# THE EVOLUTION AND MACROECOLOGICAL CONSEQUENCES OF GRAZING AND SHELL-CRUSHING IN FISHES

Thesis submitted for the degree of Doctor of Philosophy at the

University of Leicester

by

Laurent Patrick Gilbert Darras

Department of Geology

University of Leicester

### THE EVOLUTION AND MACROECOLOGICAL

## CONSEQUENCES OF GRAZING AND SHELL-

### **CRUSHING IN FISHES**

by

Laurent Patrick Gilbert Darras

### Abstract

Fishes are a major component of modern aquatic ecosystems where they regulate some key biological processes such as the proliferation of algae. Their diet is an important and highly variable aspect of their ecology and role in the environment. Yet fossil fishes are often interpreted as ecologically restricted to a single function. Fossil fishes from the order Pycnodontiformes are considered as specialised shellcrushers that drove the evolution of shelled animals in the escalation event known as the Mesozoic Marine Revolution. But this hypothesis is still untested for want of a reliable tool.

The manner in which vertebrates wear their teeth provides a direct link between a resilient, easy to fossilise structure and their feeding ecology. It has recently become possible to quantify and compare the roughness of this wear at the microscopic scale. The potential of dental microtexture analysis as a tool to compare and make inferences of dietary preferences in fishes is further investigated here. Here we show that the technique can track subtle differences in diet between morphologically similar animals and correctly assign them to a relevant trophic niche in a taxon, size and environmentindependent way.

Dental microtexture analysis is applied to teeth from pycnodontiform fishes to test their role in Mesozoic foodwebs. The hypothesis that they were specialised shellcrushers is refuted. A broader range of realised trophic ecologies is highlighted in pycnodonts. They had more generalist or herbivorous habits and potentially were among the first major consumers of macroalgae, a key ecological role filled by extant analogues which allows light to reach zooxanthellate corals on modern reefs.

The results of early investigations comparing dental microtexture between different histologies in extant and early gnathostomes are also presented, and show that trophic diversity and shell-crushing are 360-million years old phenomena that need to be considered in scenario of macro-evolution.

### Acknowledgements

Getting to the end of a Ph.D is not a personal achievement, many people provide help or support and I doubt I shall be able to acknowledge them as is really befitting. As a foreword: I may forget to mention some people, there are so many to thank, for every little helps, but know your contribution was appreciated.

I think I should start with the people I am closest to first. I mean, I will do it in a geographical manner: many thanks to my office mates Amy, Emily "deep breath", Jen, Nyree and Oliver for coping with my mood while I was writing-up. Next along the corridor are many colleague scientists, post-grads who helped in a variety of ways, and notably I should thank those who started the Ph.D adventure when I did: Mohib, Sam, Jennifer, for their support, and especially Alison for her feedback and contribution to my skills in English (There is still room for improvement, I admit it).

Now we are reaching the other hand of the corridor and the office of Prof. Mark Purnell, whom I must thank more than many others. My supervisors Professors Mark Purnell and Paul Hart gave me a chance to carry on in the world of science when I had almost given up on the idea. Their help and feedback has always been welcome and appreciated. I hope they will consider that this work is worthy of a little space on their bookshelves, and I don't mind if they open it from times to times, but I know they are quite busy. Mark must also be acknowledged for letting me have my first taste of experimental taphonomy, public outreach, whisky tasting and congratulations via text messages.

All the clerical, technical, teaching members of staff in the department of Geology at the University of Leicester are thanked for their help and advice. To make it short, you guys rock. I should not forget my fellow post-graduates and friends; thumbs

ii

up to Nina for the coffee machine, much appreciated. Thanks to all the members of the palaeobiology research group and the labgroup meetings. We had many exciting interactions and it has been a pleasure discussing science with Rob, Sarah, Rowan, Jon, David, Joe, Duncan, Laura et al. Also thanks to Jan and Mark W. I shall find the time to read your book in the near future.

Away from Leicester, many thanks to Zerina Johanson for guiding me through the collections of the Natural history museum of London; to Živile for her support. Thanks to all my colleagues and friends, especially the Bristol group, for their contribution to enjoyable ProgPal and PalAss meetings. Actually, for the PalAss meetings, thanks also to my fellow researchers, post-grads or otherwise from what I now call "overseas". Special thanks go to Zozo, Seb, Bastien, Ivan, Jean-mi, and many others, especially the special team of "Crayssacois", Adeline and the other Jean-mi.

Thanks must go to Alain Blieck at the University of Lille who gave me my first hands-on experience of palaeoichthyology, which lead ultimately to this Ph.D. Thanks also to all the people from the former department of Palaeozoic Palaeontology and Palaeogeography and all my friends from Northern France.

Last but not least, thanks to all my family. Thanks to my brothers Florian and Thierry, both role-models for me in their own special ways. Thanks to my parents Jean-Luc and Michèle for their help, their support, for giving me the taste and the resolve to do more than just the bare minimum. No matter what happens next, I shall try to make you proud (but for the castle in Spain you will have to wait, I am afraid). Many thanks to the most unique grand-mother for she never gave up, carried on through many hardships, but still kept on helping and motivating her children, grand-children and others, never stopping, never tiring, just because she could. Thanks, Cécile, none of this would have happened without you.

### Contents

I
II
IV
V
IX
1
BY
9
СК
EAR
61
ł,
100
PHIC
133
156
163
185

### List of tables

Table 1-1: Impression materials compared in this study
Table 1-2: List and description of the textural parameters compared between the
casts and gold-coated teeth as provided by Alicona or described in Scott et al 200614
Table 1.2: Analyzes of variance on data from the rough teeth (held) or smooth
Table 1-5. Analyses of variance on data from the fough tooth (bold) of shiooth
tooth 17
Table 1-4: Tukey HSD's pairwise comparisons on data from the rough tooth
(bold) or smooth tooth
Table 1-5: Analyses of variance on data from both teeth after fitting by
generalized least-squares
Table 1-6: Pairwise t-tests on data from both teeth after fitting by generalized
least-squares
Table 1-7: Analyses of variance on data from both teeth limited to the
polyvinylsiloxanes (AccuTrans and Speedex) and the specimen after fitting by
generalized least-squares
Table 1-8: Parameters highlighting a difference in pairwise t-tests on data from
the both teeth limited to the polyvinylsiloxanes (AccuTrans and Speedex) and the
specimen after fitting by generalized least-squares
Table 2-1: Mean percentage (± S.E.) volumetric contribution of prey categories
consumed by A. probatocephalus at Mosquito and Indian River lagoons. (modified from
Cutwa and Turingan, 2000)
Table 2-2: Extant fish samples from which dental surfaces were compared, with
their known diet

 Table 2-3: Textural parameters provided by the SurfStand software (ISO 25178)

and at htt	p://www.michmet.com	n/3d filt	Itering.htm	7

Tabl	e 2-4:	Summary	of	Welch-Satterwaithe	t-tests	comparing	the	two
populations	of Arch	osargus pro	bato	cephalus				42

 Table 2-5: Summary of Welch-Satterwaithe t-tests comparing the two mesowear

 categories of Anarhichas lupus.

