanoi*, the L_∞ ranged from 64.53 to 72.43 mm (67.07 ±

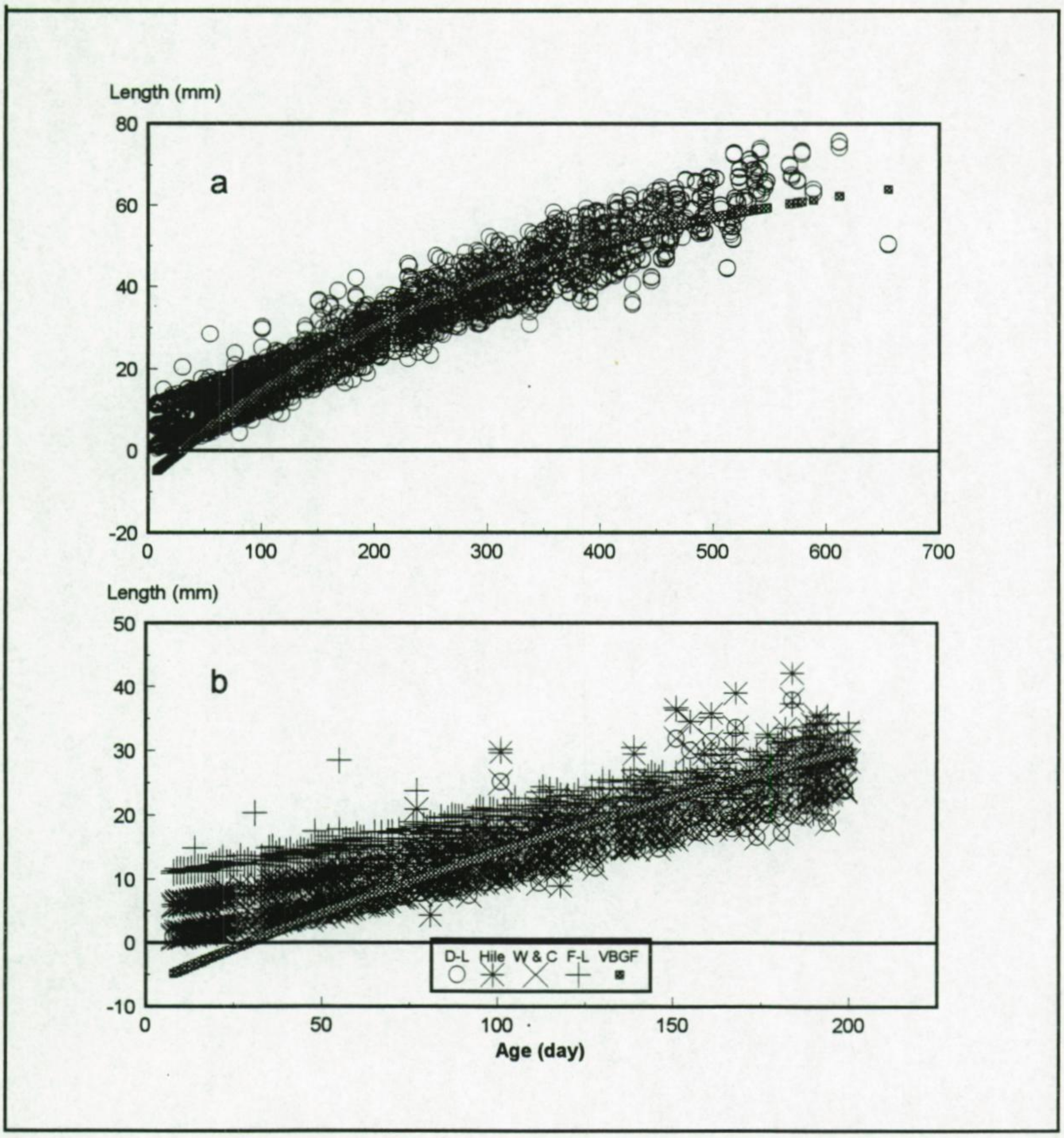


Figure 6.3. *Pomatoschistus minutus*. Age-length data derived from different back-calculation equations with superimposed von Bertalanffy growth curve (VBGF). a: whole sample, b: sample up to 200 days, D-L: Dahl-Lea, W & C: Whitney-Carlander, F-L: Fraser-Lee.

3.73), *K* from 1.12 to 1.41 y^{-1} (1.31 ± 0.14) and t_0 from -0.02 to 0.18 (0.12 ± 0.10). In female *P. lozanoi*, the L_∞ ranged from 64.03 to 68.38 mm (65.01 ± 3.73), *K* from 1.24 to 1.43 y^{-1} (1.37 ± 0.11) and t_0 from 0.01 to 0.17 (0.08 ± 0.11 SD). The data points used for estimating L_∞ and K of both sexes ranged from about 20 to 40 mm SL (Figures 6.4a-d). The back-calculated lengths of *P. lozanoi* derived from the different equations

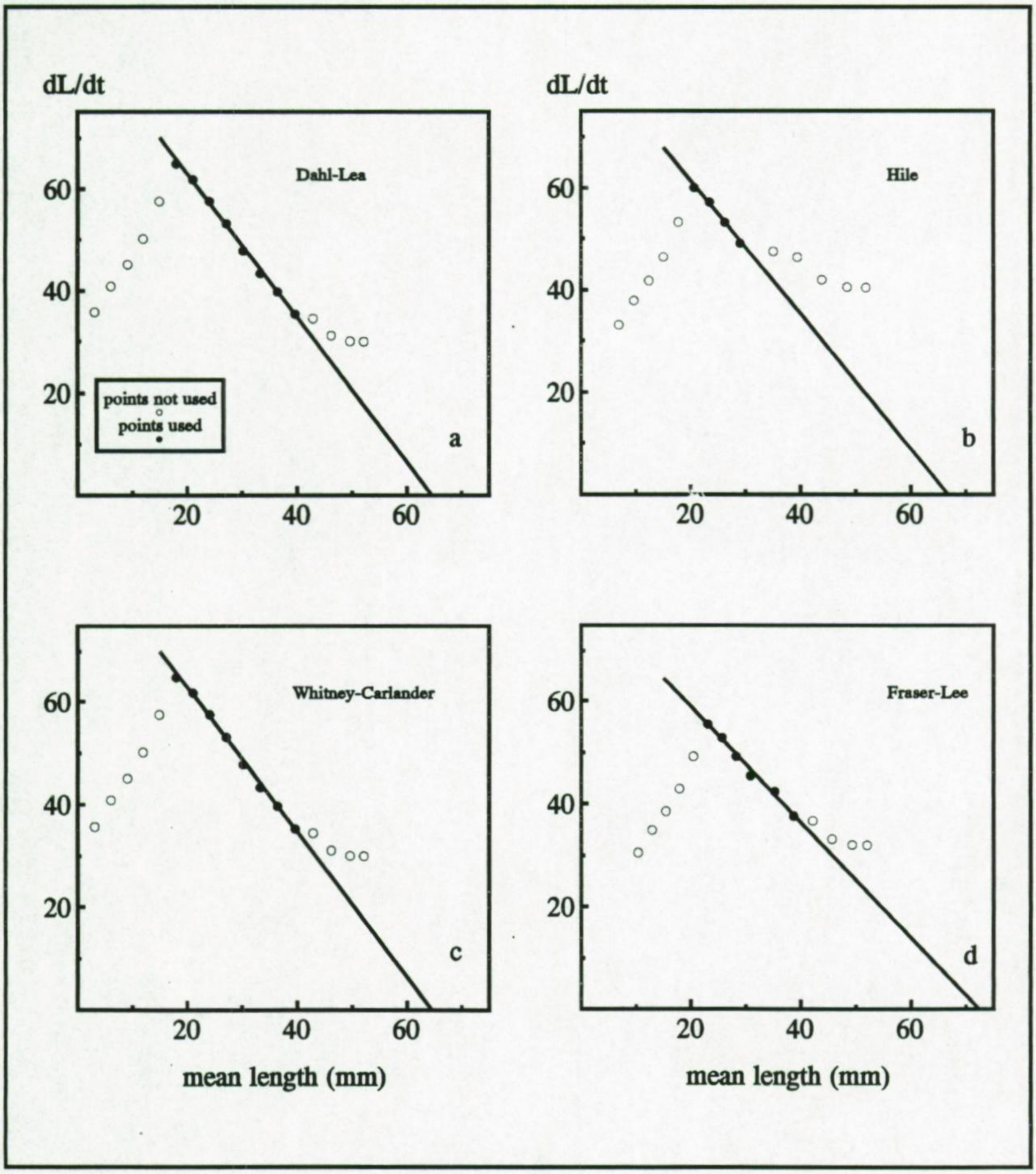


Figure 6.4. *Pomatoschistus lozanoi*. Gulland and Holt plot of mean length against growth rate for the estimation of the growth parameters of the von Bertalanffy growth model using the age-length data derived from 4 different back-calculation models.

and the fitted VBGF curves are shown in Figures 6.5a-b.

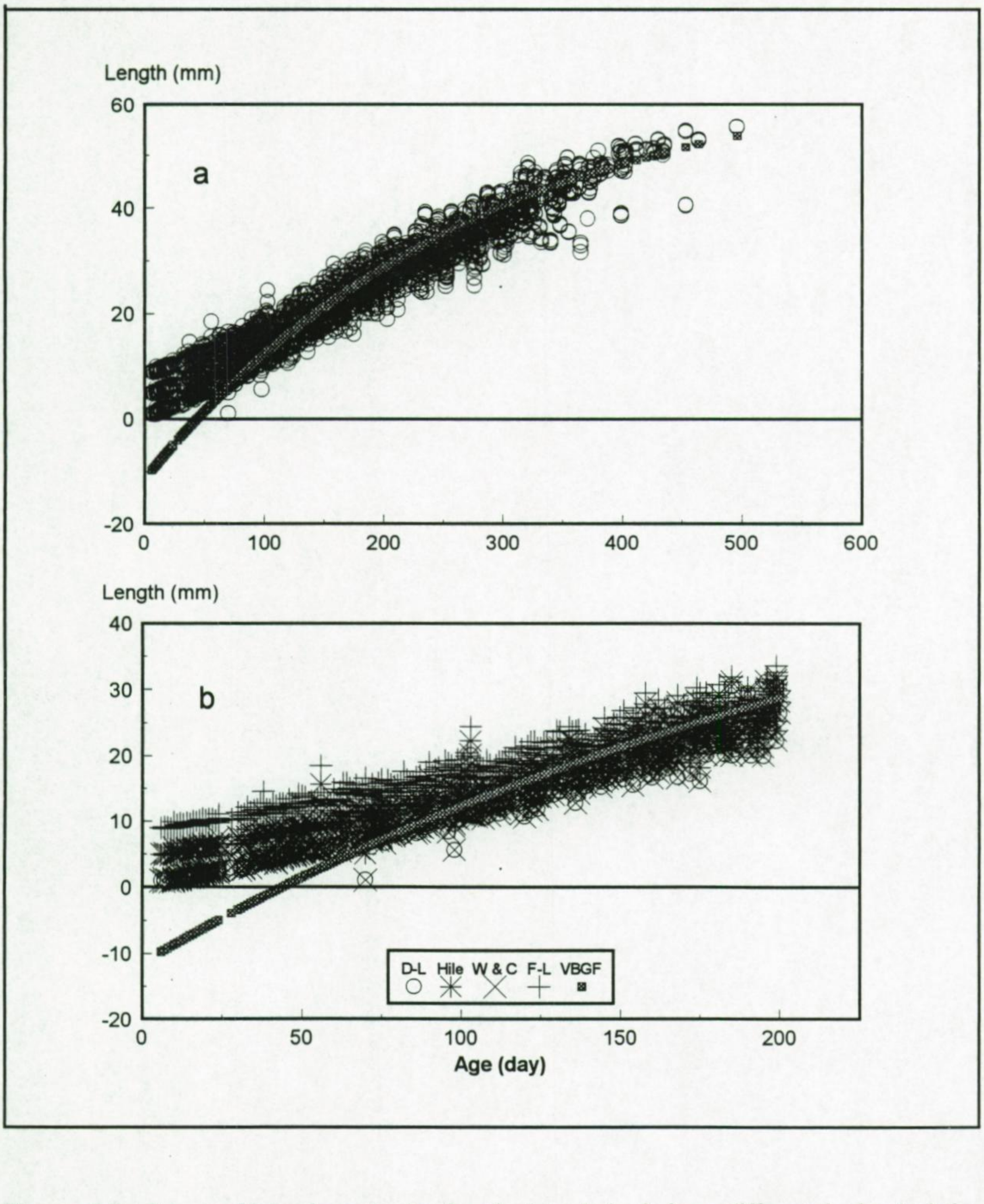


Figure 6.5. *Pomatoschistus lozanoi*. Age-length data derived from different back-calculation equations with superimposed von Bertalanffy growth curve (VBGF). a: whole sample, b: sample up to 200 days, D-L: Dahl-Lea, W & C: Whitney-Carlander, F-L: Fraser-Lee.

In the smallest length groups, the back-calculated lengths estimated with the Dahl-Lea and Whitney-Carlander equations were similar, but they were lower than those estimated with the other equations. The Fraser-Lee equation gave the highest estimates. All models yielded highly similar back-calculated lengths from about 30 mm SL onwards (Figures 6.3b & 6.5b).

The growth parameters for the radius of the sagitta pararostral of both sexes of *Pomatoschistus minutus* and *P. lozanoi* are summarised in Table 6.3. In male *P. minutus*, the R_∞ was 1314.15 μm, K was 1.03 y^{-1} and t_0 was 0.14, while in females the R_∞ was 1333.74 μm, K was 1.41 y^{-1} and t_0 was 0.39. In male *P. lozanoi*, the R_∞ was 1101.05 μm, K was 1.33 y^{-1} and t_0 was 0.17, while in females the R_∞ was 1132.52 μm, K was 1.23 y^{-1} and t_0 was 0.14. The data points used for the estimation of the growth parameters ranged from about 600 to 1000 μm for both sexes of *P. minutus* (Figures 6.6a-b) and from about 300 to 700 μm for both sexes of *P. lozanoi* (Figures 6.6c-d).

Table 6.3. *Pomatoschistus minutus* and *P. lozanoi*. Growth parameters of the von Bertalanffy growth model estimated from the age-otolith radius back-calculated with Dahl-Lea equation. R_∞: asymptotic pararostral, K: growth constant, t_o: age at zero pararostral, r^2: coefficient of determination.

Species/Sex	**R_∞(μm)**	**K(y^{-1})**	**t_o**	**r^2**
P. minutus				
Male	1314.15	1.03	0.14	0.91
Female	1333.74	1.41	0.39	0.97
P. lozanoi				
Male	1101.05	1.33	0.17	0.98
Female	1132.52	1.23	0.14	0.98

The daily growth rates of the sagitta pararostral and the standard length of both sexes of *Pomatoschistus minutus* and *P. lozanoi* are shown in Figure 6.7. In both species,

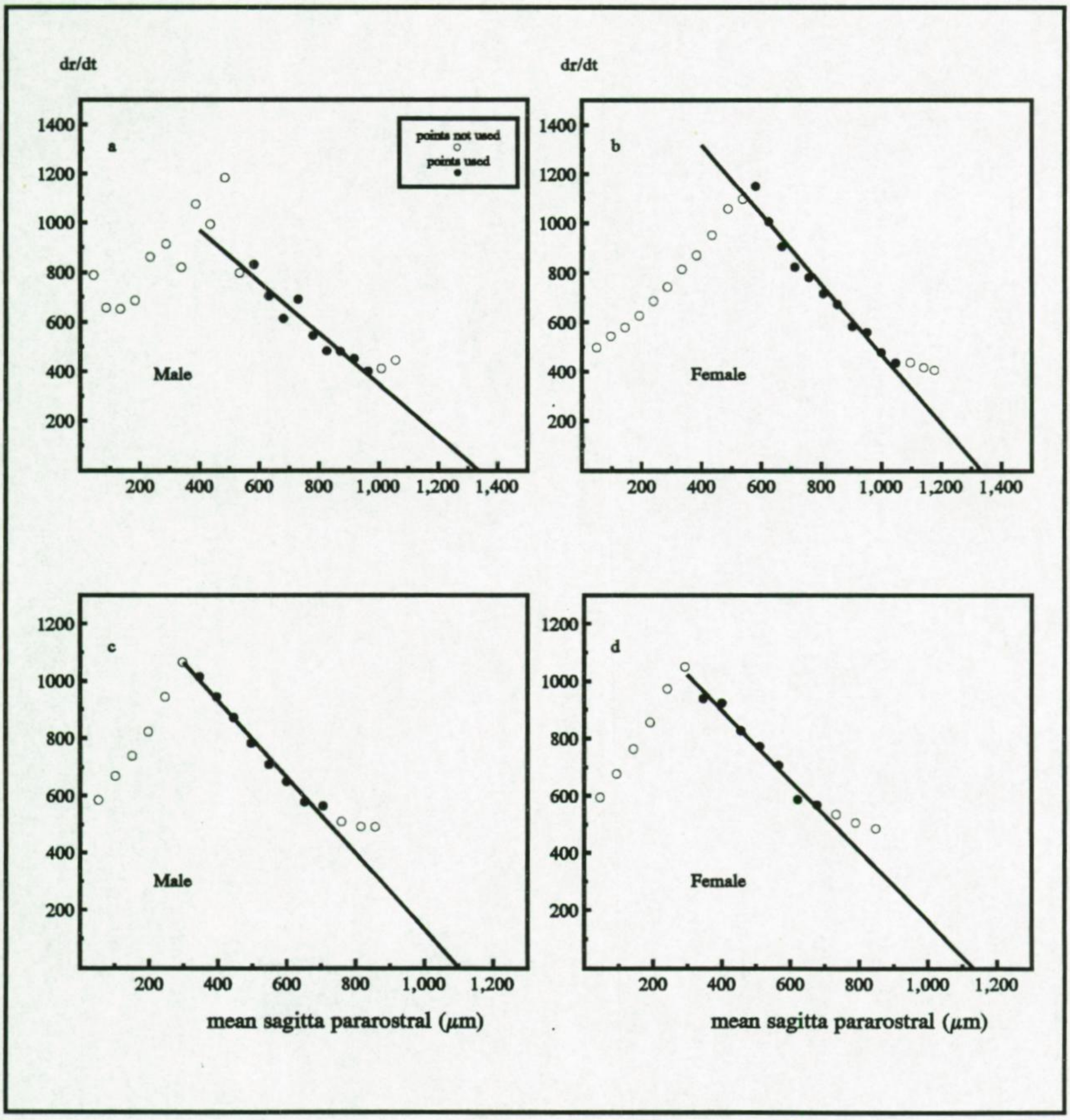


Figure 6.6. *Pomatoschistus minutus* (a,b) and *P. lozanoi* (c,d). Gulland and Holt plot of mean sagitta pararostral against growth rate for the estimation of the growth parameters of the von Bertalanffy growth model using the age-length data derived from Dahl-Lea back-calculation models.

the daily growth in SL was directly proportional to the daily growth of the pararostral. In *P. minutus*, the growth rates of the pararostral ranged from 1.25 to 3.5 μm d^{-1} (1.93 ±

0.60 μm d^{-1} for males; 1.96 ± 0.62 μm d^{-1} for females), while the growth rates of the fish ranged from 65 to 180 μm d^{-1} (109.83 ± 36.14 μm d^{-1} for males; 107.92 ± 34.07 μm d^{-1} for females). The daily growth rates of increased until day 200-300 and then decreased until day 500 after which they remained more or less constant (Figures 6.7a-b).

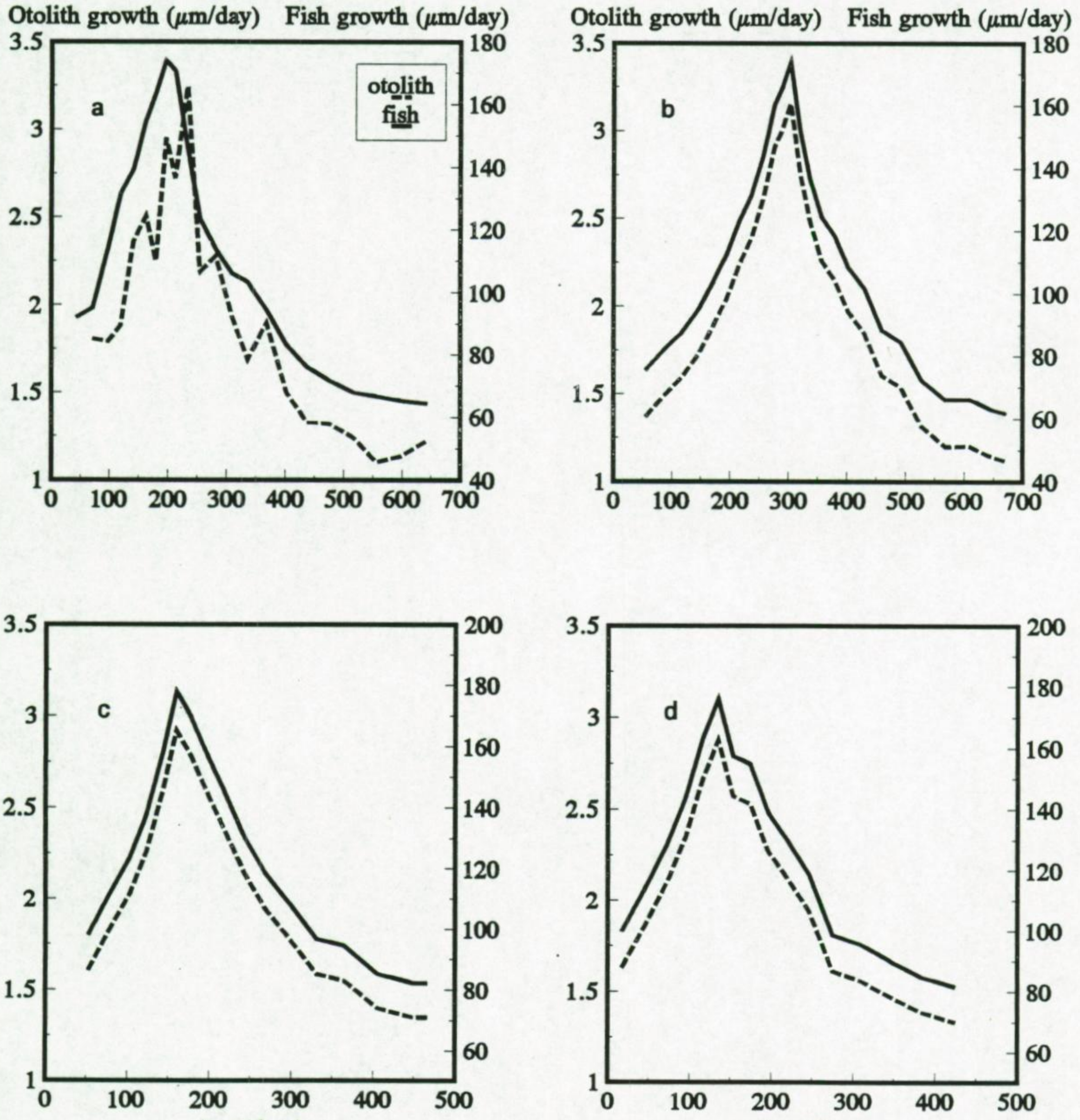


Figure 6.7. *Pomatoschistus minutus* (a,b) and *P. lozanoi* (c,d). Daily growth rates of sagitta pararostral and fish length.

In *P. lozanoi*, the growth rates of the pararostral ranged from 1.50 to 3.0 μm d^{-1} (2.01 ± 0.52 μm d^{-1} for males; 2.02 ± 0.49 μm d^{-1} for females), while the growth rates of the fish ranged from 75 to 175 μm d^{-1} (122.26 ± 31.22 μm d^{-1} for males; 123.40 ± 30.12 μm d^{-1} for females). The daily growth rates increased until day 150 and then decreased until day 300 after which they remained more or less constant (Figs 6.7c-d).

6.3.3. Mortality

The monthly mortalities of both sexes of *Pomatoschistus minutus* and *P. lozanoi* in the Grevelingen area are shown in Figure 6.8. In male *P. minutus*, the Z ranged from

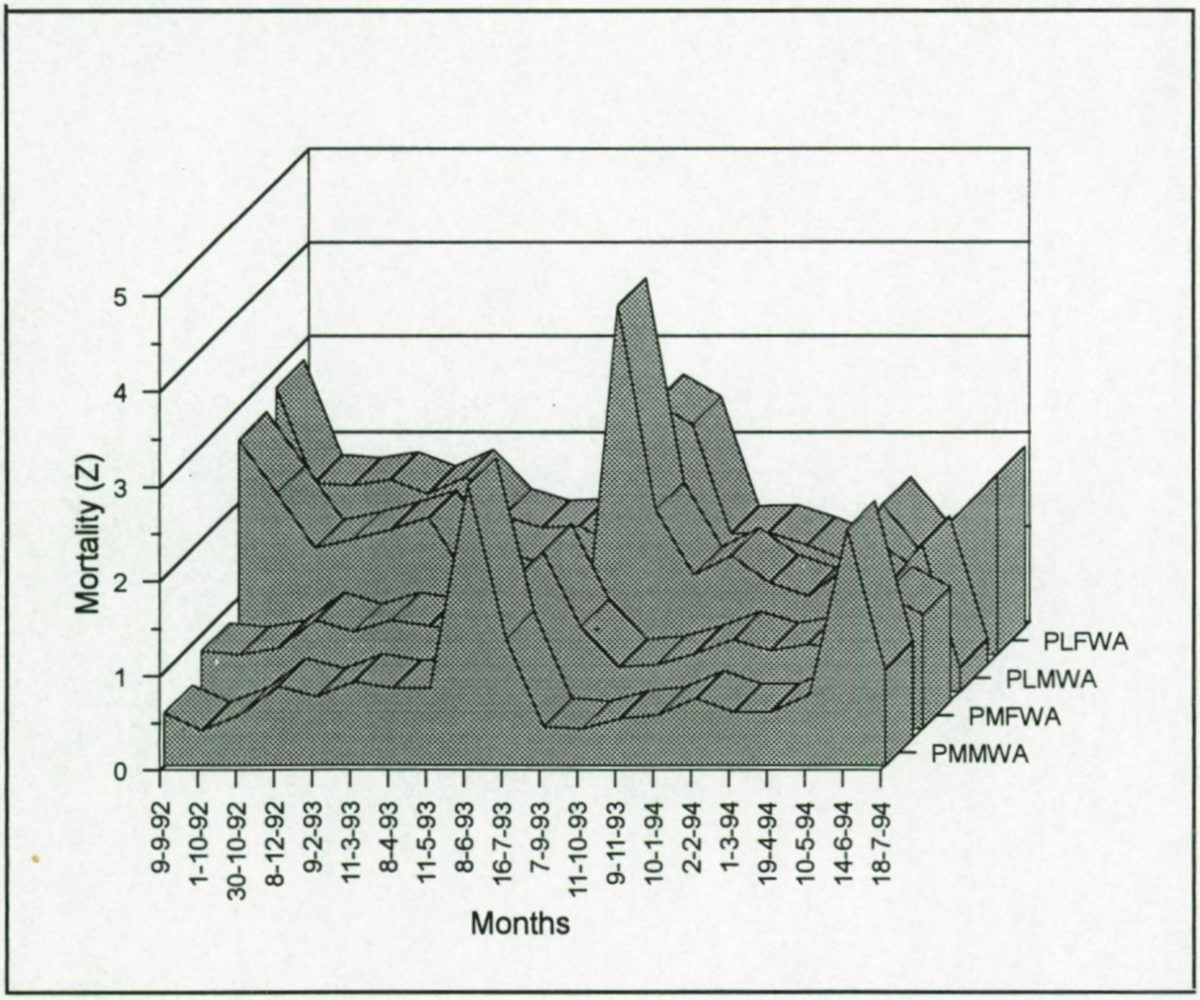


Figure 6.8. *Pomatoschistus minutus* (PM) and *P. lozanoi* (PL). Monthly mortality. M: male, F: female, WA: whole area.

0.36 (October 1992) to 2.96 (June 1994), while female Z ranged from 0.65 (October 1993) to 1.87 (July 1993). Highest mortalities were observed in spring and early summer. The mortalities in other seasons were lower and more or less constant. In *P. lozanoi*, the Z of males ranged from 0.26 (July 1994) to 4.08 (September 1993), while that of females ranged from 0.99 (March 1994) to 2.8 (September 1992). Highest mortalities were observed in late summer-early autumn. From November onwards, mortality gradually decreased up to July of the next year.

Mean mortalities of both sexes and of both species are shown in Table 6.4. *Pomatoschistus lozanoi* had significantly higher mortalities than *P. minutus* (both sexes).

Table 6.4. *Pomatoschistus minutus* (PM) and *P. lozanoi* (PL). Comparison of mean mortalities (Z) between species and between sexes by Kruskal-Wallis ANOVA test. M: male; F: female; (*** = $p<0.001$; ** = $p<0.01$; NS = $p>0.05$).

Species/Sex	**Kruskal-Wallis ANOVA test**	
	Z_1:Z_2	
PM vs PL	0.95:1.57	***
PMM vs PMF	0.89:1.02	**
PLM vs PLF	1.51:1.64	NS
PMM vs PLM	0.89:1.51	**
PMF vs PLF	1.02:1.64	***

Male *P. minutus* had a significantly lower mortality than females of the same species, while no significant differences were observed between the sexes in *P. lozanoi*.

The annual mortalities were estimated from the time of emergence of a cohort until the time of emergence of the next cohort (Table 6.5, transformed to percentage using $1-e^{-Z}$). The annual percentage of death in the *Pomatoschistus minutus* population was 44% for males and 61% for females; in *P. lozanoi* 86% of both sexes died. The annual percentage of death in *P. lozanoi* was on average 34% higher than in *P. minutus*.

Table 6.5. *Pomatoschistus minutus* and *P. lozanoi*. Annual mortalities (Z) translated to percentages.

Species/Sex	**Z**	**Percent**
P. minutus		
Male	0.57	44
Female	0.94	61
Both sexes	0.73	52
P. lozanoi		
Male	2.02	86
Female	1.95	86
Both sexes	1.99	86

6.4. Discussion

6.4.1. Sources of bias

Most parameters estimated in this study are subjected to sources of bias. Growth increment counts are influenced by the clarity of the counting path and other light artifacts. The estimates of the growth of otolith radii and fish lengths are influenced by the selection of the data points used for the regressions and the back-calculation plots. Selection of the data points is sometimes quite subjective and may lead to either over- or underestimates of the growth parameters. Estimates of back-calculated lengths using the equations of Hile, Fraser-Lee and Whitney-Carlander are further affected by the estimates of the intercepts and slopes of the regressions which can be influenced by the uncoupling of fish and otolith growth (Arellano *et al.* submitted b).

For older fish (>30 mm SL), the back-calculation of length from growth increment width yielded highly similar results with all 4 back-calculation equations. The back-calculated lengths of fish smaller than about 30 mm (this corresponds to the length at which sexual maturity is reached, Arellano *et al.*, submitted b) differed in some instances between the different models used. The Dahl-Lea and Whitney-Carlander equations yielded very similar estimates for the juveniles, but these were lower than those obtained with the other 2 models. This similarity is due to the fact that the Dahl-Lea and Whitney-Carlander equations are both based on the hypothesis that otolith growth is in exact proportion to fish growth (Francis 1990). For this reason, the results obtained with these equations are probably more reliable than those obtained with the Fraser-Lea and Hile models, and we recommend their use for estimation of length for the smallest size classes.

The estimates of the total mortality are influenced by sampling gear selection. Indeed, in the Beverton and Holt model, the estimate of Z will depend on the mean length in the catch, the length under full exploitation and the estimates of the growth parameters. Changes in the behaviour of male gobies during the spawning season (nest guarding) will result in a lower catchability of larger individuals and will thus lead to an underestimation of mean length and an overestimation of total mortality in the population. The selection of the L' (length under full exploitation) is very subjective because no mesh selection curve experiments are available for the species under consideration. For this study we simply used the smallest abundant size class caught for each species (20 mm SL for both species). One of the assumptions of the Beverton and Holt model, i.e. that the lengths under full exploitation have uniform mortalities, could thus not be validated.

The slopes of the regressions between LM and SEM growth increment counts did not deviate from a 1:1 correspondence. It is concluded that both microscopic techniques can be used for ageing.

6.4.2. Age distribution

In the study area, *Pomatoschistus minutus* spawn from March until June. Males can survive until the second spawning season, while females will die before that time. *P. lozanoi* spawn from June until August. Both sexes will die before their second spawning period. The maximal age of both *P. minutus* and *P. lozanoi* never exceeded 2 years. This is in close accordance with the age estimates reported for *P. minutus* by Shann (1910), Fonds (1971, 1973) and Healey (1971), for populations in Scottish waters, the Dutch Wadden Sea and the Ythan estuary, respectively. The age of both goby species, as deduced from the spawning time and maximum recorded length, never exceeded 2 years in the Wadden Sea (Fonds 1971, 1973). Most of the old gobies were found to disappear within a few months after their first spawning season. Studying gonad development and fecundity during the breeding season Healey (1971) concluded that *P. minutus* survived for a maximum of about 22 months. Shann (1910) collected *P. minutus* from several locations along the Scottish coasts and, using his extensive knowledge on the life history of the species, also concluded that they never grew older than 24 months. All studies mentioned above dealt with populations from temperate waters. In the Tagus estuary (Portugal) the maximum age of *P. minutus* was estimated at 32 months, i.e. well over 2 years (Moreira *et al.* 1991). This differences seems very striking because the gobies from the Tagus had the same maximum size as those from our samples (72 mm SL for both sexes). Furthermore, temperature recorded in the Tagus ranged from about 10°C to 24°C, while the waters of the Grevelingen area were significantly colder (4°C to 18°C). The maximal age of *P. minutus* is then expected to be lower in the Tagus, since fish living in warmer areas generally live shorter than their temperate counterparts. However, the different estimates of longevity are probably due to the use of different ageing techniques: Moreira *et al.* (1991) derived age from scale readings, which have been shown to be less reliable for age and growth estimates than otoliths as used in the present study. The number of concentric rings on the scales of *P. minutus* is highly variable: scales of gobies from 28 to 81 mm total length were reported to display 4 to 46 rings by Shann (1910). This author further remarked that the numbers of rings strongly differed between adjacent scales and that there was absolutely no relationship between the number of concentric rings on the scale and the length of fish. The use of otoliths has some obvious advantages over scales in age and growth studies: scale growth ceases under e.g. food deprivation and resorption may occur under severe stress, whereas otoliths will continue to grow (Marshall & Parker 1982, Campana 1983a & b, Campana & Neilson 1985). Additionally, otoliths are often the first calcified structure that appear during the early development of teleosts (Campana & Neilson 1985, see also below).

6.4.3. Growth

The proportionality of otolith growth to fish growth is one of the basic assumptions in otolith microstructure analyses (Campana & Neilson 1985). This was clearly the case in the relationship between sagitta radius and standard length of *Pomatoschistus minutus* and *P. lozanoi*.

Growth parameter estimates from age-length data derived from the back-calculation equations were highly similar. This is due to the exclusion of the first (ascending) and the last (tapering) length groups of the curves (Figure 6.2). The ascending part of the curve (representing young animals) was not included for the regression because young fish do not always grow according to the VBGF and are presumably not caught efficiently by the sampling gear. The last length groups (representing older animals) were also excluded because, when approaching L_∞, the relationship between age and length becomes uncertain (Pauly 1984, Sparre *et al.* 1989). Therefore, only the data points that appear in a straight line and are corresponding to a decreasing growth rate have been used for the estimation of growth parameters. In this size range, the back-calculated lengths from the different equations are very similar (see higher).

For the smaller size classes (< 32 mm SL for *P. minutus* and < 30 mm SL for *P. lozanoi*), regressions between length and age were established separately (Table 6.6).

Table 6.6. *Pomatoschistus minutus* and *P. lozanoi*. Relationship between fish length (SL) and age of juveniles. a: intercept, b: slope, r^2: coefficient of determination, N: number of samples, M: males, F: females.

Relationship/Sex	**a**	**b**	**r^2**	**$p<0.01$**	**N**
P. minutus					
SL-Age (M)	6.728	7.809	0.988	***	657
SL-Age (F)	7.575	7.843	0.969	***	661
P. lozanoi					
SL-Age (M)	8.180	7.667	0.975	***	775
SL-Age (F)	10.152	7.727	0.975	***	608

The intercept of the scale radius-fish length regression has been interpreted as the length of the fish at the time of first scale formation (e.g. Fraser 1916, Lee 1920). Adhering the same biological meaning to the intercept of the SL - age regressions of *P. minutus* and *P. lozanoi*, we can hypothesise that the sagittae were already formed 7-10 days before the time of hatching. This hypothesis is supported by the observation of hatching marks in the otoliths (Arellano *et al.* submitted c). The formation of daily growth rings before the time of hatching has recently been observed in several other species, e.g. plaice *Pleuronectes platessa* (Karakiri & von Westernhagen 1989), juvenile galaxiids (McDowall *et al.* 1994), and *Sardina pilchardus* (Alemany & Alvarez 1994).

The growth parameters differed between sexes and between species. In both species, females have higher K values than males. This is related to their shorter life span and lower L_∞. K is known as a 'curvature parameter' which determines how fast the fish approaches L_∞ (Sparre *et al.* 1989). The higher K value of the females suggests that they reach L_∞ faster than males; the higher K values of *Pomatoschistus lozanoi* as compared to *P. minutus* suggests that the former species is faster to reach its asymptotic length. Despite the fact that both sexes of both species never live longer than 2 years - and are considered to be short-lived - the lower number of daily growth increments in the sagittae of females and the shorter life span of *P. lozanoi* is thus reflected in higher K values. The L_∞s of male and female *P. minutus* are higher than those of male and female *P. lozanoi*. *P. minutus* thus attain bigger sizes than *P. lozanoi*. This morphological difference has also been observed in other studies (e.g. Fonds 1973, Nijssen & de Groot 1983, Hamerlynck 1990).

The K values of *Pomatoschistus minutus* reported here (1.19) are higher than those reported for the Dutch Wadden Sea population (0.9, Fonds 1973). This difference suggests that the growth characteristics of *P. minutus* are either stock-specific or vary between years. The K values estimated from the sagittae (this study) are slightly lower than those estimated from the length-frequency distributions of the same population (Arellano *et al.* submitted e). This difference is probably partly due to the model used: in the latter study, the growth parameters were derived from a seasonally oscillating version of the VBGF (Gayanilo *et al.* 1989). Still, the growth parameters derived from the otoliths are argued to be more reliable than those derived from length-frequency data: in the analysis of a time series of length-frequency data, the detection of modes (mean lengths of the same cohort) is often quite subjective. Further, variable cohort strength and gear selection are also sources of error (Longhurst & Pauly 1987). Despite the discrepancies, both methods are complementary: each method gives specific information on the growth history of the species (e.g. growth oscillations cannot be derived from otolith studies).

Increasing daily growth rates were observed during the first 4 to 5 months in *Pomatoschistus lozanoi* and during the first 6 to 10 months in *P. minutus*. Afterwards, growth rates decreased. Fastest growth was observed during summer and autumn and slowing growth was observed during winter and during the spawning period. Additionally, the start of declining growth rates coincided with the reaching of age/length at sexual maturity, which was found to differ between sexes and species. The fastest growth of *P. minutus* and *P. lozanoi* coincided with highest water temperature. Temperature is one of the most important exogenous factors to affect fish growth, and its effect is especially evident at higher latitudes where growth is usually reduced during winter (Wootton 1990). Slower growth during the spawning season is correlated with the allocation of energy for reproduction and reproductive behaviour. Gobies have a complex reproductive behaviour, which is composed of 5 main patterns: establishment of a territory, nest preparation, courtship, spawning, and parental care, 4 of which are performed by males (Reese 1964, Fonds 1973, Miller 1984). All of these behaviours will reduce somatic growth (Stanley 1983, Miller 1984, Wootton 1990). It can thus be expected that males have slower growth rates and attain smaller sizes than females. Since the opposite trends are observed it is clear that spawning females will allocate a lot more energy in reproduction than males: egg production represents a massive cytoplasmic investment as compared to sperm production (Wootton 1990).

6.4.4. Mortality

The *Pomatoschistus lozanoi* population, with higher K and lower L_∞ values, was characterised by a much higher total mortality than *P. minutus*. Since gobies have no commercial value, fishing mortality can be assumed to be negligeable and total mortality (Z) is thought to be equal to natural mortality (M). The higher mortalities in *P. lozanoi* may then indicate that they are more susceptible to predation, disease, or spawning stress than *P. minutus*. Of all young *P. lozanoi* which recruited in the samples after the production of a new cohort, 86% were found to die annually. They were first encountered by the beam trawl in September and highest population mortality was found to occur during the first year of their live. For both species, highest monthly mortality estimates coincided with the emergence of a new cohort and the month after, which suggests that the bulk of the gobies die shortly after spawning (e.g. only about 15% of the *P. lozanoi* cohort survive). This was also observed in the *P. minutus* and *P. lozanoi* populations in the Dutch Wadden Sea (Fonds 1971, 1973). High mortalities also coincided with the increase in densities of predators, notably bib *Trisopterus luscus* and whiting *Merlangius merlangus* (Hamerlynck & Hostens 1993). In June and July gobies were found to provide a substantial proportion (14% for bib and 35% for whiting) of these species' energy requirements (Hamerlynck & Hostens 1993). Increased water transparency during the high mortality period makes gobies more susceptible to visual predation. Also, from April to July, the diet of *P. lozanoi* was found to consist to a large extent of juvenile *P. minutus* (Hamerlynck *et al.* 1990) and male *P.minutus* consumed high numbers of *Pomatoschistus* eggs during the spawning season (Hamerlynck & Cattrijsse 1994).

Thus, the high mortalities observed during the months of emergence of a new cohort are probably correlated with (1) high predation pressure on small gobies and (2) death of large gobies due to old age.

CHAPTER 7.

Population dynamics of two sympatric gobies in a shallow coastal area: *Pomatoschistus minutus* and *P. Lozanoi* (Pisces, Gobiidae).

Abstract

The *Pomatoschistus minutus* and *P. lozanoi* populations of the ebb-tidal delta of the Grevelingen area (Delta area, SW Netherlands) were sampled monthly from September 1992 to July 1994 in 2 depth strata (-5 and -10 m). Densities, biomass and length-frequency distributions of males and females of both species were recorded separately and their longevity, growth, population structure and secondary production were studied. Cohorts were identified and separated with the Bhattacharya method and modal progression analyses. Age and growth were described with a seasonally oscillating version of the von Bertalanffy growth equation. Allometry was assessed from the slopes of the length-weight regressions. Production was estimated with the size-frequency method. Since both species are morphologically very similar, the effects of different levels of pooling (sexes and species) on the estimates of growth parameters and secondary production were also assessed.

The restructured length-frequency data displayed 3 cohorts for *Pomatoschistus lozanoi* and 4 for *P. minutus* and the monthly pooled data revealed that both species had a life span of less than 2 years. Only one cohort was produced per year which emerged in June for *P. minutus* and in September for *P. lozanoi*. In *P. minutus*, males attained a theoretical maximum longevity (TML) of 620 days for a theoretical maximum size (TMS) of 79 mm standard length (SL), while females attained a TML of 650 days for a TMS of 70 mm SL. In *P. lozanoi*, the TMS of males of 59 mm SL corresponded to a TML of 600 days, while for females the TMS of 49 mm SL corresponded to a TML of 650 days. Pooling data of both sexes yielded a TMS of 63 mm SL with a corresponding TML of 650 days. Pooling data of both species yielded a TMS of 71 mm SL with a corresponding TML of 720 days.