 43

Table 2-6: Summary of Welch-Anovas comparing the two populations ofArchosargus probatocephalus and the lesser worn Anarhichas lupus.44

Table 2-8: Attribution of single teeth data from the model populations
(Archosargus and Anarhichas) to trophic categories in LDA performed on the
parameters Sdq, Sdr, Vmc, Vvv, Sk, Smr1, Sa and Vvc/Vmc
Table 2-9: Attribution of single teeth data from Cichlidae to trophic categories in
LDA performed on the parameters Sdq, Sdr, Vmc, Vvv, Sk, Smr1, Sa and Vvc/Vmc 49
Table 2-10: Probability of attribution for cichlid's teeth to a trophic category in
LDA
Table 2-11: Comparison of automatic clusters based on dental microwear texture

Table 3-1:Sampled fossil specimens, indicating the tooth-bearing boneanalysed, geographic origin and institution where the material is stored.65Table 3-2:Results of Welch-anova for the genus *Proscinetes*.72Table 3-3:Results of Welch-anova for the genus *Eomesodon*.76Table 3-4:Results of Welch-anova for the genus *Gyrodus*.77Table 3-5:Results of Welch-anova for the family Pycnodontidae.80

Table 3-6: Results of Welch-anova for the sub-Order Pycnodontoidei
Table 3-7: Results of Welch-anova for the Order Pycnodontiformes
Table 4-1: Sampled fossil specimens, with geographic origin and group as
described in chapter 3
Table 4-2: Results of Welch-anova testing hypothesis H0 in Pycnodontoidei and
extant fishes
Table 4-3: Parameters found to display a significant difference between trophic
categories in pairwise Welch-Satterwaithe t-tests for groups of Pycnodontoidei or extant
fishes
Table 4-4: Results of Welch-anova testing hypothesis H0 in Gyrodontoidei and
extant fishes 117
Table 4-5: Parameters found to display a significant difference between trophic
categories in pairwise Welch-Satterwaithe t-tests for Gyrodontoidei or extant fishes. 118
Table 5-1: Specimens of placoderms compared with extant fishes, locality and
housing institution
Table 5-2: Summary of Welch-Satterwaithe t-tests comparing the two histologies
on toothplates of <i>Protopterus annectens</i> 142
Table 5-3: Summary of Welch-Anovas comparing the two populations of
Archosargus probatocephalus, the lesser worn Anarhichas lupus and petrodentine from
Protopterus annectens
Table 5-4: Parameters displaying a significant difference in pairwise t-tests 143
Table 5-5: Summary of Welch-Anovas comparing the two clusters of
Compagopiscis croucheri with dentine from a specialised shell-crusher Protopterus
annectens

Table 5-6: Parameters displaying a significant difference in pairwise t-tests

comparing the two clusters of Compagopiscis	<i>croucheri</i> with	dentine from	a specialised
shell-crusher Protopterus annectens			

Table 5-7: Summary of Welch-Anovas comparing the two specimens of
ptyctodontid placoderms with dentine from a specialised shell-crusher Protopterus
annectens
Table 5-8: Parameters displaying a significant difference in pairwise t-tests 149
Table A-1: Summary of the results of pairwise t-tests between treatments 168
Table A-2: Summary of the results of t-tests between populations of Archosargus
probatocephalus
Table A-3: Summary of the results of anova on different parts of the dentition of
Archosargus probatocephalus specimens SH-IR01 and SH-PC02
Table A-4: Summary of the results of anova on different parts of the dentition of
Archosargus probatocephalus for all specimens in each population
Table A-5: Summary of the results of anova comparing populations of
Archosargus probatocephalus
Table A-6: Summary of Welch-Satterwaithe t-tests comparing the two
populations of durophagous Archosargus probatocephalus and lesser-worn Anarhichas
<i>lupus</i>
Table A-7: Summary of Welch-Satterwaithe t-tests comparing the two
populations of herbivorous Archosargus probatocephalus and lesser-worn Anarhichas
lupus

Table B-1: welch t-tests for Pycnodus.    179	)
---	---

### List of figures

Figure 1-2: Examples of surfaces of casts after scanning at x50 (A and C) or x100 (B and D) magnification. All images represent the quadrant C from the rough tooth. A and B: cast from a Microset 101RF mould; C and D: cast from a Speedex mould.

Figure 2-1: Bodies (A, D, G), jaws (B, E, H) and dental surfaces (C, F, I) in compared fishes.

Figure 2-2: Calculated predictions of the Linear Discriminant Analysis on

Figure 3-1: Multivariate analysis of microtextural parameters in *Proscinetes*...73

Figure 3-2: Multivariate analysis of microtextural parameters in *Eomesodon*...75

Figure 3-3: Multivariate analysis of microtextural parameters in *Gyrodus*.......78

Figure 3-4: Multivariate analysis of microtextural parameters in Pycnodontidae

### Introduction

Aquatic ecosystems can be regarded as both simple and complex. They are simple in comparison to tropical rainforests, in which every part of a single tree has its own associated ecosystem of plants, fungi, arthropods, vertebrates and more, but they are also complex in that reefs and lakes are topographically heterogeneous environments, containing a variety of seagrasses, macroalgae and algal turf that provide food and shelter for many soft-bodied or shelled "invertebrates", all of which are potential prey for vertebrates. Coral reefs provide colourful examples of heterogeneous aquatic environments in which thousands of species exist in a limited space sharing limited resources (Bellwood et al., 2006; Choat et al., 2004; Harborne et al., 2009; Mantyka and Bellwood, 2007; Opitz, 1996; Silverstein et al., 2012).