All growth curves showed a pronounced oscillation and growth stopped once a year. Growth was fastest from July to October and slowest from January to March. Allometry was positive in both sexes of both species, and the growth of males was faster

than that of the females. In *Pomatoschistus minutus*, the estimated growth parameters (L_∞, K, C and WP) were 79.4 mm, 1.9 y^{-1}, 1 and 0 for males, and 73.9 mm, 1.65 y^{-1}, 1, and 0.12 for females. When both sexes were pooled, the analyses yielded values of 79.20 mm, 1.70 y^{-1}, 1 and 0.03. In *P. lozanoi*, the growth parameters were 65.30 mm, 1.5 y^{-1}, 1 and 0.13 for males, 54.85 mm, 1.35 y^{-1}, 1, and 0.27 for females, and 64.90 mm, 1.90 y^{-1}, 1 and 0.12 for both sexes pooled. When the data of both species were lumped, the K values were underestimated and L_∞'s were overestimated: the L_∞, K, C and WP became 83.50 mm, 0.94 y^{-1}, 1 and 0.15, respectively.

The production of *Pomatoschistus minutus* (0.427 g AFDW m^{-2} yr^{-1}) was higher than that of *P. lozanoi* (0.046 and 0.091 g AFDW m^{-2} yr^{-1} for the 1992 and 1993 cohorts, respectively). Lumping the length-frequency data of different strata, sexes and species seriously overestimated production.

7.1. Introduction

Gobies, especially species of the genus *Pomatoschistus*, are the most abundant demersal fishes in the Mediterranean and in the Atlantic coastal waters of northwestern Europe (Fonds 1973, Miller 1973, Webb 1980, Claridge *et al.* 1985, Henderson 1989, Hamerlynck *et al.* 1990). More than 20 species of gobies have been recorded from the European Atlantic coast (Fonds 1973, Miller 1986). Only 6 of these occur in the shallow coastal waters of the Netherlands and Belgium: the transparent goby *Aphia minuta* Risso 1810, the black goby *Gobius niger* Linneaus 1758, the painted goby *Pomatoschistus pictus* Malm 1865, the common goby *P. microps* Krøyer 1838, the sand goby *P. minutus* Pallas 1769, and lozano's goby *P. lozanoi* de Buen 1923 (Fonds 1973, Nijssen & de Groot 1983, Hamerlynck *et al.* 1990, Arellano 1991).

Pomatoschistus minutus and *P. lozanoi* are the dominant goby species in the Voordelta (Delta area, southwest Netherlands) (Hamerlynck *et al.* 1993b, Hostens & Hamerlynck 1994), where they constitute one of the main prey items for the O-group gadoids bib *Trisopterus luscus* and whiting *Merlangius merlangus* (Hamerlynck & Hostens 1993). Because of their high abundances throughout the year and their ecological importance in the food webs of marine ecosystems, these sympatric goby species have been used in different types of research. Information is available on their feeding ecology, morphology, physiology, parasites infection and load, seasonal abundance, spawning in relation to nest availability and depth, gonad development and fecundity, age, growth and production (e.g. Fonds 1971, 1973, Healey 1971, 1972, Morawski 1978, Hesthagen & Koefoed 1979, Miller 1984, Claridge *et al.* 1985, Hamerlynck *et al.* 1986, 1989, 1990, 1992, 1993a, 1993b, Hamerlynck 1990, Moreira *et al.* 1991, Petersen 1992, Hamerlynck & Hostens 1993, Nellbring 1993, Del Norte-Campos & Temming 1994, Hamerlynck & Cattrijsse 1994, Hostens & Hamerlynck 1994). Still, *Pomatoschistus minutus* is more intensively studied than *P. lozanoi*; only occasionally both species were considered together (e.g. Fonds 1971, 1973, Nijssen & de Groot 1983, Claridge *et al.* 1985, Hamerlynck *et al.* 1989, Hamerlynck 1990, Hamerlynck & Cattrijsse 1994, Hostens & Hamerlynck 1994). In the coastal waters of Belgium, *P. minutus* spawns from March to June. It is a generalist species which feeds mostly on endo- and epibenthic animals and seems to be well adapted to exposed conditions. *P. lozanoi* spawns from June to August. This species is a specialist which predominantly feeds on hyperbenthic organisms and is better adapted to the neritic environment (Hamerlynck 1990, Hamerlynck *et al.* 1986, 1990, Hamerlynck & Cattrijsse 1994).

Studies estimating the growth parameters, allometric growth, population structure, and production of both sexes of *Pomatoschistus lozanoi* and *P. minutus* are not yet available. Growth parameter estimates of *P. minutus* in the Dutch Wadden Sea were derived from lumped sexes and the data were also lumped with those of *P. lozanoi* (Fonds 1973). Also for production estimates, the sexes and species are invariably lumped.

Production, one of the parameters in the energy budget model, is mainly used to quantify the energy flows in the aquatic ecosystem (Crisp 1984). Accurate estimates depend on the age and growth determination, the identification and separation of cohorts, the representativeness of the sampled population (Crisp 1984) and the use of appropriate production models (Mees *et al.* 1994).

With appropriate mathematical growth models, length-frequency data convey information about the relative age, growth, population structure and production of cohorts (MacDonald 1987, Pauly 1987, Arellano 1989, Mees *et al.* 1994). The main constraint for using these data, however, is correlated with the occurrence of overlapping components of different cohorts as a result of recruitment, growth, mortality, and gear selection (MacDonald 1987, Arellano 1988). Separation and detection of cohorts, using paper and pencil methods, is a difficult, tedious and often subjective task, especially in the older parts of the population. With the availability of software packages in fisheries biology (e.g. ELEFAN), the difficulty of separating cohorts is minimised.

The aims of the present study are (1) to describe and compare some aspects of the population dynamics (relative age, growth, population structure and production) of both sexes of *Pomatoschistus lozanoi* and *P. minutus*; (2) to assess the effects of different levels of pooling of data (depth strata, sexes and species) on the estimates of growth parameters and production.

7.2. Materials and methods

7.2.1. Study area

The Voordelta is the shallow coastal area at the mouth of the delta of the rivers Rhine, Meuse and Scheldt in the Southern Bight of the North Sea (Figure 7.1). It

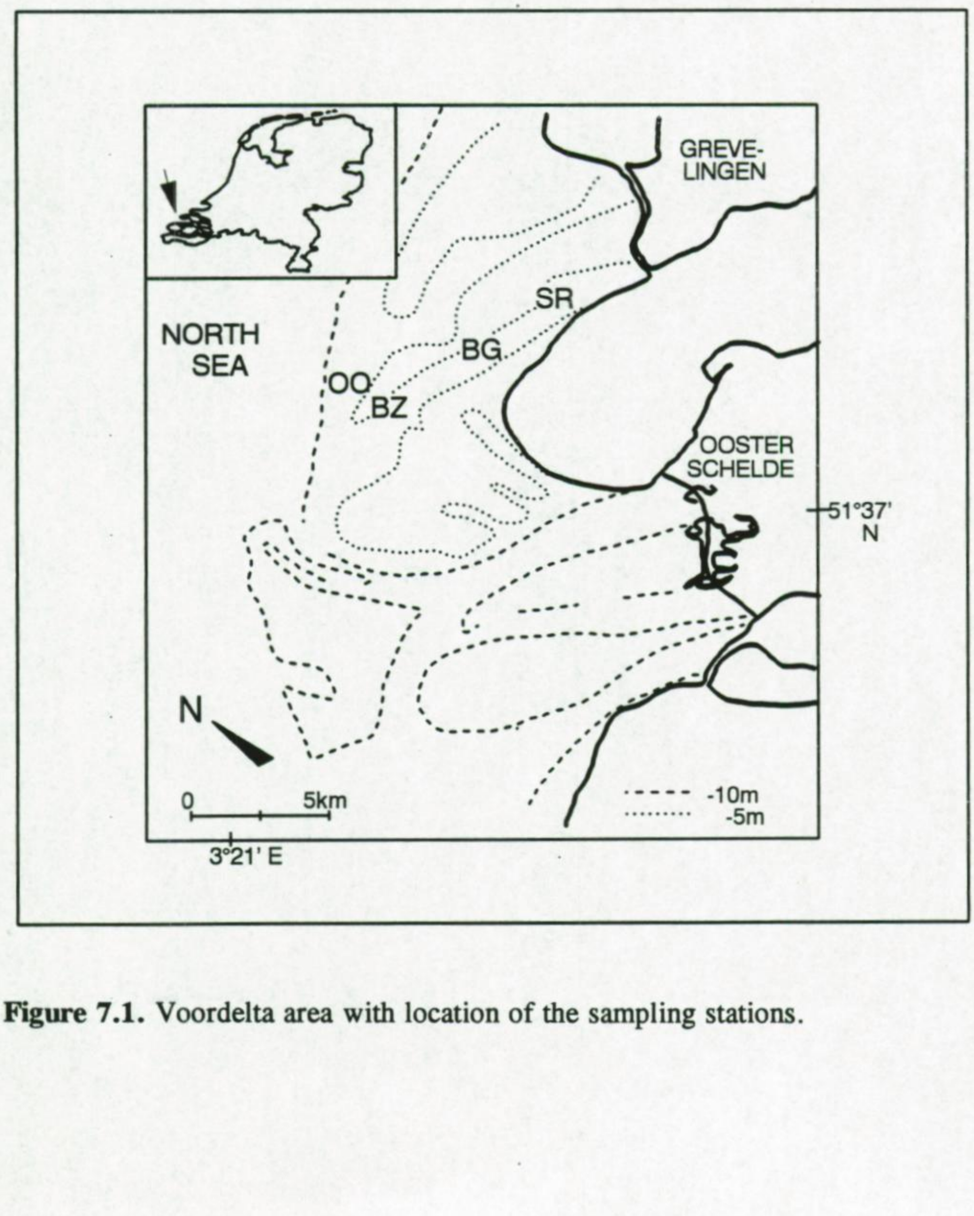


Figure 7.1. Voordelta area with location of the sampling stations.

stretches from the Belgian-Dutch border in the south to the Hoek van Holland in the north. Its marine boundary is arbitrarily defined by the -15 m depth isobath. River outflow, tidal currents and wave action have created a complex pattern of gullies and sandbanks. Due to major engineering works, the hydrodynamical regime and the bottom morphology are still changing (Elgershuisen 1981, Bergh 1984). The mean tidal amplitude is ca. 3 m. For a detailed description of the physical environment we refer to Kohsiek & Mulder (1988).

For this study 4 sampling stations were covered in the ebb-tidal delta of the former Grevelingen estuary. Three stations were located in the main tidal channel: the 'Schaar van Renesse' station (SR) close to the Brouwersdam, the 'Brouwershavense Gat' station (BG) in the middle of the tidal channel and the 'Bollen van het Zand' station (BZ) at the mouth of the tidal channel. The 'Ooster' station (OO) was situated on the seaward side of the Ooster bank that forms the northern edge of the main tidal channel.

7.2.2. Sampling

Gobies were collected monthly from the 4 sampling stations from 9 September 1992 to 18 July 1994. In each station, 2 depth strata were sampled (-5 and -10 m isobath).

Samples were taken during daytime with a beam trawl, operated from the port side of the R.V. 'Luctor' (34 m, 500 hp). The trawl had an opening of 3 m x 50 cm, was 6 m long, and had a 10 mm codend mesh size. A tickler chain was attached to the groundrope. Trawling was always done for 1000 m (radar readings from fixed points) with the tide, at an average ship speed of 4.5 knots relative to the bottom. Gobies were preserved in neutralized formaldehyde (7%) within fifteen minutes after capture. Gobies for allometric growth analyses were taken from additional trawls or from excess subsamples. Standard length (SL in mm) and wet weight (WW in g) were measured to the nearest mm and mg, respectively.

Salinity, temperature, dissolved oxygen concentration, Secchi disc depth, pH and conductivity were recorded at the end of each trawl (Table 7.1).

Table 7.1. Environmental variables recorded from the ebb-tidal delta of the former Grevelingen estuary.

Date	Salinity (PSU)		Temperature (°C)		Oxygen (mg l^{-1})		pH		Conductivity (μS cm^{-1})		Secchi (cm)	
	10	5	10	5	10	5	10	5	10	5	10	5
09 Sep 92	36.2	-	15.6	-	7.5	-	7.9	-	54.5	-	111.3	-
01 Oct 92	34.3	34.3	16.4	16.4	7.1	7.3	7.8	7.8	52.0	52.0	200.0	112.5
30 Oct 92	34.4	34.4	9.7	9.9	8.1	8.2	7.7	7.7	52.1	52.2	125.0	76.7
12 Dec 92	34.2	34.3	7.4	7.4	8.6	8.7	7.6	7.6	51.9	51.9	67.5	50.0
09 Feb 93	33.5	33.8	6.0	6.0	9.4	9.5	7.9	7.9	50.9	50.7	65.0	50.0
11 Mar 93	33.1	32.7	5.4	5.6	9.4	9.4	7.8	7.8	50.4	49.8	145.0	90.0
08 Apr 93	33.8	33.5	7.4	7.4	9.7	9.6	7.9	7.8	51.4	51.0	150.0	70.0
11 May 93	31.3	30.8	11.7	12.1	9.6	10.3	7.9	8.0	47.9	47.3	20.0	62.5
08 Jun 93	-	33.0	-	16.8	-	8.5	-	8.2	-	50.2	-	175.0
16 Jul 93	33.7	33.4	17.7	17.8	7.2	7.2	8.0	8.0	51.2	50.7	262.5	172.5
07 Sep 93	31.9	31.3	16.5	16.4	8.4	7.9	7.9	7.9	48.7	48.0	157.5	105.0
11 Oct 93	33.8	33.8	14.1	14.1	8.1	8.2	7.7	7.7	51.3	51.3	300.0	240.0
09 Nov 93	32.3	32.8	10.2	10.5	8.1	8.4	8.0	8.0	49.3	49.8	262.5	227.5
10 Jan 94	33.5	33.5	5.1	5.0	9.3	9.5	7.8	7.8	51.0	50.9	75.0	47.5
02 Feb 94	32.8	32.3	5.8	5.8	-	-	7.8	7.8	50.0	49.2	57.5	32.5
01 Mar 94	33.5	33.4	4.4	4.5	10.1	10.5	7.7	7.7	50.9	50.8	130.0	80.0
19 Apr 94	30.9	30.5	8.2	8.2	9.3	9.6	8.2	8.2	47.7	46.7	102.5	105.0
10 May 94	32.6	32.6	12.1	12.1	10.8	11.0	8.5	8.5	49.7	49.6	175.0	125.0
14 Jun 94	31.0	29.7	14.4	14.6	8.1	8.1	8.0	8.0	47.5	45.8	150.0	262.5
Mean	33.1	32.8	10.4	10.6	8.7	8.9	7.9	7.9	50.4	49.9	142.0	115.8
SD	1.3	1.4	4.5	4.6	1.0	1.1	0.2	0.2	1.8	1.9	76.6	70.8

7.2.3. Treatment of samples

Within 24 hours after sampling, the gobies were transferred to 96% ethanol for long storage. After 3 months, when the length and weight reduction due to fixation was stabilised (Arellano *et al.* submitted a), the standard lengths of the gobies were measured to the nearest mm. Species and sex were identified using the keys of Guitel (1892) and Hamerlynck (1990). Preserved lengths (PL) were converted to fresh lengths (FL) using the following regression equations (Arellano *et al.* submitted a): for *Pomatoschistus minutus* $FL = 2.713 + 0.997 * PL$ ($N = 97$, $r^2 = 0.99$, $p<0.01$) and for *P. lozanoi* $FL = 0.589 + 1.024 * PL$ ($N = 86$, $r^2 = 0.98$, $p<0.01$).

7.2.4. Data analysis

Densities of the populations are further expressed as numbers (N) of individuals per 1000 m^2, assuming a net efficiency of 20% (Kuipers 1975). Biomass, expressed in g ash-free dry weight (AFDW) per 1000 m^2, was estimated from the length-frequency data and SL-AFDW regressions (Hostens, unpublished data): $\ln AFDW = -7.851 + 3.460 * \ln SL$ for *Pomatoschistus minutus*, and $\ln AFDW = -7.842 +3.448 * \ln SL$ for *P. lozanoi*.

The spatial variation in density, biomass, sex ratio, size distribution and environmental variables, i.e. possible differences between depth strata, were tested with either parametric or nonparametric statistical methods (t = Student's t-test, z = Z-test, U = Mann-Whitney U-Test) (Siegel 1956, Sokal & Rohlf 1981, Walpole 1982).

The allometry of growth of male and female *Pomatoschistus minutus* and *P. lozanoi* was determined using the slopes (*b*) of the SL-WW regression equations (Table 7.2). To evaluate allometry of growth (isometry, positive or negative allometry) the model of Pauly (1984) was used: When $b=3$, the growth in weight proceeds in the same dimension as the cube of length (isometry). When $b>3$, this implies that there is a differential increase in weight relative to length (positive allometry). When $b<3$ the weight/length ratios decrease with increasing length (negative allometry).

Since exploratory investigation of the length-frequency data revealed that population densities, lengths and growth rates differed between sexes, cohort analyses and growth parameter estimations were performed for male and female *Pomatoschistus* *minutus* and *P. lozanoi* separately. Next, the length-frequency data were pooled to determine the effect of lumping of sexes and species on the estimates of growth parameters, growth performance indices and production values.

Table 7.2. *Pomatoschistus minutus* and *P. lozanoi*. Length (ln SL)-weight (ln WW) relationship. ln: natural logarithm, SL: standard length, WW: wet weight, a: intercept, b: slope, r: correlation coefficient, N: number of samples.

Species/Sex	**a**	**b**	**r**	**$p < 0.001$**	**N**
P. minutus					
Male	-13.244	3.50	0.991	***	174
Female	-12.450	3.29	0.991	***	90
Both sexes	-12.983	3.44	0.983	***	264
P. lozanoi					
Male	-13.210	3.46	0.983	***	63
Female	-12.690	3.31	0.988	***	44
Both Sexes	-13.000	3.40	0.971	***	107
Both species	-13.720	3.62	0.986	***	371

Unless otherwise specified, the ELEFAN program (Gayanilo *et al.* 1989) was used for cohort separation and for the estimation of growth parameters. Modal progression analysis and the Bhattacharya method (Bhattacharya 1967) were used to determine the number of cohorts and to separate cohorts. The former method several length-frequency data are plotted sequentially and the growth of a cohort is inferred from the apparent shift of modes, while the latter method splits monthly composite length-frequency distributions into separate normal distributions (Sparre *et al.* 1989). To determine the life span of the species, a cohort separation analysis was also performed on the pooled monthly length-frequency data.

Growth parameters were estimated for the seasonally oscillating version of the von Bertalanffy growth function (VBGF: Pauly & Gaschutz 1979). It is expressed as:

$$L_t = L_\infty [1 - \exp^{(-K(t - t_o) - \frac{CK}{2\pi}(\sin 2\pi (t - t_s) - \sin 2\pi (t_o - t_s))}]$$

where L_t is the predicted standard length at time t, L_∞ is the asymptotic length, K is a growth constant, C is the amplitude of the seasonal oscillation, t_o is the age at zero length and t_s is the starting point of the oscillation with respect to t_o (in ELEFAN t_s is replaced by the winter point (WP) corresponding to the time of the year with the slowest growth ($WP = t_s + 0.5$)).

Initial estimates of the different growth parameters were seeded in the 'Direct Search for Optimum Parameters Combination' (one of the options in ELEFAN 1) to find the optimal combination of L_∞, K, C and WP. The initial value of L_∞ was estimated with a modified Wetherall method (Pauly 1986). The initial value of K was based on the K value reported for the *P. minutus* population in the Wadden Sea (Fonds 1973). The initial value of C was obtained from the general relationship between the amplitude of the seasonal growth oscillation (C) of a great variety of fish and shrimp species and the difference between highest and lowest mean monthly temperature of their habitats [ΔT(°C)] (Pauly 1984). The initial value of the WP was 0.25, because lowest temperatures were recorded in March (Table 7.1).

The growth performance indices (ϕ) (Pauly & Munro 1984) for male and female *Pomatoschistus minutus* and *P. lozanoi* were estimated as:

$$\phi = \log_{10} K + 2 \log_{10} L_\infty$$

where K and L_∞ are the growth parameters of the VBGF.

Annual production values of males and females of both species and of the different cohorts were calculated from the length-frequency data and the SL-WW regressions. The predicted WW was converted to g AFDW using the regressions presented in Table 7.2.

For *Pomatoschistus minutus*, only the production of 1993 cohort was estimated (the data sets on the other cohorts were incomplete, see below). The data from June 1993 to May 1994 were used. For *P. lozanoi*, the length-frequency data from September 1992 to July 1993 were used for the production estimate for the 1992 cohort. For the 1993 cohort, the data from September 1993 to July 1994 were used. Also, the data of the 1993 cohorts were pooled to assess the influence of lumping of sexes and species on production estimates.

The production model used was the size-frequency method (Menzie 1980), which is expressed as:

$$P = [i\sum_{j=1}^{i} (\overline{n}_j - \overline{n}_{j+1})(W_j W_{j+1})^{1/2}] \times 365/CPI$$

where P is the annual production, i is the number of size classes, $\bar{n}_j$ is the number of individuals that have developed into a particular size category j during the year, W_j is the mean weight of an individual in the jth size category, and *CPI* is the cohort production interval (the number of days from the date of birth of the cohort to the attainment of the largest size). A detailed explanation of the different parameters is given in Hynes & Coleman (1968), Menzie (1980) and Mees *et al.* (1994). The values of the CPI were estimated by seeding the growth parameters of males and females of both species (or lumped categories) into the seasonally oscillating version of the VBGF. A relative age which results in a length (L_t) close to the asymptotic length (L_∞) was used to estimate the CPI.

7.3. Results

7.3.1. Environmental variables

Salinity ($t = 0.66$, $p > 0.05$), temperature ($t = -0.13$, $p > 0.05$), dissolved oxygen concentration ($t = -0.55$, $p > 0.05$), pH ($t = 0$, $p > 0.05$) and conductivity ($t = 0.81$, $p > 0.05$) showed no significant differences between the two sampling strata (Table 7.1). Water temperature changed seasonally (range from 4.4 to 17.8°C). The highest water temperatures were observed in summer (July-September). Water temperature was around

16°C at the beginning of sampling in September 1992. It decreased to 10°C by the end of October and reached a minimal value of 5.5°C in March 1993 and then increased again to maximum value of nearly 18°C in July 1993. From September 1993 onward it decreased again to 4.5°C in March 1994 and gradually increased to 14.5°C by the end of sampling in June 1994. Salinity varied between 29.7 and 36.2 PSU with an average of 32.9 ± 1.35 PSU. Highest dissolved oxygen concentrations were recorded in February-May (9.4 to 10.5 mg l^{-1}); the average was 8.8 ± 1.05 mg l^{-1}. Average conductivity and pH were 50.2 ± 1.85 μS cm^{-1} and 7.9 ± 0.2, respectively. The water transparency (Secchi disc depth) was significantly higher ($U = 66$, $p<0.001$) in the -10 m depth stratum (142.0 ± 76.6 cm) than at -5 m depth stratum (115.8 ± 70.8 cm). Secchi disc readings ranged from 20 to 300 cm and were generally higher in the seaward stations than in the sampling stations near the coast. Lowest Secchi disc readings were recorded in December-February and in May 1993 (20 to 75 cm).

7.3.2. Patterns in density, biomass and population structure

A total of 22435 *Pomatoschistus minutus* (12075 males: 9119 from -10 m and 2956 from -5 m with a size range from 16 to 76 mm SL; and 10360 females: 6953 from -10 m and 3407 from -5 m with a size range from 19 to 72 mm SL) and a total of 6119 *P. lozanoi* (3684 males: 1767 from -10 m and 1917 from -5 m with a size range from 19 to 60 mm SL; and 2435 females: 978 from -10 m and 1457 from -5 m with a size range from 20 to 55 mm SL) were used in this study.

The temporal and spatial patterns of density and biomass of the two species are shown in Figures 7.2 & 7.3. Density and biomass of *Pomatoschistus minutus* in the Grevelingen area fluctuated seasonally (Figure 7.2a). The density and biomass density reached minimal values (30-85 individuals or 7-22 g AFDW per 1000 m^2) in March-July and reached maximum values (410-660 individuals or 160-250 g AFDW per 1000 m^2) in September-February. No significant differences were observed between males and females in the -5 m depth stratum ($z = 1.36$, $p>0.05$ for density; $z = 0.436$, $p>0.05$ for biomass) while in the -10 m depth stratum males occurred at significantly higher densities and biomass ($z = 7.02$, $p<0.005$ for density; $z = 7.65$, $p<0.005$ for biomass). The overall density and biomass of males were significantly higher than those of females ($z = 5.66$, $p<0.005$ for density; $z = 7.44$, $p<0.005$ for biomass). The total density and biomass of gobies in the -10 m depth stratum were significantly higher than in the -5 m

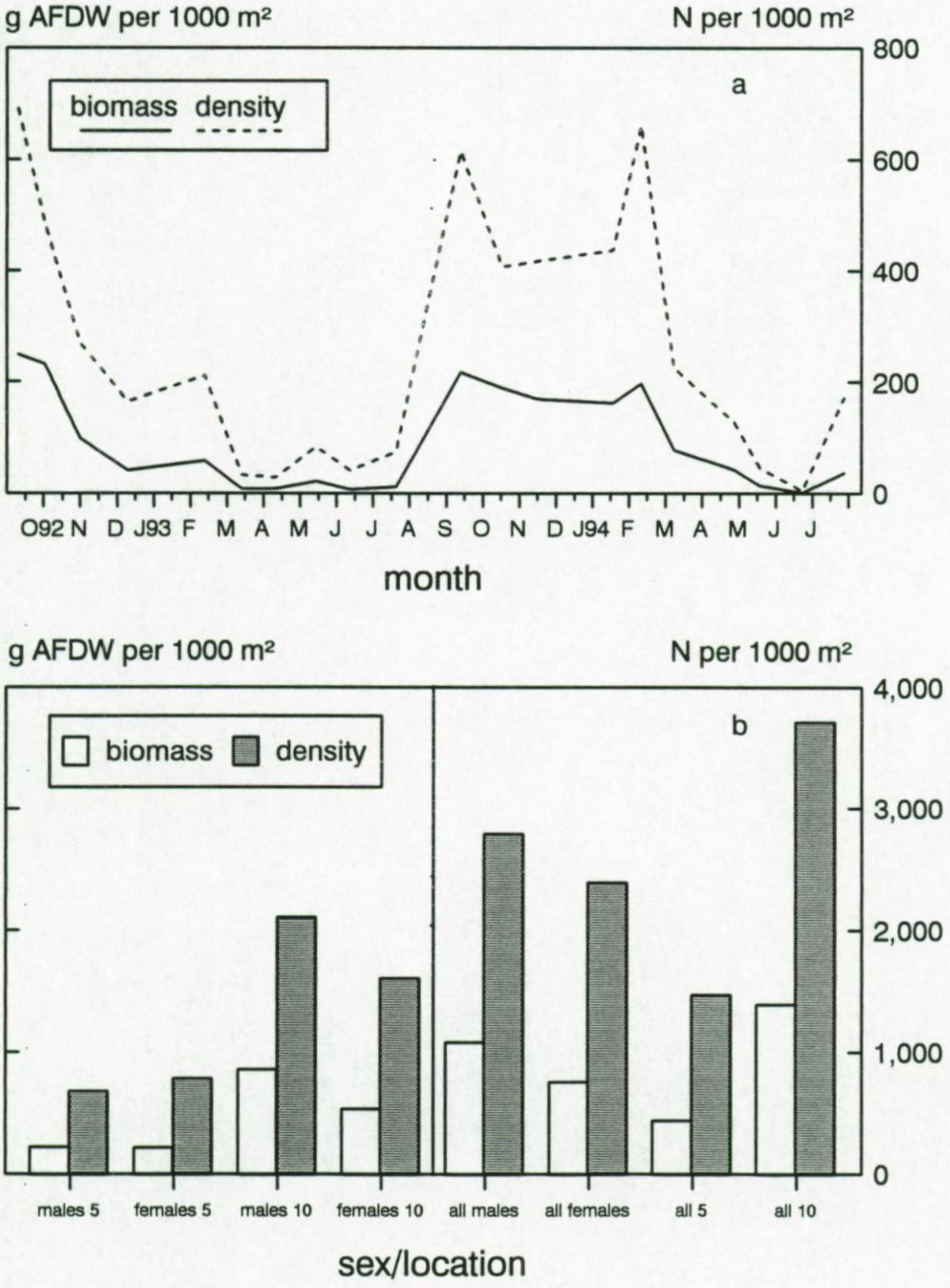


Figure 7.2. *Pomatoschistus minutus*. Temporal (a) and spatial (b) variation of biomass and density.

depth stratum ($z = 24.88$, $p<0.005$ for density; $z = 17.16$, $p<0.005$ for biomass).

Density of *Pomatoschistus lozanoi* ranged from 0.46 to 353 individuals per 1000 m² (Figure 7.3a). Most monthly densities were below 100 individuals per 1000 m². The

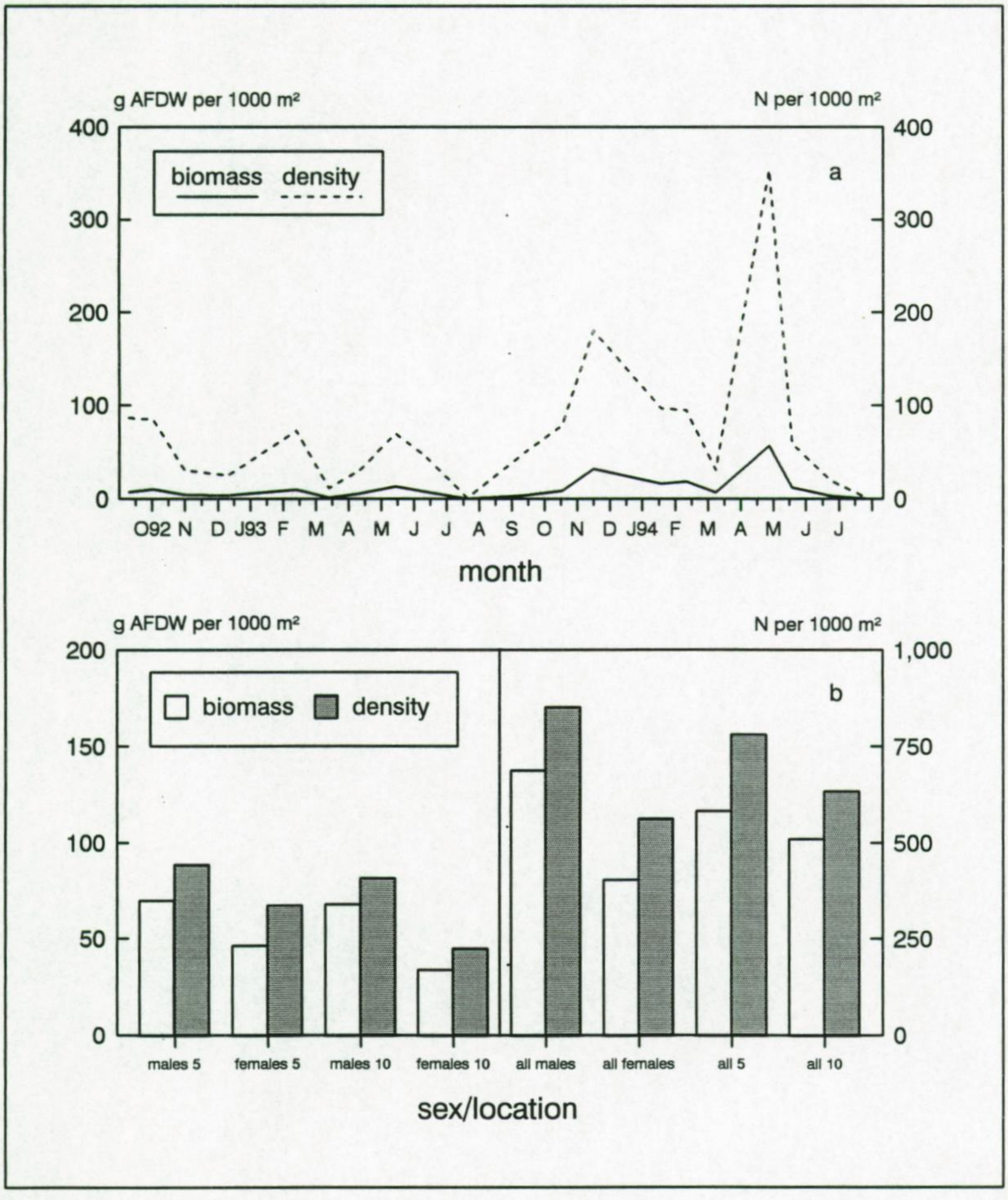


Figure 7.3. *Pomatoschistus lozanoi.* Temporal (a) and spatial (b) variation of biomass and density.

lowest densities were observed in July 1993 and July 1994. Densities higher than 100 individuals per 1000 m^2 were only observed in November 1993 (180) and April 1994 (353). Biomass ranged from 0.09 (July 1993) to 57 (April 1994) g AFDW per 1000 m^2. Biomass higher than 25 g AFDW per 1000 m^2 was only observed in November 1993 (32 g AFDW per 1000 m^2) and April 1994 (57 g AFDW per 1000 m^2). Pooled densities of *P. lozanoi* over the entire period of sampling (1992-1994) were significantly higher ($z = 3.74$, $p<0.005$) in the shallow than in the deep stratum, while biomass was not significantly different between the 2 depth strata ($z = 0.89$, $p>0.05$). Density and biomass of males were significantly higher ($z = 7.37$, $p<0.005$ for density and $z = 3.72$, $p<0.005$ for biomass) than those of females. Density and biomass of males did not differ significantly between the -5 m and -10 m sampling strata ($z = 1.17$, $p>0.05$ for density and $z = 0.34$, $p>0.05$ for biomass). Density of females was significantly higher ($z = 4.65$, $p<0.005$) in the 5 m depth stratum, while biomass was not ($z = 1.42$, $p>0.05$). Male gobies were more abundant than females in both sampling strata ($z = 3.88$, $p<0.005$ for -5 m and $z = 6.75$, $p<0.005$ for -10 m depth).

Sex ratios of *Pomatoschistus minutus* and *P. lozanoi* were observed to fluctuate seasonally (Figures 7.4a & 7.4b). In *P. minutus* males constituted nearly 60% of the total population at the beginning of sampling in September 1992. The percentage decreased to around 45% by the end of October and reached minimal values of less than 25% in April and May 1993. From June 1993 onward, it increased and reached maximum values of more than 60% from July to November 1993 after which it again gradually decreased to a minimum value of 15% in June 1994. In *P. lozanoi*, the male:female sex ratio at the beginning of sampling in September 1992 was about 60% and it remained higher than 50% up to April 1993 (except in December 1992: 47%). In May 1993 it decreased, reaching a value of 0% in July 1993. In September 1993 the sex ratio was 58% and increased to a maximum value of 81% in November 1993. Afterward, it decreased again to 65% and stayed higher than 50% until the end of sampling in July 1994.

In *Pomatoschistus minutus*, densities and biomass of both sexes were generally highest in the deeper water stratum, while in *P. lozanoi* neither males or females showed a clear defined depth preference. Male gobies were most abundant in the deeper stations in September 1992, April, May, October and November 1993, and March and May 1994. No males were caught in July 1993 and only two males were caught in the -5 m depth stratum in July 1994. Females were most abundant in the deeper stations in December

1992, April, May, October and November 1993, and January, March and May 1994. No females were caught at -10 m depth in July 1993 and July 1994.

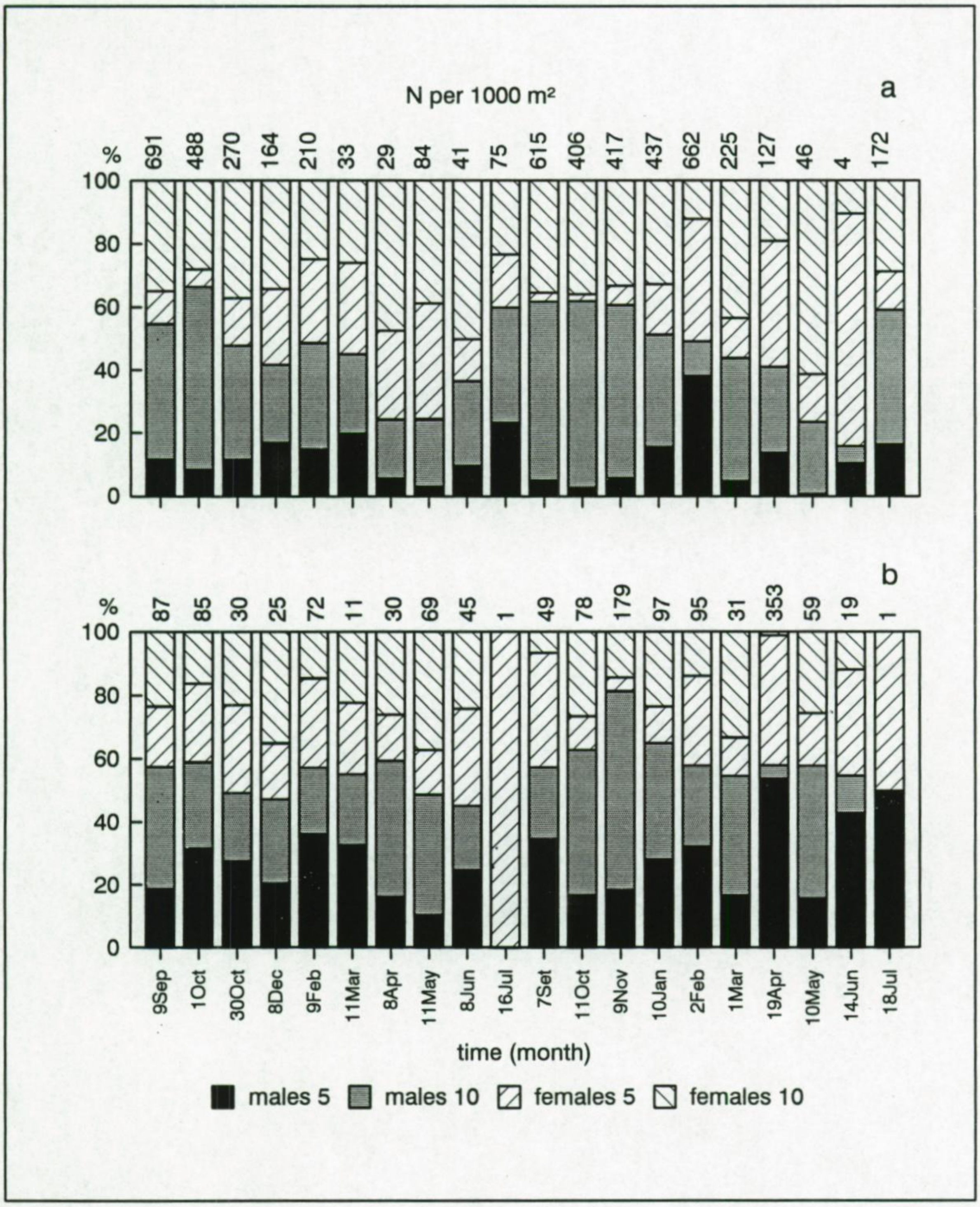


Figure 7.4. *Pomatoschistus minutus* (a) and *P. lozanoi* (b). Population structure on each sampling date.

7.3.3. Size distribution

The relative length-frequency distributions of male and female *Pomatoschistus minutus* and *P. lozanoi* in both sampling strata (the 8 stations and all months pooled) are shown in Figures 7.5 & 7.6. In *P. minutus*, female size classes from 30 to 75 mm SL (Figure 7.5a) and male size classes from 35 to 80 mm SL (Figure 7.5b) were relatively

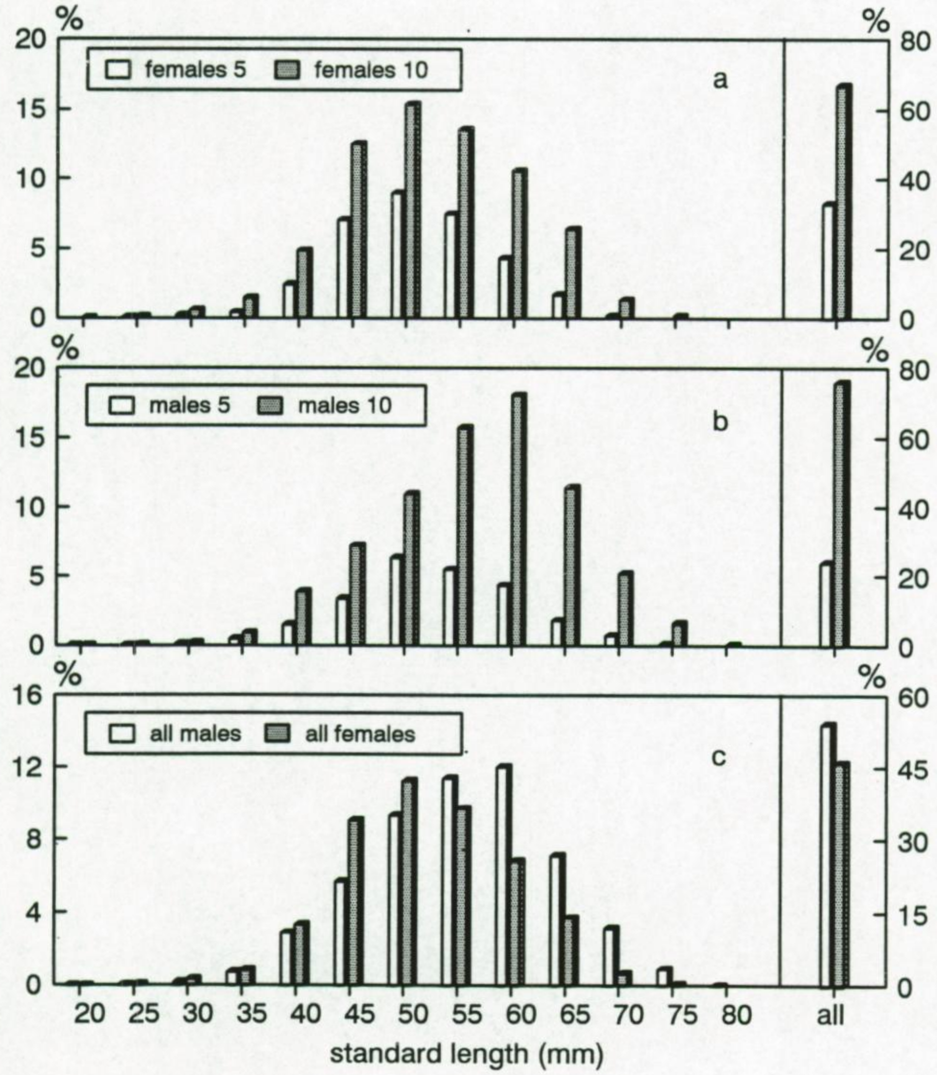


Figure 7.5. *Pomatoschistus minutus*. Size distribution of males and females in the two different sampling strata and in the whole area.