Fishes play an important role in those ecosystems as a group of predators and provide many examples of how plastic the trophic ecology can be in vertebrates (Binning et al., 2009; Mendes et al., 2009; Ruehl and DeWitt, 2007; Swanson et al., 2003). Seasonality, locality, interactions with other species and physical factors all influence the resources to which fishes have access (Binning and Chapman, 2010; Binning et al., 2009; Castillo-Rivera et al., 2007; Choat et al., 2004; Cutwa and Turingan, 2000; Kerschbaumer et al., 2011; Komiya et al., 2011; Langerhans et al., 2007; Schaack and Chapman, 2004). The rule in trophic ecology is that a morphologically specialised animal behaves as an ecological generalist in the wild and feeds on a prey for which it is "specialised" only in times of resource scarcity, an observation coined Liem's paradox (Binning and Chapman, 2010; Binning et al., 2009; Liem, 1973, 1980; Robinson and Wilson, 1998; Summers et al., 2004; Ungar et al., 2008) Fossil fishes on the other hand are often interpreted as performing a single function in the food web of their respective past ecosystems. Trophically specialised fishes in modern environments focus on a type of prey in a qualitative rather than taxonomic manner. Chimaeroid chondrichthyans (the ratfishes) or Anarhichadid teleosteans (the wolffishes or catfishes) are specialised durophages: most of their food intake is composed of crushed invertebrates (Gill, 1911; Huber et al., 2008; Liao and Lucas, 2000a). Durophages can have a wide range of prey: bivalves, gastropods, brachiopods, crinoids, sea urchins, arthropods (in their own or borrowed shells). This range is limited in turn by mechanical constraints: gape-size, skills related to preygrasping and manipulation, maximum force exerted by the jaws, and other factors as trivial as the presence of this type of invertebrate or the presence of an easier to process resource (Binning et al., 2009; Hoey et al., 2012; Huber et al., 2008; Hulsey et al., 2006; Hulsey et al., 2008; Kolmann and Huber, 2009).

It is not possible for any researcher or research group to take all factors into account when realising macro-evolutionary studies, but it is possible to carry out investigations into the diet of fossil fishes at a few points in time and assess whether our assumptions about their ecologies are met and the interpretations based on these are correct.

In his 1977 study on fossil gastropods, Vermeij (1977) introduced the notion of the Mesozoic Marine Revolution (MMR), a global scale escalation event which encompassed the antagonistic evolutionary innovations from the Mesozoic to the Cenozoic. Exoskeletonized animals displayed changes in both their shell properties and their ecologies in response to the emergence of new predators and changing environments (Aberhan et al., 2006; Baumiller et al., 2010; Finnegan et al., 2011; Harper, 2003; Hautmann, 2004; McRoberts, 2001; Vermeij, 1977). Pycnodont fishes, a

group of neopterygian actinopterygians, have been regarded as a potential driver of this escalation mostly because of their molariform "crushing" dentition (Delsate and Kriwet, 2004; Nursall, 1993a, 1999). A range of ecologies has recently been proposed for the Pycnodontiformes, such as grazers or shell-crushers (Baines, 2010; Kriwet, 2001; Nursall, 1993a; Poyato-Ariza, 2005), but those hypotheses still need to be tested before their role in the MMR can be confirmed or reconsidered.

Fishes with potential shell-crushing habits were present in aquatic ecosystems long before the apparent increase in durophagy of the Mesozoic. Gnathostomes such as ptyctodontid and arthrodire placoderms (Denison, 1978; Janvier, 1996), dipnoan lungfishes (Janvier, 1996; Turner et al., 1995) and holocephalan chondrichthyans (Darras et al., 2008; Stahl, 1999) may have played a part in the evolution of shelled invertebrates such as crinoids (Gorzelak et al., 2011; Sallan et al., 2011).

Until recently, most studies in the trophic ecology of fishes were restricted to interpretations of gut content (Kriwet, 2001; Nursall, 1993a) and ecomorphological comparisons (Anderson, 2010; Anderson, 2008; Bellwood, 2003; Goatley et al., 2010). However, the former is rare and may represent an exceptional feeding event or the cause of death, whilst the latter provides insights only into the potential rather than into the realised diet of the animal (Lauder, 1995). Fortunately, new tools are providing ways to test our assumptions regarding feeding habits in fossil fishes. In particular, microwear texture analysis (A.K.A microtexture analysis, surface metrology or tribology Calandra, 2011; Purnell et al., 2012; Schulz et al., 2010; Scott et al., 2006) of the food-processing parts in fishes can be used in order to assess their quality as grazers or shell-crushers.

The manner in which vertebrates wear their teeth provides a direct insight into their trophic ecology and so a proxy which can be applied to both extant and extinct animals. A variety of techniques has thus been applied to dental surfaces in a number of mammals, dinosaurs, fishes and other vertebrates (Baines, 2010; Daegling, 1999; Fraser and Theodor, 2011; Goillot et al., 2009; Macho and Shimizu, 2010; Merceron, 2005; Merceron et al., 2010; Mihlbachler et al., 2012; Osi and Weishampel, 2009; Peigne et al., 2009; Purnell, 1995; Purnell et al., 2006; Ramdarshan et al., 2012; Rivals and Solounias, 2007; Rodrigues et al., 2009; Romero et al., 2009; Rots et al., 2011; Schmidt, 2010; Schubert et al., 2010; Scott et al., 2006; Scott et al., 2005; Teaford and Walker, 1983; Todd et al., 2007; Ungar et al., 2003; Walker et al., 1978; Whitlock, 2011; Williams et al., 2009). There is a rising interest in developing the available techniques in order to 1) bypass the traditional user-error linked to semi-automated counting of microwear features by using fully automated techniques, and 2) accurately link together the quantified wear and the different aspects of trophic ecology: chewing mechanisms, digestive strategies and diet. As microwear texture analysis has the advantage of being automated, investigations carried with this technique can focus on the link between wear and trophic ecology.

In this thesis, microtexture analysis is applied to teeth or food-processing parts of extant fishes with known dietary habits, fossil pycnodonts from the Late Jurassic and Devonian placoderms. The roles of those animals as predators in their ecosystem are compared and the diet of the fossil forms inferred.

#### Aims and objectives

The aims of this thesis are to understand:

- How to record and compare the effects of grazing and shell-crushing in fishes and
- Whether this signal can be used to test hypotheses concerning the diet of fossil fishes.

In order to achieve these aims, a series of investigations has been carried which poses these objectives as a series of questions to be answered in this thesis:

- Can the three-dimensional surface of a tooth be recorded accurately at the microscopic scale by a moulding compound?
- Does microwear texture analysis highlight differences between the surfaces of teeth from fish with grazing or shell-crushing habits?
- Can microwear texture analysis be used to make inferences of trophic ecology in fossil fishes?