more abundant in the -10 m depth stratum. Sixty seven percent of the females (Figure 7.5a) and 76% of the males (Figure 7.5b) were observed in the -10 m depth stratum. Gobies smaller than 30 mm SL did not show consistent patterns (inadequacy of samples of these size classes due to mesh selection). Pooling the data of both sexes (Figure 7.5c) revealed that size classes larger than 55 mm SL were dominated by males, and that gobies from the 45 to 50 mm SL size classes were dominated by females. Overall, the population was dominated by males (54%). In *P. lozanoi*, the female size classes from 26 to 47 mm SL (Figure 7.6a) and the male size classes from 38 to 47 mm SL (Figure 7.6b)

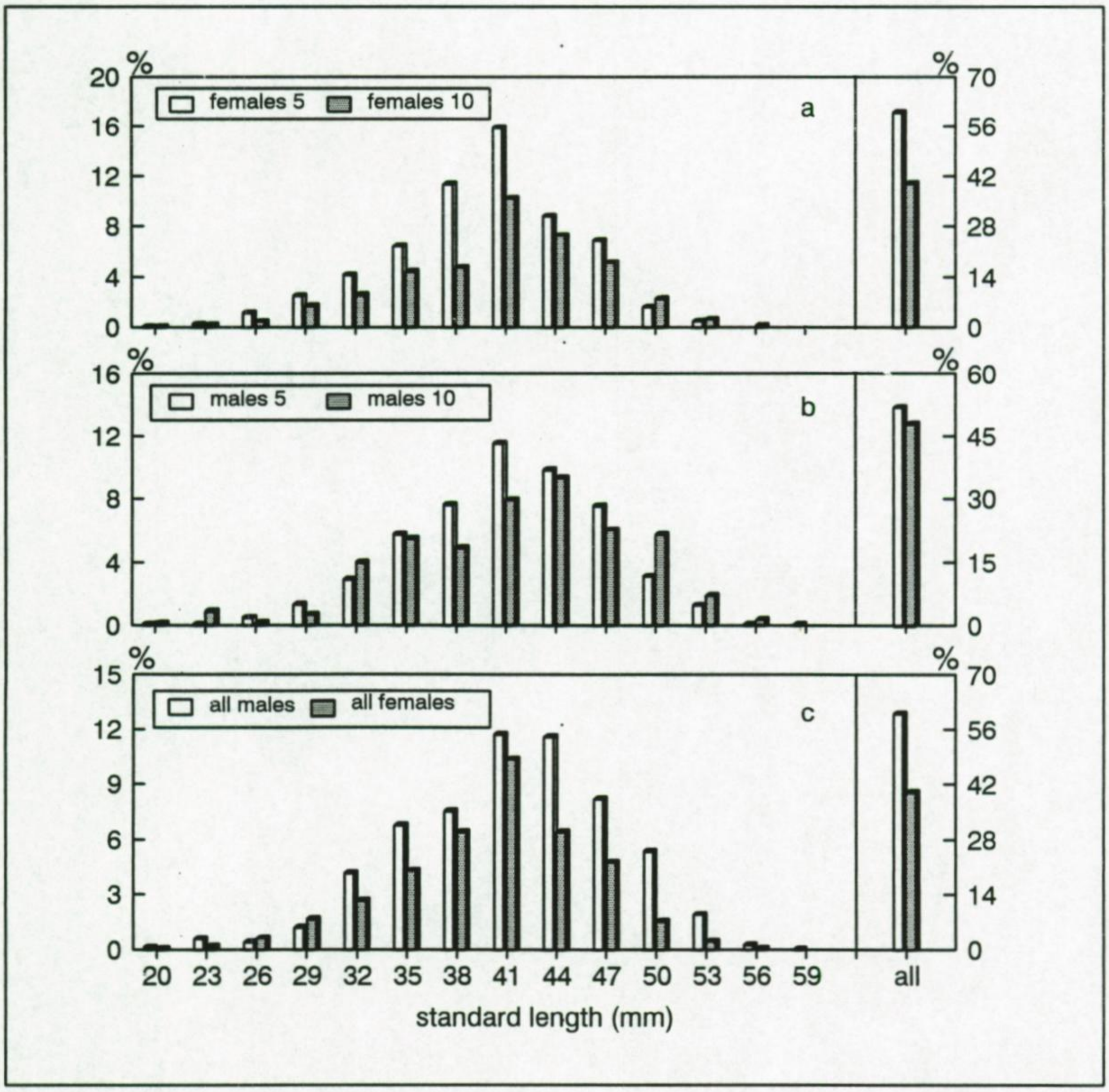


Figure 7.6. *Pomatoschistus lozanoi*. Size distribution of males and females in the two different sampling strata and in the whole area.

were relatively more abundant in the -5 m depth stratum. Gobies larger than 47 mm SL were more abundant in deeper water, while smaller gobies ($<$26 mm SL for females and $<$38 mm SL for males) did not show a clear preference for either of the two depth strata. Sixty percent of all females and 52% of all males were caught in shallow waters. Pooling the data of both sexes (Figure 7.6c) revealed that males from 32 to 59 mm SL were more abundant than females of the same size range. Overall, the population was again dominated by males (60%). In July 1993, only 2 female *P. lozanoi* (class midpoint, CM, of 44 mm SL) were caught in the -5 m depth stratum. In July 1994, only 4 individuals of this species (2 males with CM of 59 mm SL and 2 females with CM of 38 mm SL) were caught in the same sampling stratum.

The monthly and pooled length-frequency data of male and female *Pomatoschistus minutus* and *P. lozanoi* collected from the two sampling strata are shown in Figures 7.7 & 7.8 and Figures 7.9 & 7.10, respectively. In both species, both sexes showed similar patterns. The emergence of a young cohort of *P. minutus* was observed in June 1993, and again in June 1994. At this time small (young cohort) and large gobies (old cohort) co-occurred. The young cohort grew rapidly from June to October after which growth seemed to stop. Generally, the distributions were wide from September to January, and narrow from February to June. The modal points of the monthly length-frequency data reveal that small *P. lozanoi* (both sexes) appeared in September 1992 and again in September 1993. Subsequently, the modes shifted abruptly to higher size classes in October and November and then stabilised up to June. Small gobies were also frequently observed in other months. The pooled data (both species and both sexes) always revealed unimodal patterns.

7.3.4. Growth

Allometric growth of male and female *Pomatoschistus minutus* and *P. lozanoi* was described by the SL-WW regressions (Table 7.2). All SL-WW regressions were highly significant. In both species the slopes of the regressions for males and females were significantly greater than 3: allometry was thus always positive ($t = 19.48$, $N=174$, $p<0.005$ for male *P. minutus*, and $t = 8.89$, $N=90$, $p<0.005$ for female *P. minutus*; $t = 3.97$, $N=44$, $p<0.005$ for females *P. lozanoi* and $t = 5.60$, $p<0.005$ for male *P. lozanoi*). In *P. minutus* the slope of regression of males was significantly greater than the

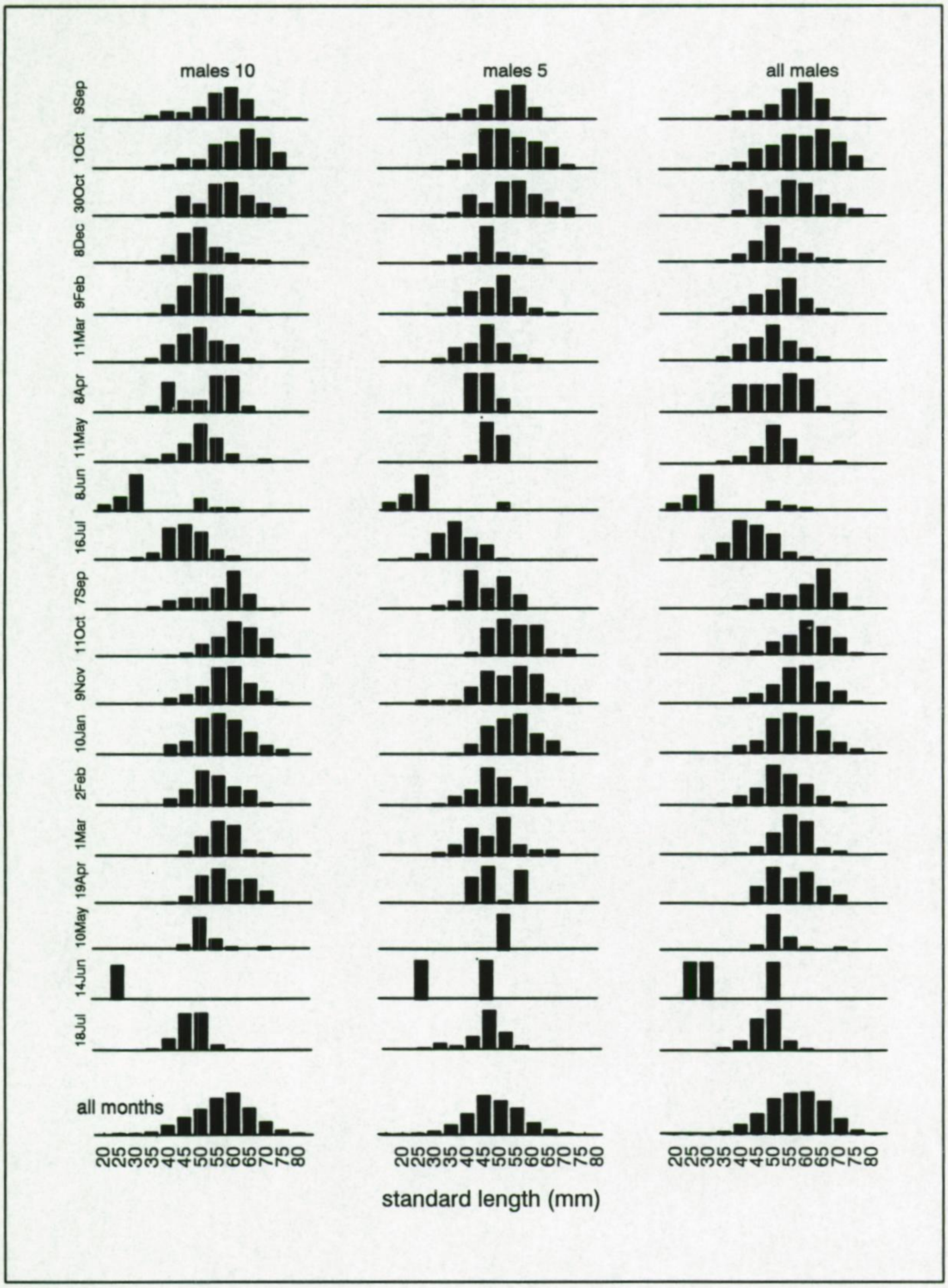


Figure 7.7. *Pomatoschistus minutus.* Length-frequency distribution of males in the two different strata and in the whole area from September 1992 to July 1994.

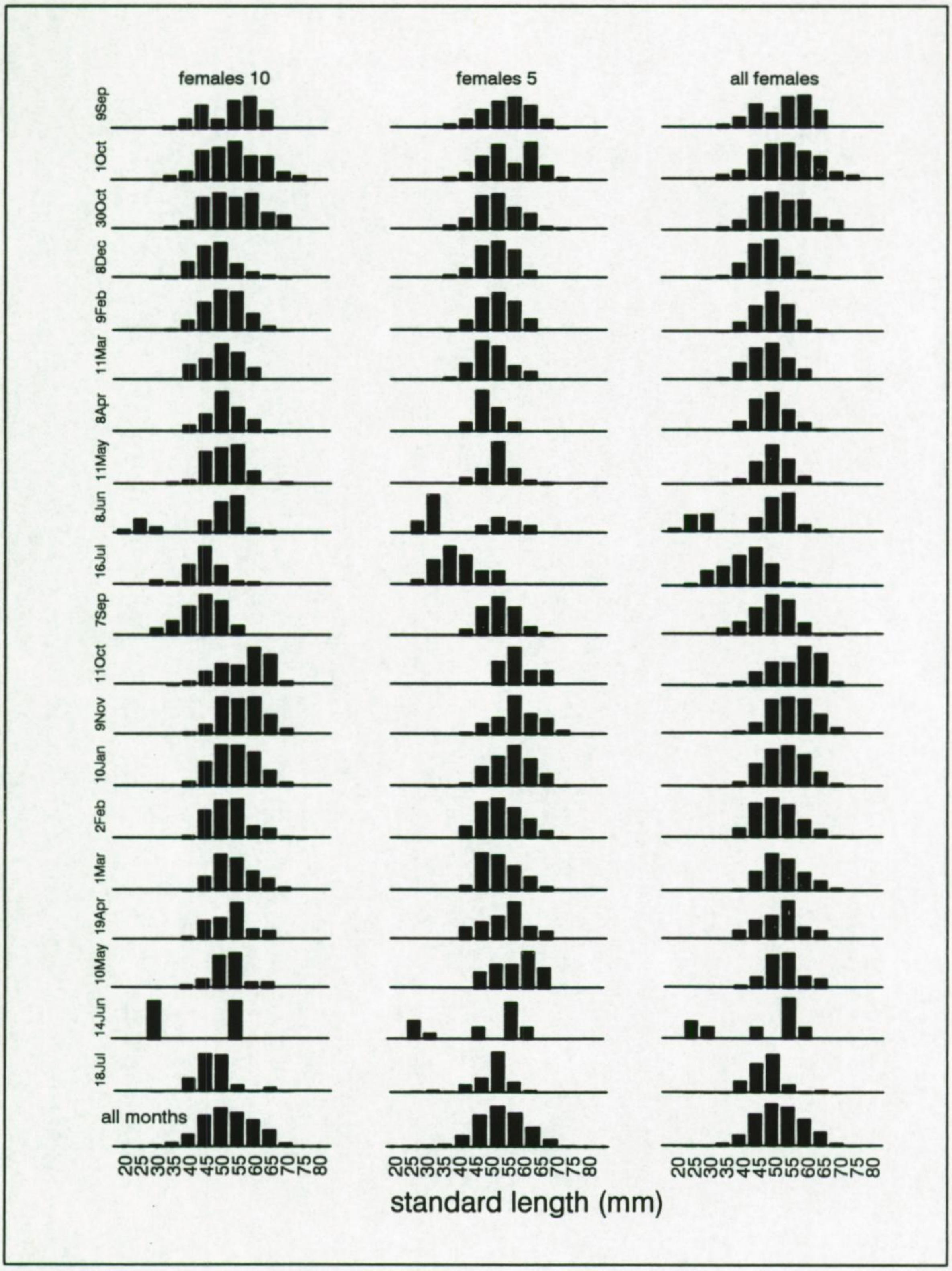


Figure 7.8. *Pomatoschistus minutus.* Length-frequency distribution of females in the two different strata and in the whole area from September 1992 to July 1994.

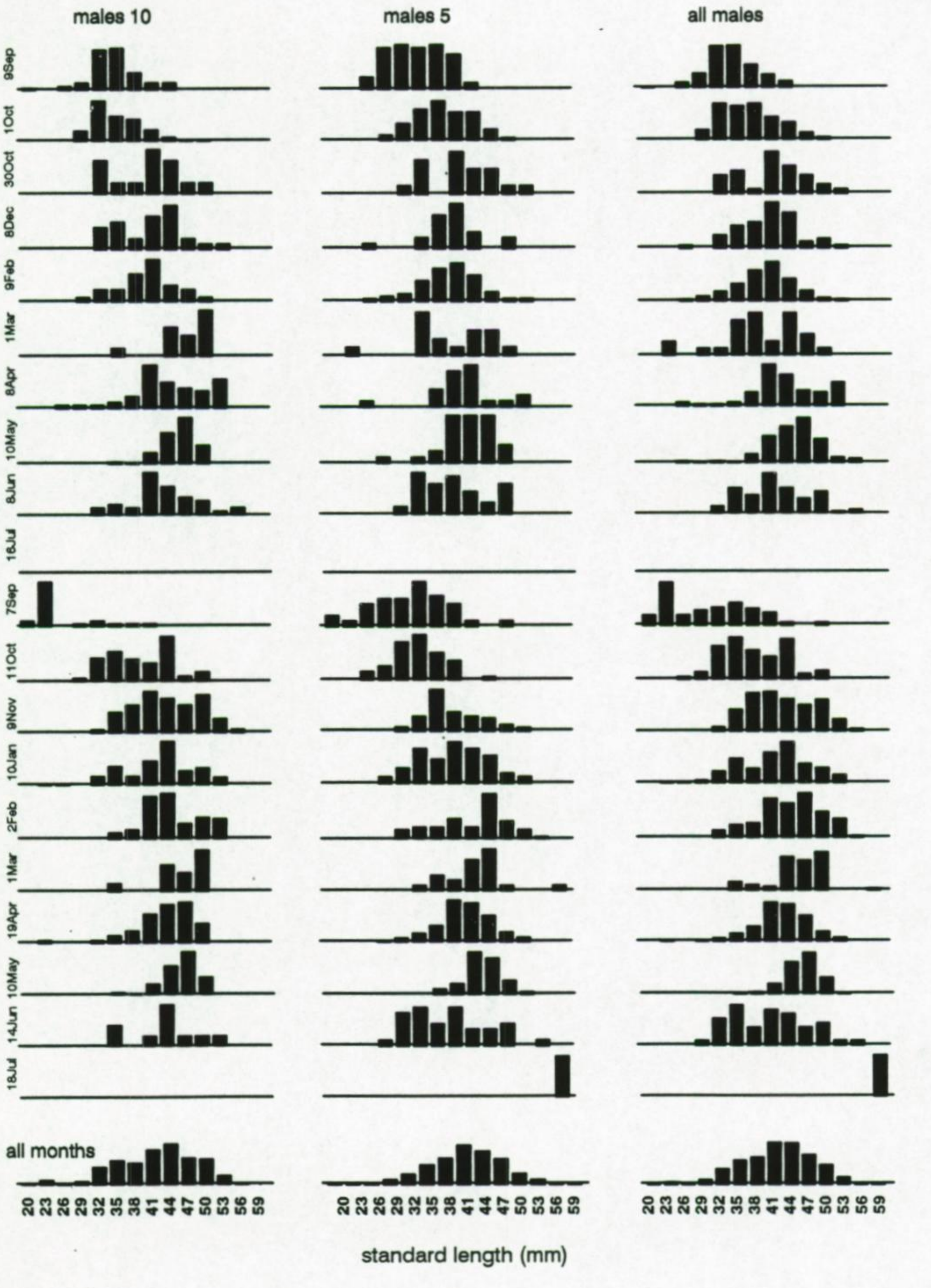


Figure 7.9. *Pomatoschistus lozanoi.* Length-frequency distribution of males in the two different strata and in the whole area from September 1992 to July 1994.

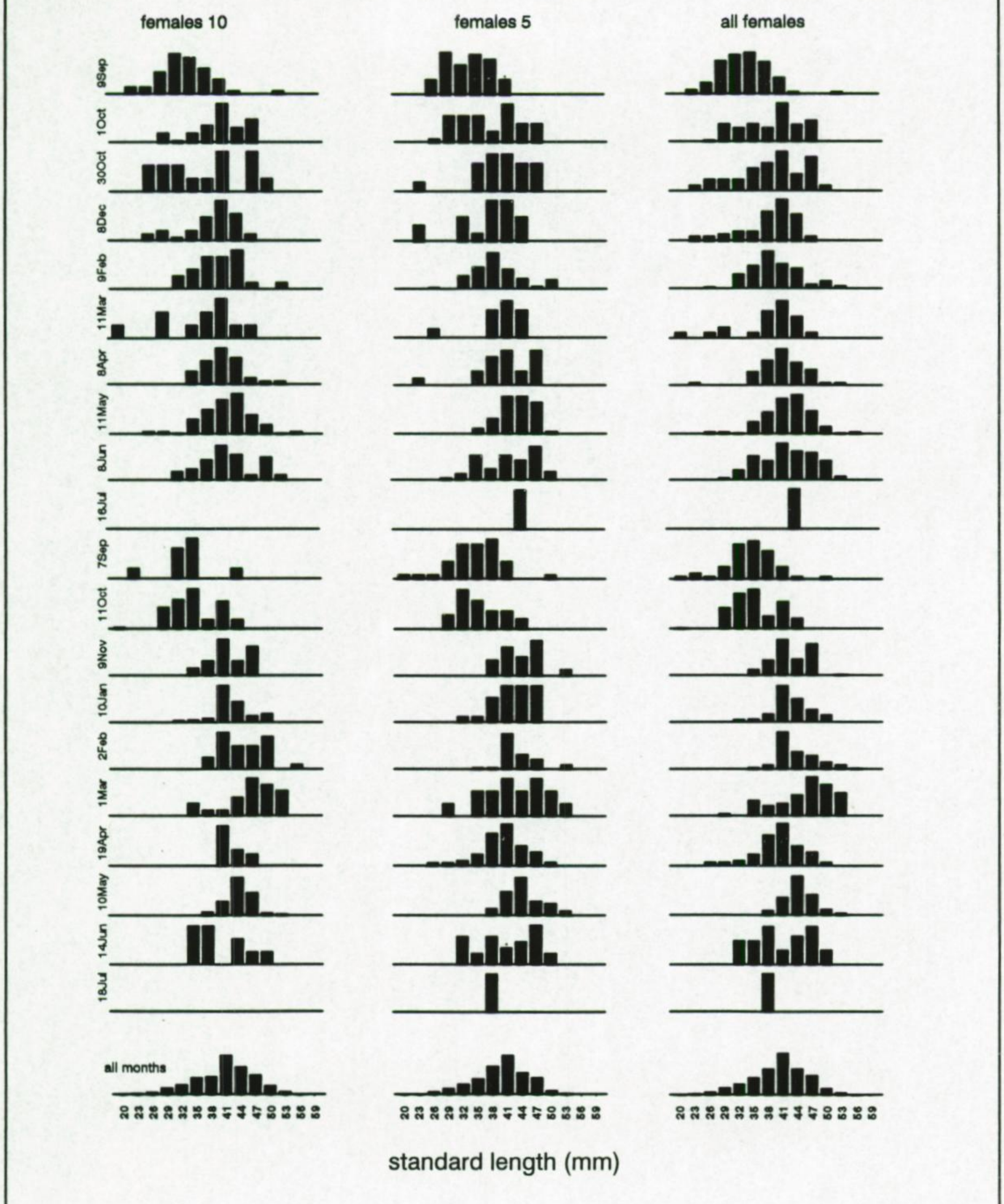


Figure 7.10. *Pomatoschistus lozanoi.* Length-frequency distribution of females in the two different strata and in the whole area from September 1992 to July 1994.

slope of the regression of females ($t = 3.44$, $N = 264$, $p<0.005$), while in *P. lozanoi* the slopes of the female and male regressions were not significantly different ($t = 1.31$, $N = 107$, $p>0.05$). Thus, in *P. minutus* males grew faster than females. When both species were pooled, the allometry was still positive but the slope was overestimated.