### **Thesis structure**

The main results of investigations concerning dental microwear texture analysis in a range of extant and fossil fishes are presented in the following chapters. The first chapter represents a necessary investigation in how to better replicate roughness from the surface of teeth at the studied scale. The following chapters apply a methodology similar to that of Purnell et al. (2012): Comparing microtextural parameters through statistical tests of hypotheses and multivariate analyses. Hypotheses were tested using Analysis of Variance (Anova) after assessment of the normality of the data using Shapiro-Wilk's test. If the data failed to meet the assumption for Anova, it was either fitted using generalised least squares, log-transformed or rank-transformed before testing. Rank-transformation does not provide normally distributed data but allows parametric testing of a power equivalent to that of non-parametric alternatives (Conover and Iman, 1981; Zimmerman, 2012). Post-hoc comparisons were realised using Tukey's Honest Significant Difference in the first chapter or pairwise comparison using Welch-Satterwaithe's version of Student's t-test in order to account for heteroscedasticity. Principal Components Analysis and Linear Discriminant Analysis were performed on normal or log-transformed data unless otherwise stated.

Chapter 1: Surface roughness of tooth replicas scanned by focus-variation microscopy

The first chapter addresses the issue of finding which impression material provides the most accurate replication of the texture at the surface of molariform teeth. ISO-standard roughness parameters from the surface of black epoxy casts prepared from a range of moulds using different compounds are compared with the gold-coated teeth they replicate.

## Chapter 2: Can dental microwear texture analysis track differences in diet between populations of extant fishes?

Here the potential of dental microwear texture analysis to highlight interdemic differences linked to different feeding habits is investigated. Groups of fishes from varied environment and sizes are compared in order to highlight differences and convergences in dental microwear texture and their link to diet.

## Chapter 3: Trophic ecology of Late Jurassic Pycnodontiformes (Fish, Neopterygii): insights from microwear texture analysis

In this chapter we investigate aspects of trophic ecology in Pycnodontiformes so as to explain the co-occurrence of several "specialised shell-crushers" in an environment by testing for trophic specialisation or diversity. Dental microwear analysis is applied to individual pycnodonts in different genera or different branches of their cladogram. Trophic diversity as observed in different taxa is compared to assess the range of realised diets in Pycnodontiformes.

## Chapter 4: Inferences of diet in Pycnodontiformes (Fish, Neopterygii) via dental microwear texture analysis

Most of the fossil pycnodonts studied in this thesis come from the Late Jurassic of the Tethys, i.e. the "consolidation" time of the MMR. It would be expected under the classic escalation scenario that during that time the animals were all well settled in their respective ecological niches. The technique developed by Purnell et al. (2012) has been shown to allow for discrimination between soft and hard-objects feeders, as well as between specialised durophages and more generalist animals in chapter 2 and provides an ideal tool to test this hypothesis of Pycnodontiformes as shell-crushers.

## Chapter 5: Microwear texture analysis highlights trophic diversity in early jawed vertebrates (Placodermi)<sup>1</sup>

In this study microwear texture analysis is performed on gnathal plates of 2 unidentified ptyctodontid placoderms and seven individuals of *Compagopiscis croucheri*. Here is tested the hypothesis that arthrodires and ptyctodonts were specialised predators with a specific ecological function, by comparison with the feeding apparatus of an extant shell-crusher: *Protopterus annectens*. The petrodentine and dentine from the toothplates of *Protopterus* was used as it remains unclear whether the dentine at the surface of gnathal plates from placoderms was of comparable hardness to the tissues covering the teeth of extant actinopterygians studied previously.

<sup>&</sup>lt;sup>1</sup> Here are presented early results from a work in progress undertaken for some parts as a research assistant to Prof. Mark Purnell.

"For if we confine our attention either to the living or to the extinct alone, the series is far less perfect than if we combine both into one general system"

Charles Darwin "On the Origin of Species by Means of Natural Selection"

### Chapter 1: Surface roughness of tooth replicas scanned by focusvariation microscopy

#### Abstract

The ability of different moulding compounds to replicate dental surfaces is assessed by comparing standard estimates of roughness derived from casts and the original replicated surface. Comparisons and inferences of diet in fossil vertebrates have long been based on measurements derived from 2-dimensional representations of dental surfaces marked with microwear features (gouges, scratches, pits), linked to diet or jaw motion. This technique relies on the development of identifiable microwear features over flat surfaces in order to avoid biases in measured dimensions. Both conditions are rarely met in jawed vertebrates, mammals being the exception. Recent development of three-dimensional imaging and surface analysis offers an opportunity to study the ecology of such animals through dental microtexture analysis. In order to carry informative investigations in the future, dental roughness as replicated by several moulding compounds replicating teeth from the sheepshead seabream (Archosargus probatocephalus, Fish, Sparidae) are compared between them and with the original surfaces. Investigations show that high-viscosity compounds are less accurate when compared with the more fluid material, for both mean values and variability in the parameters. Consistent use of a low-viscosity, polyvinylsiloxane-based replicating medium prior to the preparation of casts is thus advocated for investigations regarding the diet of extant and fossil organisms with rounded dental surfaces.

### Introduction

Dental microwear is formed when teeth contact food (abrasion) or other teeth (attrition) and therefore is the blueprint of diet and feeding mechanics for both living and extinct animals. Analysis of dental microwear is widely used in palaeontology, particularly for testing hypotheses of feeding and diet in mammals (for reviews see Galbany et al., 2006; Scott et al., 2009), but also dinosaurs (Williams and Doyle, 2010; Williams et al., 2006; Williams et al., 2009) and fishes (Purnell et al., 2007; Purnell et al., 2012; Purnell et al., 2006). It often requires for moulds and/or casts to be prepared, since museum curators are usually unwilling to lend specimens that will be permanently gold-coated for study by scanning electron microscopy (SEM). Solutions exist to remove gold from fossil specimens (see Jones et al., 2012 and references therein), but easy access to casts or moulds facilitates the exchange of material between different institutions (Galbany et al., 2006). Recently different new techniques have been used to analyse dental microwear, including white light-based techniques, such as stereomicroscopy (Goillot et al., 2009; Merceron, 2005; Merceron et al., 2004; Semprebon et al., 2004b) or focus-variation scanning microscopy (Calandra, 2011; Merceron et al., 2010; Merceron et al., 2009; Schubert et al., 2010; Schulz et al., 2010; Scott et al., 2009; Ungar et al., 2003; Ungar et al., 2007), but these cannot be applied on teeth with slightly translucent enameloid, the image of the surface being incomplete or inaccurate (pers. obs.) and not consistent between modern and fossil samples (Kallaste and Nemliher, 2005). For most studies, a good replication of the features at the surface of the teeth, such as pits and scratches, is enough to measure and compare them between specimens. Most frequently polyvinylsiloxane materials are used to prepare moulds and epoxy or polyurethane to make casts (Galbany et al., 2006; Williams et al., 2006), though most institutions use their own standard techniques and materials and there is no consensus regarding the most accurate replication material.