The estimated growth parameters of the seasonally oscillating version of the VBGF and the growth performance indices of males and females and of both sexes and species pooled are summarised in Table 7.3. The L_∞ and *K* of males were always higher than those of females. Pooling of the length-frequency data of both sexes resulted in an overestimate of *K* in *Pomatoschistus lozanoi*. Pooling of both species resulted in an

Table 7.3. *Pomatoschistus minutus* and *P. lozanoi*. Growth parameters of the von Bertalanffy growth curves of males, females, both sexes and species pooled. L_∞: asymptotic length; *K*: growth constant; *C*: amplitude of growth oscillation; *WP*: time of the year with the slowest growth; t_s: starting point of oscillation; ϕ: growth performance index; *RN*: a measure of goodness of fit.

Species/Sex	**L_∞ (mm)**	**K (y^{-1})**	**C**	**WP**	**t_s**	**ϕ**	**Rn**
P. minutus							
Male	79.40	1.90	1.0	0.00	0.50	4.08	0.44
Female	73.90	1.65	1.0	0.12	0.62	3.95	0.34
Both sexes	79.20	1.70	1.0	0.03	0.53	4.03	0.32
P. lozanoi							
Male	65.30	1.50	1.0	0.13	0.63	3.81	0.47
Female	54.85	1.35	1.0	0.27	0.77	3.61	0.28
Both sexes	64.90	1.90	1.0	0.12	0.62	3.90	0.62
Lumped species	83.50	0.94	1.0	0.15	0.65	3.81	0.44

underestimation of *K* and an overestimation of L_∞.

The growth of both sexes and both species showed pronounced oscillations and growth stopped once a year ($C=1$). The *WP* values revealed that slowest growth occurred from January to March. Males had higher growth performance indices than females (Table 7.3).

In *Pomatoschistus minutus*, males attained a TML of 620 days for a TMS of 79 mm SL, while females attained a TML of 650 days for a TMS of 70 mm SL. Pooling of the data of both sexes yielded a TMS of 76 mm SL with a corresponding TML of 650 days. In *P. lozanoi*, the estimated TMS of males of 59 mm SL corresponded to a TML of 600 days, while for females the TMS of 49 mm SL corresponded to a TML of 650 days. Pooling data of both sexes yielded a TMS of 63 mm SL with a corresponding TML of 650 days. Pooling of the data of both species yielded a TMS of 71 mm SL with a corresponding TML of 720 days.

7.3.5. Cohort separation

The VBGFs superimposed on the restructured length-frequency of males, females and both sexes of *Pomatoschistus minutus* pooled are shown in Figure 7.11. On each occasion 4 cohorts were detected (1991, 1992, 1993 and 1994 cohorts). The 1993 and 1994 cohorts emerged in the same month (June) each year and disappeared after about 22 months. The same number of cohorts was observed when the data of both sexes were lumped. Pooled monthly length-frequency data of male *P. minutus* revealed only one clear cohort (age group 1) (Figure 7.12). The few data points beyond the regression line represent individuals of another cohort (age group 2). A regression line was fitted to these data points, but the individuals of the age group 2 overlapped with the largest individuals of age group 1. Thus, the curve of the cohort was not superimposed to age group 2. The same number of cohorts was observed for females and for both sexes lumped (not figured).

The VBGFs and the restructured length-frequency of male and female *Pomatoschistus lozanoi* are shown in Figure 7.13. Two cohorts (1992 and 1993), which emerged in the same month each year (September), were clearly identified. The same number of cohorts and time of cohort emergence were also observed in the pooled data. The last individuals of a 1991 cohort were also detected. The number of cohorts observed

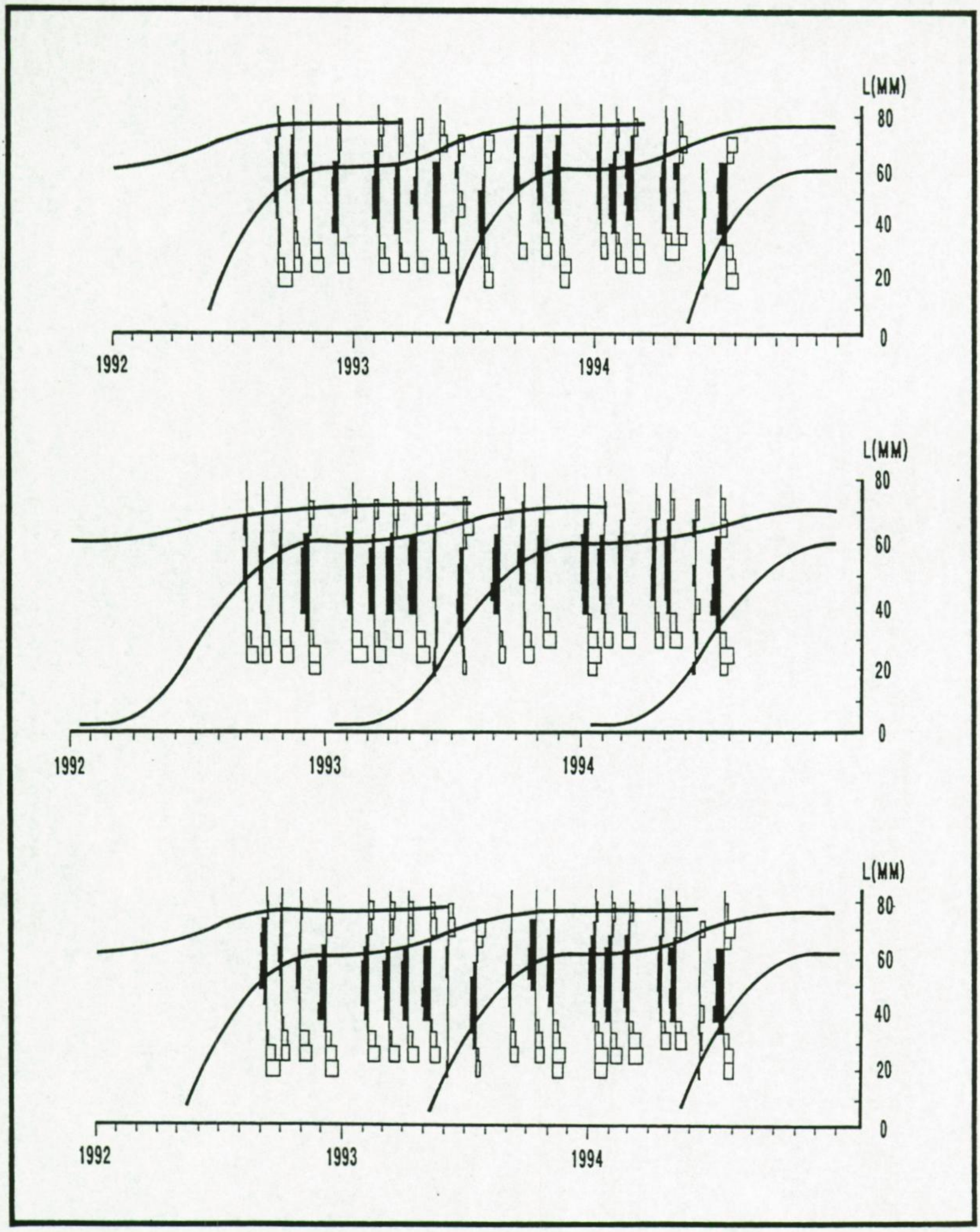


Figure 7.11. *Pomatoschistus minutus.* Growth curves superimposed on the different cohorts as estimated by the ELEFAN program. Top = males; center = females; bottom = sexes pooled.

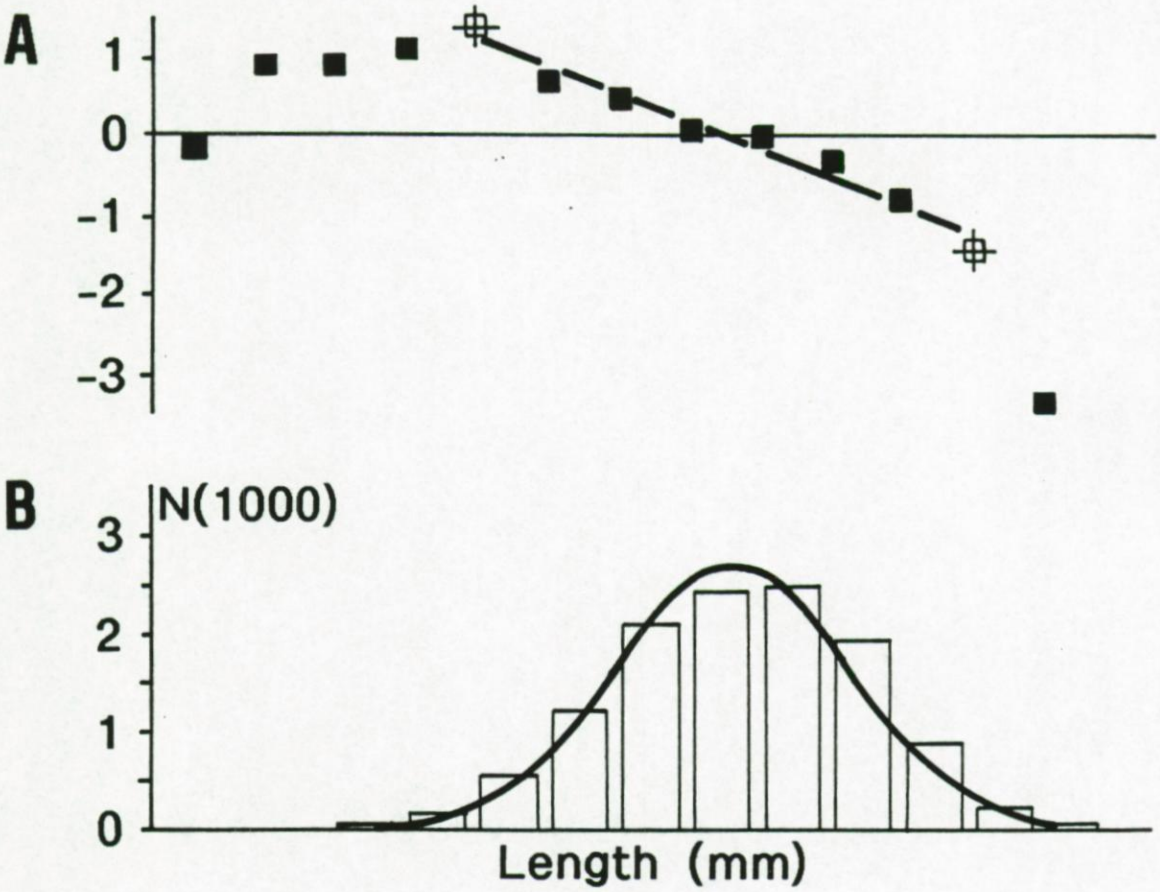


Figure 7.12. *Pomatoschistus minutus.* Number of cohorts in the monthly pooled data, separated with the use of the Bhattacharya method. a = log-transformed data; b = normal curve superimposed on the raw data.

in the pooled monthly length-frequency data of male *P. lozanoi* is shown in Figure 7.14. The results of this analysis (also for females and for both sexes pooled; not figured) are similar to these of *P. minutus*. The VBGFs superimposed on the restructured length-frequency of pooled species are shown in Figure 7.15. The result of this analysis is similar to *P. minutus*.

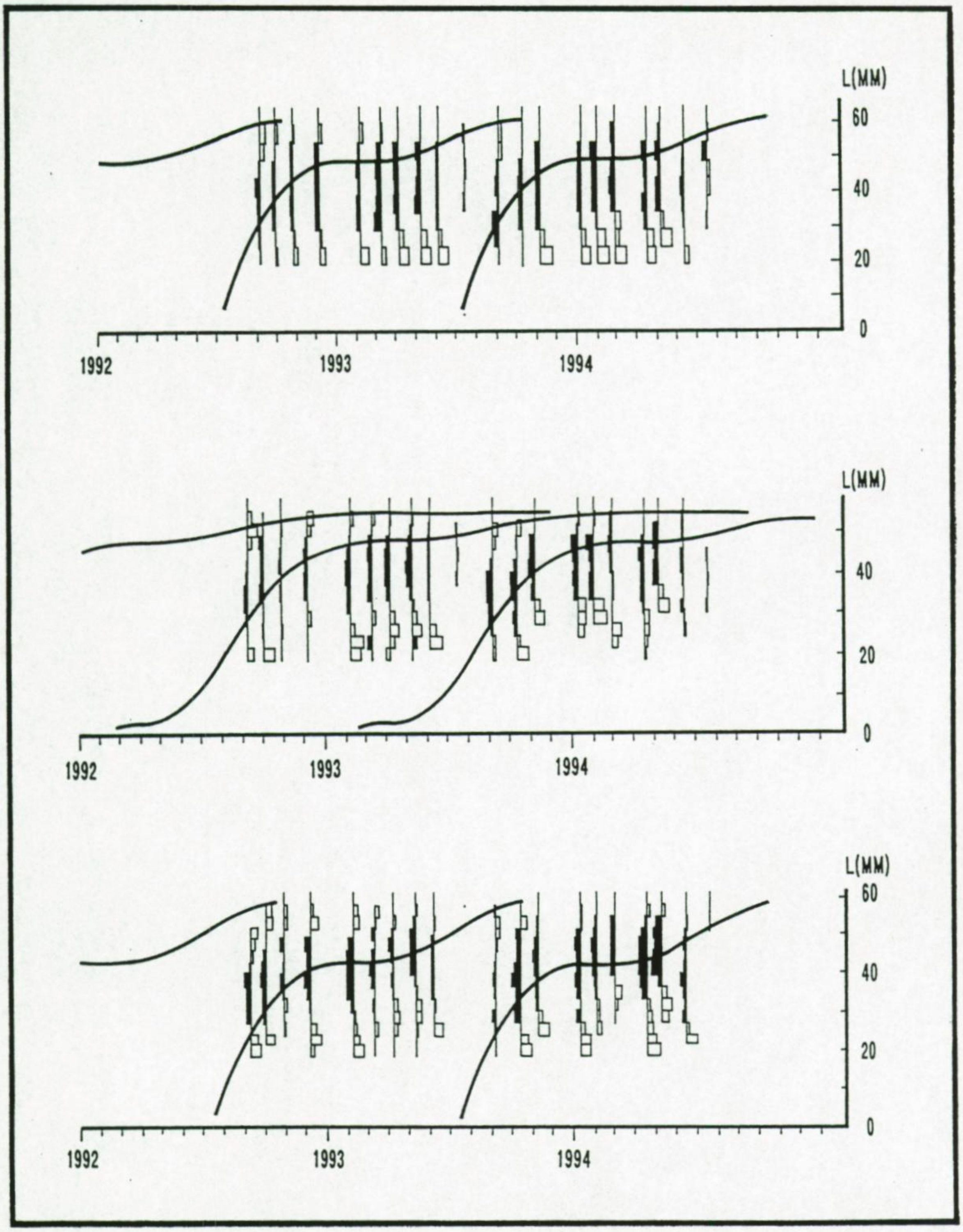


Figure 7.13. *Pomatoschistus lozanoi*. Growth curves superimposed on the different cohorts as estimated by the ELEFAN program. Top = males; center = females; bottom = sexes pooled.

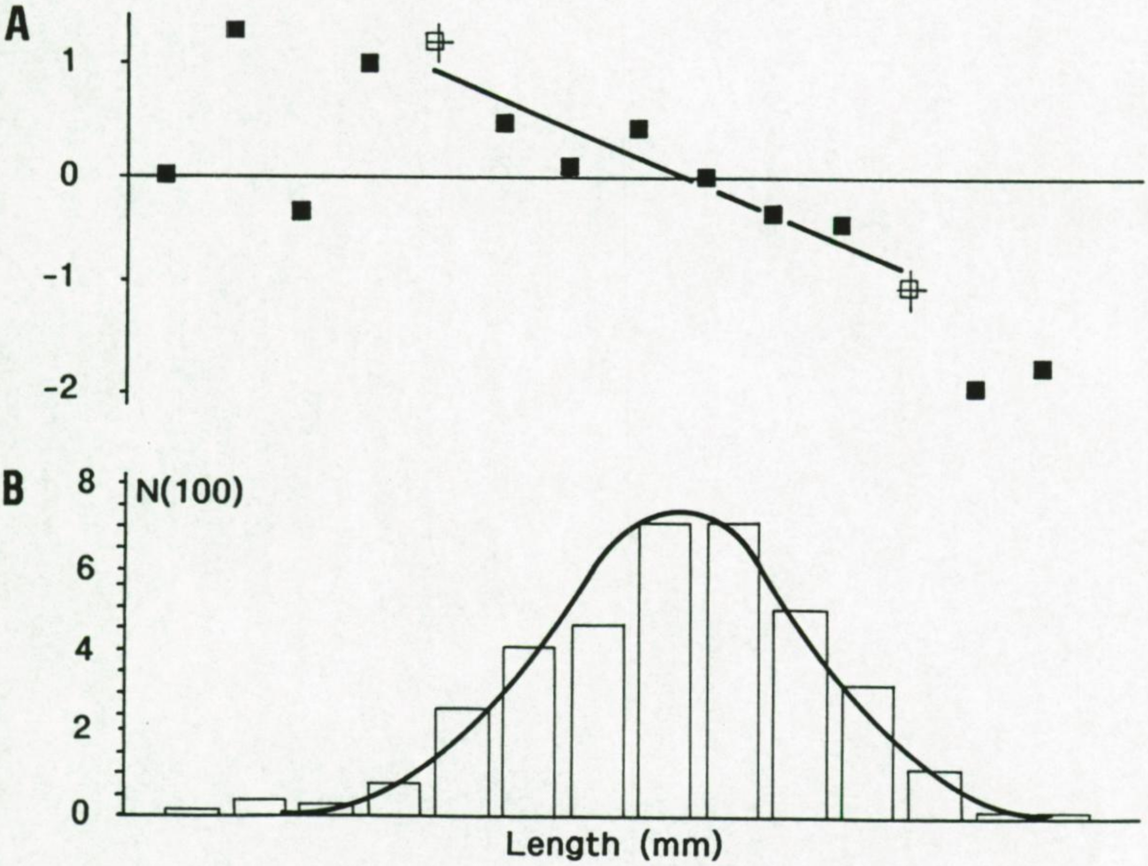


Figure 7.14. *Pomatoschistus lozanoi.* Number of cohorts in the monthly pooled data, separated with the use of the Bhattacharya method. a = log-transformed data; b = normal curve superimposed on the raw data.

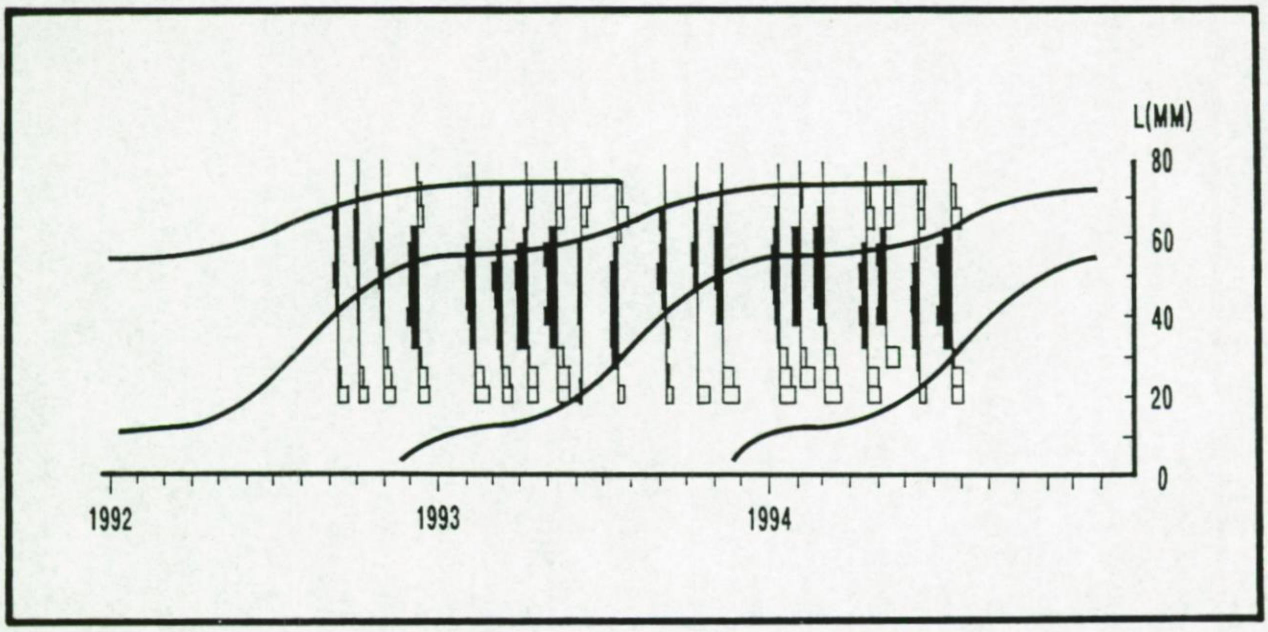


Figure 7.15. Lumped species. Growth curves superimposed on the different cohorts as estimated by the ELEFAN program.

7.3.6. Production

Estimates of the annual production of male and female *Pomatoschistus minutus* and *P. lozanoi* and of both species lumped are summarised per depth stratum in Table 7.4. In *P. minutus* and in the 1992 cohort of *P. lozanoi*, the production of males and females was highest in deeper waters and production of males was higher than that of females. In the 1993 cohort of *P. minutus*, male production was higher than that of females and total production in shallow waters (0.182 g AFDW m^{-2} y^{-1}) was lower than in deeper waters (0.245 g AFDW m^{-2} y^{-1}). In the 1993 cohort of *P. lozanoi*, male production was 4 times higher than that of females and total production of the species in shallow waters was 2.4 times higher than that in deeper waters. The production of the 1993 cohort was 2.5 times higher than that of the 1992 cohort. In deeper waters, the total production of the 1992 cohort (0.028 g AFDW m^{-2} y^{-1}) was more or less equal to that of the 1993 cohort (0.027 g AFDW m^{-2} y^{-1}), while in shallow waters, the 1992 cohort (0.018 g AFDW m^{-2} y^{-1}) produced 3.5 times less than the 1993 cohort (0.064 g AFDW m^{-2} y^{-1}).

Compared to production estimates obtained from the maximally disaggregated length-frequency data, lumping of data resulted in serious overestimates of production. Lumping of data of both depth strata generally yielded overestimates in both sexes of both

Table 7.4. *Pomatoschistus minutus* and *P. lozanoi*. Production estimates (g AFDW m^{-2} y^{-1}) obtained from the different cohorts, strata, sexes and lumped data.

Species/Sex/location	Production	Biomass	P/B ratio
P. minutus			
1993 cohort			
Males 5m	0.082	0.035	2.370
Males 10m	0.134	0.061	2.220
Sum	0.216	0.096	2.250
Strata pooled	0.370	0.153	2.414
Females 5m	0.100	0.050	1.988
Females 10m	0.111	0.055	2.037
Sum	0.211	0.105	2.009
Strata pooled	0.236	0.103	2.279
Sum of sexes	0.427	0.201	2.124
Sexes pooled	0.891	0.391	2.282
P. lozanoi			
1992 cohort			
Males 5m	0.012	0.006	2.067
Males 10m	0.017	0.009	1.928
Sum	0.029	0.015	1.933
Strata pooled	0.030	0.015	1.970
Females 5m	0.006	0.004	1.458
Females 10m	0.011	0.006	1.769
Sum	0.017	0.010	1.700
Strata pooled	0.019	0.011	1.693
Sum of sexes	0.046	0.025	1.84
Sexes pooled	0.052	0.029	1.809
1993 cohort			
Males 5m	0.042	0.016	2.590
Males 10m	0.021	0.010	2.084
Sum	0.063	0.026	2.423
Strata pooled	0.092	0.039	2.341
Females 5m	0.022	0.013	1.756
Females 10m	0.006	0.004	1.633
Sum	0.028	0.017	1.647
Strata pooled	0.023	0.016	1.460
Sum of sexes	0.091	0.043	2.116
Sexes pooled	0.129	0.062	2.095
Both species			
Total prod. 1993 cohort	0.518	0.244	2.123
Species pooled	1.212	0.582	2.082

species: 42% and 11% overestimate for male and female *P. minutus*, respectively; 3% and 10% overestimate for male and female *P. lozanoi* of the 1992 cohort; 32% overestimate and 18% underestimate for male and female *P. lozanoi* of the 1992 cohort. Lumping of sexes resulted in 52% (*P. minutus*, 1993 cohort), 29% (*P. lozanoi*, 1993 cohort) and 12% (*P. lozanoi*, 1992 cohort) overestimations of production. Lumping of species (all data) resulted in a 57% overestimation of production (cohorts of 1993).

7.4. Discussion

7.4.1. Estimates of density

Published estimates of the net efficiency of beam trawls vary between 10 and 60% (Riley & Corlett 1966, Edwards & Steele 1968, Kuipers 1975, Doornbos & Twisk 1984, Rogers & Lockwood 1989). It is most likely that the net efficiency for demersal fish is between 20 and 35% (e.g. Elliot & Taylor 1989, Hostens & Hamerlynck 1994). In this study, we used a 20% net efficiency, which is Kuipers' (1975) efficiency for plaice, independent of size class. Still, catchability of fish is known to vary greatly for different species and, within a species, between different size classes. For instance, because the beam trawl was operated on the sea bottom with a headrope clearance of about 50 cm the more pelagic feeding activity of *Pomatoschistus lozanoi* can well have resulted in a lower catch efficiency and thus in lower production estimates. Also, the absence of gobies smaller than 20 mm SL and the low densities of small gobies in June are most probably due to the sampling gear selection.

7.4.2. Spatial and seasonal abundance patterns, sex ratio's and spawning

The observed temporal and spatial fluctuations in the abundance of gobies are correlated with the species' growth, reproduction, mortality, recruitment, migration, spawning behaviour and changes in environmental conditions.

Pomatoschistus minutus

The higher biomass of *Pomatoschistus minutus* in deeper waters is due to the higher densities of large males and females and to higher recruitment of juveniles in this stratum. The higher recruitment of gobies in deeper waters is correlated with the breeding behaviour of males. It is known that males guard the eggs which are deposited under bivalve shells during the spawning season (Miller 1984). The significantly higher abundance of males during the spawning season (March-May) in deeper waters (75% in the -10 m depth stratum versus 25% in the -5 m depth stratum) ($z = 5.86$, $p < 0.005$) is an indication of a spawning preference of *P. minutus* in deeper waters. This finding is in agreement with observation on the sand goby population in the Wadden Sea (Fonds 1973) and in the Baltic (Nellbring 1993). The males further have a higher growth performance index than females (they attain a higher L_∞ and grow faster). An increase in mean size of fish with depth has often been noted (e.g. Heincke 1913, Harden-Jones 1968, Johannes 1981). In Gdańsk Bay, sand gobies were even more concentrated at depths of 70-80 m (Morawski 1978). Also in the Belt Sea sand gobies have been found to prefer deeper waters (28-32 m) than other goby species (Hesthagen 1971). The marked reduction of the densities of male *P. minutus* in April and in May (the actual spawning time) was probably due to the lower efficiency of the beam trawl in catching males (the males are still hiding under shells or stones to guard the nests). Nellbring (1993) stated that male *P. minutus* used almost all available shells at the peak of the spawning period. Goby egg surveys conducted by Fonds (1973) in the Wadden Sea and adjacent areas in the North Sea revealed that a beam trawl is less effective than a dredge to collect shells (*Mya arenaria* and *Ostrea* species) to which the eggs were attached. Additionally, spawning migration of males to areas deeper than 10 m, and the presence of 'sneaker males' (smaller size male breeders which lack nuptial coloration) (Miller 1984) may also contribute to the lower abundances recorded during the breeding season. The increase of the densities and biomass of *P. minutus* observed in July was correlated with increasing water temperature, the emergence of a new cohort, increased growth rates and the re-emergence of male breeders from the spawning sites. The seasonal pattern in abundance of *P. minutus* was similar to that found by Swedmark (1958) for a Swedish population and Fonds (1971) for the Wadden Sea population.

In the Dutch Wadden Sea young *Pomatoschistus minutus* recruited in the beam trawl in July (Fonds 1971). Recruitment was earlier in the Grevelingen, which indicates that the species spawns earlier in this area. In the Tagus estuary, Portugal, the youngs of *P. minutus* were already caught in May (Moreira *et al.* 1991). In the Askö area, northern

Baltic proper, *P. minutus* spawned up to the beginning of August (Nellbring 1993).

Pomatoschistus lozanoi

Temporal variations in the density estimates of *Pomatoschistus lozanoi* revealed that the 1993 cohort was a stronger year class than 1992 cohort. Irregular patterns in the abundances recorded in different months are probably an indication of migrations in and out of the sampling area. Most monthly sex-ratios did not depart much from a 1:1 ratio, except during the spawning season. The emergence of high numbers of young (small) individuals in September, suggests that highest spawning intensity occurred before August. Additionally, the abrupt decline of gobies in June and July and the disappearance of males in July are also manifestations of peak spawning in these months. As in *P. minutus*, males are known to guard the eggs which are deposited under bivalve shells, thus decreasing their catchability (Miller 1984). Indeed, the nests have been shown not to be disturbed by the tickler chain of a small beam trawl (Hesthagen 1975, 1977). After September, juveniles continued to occur in the samples up to April, suggesting that *P. lozanoi* has an extended spawning period. Spawning probably continues up to October. Water temperatures recorded in October (12.4 ± 3.7 °C) were well within the range where *P. lozanoi* can spawn. Indeed, experimental studies have shown that *P. lozanoi* can spawn at temperatures between 10°C and 15°C while no nesting behaviour or spawning were observed between 4°C and 9°C (Fonds 1973). Furthermore, females have been shown to be batch spawners, laying eggs several times every spawning season (Miller 1984). Thus, spawning intensity in October was low and fewer individuals appeared in April. After October spawning is unlikely to occur due to low temperatures. Juveniles produced in this month will overwinter as juveniles until spring. In the Belgian and Dutch coastal waters, spawning was thought to occur from June to August, juveniles being only observed in September and October (Hamerlynck 1990). However, the juveniles that appeared in April of 1993 and 1994, indicate that the spawning period may be extended up to October. Additionally, on the basis of growth, gobies recruited in September are likely to be adult in April because they grow fast.

Pomatoschistus lozanoi was more abundant in the shallower parts of the ebb-tidal tidal of the Grevelingen, particularly the small females. This higher abundance in shallow waters is probably correlated with the advantages of lower water transparencies (cf. lower visual predation pressure, higher densities of hyperbenthic prey). The dominance of young *P. lozanoi* in the shallow stratum and the dominance of old gobies in the deeper stratum suggests that most spawning sites are located in the shallow areas and that old gobies migrate from shallow to deeper waters.

7.4.3. Growth

Inferences on growth using only one method, e.g. ELEFAN 1, are not necessarily justified unless the results are compared with those obtained with other methods, e.g. allometric growth analysis. Thus, allometry is important in the generalisation and validation of the overall growth. The parameters of the length-weight regression are sources of information on the allometry of growth and the representativeness of the sample size (Weatherley 1972, Pauly 1984). Pauly (1984) stated that L-W regressions with a slope outside the range of 2.5 to 3.5 indicate that the sample is not well representative of the population size. The sample sizes used in this study for the assessment of allometric growth of *Pomatoschistus minutus* and *P. lozanoi* are thus representative of the population. The slope of the L-W regression of the lumped species, however, is outside this range. Allometric growth of *P. minutus* and *P. lozanoi* was not yet reported despite extensive use of this species in many ecological studies. The growth in weight of both male and female *P. minutus* is faster than the increase in SL (positive allometry, expect for small gobies). Still, males grow faster than females (cf. the higher slopes of the SL-WW). Additionally, the body structure of males is more robust than that of the females of the same mean length (Hamerlynck 1990). This sex-specific somatic growth pattern might not be the same throughout the species' life. Rogers (1988) stated that the gonado-somatic index of female *P. microps* increased quite dramatically about 2 months before the start of spawning and decreased when spawning started. Since *P. minutus* showed a similar reproductive behaviour as *P. microps* (Fonds 1973), it is assumed that the somatic growth of males is slower than that of the females before spawning. Ovary weight of female *P. minutus* increased rapidly before the spawning period, while an increase in the testes weight of males was not obvious (Healey 1971). By the end of spawning in June a dramatic reduction in the size of the ovaries was observed, while the testes only showed a small reduction in size (Healey 1971). The increase in weight of both sexes of *P. lozanoi* is also faster than the increase in length. The higher slope value of the SL-WW regression of males than that of females is indicative of a faster male growth rate, although this difference was not significant.

The description of the somatic growth of male and female *Pomatoschistus minutus* and *P. lozanoi* using ELEFAN 1 strongly supports the result of the allometric growth analysis. The growth of males and females of both species is described well with use of seasonally oscillating version of the VBGF. The growth curves superimposed on the monthly restructured length-frequency data of the different cohorts are the mirror image of one another. Thus, one of the assumptions for a valid application of the VBGF (the growth of cohorts has to be similar from one year to the next) is well met.

Correlating the water temperatures with the growth curves, it is obvious that fast growth (June to October) and slow growth (December to April) of cohorts coincided with increasing and decreasing water temperatures, respectively. The values of the 'winter point' (ranging from 0.0 to 0.12; that is from January to February) revealed that slowest growth coincided with the winter months. The values of *C* (which were all equal to 1) indicated a pronounced growth oscillation and one complete growth stop per year. This suggests that water temperature is the main factor that controls growth.

Fonds (1973) estimated the growth parameters of the general VBGF of *Pomatoschistus minutus* in the Wadden Sea and the North Sea and found a *K* value of around 0.9 and an L_∞ of about 90 mm Total Length (TL). This is equivalent to 76.4 mm SL (SL-TL regression, Hostens unpublished). Growth parameters of *P. minutus* estimated from the length-frequency data (males and females are lumped) collected in the Voordelta in 1989 (Arellano, unpublished) yielded a *K* value of about 1.4 and an L_∞ of about 80 mm SL. The *K* value reported by Fonds (1973) is quite low and is remarkably close to the *K* value found for the lumped data of *P. minutus* and *P. lozanoi* (this study), while the *K* value of Arellano (unpublished) is very close to the *K* values estimated for the 1993 cohort in this study (1.9 for males and 1.65 for females). The growth of *P. minutus* is thus stock and sex-specific. The lumping of data of males and females and of different species results in an underestimation of *K* values and overestimation of L_∞.

7.4.4. Cohort separation

Cohort separation of the pooled monthly length-frequency data and the restructured monthly length-frequency data revealed that male and female *Pomatoschistus minutus* and

P. lozanoi have a lifespan of less than 2 years. If the gobies live longer, all 3 (*P. lozanoi*) or 4 (*P. minutus*) cohorts observed in the restructured length-frequency data should be detectable in the pooled length-frequency data. Since a cohort only lives for 2 years or less, the 1991 and 1992 cohorts can not be detected in the pooled length-frequency data because all members died before the last sampling month (July 1994). Additionally, the graphs of the log transformed pooled length-frequency data also suggest that the gobies live less than 2 years: the data points beyond the first regression line (age group 1) indicate the presence of a second cohort. Age group 2 is not clearly separated because it contains few individuals (inadequacy of sample size due to higher mortality attributed to old age and overlap of age group 2 with the larger individuals of age group 1).

Seeding the estimated growth parameters into the seasonally oscillating version of the VBGF with t_o set to 0, male *Pomatoschistus minutus* attain a TMS of 79 mm SL for a TML of 620 days, while females attain a TMS of 70 mm SL for a TML of 650 days. In *P. lozanoi*, males and females of the 1992 cohort attain a TMS of 59 mm SL for a TML of 600 days and a TMS of 49 mm SL for a TML of 650 days, respectively. Pooling the data of both sexes of *P. lozanoi* yielded a TMS of 63 mm SL with a corresponding TML of 650 days. Pooling data of both species yielded a TMS of 71 mm SL with a corresponding TML of 720 days. These predicted values are remarkably close to the age counts using daily growth increments in the sagittal otoliths of the same samples (Arellano *et al.*, submitted d).

7.4.5. Production

The production of *Pomatoschistus minutus* in the Grevelingen area is higher than that reported for the Oosterschelde population (0.02 g AFDW m^{-2} y^{-1} with a P/B of 2.5) (Hostens & Hamerlynck 1994). This difference is due to the much higher abundances of sand gobies in the Voordelta: average density of sand gobies in the Grevelingen is 6.2 individuals per m^2 versus 0.4 in the Oosterschelde (Hostens & Hamerlynck 1994). Production of sand gobies in the Ythan estuary ranged from 0.09 to 0.17 g m^{-2} y^{-1} (Healey 1972) which is within the range of our estimates.

Lumping the data of different depth strata, sexes and species resulted in overestimates of production. This is due to the differential occurrence of different size classes at different depths and to sex- and species- related differences in allometric growth and size. For this reason, one of the assumptions of the size-frequency method is that all sexes and cohorts must attain the same maximum size (Hamilton 1969). The high production of males and females in the deeper waters are due to the intra-migration of larger goby in the population from shallow to deeper water. Since the production estimates reported in this study were derived from maximally disaggregated size-frequency distributions, they are thought to be quite reliable. Further, the CPI's used for the production estimates are very close to the age counting values derived from otoliths. Still, production values reported in this study are likely to be underestimates on the account of sampling gear selection and male breeding behaviour. Further, cohort separation analysis are not completely objective, and the estimates of growth parameters and CPI's are likely to influence the production estimates.

7.4.6. Comparison of the life histories of *Pomatoschistus minutus* and *P. lozanoi*

Since the juveniles of *Pomatoschistus minutus* recruit earlier in the samples (June) than those of *P. lozanoi* (September), the former species spawns earlier in the Voordelta. This has also been observed from other areas (Fonds 1973, Hamerlynck 1990). The absence of juvenile *P. minutus* in April further suggests that this species has a shorter spawning period (March to May) while that of *P. lozanoi* is extended (June to October). This finding is contrary to the findings of Hamerlynck (1990). Further, there is a spatial segregation of the species: *P. minutus* lives in the deeper parts of the area. This habitat segregation is probably linked with the species' feeding ecology and social behaviour. The two species show food resource partitioning. *P. lozanoi* feeds predominantly on hyperbenthic animals and *P. minutus* feeds mostly on endobenthic and epibenthic animals (Hamerlynck *et al.* 1986, 1990). Visual predation avoidance is probably more important for a hyperbenthos feeder than for an epibenthos feeder. From all gobies collected 79% were *P. minutus*, 72% of which were caught in deeper waters. 55% of the *P. lozanoi* landed were caught in shallow waters. Almost all samples were dominated by *P. minutus* except for those taken from April to June. In the Wadden Sea, *P. minutus* dominated the samples collected in March, whereas *P. lozanoi* dominated in April (Fonds 1973). *P. minutus* spawns in deeper waters (Nellbrings 1993). It is most probable that this species dominates in the deep waters and that *P. lozanoi* rather uses the shallower areas as spawning grounds.

Allometric growth of both species is similar. However, the higher *b* values (both sexes) of *Pomatoschistus minutus* are indicative of a faster growth in weight than *P. lozanoi*. *P. minutus* is a more robust species than *P. lozanoi* (Hamerlynck 1990) and males of both species are larger than females.

The amplitude and starting point of the growth oscillations are very similar in both species. However, the L_∞, K and ϕ are higher in *P. minutus*, which strongly supports the results of the allometric growth analysis. The production estimate of *P. lozanoi* is lower than that of *P. minutus*. This difference is attributed to the differences in growth and abundance. The life span of *Pomatoschistus lozanoi* and *P. minutus* never exceeded 2 years. However, *P. minutus* reached an older age than *P. lozanoi*.