In the next chapters different hypotheses regarding feeding and diet in fossil fishes will be tested, but this comes with an additional difficulty. In most species, fishes

wear their teeth down to the point where microwear features (pits and scratches) are recognizable no more. Recent developments in the fields of three-dimensional imaging techniques and automated surface texture analysis, accounting for not only the patterns of microwear, but also the differences in roughness associated with different diets, offer a solution. Tandem scanning confocal microscopy, a useful tool for the study of mammalian teeth, while very accurate on flat surfaces (Calandra, 2011; Schulz et al., 2010) is of limited use for curved surfaces, as on teeth from most jawed vertebrates.

Here the technique developed in Purnell et al. (2012) using an Alicona Infinite Focus microscope G4b (IFM; software v. 2.1.2), is applied to record high-resolution three-dimensional surfaces. It has the advantage of accurately recording the texture of curved objects at the microscopic scale. It already proved useful to investigate dental growth patterns (Bocaege et al., 2010) and cutmark micromorphology (Bello et al., 2009; Bello and Soligo, 2008; Stevens et al., 2010).

Here I address the issue of finding which impression material provides the most accurate replication of the texture at the surface of molariform teeth from fishes. Statistical comparisons are performed on ISO-standard roughness estimates gathered from the surface of black epoxy casts. The casts were prepared from different moulds of polyvinylsiloxane (Coltène-Whaledent: Speedex light body and AccuTrans), Microset 101RF, room temperature vulcanising (RTV) rubber (acc silicones MM913), and MM240TV (acc silicones). The casts were also compared with the gold-coated teeth they replicate.

### Materials and methods

### Preparation of moulds & casts

A formwork has been prepared on a right dentary (lower jaw) of *Archosargus probatocephalus*, the sheepshead seabream, in order to isolate two teeth with visually

different surface textures, one smooth, with enameloid (composed near-exclusively of well-ordered hydroxyapatite crystals) on most of the surface, the other one rough, with enameloid removed by natural wear processes and the underlying dentine (hydroxyapatite crystals, organic matter and water) exposed. Needle-made scratches effectively divided each tooth into 4 quadrants designated from A to D on the rough tooth (dentine) and E to H on the smooth tooth (enameloid).

Impression Manufacturer Consistency Colour material Microset 101RF Isomark Heavy Black MM240TV ACC silicones Heavy Light blue MM913 ACC silicones Light Transparent Coltène-Whaledent Brown AccuTrans Light Speedex light body Coltène-Whaledent Light Blue

Each of the impression materials (Speedex light body, AccuTrans, Microset

Table 1-1: Impression materials compared in this study.

101RF, MM913, and MM240TV) was prepared following the manufacturer's instructions then delicately poured into the formwork. Colour and transparency of replication materials can affect the digitization of surfaces (Rodriguez et al., 2009), so black epoxy resin casts (EpoTek 320 LV) were prepared from each mould. This resin is black, opaque and does not necessitate gold-coating to be studied under white light. The only variable element in the protocol is thus the mould used to prepare the casts, and from this point on the cast obtained from a mould will be referred to by the name of the replication material used. Once all the moulds had been prepared, the original teeth were sputter-coated with gold (Emitech K500X, 3 minutes).

### Digitization of surface by the Alicona IFM and data acquisition

Digitized representations of the surfaces were obtained for all casts and the specimen using an Alicona Infinite Focus microscope G4b (IFM; software v. 2.1.2) with a x100 objective, providing high-resolution three-dimensional surfaces for a 145 x 110  $\mu$ m field of view. Exposure and contrast (gamma) settings were set for each scan in

order to optimise the quality of the 3D data. Images captured at even intervals along the Y-axis are automatically analysed, separating under-illuminated and over-illuminated areas, and the coordinates of in-focus parts of the surface recorded. All coordinates are then assembled as a composite detailed three-dimensional mesh which is an exact (notwithstanding scanning errors) virtual reproduction of the surface of the scanned object.

Care was taken to orient the casts under the objective lens as horizontally as possible and each quadrant was scanned at the same location, based on easily recognizable features of the surface. Any errors of surface measurements were removed by manually editing the data with the "3D editor" software supplied with the microscope (InfiniteFocus 2.1.2, IFM software version 2.1.2). The "cleaned" point clouds were then automatically tilted by adjusting the reference plane to the parts of the surface analysed and interpreted as even by the software.

Fishes shed their teeth and replace them through their life. As a result, the teeth studied here still have some of their conical shape, a condition different from mammals, where mastication processes lead to the development of flat wear facets. The application of a gaussian wavelength filter virtually removes the tooth's own volume from the point clouds. The wavelength needs to be set manually, and all the point clouds from a similar quadrant on different casts have been treated using the same wavelength for consistency purpose and to focus on roughness, independently of any potential operator bias.

 Table 1-2: List and description of the textural parameters compared between the casts

and gold-coated teeth as provided by Alicona or described in Scott et al., 2006.

Name	Description
Sa	Average height of selected area. This parameter is, like Sq, an often used
	value to describe surfaces.
Sq	Root-Mean-Square height of selected area
Sp	Maximum peak height of selected area. This parameter describes the
	height of the highest peak in relation to the zero level. This also means that
	the parameter will be affected by a single measure point. A more robust
	peak value is the Spk value calculated from the bearing ratio curve.
$\mathbf{Sv}$	Maximum valley depth of selected area. This value is calculated similar to
	the Sp value.
Sz	Maximum height of selected area. This value is equivalent to the sum of
	Sp and Sv. Like Sv and Sp this value is also influenced by a single value.
<u> </u>	A more robust version of this dimension is the S10z parameter.
510z	Ten point neight of selected area, based on the average 10 nighest and 10
Calz	deepest points.
SSK	Skewness of selected area. This parameter describes whether more values
	are below of above the zero plane. This value is calculated from all 5D
Slav	points of the surface and is therefore an average value of the surface.
<u>Sku</u>	Ruitosis of selected alea
Suq	surface. If this parameter is high the surface is composed of steep surface
	parts. If the parameter is small, the surface consists mainly of flat surface
	parts. If the parameter is small, the surface consists manny of that surface
Sdr	Developed interfacial area ratio
Sk	Core roughness denth Height of the core material
Snk	Reduced neak height mean height of the neaks above the core material
Syk	Reduced valley height mean depth of the valleys below the core material
Smr1	Peak material component the fraction of the surface which consists of
21111	peaks above the core material
Smr2	Peak material component, the fraction of the surface which will carry the
	load
Vmp	Peak material volume of the topographic surface (ml/m <sup>2</sup> )
Vmc	Core material volume of the topographic surface (ml/m <sup>2</sup> )
Vvc	Core void volume of the surface (ml/m <sup>2</sup> )
Vvv	Valley void volume of the surface (ml/m <sup>2</sup> )
Vvc/Vmc	Ratio of Vvc parameter to Vmc parameter
Str	Texture aspect ratio ( $0 \le \text{Str} \le 1$ ). Ratio from the distance with the
	fastest to the distance with the slowest decay of the autocorrelation
	function to the value 0.2. Str $< 0.3$ : strong directional structure. Str $> 0.5$ :
	uniform texture.
Std	Iexture direction (°). Derived from the maximum of the angular power
	spectrum. Std = $90^{\circ}$ means a dominant lay parallel to the y-axis.
Stdı	Texture direction index ( $0 \le \text{Stdi} \le 1$ ). Smaller values correspond to
0	stronger directional structures.
astc	Area-scale fractal complexity. This parameter has been described in Scott
	et al. (2006)

The Area Analysis software (InfiniteFocus 2.1.2, IFM software version 2.1.2) automatically calculates roughness-related parameters (EN ISO 4287) from those surfaces. The different parameters related to the surface texture (Sa, Sq, Sp, Sv, Sz, S10z, Ssk, Sku, Sdq, Sdr), the bearing area curve (Firestone-Abbott curve, Sk, Spk, Svk, Smr1, Smr2, Vmp, Vmc, Vvc, Vvv, Vvc/Vmc), the autocorrelation of the surface structures (Sal, Str, Std, Stdi) and the fractal dimension (asfc) were collected for each mesh and compared.

The parameters from the "Surface texture" table of the software are amplituderelated descriptors of the roughness of the mesh. Profilometry studies already use similar parameters (restricted to a single profile or averaged over several profiles along a defined area) for a range of applications (Bigerelle et al., 2008; Rodriguez et al., 2009), including comparison of microwear textures in mammals (Kaiser and Brinkmann, 2006). Data from the bearing area curve highlighted differences between teeth or areas with clearly different roughness (Las Casas et al., 2008), it is expected to also highlight more subtle differences. In the same study, the texture aspect helped elucidate the active wear mechanisms that affected the surface of teeth. Many recent studies have analysed the area-scale fractal complexity as a potential indicator of differences in diet between and within species or to characterize food surfaces (El-Zaatari, 2008; Merceron et al., 2010; Pedreschi and Aguilera, 1999; Pedreschi et al., 2002; Schubert et al., 2010; Scott et al., 2009; Scott et al., 2005; Ungar et al., 2007; Ungar et al., 2010). An overview of the parameters examined in this study is provided in Table 1-2.

#### Data Analysis

The compiled data was first visually examined via Principal Components Analysis (PCA) before analysis via one-way analyses of variance (ANOVA) on the

complete data set, then for each tooth separately. The homoscedasticity was assessed by Bartlett's test and the normality by Shapiro-Wilk's test, with a risk of 5%. The parameters found to show significant differences were further analysed with Tukey's Honest Significant Difference (HSD) post-hoc test.

As many ANOVAs could not be conducted for the requirements of normality and homoscedasticity were not respected, the original data was fitted using a generalised-least-squares model (gls) of the parameter as a function of the quadrants (A to H), with correlations between similar teeth considered, thus preserving the betweenmoulds variability while limiting the between-teeth and between-quadrant variability. Analyses of variance (F-test) and between-moulds comparisons (pairwise t-tests with Bonferroni corrections) were run on the residuals of the fitted model.

### Results

The PCA on the ensemble of the roughness parameters (Figure 1-1 A) shows a clear separation between data from the dentine and from the enameloid, the least-organised tissue having data more scattered than the enameloid. Despite this clustering of the data, the first 2 axes (accounting respectively for 45.07 and 21.32% of the variance) show a clear overlap of the data from the polyvinylsiloxanes (AccuTrans and Speedex) with that from the original teeth surfaces, whereas the other samples are separated from this cluster either based on the first (Microset 101RF, MM240TV) and/or the second axis (MM913, Microset 101RF, and MM240TV). Most parameters weighted evenly on PC1 with the exception of Sku, Smr1, Smr2, Vvc/Vmc, Std and Stdi. Parameters Sq, Smr1, Vvc/Vmc, Std and Stdi contributed the least to PC2.

Parametric analyses of variance highlighted no significant differences between the moulds for the complete dataset (including data from both teeth), but out of the 25

parameters, only 3 (Sk, Smr1, Stdi) respected the assumptions of normality and homoscedasticity.

Comparing data from only each tooth separately (Table 1-3) significant between-moulds differences were found for the Str parameter for the rough tooth. On the smooth tooth, the parameters Sa, Sk, Vvc and Sal highlight between-moulds differences.

parameter	df	F-value	p-value
Str	5	5.6857	0.0026
Sa	5	3.3915	0.0247
Sk	5	3.7108	0.0175
Vvc	5	4.3493	0.0090
Sal	5	2.8056	0.0481

Table 1-3: Analyses of variance on data from the rough tooth (bold) or smooth tooth.

Post-hoc tests show that MM240TV and MM913 are different from any other cast and the specimen, based on Sk and Vvc on the smooth tooth (Table 1-4). On the rough tooth only the cast from the MM240TV mould is different from other casts and the specimen with the exception of the Microset 101RF mould. Due to the very small sample size when considering the teeth separately (4 points per tooth for each cast), these results are mostly indicative, although (Purnell et al., 2012) showed that a very limited number of points were sufficient to highlight diet-related differences in roughness from the dental surfaces in modern fishes.