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Length Frequency
Pomatoschistus minutus
Voordelta
September 9 1992 to July 18 1994

Both male and female

Length(mm)	9-9-92	1-10-92	30-10-92	8-12-92	9-2-93	11-3-93	8-4-93	11-5-93	8-6-93	16-7-93	7-9-93	11-10-93	9-11-93	10-1-94	2-2-94	1-3-94	19-4-94	10-5-94	14-6-94	18-7-94	Sum
20	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	0	0	9
25	0	0	0	0	0	0	0	0	27	3	0	0	0	0	0	0	0	0	4	2	36
30	1	0	0	2	0	0	0	0	47	21	0	0	2	2	0	0	0	0	3	4	82
35	72	37	16	12	7	3	1	4	0	44	48	8	2	2	16	1	0	0	0	13	286
40	208	72	54	75	65	20	13	20	0	88	134	24	52	66	193	13	28	4	0	82	1211
45	354	209	214	184	183	34	32	75	12	89	334	96	125	206	530	117	83	20	2	241	3140
50	337	249	205	248	260	44	38	142	37	60	504	228	311	420	831	248	129	77	1	311	4680
55	710	361	234	115	253	25	25	89	37	14	446	291	456	474	662	291	154	62	7	69	4775
60	815	345	224	50	108	14	13	26	8	6	417	504	460	402	398	207	88	19	2	11	4117
65	443	414	117	17	27	2	2	2	2	0	547	416	258	213	179	70	53	13	0	8	2783
70	27	279	78	7	4	0	0	4	0	0	208	172	122	78	44	24	14	2	0	2	1065
75	8	140	24	0	0	0	0	0	0	0	17	16	12	24	2	0	0	0	0	0	243
80	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
sum	2983	2106	1166	710	907	142	124	362	179	325	2655	1755	1800	1887	2855	971	549	197	19	743	22435

Length Frequency
Pomatoschistus minutus
Voordelta
September 9 1992 to July 18 1994

Male

Length(mm)	9-9-92	1-10-92	30-10-92	8-12-92	9-2-93	11-3-93	8-4-93	11-5-93	8-6-93	16-7-93	7-9-93	11-10-93	9-11-93	10-1-94	2-2-94	1-3-94	19-4-94	10-5-94	14-6-94	18-7-94	Sum
20	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0	6
25	0	0	0	0	0	0	0	0	13	1	0	0	0	0	0	0	0	0	1	1	16
30	1	0	0	0	0	0	0	0	32	6	0	0	2	0	0	0	0	0	1	1	43
35	42	19	4	6	5	2	1	2	0	25	0	0	2	0	16	1	0	0	0	12	137
40	103	36	18	25	29	9	5	6	0	58	36	8	42	42	98	4	1	0	0	44	564
45	111	90	90	65	78	13	5	15	0	51	127	30	74	71	182	29	28	4	0	144	1207
50	187	108	66	114	97	20	5	35	8	38	200	116	141	198	428	82	60	28	1	186	2118
55	395	217	126	42	144	11	7	22	4	11	178	180	273	230	327	150	42	10	0	43	2412
60	484	232	114	27	62	7	6	6	2	4	329	316	287	213	221	124	52	2	0	7	2495
65	267	322	73	12	21	2	1	0	0	0	541	261	162	128	96	24	28	0	0	0	1938
70	26	249	42	5	4	0	0	2	0	0	206	156	94	62	32	12	14	2	0	0	906
75	8	124	24	0	0	0	0	0	0	0	17	16	12	24	0	0	0	0	0	0	225
80	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
sum	1632	1397	557	296	440	64	30	88	65	194	1634	1083	1089	968	1400	426	225	46	3	438	12075

Length Frequency
Pomatoschistus minutus
Voordelta
September 9 1992 to July 18 1994
Ten meters depth

Male

Length(mm)	9-9-92	1-10-92	30-10-92	8-12-92	9-2-93	11-3-93	8-4-93	11-5-93	8-6-93	16-7-93	7-9-93	11-10-93	9-11-93	10-1-94	2-2-94	1-3-94	19-4-94	10-5-94	14-6-94	18-7-94	Sum
20	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	4
25	0	0	0	0	0	0	0	0	9	0	0	0	0	0	0	0	0	0	1	1	11
30	0	0	0	0	0	0	0	0	23	2	0	0	0	0	0	0	0	0	0	0	25
35	38	17	4	4	4	1	1	2	0	7	32	0	0	0	0	0	0	0	0	4	114
40	86	28	10	13	21	5	5	6	0	32	118	8	40	40	17	0	1	0	0	40	470
45	78	76	58	49	53	8	2	14	0	36	157	29	64	52	41	19	8	4	0	125	873
50	140	70	36	58	68	10	2	29	8	28	155	107	121	144	89	75	32	28	0	126	1326
55	300	179	94	26	101	6	6	18	2	10	293	168	256	162	75	136	40	9	0	18	1899
60	372	202	98	16	43	5	6	6	2	4	525	306	264	135	48	120	27	2	0	3	2184
65	228	296	60	6	14	1	1	0	0	0	206	251	144	88	38	22	28	0	0	0	1383
70	24	229	38	5	2	0	0	2	0	0	17	154	88	36	6	10	14	2	0	0	627
75	8	120	24	0	0	0	0	0	0	0	0	14	9	20	0	0	0	0	0	0	195
80	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8
sum	1282	1217	422	177	306	36	23	77	48	119	1503	1037	986	677	314	382	150	45	1	317	9119

Length Frequency
Pomatoschistus minutus
Voordelta
September 9 1992 to July 18 1994
Five meters depth

Male

Length(mm)	9-9-92	1-10-92	30-10-92	8-12-92	9-2-93	11-3-93	8-4-93	11-5-93	8-6-93	16-7-93	7-9-93	11-10-93	9-11-93	10-1-94	2-2-94	1-3-94	19-4-94	10-5-94	14-6-94	18-7-94	Sum
20	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2
25	0	0	0	0	0	0	0	0	4	1	0	0	0	0	0	0	0	0	0	0	5
30	1	0	0	0	0	0	0	0	9	4	0	0	2	0	0	0	0	0	1	1	18
35	4	2	0	2	1	1	0	0	0	18	4	0	2	0	16	1	0	0	0	8	59
40	17	8	8	12	8	4	0	0	0	26	9	0	2	2	81	4	0	0	0	4	185
45	33	14	32	16	25	5	3	1	0	15	43	1	10	19	141	10	20	0	0	19	407
50	47	38	30	56	29	10	3	6	0	10	23	9	20	54	339	7	28	0	1	60	770
55	95	38	32	16	43	5	1	4	2	1	36	12	17	68	252	14	2	1	0	25	664
60	112	30	16	11	19	2	0	0	0	0	16	10	23	78	173	4	25	0	0	4	523
65	39	26	13	6	7	1	0	0	0	0	0	10	18	40	58	2	0	0	0	0	220
70	2	20	4	0	2	0	0	0	0	0	0	2	6	26	26	2	0	0	0	0	90
75	0	4	0	0	0	0	0	0	0	0	0	2	3	4	0	0	0	0	0	0	13
80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sum	350	180	135	119	134	28	7	11	17	75	131	46	103	291	1086	44	75	1	2	121	2956

Length Frequency
Pomatoschistus minutus
Voordelta
September 9 1992 to July 18 1994

Female

Length(mm)	9-9-92	1-10-92	30-10-92	8-12-92	9-2-93	11-3-93	8-4-93	11-5-93	8-6-93	16-7-93	7-9-93	11-10-93	9-11-93	10-1-94	2-2-94	1-3-94	19-4-94	10-5-94	14-6-94	18-7-94	Sum
20	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	3
25	0	0	0	0	0	0	0	0	14	2	0	0	0	0	0	0	0	0	3	1	20
30	0	0	0	2	0	0	0	0	15	15	0	0	0	2	0	0	0	0	2	3	39
35	30	18	12	6	2	1	0	2	0	19	48	8	0	2	0	0	0	0	0	1	149
40	105	36	36	50	36	11	8	14	0	30	98	16	10	24	95	9	27	4	0	38	647
45	243	119	124	119	105	21	27	60	12	38	207	66	51	135	348	88	55	16	2	97	1933
50	150	141	139	134	163	24	33	107	29	22	304	112	170	222	403	166	69	49	0	125	2562
55	315	144	108	73	109	14	18	67	33	3	268	111	183	244	335	141	112	52	7	26	2363
60	331	113	110	23	46	7	7	20	6	2	88	188	173	189	177	83	36	17	2	4	1622
65	176	92	44	5	6	0	1	2	2	0	6	155	96	85	83	46	25	13	0	8	845
70	1	30	36	2	0	0	0	2	0	0	2	16	28	16	12	12	0	0	0	2	159
75	0	16	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	18
sum	1351	709	609	414	467	78	94	274	114	131	1021	672	711	919	1455	545	324	151	16	305	10360

Length Frequency
Pomatoschistus minutus
Voordelta
September 9 1992 to July 18 1994
Ten meters depth

Female

Length(mm)	9-9-92	1-10-92	30-10-92	8-12-92	9-2-93	11-3-93	8-4-93	11-5-93	8-6-93	16-7-93	7-9-93	11-10-93	9-11-93	10-1-94	2-2-94	1-3-94	19-4-94	10-5-94	14-6-94	18-7-94	Sum
20	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	3
25	0	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	11
30	0	0	0	2	0	0	0	0	5	4	48	0	0	0	0	0	0	0	1	1	61
35	20	16	6	2	0	0	0	2	0	2	98	8	0	0	0	0	0	0	0	0	154
40	82	30	20	36	16	5	4	4	0	17	203	16	8	18	7	4	3	4	0	29	506
45	196	99	78	72	40	7	10	36	10	32	284	66	40	92	74	50	20	12	0	80	1298
50	82	111	90	80	88	12	23	40	25	16	241	102	153	156	101	130	23	43	0	78	1594
55	236	130	78	32	52	9	14	43	30	3	68	95	144	154	103	116	39	46	1	14	1407
60	274	81	88	13	24	4	7	14	4	2	0	182	152	129	32	69	11	8	0	2	1096
65	154	80	40	5	6	0	1	0	2	0	0	149	80	58	25	42	9	8	0	8	667
70	0	28	34	2	0	0	0	2	0	0	0	16	24	14	4	12	0	0	0	2	138
75	0	16	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	18
sum	1044	591	434	244	226	37	59	141	90	76	942	634	601	621	348	423	105	121	2	214	6953

Length Frequency
Pomatoschistus minutus
Voordelta
September 9 1992 to July 18 1994
Five meters depth

Female

Length(mm)	9-9-92	1-10-92	30-10-92	8-12-92	9-2-93	11-3-93	8-4-93	11-5-93	8-6-93	16-7-93	7-9-93	11-10-93	9-11-93	10-1-94	2-2-94	1-3-94	19-4-94	10-5-94	14-6-94	18-7-94	Sum
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	3	2	0	0	0	0	0	0	0	0	3	1	9
30	0	0	0	0	0	0	0	0	10	11	0	0	0	2	0	0	0	0	1	2	26
35	10	2	6	4	2	1	0	0	0	17	0	0	0	2	0	0	0	0	0	1	45
40	23	6	16	14	20	6	4	10	0	13	4	0	2	6	88	5	24	0	0	9	250
45	47	20	46	47	65	14	17	24	2	6	20	0	11	43	274	38	35	4	2	17	732
50	68	30	49	54	75	12	10	67	4	6	27	10	17	66	302	36	46	6	0	47	932
55	79	14	30	41	57	5	4	24	3	0	20	16	39	90	232	25	73	6	6	12	776
60	57	32	22	10	22	3	0	6	2	0	6	6	21	60	145	14	25	9	2	2	444
65	22	12	4	0	0	0	0	2	0	0	2	6	16	27	58	4	16	5	0	0	174
70	1	2	2	0	0	0	0	0	0	0	0	0	4	2	8	0	0	0	0	0	19
75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sum	307	118	175	170	241	41	35	133	24	55	79	38	110	298	1107	122	219	30	14	91	3407

Length Frequency
Pomatoschistus lozanoi
Voordelta
September 9 1992 to July 18 1994

Both male and female

Length(mm)	9-9-92	1-10-92	30-10-92	8-12-92	9-2-93	11-3-93	8-4-93	11-5-93	8-6-93	16-7-93	7-9-93	11-10-93	9-11-93	10-1-94	2-2-94	1-3-94	19-4-94	10-5-94	14-6-94	18-7-94	Sum
20	2	0	0	0	0	1	0	0	0	0	10	1	0	0	0	0	0	0	0	0	14
23	4	0	2	2	0	2	1	0	0	0	38	0	0	1	0	0	1	0	0	0	51
26	18	2	4	3	3	1	2	4	0	0	10	3	0	1	0	0	16	0	0	0	67
29	50	32	4	3	6	3	1	3	1	0	20	26	0	4	0	1	24	0	1	0	179
32	94	62	12	8	26	1	1	3	10	0	36	67	13	22	9	0	46	0	11	0	421
35	97	63	18	11	45	6	8	14	29	0	42	81	74	45	18	11	103	2	14	0	681
38	62	62	12	20	77	11	18	31	22	0	31	43	136	36	25	7	240	9	12	2	856
41	33	70	32	31	75	9	37	62	45	0	18	48	168	107	123	7	442	36	11	0	1354
44	12	41	18	22	48	10	26	75	32	2	4	54	120	104	76	28	326	91	13	0	1102
47	0	31	20	4	17	4	15	66	25	0	0	6	123	51	80	33	227	82	12	0	796
50	0	3	6	3	11	1	8	30	24	0	4	9	96	35	45	35	77	30	8	0	425
53	2	0	2	1	4	0	13	6	3	0	0	0	38	12	30	9	24	4	1	0	149
56	0	0	0	0	0	0	0	6	2	0	0	0	8	0	4	0	0	0	1	0	21
59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	3
sum	374	366	130	108	312	49	130	300	193	2	213	338	776	418	410	132	1526	254	84	4	6119

Length Frequency
Pomatoschistus lozanoi
Voordelta
September 9 1992 to July 18 1994

Male

Length(mm)	9-9-92	1-10-92	30-10-92	8-12-92	9-2-93	11-3-93	8-4-93	11-5-93	8-6-93	16-7-93	7-9-93	11-10-93	9-11-93	10-1-94	2-2-94	1-3-94	19-4-94	10-5-94	14-6-94	18-7-94	Sum
20	2	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	10
23	0	0	0	0	0	2	0	0	0	0	34	0	0	1	0	0	1	0	0	0	38
26	8	0	0	1	2	0	2	2	0	0	8	3	0	1	0	0	0	0	0	0	27
29	21	13	0	0	6	1	1	1	0	0	12	8	0	4	0	0	8	0	1	0	76
32	60	47	8	4	12	1	1	2	4	0	14	37	13	18	9	0	20	0	6	0	256
35	61	44	10	7	22	5	2	1	14	0	18	47	66	40	15	5	48	2	9	0	416
38	34	47	2	8	39	6	7	8	10	0	13	32	115	23	17	3	92	3	4	0	463
41	19	30	18	14	50	2	21	25	22	0	10	25	119	50	47	2	241	15	8	0	718
44	10	23	12	11	28	6	16	34	14	0	2	45	98	67	41	20	230	45	7	0	709
47	0	9	8	2	13	3	8	42	8	0	0	6	80	31	53	18	160	58	4	0	503
50	0	3	4	3	4	1	7	22	12	0	2	9	96	24	31	23	60	23	5	0	329
53	0	0	2	1	2	0	12	5	1	0	0	0	36	12	22	0	24	1	1	0	119
56	0	0	0	0	0	0	0	4	2	0	0	0	8	0	2	0	0	0	1	0	17
59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	3
sum	215	216	64	51	178	27	77	146	87	0	121	212	631	271	237	72	884	147	46	2	3684

Length Frequency
Pomatoschistus lozanoi
Voordelta
September 9 1992 to July 18 1994
Ten meters depth

Male

Length(mm)	9-9-92	1-10-92	30-10-92	8-12-92	9-2-93	11-3-93	8-4-93	11-5-93	8-6-93	16-7-93	7-9-93	11-10-93	9-11-93	10-1-94	2-2-94	1-3-94	19-4-94	10-5-94	14-6-94	18-7-94	Sum
20	2	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	6
23	0	0	0	0	0	1	0	0	0	0	32	0	0	1	0	0	1	0	0	0	35
26	4	0	0	0	0	0	1	2	0	0	0	0	0	1	0	0	0	0	0	0	8
29	8	9	0	0	2	1	1	0	0	0	2	3	0	0	0	0	0	0	0	0	26
32	46	35	6	4	6	1	1	2	2	0	4	23	8	9	0	0	1	0	0	0	148
35	48	22	2	5	6	0	2	0	3	0	2	30	48	20	4	4	3	2	2	0	203
38	20	19	2	2	14	4	4	6	2	0	2	22	64	9	6	0	5	1	0	0	182
41	8	10	8	6	21	1	15	17	12	0	2	18	96	26	28	0	12	11	1	0	292
44	8	3	6	8	8	3	9	26	8	0	0	45	80	47	30	14	16	30	4	0	345
47	0	1	2	2	6	0	7	34	5	0	0	5	64	15	10	10	17	45	1	0	224
50	0	1	2	1	2	0	6	19	4	0	0	9	88	18	14	22	8	18	1	0	213
53	0	0	0	1	0	0	10	5	1	0	0	0	32	8	13	0	0	0	1	0	71
56	0	0	0	0	0	0	0	4	2	0	0	0	8	0	0	0	0	0	0	0	14
59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sum	144	100	28	29	65	11	56	115	39	0	48	155	488	154	105	50	63	107	10	0	1767

Length Frequency
Pomatoschistus lozanoi
Voordelta
September 9 1992 to July 18 1994
Five meters depth

Male

Length(mm)	9-9-92	1-10-92	30-10-92	8-12-92	9-2-93	11-3-93	8-4-93	11-5-93	8-6-93	16-7-93	7-9-93	11-10-93	9-11-93	10-1-94	2-2-94	1-3-94	19-4-94	10-5-94	14-6-94	18-7-94	Sum
20	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	4
23	0	0	0	0	0	1	0	0	0	0	2	0	0	0	0	0	0	0	0	0	3
26	4	0	0	1	2	0	1	0	0	0	8	3	0	0	0	0	0	0	0	0	19
29	13	4	0	0	4	0	0	1	0	0	10	5	0	4	0	0	8	0	1	0	50
32	14	12	2	0	6	0	0	0	2	0	10	14	5	9	9	0	19	0	6	0	108
35	13	22	8	2	16	5	0	1	11	0	16	17	18	20	11	1	45	0	7	0	213
38	14	28	0	6	25	2	3	2	8	0	11	10	51	14	11	3	87	2	4	0	281
41	11	20	10	8	29	1	6	8	10	0	8	7	23	24	19	2	229	4	7	0	426
44	2	20	6	3	20	3	7	8	6	0	2	0	18	20	11	6	214	15	3	0	364
47	0	8	6	0	7	3	1	8	3	0	0	1	16	16	43	8	143	13	3	0	279
50	0	2	2	2	2	1	1	3	8	0	2	0	8	6	17	1	52	5	4	0	116
53	0	0	2	0	2	0	2	0	0	0	0	0	4	4	9	0	24	1	0	0	48
56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	3
59	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	3
sum	71	116	36	22	113	16	21	31	48	0	73	57	143	117	132	22	821	40	36	2	1917

Length Frequency
Pomatoschistus lozanoi
Voordelta
September 9 1992 to July 18 1994

Female

Length(mm)	9-9-92	1-10-92	30-10-92	8-12-92	9-2-93	11-3-93	8-4-93	11-5-93	8-6-93	16-7-93	7-9-93	11-10-93	9-11-93	10-1-94	2-2-94	1-3-94	19-4-94	10-5-94	14-6-94	18-7-94	Sum
20	0	0	0	0	0	1	0	0	0	0	2	1	0	0	0	0	0	0	0	0	4
23	4	0	2	2	0	0	1	0	0	0	4	0	0	0	0	0	0	0	0	0	13
26	10	2	4	2	1	1	0	2	0	0	2	0	0	0	0	0	16	0	0	0	40
29	29	19	4	3	0	2	0	2	1	0	8	18	0	0	0	1	16	0	0	0	103
32	34	15	4	4	14	0	0	1	6	0	22	30	0	4	0	0	26	0	5	0	165
35	36	19	8	4	23	1	6	13	15	0	24	34	8	5	3	6	55	0	5	0	265
38	28	15	10	12	38	5	11	23	12	0	18	11	21	13	8	4	148	6	8	2	393
41	14	40	14	17	25	7	16	37	23	0	8	23	49	57	76	5	201	21	3	0	636
44	2	18	6	11	20	4	10	41	18	2	2	9	22	37	35	8	96	46	6	0	393
47	0	22	12	2	4	1	7	24	17	0	0	0	43	20	27	15	67	24	8	0	293
50	0	0	2	0	7	0	1	8	12	0	2	0	0	11	14	12	17	7	3	0	96
53	2	0	0	0	2	0	1	1	2	0	0	0	2	0	8	9	0	3	0	0	30
56	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	4
sum	159	150	66	57	134	22	53	154	106	2	92	126	145	147	173	60	642	107	38	2	2435

Length Frequency
Pomatoschistus lozanoi
Voordelta
September 9 1992 to July 18 1994
Ten meters depth

Female

Length(mm)	9-9-92	1-10-92	30-10-92	8-12-92	9-2-93	11-3-93	8-4-93	11-5-93	8-6-93	16-7-93	7-9-93	11-10-93	9-11-93	10-1-94	2-2-94	1-3-94	19-4-94	10-5-94	14-6-94	18-7-94	Sum
20	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	2
23	4	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	6
26	4	0	4	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	12
29	12	5	4	3	0	2	0	2	0	0	0	14	0	0	0	0	0	0	0	0	42
32	22	1	4	1	4	0	0	1	3	0	6	19	0	2	0	0	0	0	0	0	63
35	20	5	2	3	6	1	4	11	4	0	8	26	8	3	0	4	0	0	3	0	108
38	14	9	2	7	10	2	7	18	7	0	0	6	16	5	5	2	0	3	3	0	116
41	8	20	6	12	10	3	11	25	12	0	0	18	40	45	16	2	10	11	0	0	249
44	2	8	0	8	12	1	8	29	9	0	2	6	16	25	10	6	4	30	2	0	178
47	0	12	6	2	2	1	2	14	2	0	0	0	32	8	10	12	3	18	1	0	125
50	0	0	2	0	0	0	1	7	8	0	0	0	0	11	14	10	0	2	1	0	56
53	2	0	0	0	2	0	1	1	2	0	0	0	0	0	0	8	0	1	0	0	17
56	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2	0	0	0	0	0	4
sum	88	60	30	38	46	11	34	112	47	0	18	90	112	99	57	44	17	65	10	0	978

Length Frequency
Pomatoschistus lozanoi
Voordelta
September 9 1992 to July 18 1994
Five meters depth

Female

Length(mm)	9-9-92	1-10-92	30-10-92	8-12-92	9-2-93	11-3-93	8-4-93	11-5-93	8-6-93	16-7-93	7-9-93	11-10-93	9-11-93	10-1-94	2-2-94	1-3-94	19-4-94	10-5-94	14-6-94	18-7-94	Sum
20	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	2
23	0	0	2	2	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	7
26	6	2	0	0	1	1	0	0	0	0	2	0	0	0	0	0	16	0	0	0	28
29	17	14	0	0	0	0	0	0	1	0	8	4	0	0	0	1	16	0	0	0	61
32	12	14	0	3	10	0	0	0	3	0	16	11	0	2	0	0	26	0	5	0	102
35	16	14	6	1	17	0	2	2	11	0	16	8	0	2	3	2	55	0	2	0	157
38	14	6	8	5	28	3	4	5	5	0	18	5	5	8	3	2	148	3	5	2	277
41	6	20	8	5	15	4	5	12	11	0	8	5	9	12	60	3	191	10	3	0	387
44	0	10	6	3	8	3	2	12	9	2	0	3	6	12	25	2	92	16	4	0	215
47	0	10	6	0	2	0	5	10	15	0	0	0	11	12	17	3	64	6	7	0	168
50	0	0	0	0	7	0	0	1	4	0	2	0	0	0	0	2	17	5	2	0	40
53	0	0	0	0	0	0	0	0	0	0	0	0	2	0	8	1	0	2	0	0	13
56	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
sum	71	90	36	19	88	11	19	42	59	2	74	36	33	48	116	16	625	42	28	2	1457