Table 1-4: Tukey HSD's pairwise comparisons on data from the rough tooth (bold) or smooth tooth. The parameters are mentioned if the test highlighted a significant

~~~
-----

	specimen	AccuTrans	Microset	MM240TV	MM913
AccuTrans					
Microset					
MM240TV	Str, Sk,	Str			
	Vvc				
MM913	Sk, Vvc			Str	



Figure 1-1: Principal Components Analysis performed on all available parameters for A: all casts and original specimen; B: casts from Low-viscosity moulds and original specimen. Grey: measurements from the smooth tooth; black: measurements from the rough tooth.

Analysis of variance on the gls-fitted data found the mould factor to be significant for the Sp, Sv, Sz, S10z, Ssk, Sku, Sdq, Sdr, Sk, Vmc, Vvc/Vmc, Str and asfc microtextural parameters. Pairwise t-tests highlighted differences between a group of the acc silicones MM240TV and Microset 101RF versus the polyvinylsiloxane moulds (AccuTrans and Speedex), MM913 or the specimen. However, most of the amplitude-related parameters (Sv, Sz, S10z) are correlated, as are parameters related to the distribution of that data (Ssk, Sku, Sdq, Sdr). Additionally, the parameters Vmc, Vvc/Vmc, and Str were correlated to the amplitude-related parameters. The only uncorrelated parameter is Sp, the maximum peak height of the area, measuring how much the highest point departs from the reference level of the points cloud.

*Table 1-5: Analyses of variance on data from both teeth after fitting by generalized least-squares.* 

parameter	df	F-value	p-value
Sp	5	9.6587	< 0.0001
Sv	5	18.395	< 0.0001
Sz	5	15.154	< 0.0001
S10z	5	8.0816	< 0.0001
Ssk	5	3.4062	0.0113
Sku	5	4.7114	0.0017
Sdq	5	4.5772	0.0020
Sdr	5	2.618	0.0378
Sk	5	3.1217	0.0174
Vmc	5	3.0294	0.0201
Vvc/Vmc	5	2.9326	0.0233
Str	5	5.1390	0.0009

For the purpose of identifying the most accurate replication medium, further

investigations focused on the casts which showed the least number of differences with the original cast and no separation observed with the PCA (i.e. the data from the AccuTrans and Speedex moulds) or the specimen. A second PCA (Figure 1-1 B) on all the roughness parameters (parameters weights are similar to the first PCA) shows a neat separation between data from the dentine and from the enameloid, again. The data from the two moulds and the specimen still overlaps on the representation of the first 2 axes (respectively for 51.07 and 16.24% of the variance) with the data from the AccuTrans mould being more scattered along both axes.

 Table 1-6: Pairwise t-tests on data from both teeth after fitting by generalized least 

 squares. The parameters are mentioned if the test highlighted a significant difference.

	specimen	AccuTrans	Microset	MM240TV	MM913
AccuTrans					
Microset	Sp, Sv, Sz,	Sp, Sv, Sz,			
	S10z, Sku, Sdq	S10z, Sku			
MM240TV	Sp, Sv, Sz,	Sp, Sv, Sz,			
	S10z, Sdq,	S10z, Sdq,			
	Str	Sk, Vmc,			
		Str			
MM913			Sp, Sv, Sz,	Sv, Sz,	
			S10z, Sku	S10z,	
				Vvc/Vmc,	
				Str	
Speedex			Sp, Sv, Sz,	Sv, Sz, Str	
			S10z		

Table 1-7: Analyses of variance on data from both teeth limited to the

polyvinylsiloxanes (AccuTrans and Speedex) and the specimen after fitting by

generalized least-squares. Df: degrees of freedom.

parameter	df	F-value	p-value
Sp	2	4.7296	0.0202
Sz	2	6.6505	0.0058
Ssk	2	5.093	0.0157
Sku	2	3.7761	0.0397
Sdq	2	8.2963	0.0022
Sdr	2	4.2749	0.0277
Spk	2	3.5941	0.0455
Vmp	2	4.0629	0.0322

Table 1-8: Parameters highlighting a difference in pairwise t-tests on data from the

both teeth limited to the polyvinylsiloxanes (AccuTrans and Speedex) and the specimen

after fitting by generalized least-squares.

	specimen	AccuTrans
AccuTrans	Sp, Sz, Ssk, Sku, Sdq, Sdr	
Speedex		Sp, Sz, Ssk, Sdq, Sdr

On that restricted dataset, parametric ANOVAs highlighted no differences. Glsfitted data (the model was run again to avoid the influence of the highly-variable moulds on the spread of the residuals) highlighted differences for the parameters Sp, Sz, Ssk, Sku, Sdq, Sdr, Spk and Vmp. Further analyses found no significant differences between the Speedex mould and the specimen, but between the AccuTrans mould and both the Speedex or specimen data (Table 1-7, Table 1-8).

### Discussion

The non-normality of the distribution when considering the complete dataset was expected, especially in the amplitude-related parameters, since we are considering one mostly flat and one very rough surface. The statistically significant heteroscedasticity (Bartlett's test, p<0.05) highlights that some replicas provide estimates of roughness significantly different in variability from the original teeth. Differences in dispersion in comparison with the original surfaces would have a negative influence on the results and conclusions of later studies if the natural variability in the texture is hidden by that related to the casting process, or underestimated. We know that dental roughness conveys trophic information (Purnell et al., 2012) so any preparation-related variability needs to be set to a minimum in order to focus on the biological information. Comparing data from only one tooth at a time, the MM240TV and Microset casts are different from the specimen (Table 1-2, Table 1-3) and it is advised to avoid their use in studies focusing on dental microtexture analysis.



Figure 1-2: Examples of surfaces of casts after scanning at x50 (A and C) or x100 (B and D) magnification. All images represent the quadrant C from the rough tooth. A and B: cast from a Microset 101RF mould; C and D: cast from a Speedex mould. Scale bars: A and C: 20 µm; B and D: 10 µm

The issues of normality of the distribution and heteroscedasticity were lessened by using a generalized least squares fitting of the data. The statistical tests highlight differences between the high-viscosity moulds (Microset 101RF, MM240TV) and the other casts or the specimen. The MM913 cast though not statistically different from the original specimen based on gls-fitted data, is clearly distinct on the results of the PCA and classical ANOVA. Fitting of the data extracted the mould-related information from the roughness parameters. The procedure showed that highly-viscous moulding media provide less accurate casts of the surfaces at the considered scale.

The results of the statistical analysis are in agreement with a visual examination of the casts under the microscope. The most accurate replications of microwear-like features were observed on the AccuTrans and Speedex casts, the Microset 101RF offered good replications of the surface features albeit inconsistently. The only moulds that could be scanned correctly were the moulds made of Microset 101RF, no accurate three-dimensional image of the other moulds could be acquired, so it is unknown whether the inaccuracy of some replicas, especially MM913 and MM240TV is genuine or the effect of a chemical interaction between the moulding and casting compounds.

An important result in the further analyses is the difference between the AccuTrans and Speedex casts, associated with limited difference in the variability of the data. Though comparisons between the rough and smooth teeth highlight differences that could be expected between fishes with different diet, these between-moulds differences show how accurate the technique can be, potentially holding enough power to find subtle differences between individuals with yet similar ecologies, i.e. to look at trophic variability between individuals drawn from the same environments.

The results from the principal component analysis show that the data from the AccuTrans cast is a little more variable than that of the specimen or the Speedex cast.

When considering the original ensemble of the data, such differences are minimal, and not statistically significant. It is important to remember that heteroscedasticity of the roughness data could diminish the significance of the results of later studies using microtextural parameters, as it may obscure some more subtle, yet ecologically informative differences.

### Conclusion

Casts obtained from different moulds compared via microtextural analysis showed statistical differences in several roughness-related, automatically-calculated parameters. Several factors influenced the measurements: location on the tooth, wear stage of the tooth and the compound used to prepare the cast from which data was derived. Careful examination of the data excluded any effect unrelated to the accuracy of the replication of a surface by the different moulding compounds. Viscosity as a factor explained several differences, suggesting that for the considered magnification highly viscous chemicals are less suited for fine replication. It appears that polyvinylsiloxanes-based replication material (Coltène-Whaledent's Speedex or AccuTrans) provided the closest replication of surface features and importantly roughness when compared with the original, gold-coated, specimen.

# Chapter 2: Can dental microwear texture analysis track differences in diet between populations of extant fishes?

### Abstract

The potential of dental microtexture analysis has been explored in mammals and fishes and this technique proved itself as a useful tool to compare trophic ecologies. Some key questions regarding the power of the technique in extant fishes remain unanswered: Can it discriminate between two morphologically similar populations but with a different diet? Do heavily worn and lesser worn teeth used to process the same food provide a consistent microtexture? Can it allocate individual fishes to relevant trophic niches in a morphology, taxon and environment-independent way? Comparisons of dental microtexture from two populations of Archosargus probatocephalus show that it has the potential to highlight subtle differences in diet. Investigations on teeth from Anarhichas lupus highlight the fact that one diet can lead to different microtexture signatures if different stages of wear are considered. Application to a sample of cichlid fishes accurately assigns different individuals to relevant and comparable trophic niches, despite differences in animal size and physical environment. Dental microtexture analysis is a very powerful tool for comparisons and inferences of trophic ecologies in extant and fossil fishes provided the level of wear is assessed prior to future analyses.

### Introduction

Functional morphology (inferring function from form) is a widely used approach for testing ecological hypotheses in extinct organisms. But morphological traits reflect environment-independent (genetic) and environment-related factors (responses to
selection, phenotypic plasticity and non-selective effects) that influenced the ontogeny of the fish. These traits form the basis of models with which to estimate prey capture and prey processing efficiency, but prey availability (as a function of predation or seasonality) and biological interactions, like intraspecific and interspecific competition for food resources, also shape the diet of fishes. Feeding in fishes actually provides examples of the limitations of functional morphology (Lauder, 1995).

The sheepshead seabream (Archosargus probatocephalus, Walbaum 1792) is supposedly a specialised shell-crusher (Hernandez and Motta, 1997 and references therein) but in some settings it can be the main plant consumer in an ecosystem (Castillo-Rivera et al., 2007) and between-populations differences in diet can be observed between lagoons from the same region (Cutwa and Turingan, 2000). Trophic relationships between fishes and niche partitioning are also influenced by the environment and the availability of prey (Mariani et al., 2002). Interspecific studies have highlighted differences in diet associated with diverging morphologies (Bellwood, 2003; Cochran-Biederman and Winemiller, 2010; Wainwright and Richard, 1995) but at the interdemic scale, diet and ecomorphology do not necessarily match (Binning and Chapman, 2010; Cutwa and Turingan, 2000). Such variability along with the observed many-to-one mapping of form to function in fishes (i.e. the fact that several morphological combinations have similar functional properties Wainwright et al., 2005) explains why a direct link between morphological features and feeding performance (Wainwright and Richard, 1995) or observed diet (Binning et al., 2009) has rarely been observed.

Most studies on the ecomorphology of fishes focus on features influenced by complex interactions of biotic and abiotic factors (Langerhans et al., 2007) during the ontogeny, such as dimensions or weights of bones and muscles. The ensuing analyses

indicate the potential diet of the organism, influenced by its life-history and development, rather than its actual diet. Hence predictions of dietary ecology based on models developed from these measurements inaccurately describe the role of an organism within the web of interactions shaping its ecosystems. In some environments, fish species regulate major ecological processes by removing macroalgae (Castillo-Rivera et al., 2007; Hoey and Bellwood, 2009a, b; Sotka and Hay, 2009) or predating on other herbivores (Harborne et al., 2009). As a consequence it is important to develop tools that accurately assess the diet of fishes before attempting palaeoecological reconstructions of trophic webs.

Quantitative analysis of dental microwear, a technique widely used in mammals (Daegling, 1999; Goillot et al., 2009; Green, 2009; Mainland, 2006; Mainland, 2003; Ramdarshan et al., 2011; Rivals and Solounias, 2007; Walker et al., 1978), has been applied on extant and fossil threespine sticklebacks (Gasterosteus aculeatus, Purnell et al., 2006). Despite biomechanical and developmental differences between actinopterygian and mammalian teeth surfaces (e.g. polyphyodonty, non-occlusive tooth contact) the technique provided a reliable guide to allocate sticklebacks to the trophic niches they belonged to. For a range of diets, such as specialised durophagy, microwear features (pits and scratches) are difficult to identify at the surface of teeth. Repeated crushing of plant material or shelled organisms over a hemispherical, regularly replaced, surface is bound to give a very different picture than that observed on flat wear facets of mammals. Three-dimensional imaging and analysis techniques, accounting for not only the patterns of microwear, but also the differences in roughness related to different diets, offer a solution. Non-contact surface metrology and focus-variation microscopy provide 3D representations of the surface of objects. It has the advantage of accurately recording the texture of curved surfaces as present on most teeth. It already proved

useful to investigate dental growth patterns (Bocaege et al., 2010), cutmark micromorphology (Bello et al., 2009; Bello and Soligo, 2008) and trophic plasticity between groups of cichlid fishes (Purnell et al., 2012).

Here the potential of focus-variation scanning techniques to highlight betweenpopulations differences in diet in several wild-caught populations of fishes is investigated.

# Materials and Method.

#### Wild-caught animals

In order to test hypotheses of diet one first needs to evaluate the potential of microwear texture analysis to discriminate between realised diets in extant samples where they are known. Such work has already been carried on small samples of cichlids (Purnell et al., 2012). Here the technique is applied to 6 groups of fishes that differ markedly in diet, sampled from different environments (bathymetry, temperature, salinity): two populations of *Archosargus probatocephalus* (Teleostei, Sparidae, n=6 for each population), one group of *Anarhichas lupus* (Teleostei, Anarrhichadidae, n=7), and three samples of wild-caught or laboratory-reared Alluaud's haplo (*Astatoreochromis alluaudi*, Teleostei, Cichlidae, n=3 for each sample).

Fishes from the family Sparidae can have a broad dietary repertoire. Usually, animal prey such as polychaetes, decapods and bivalves compose the most of it but consumption of plant matter or detritus occurs in many species (e.g. Mariani et al., 2002). The two populations of *Archosargus* studied here are from the Indian River (referred to as IR for brevity) and Port Canaveral (PC) areas mentioned in Cutwa and Turingan (2000) and were provided courtesy of Dr. Ralph Turingan. Both populations are dietary generalists the PC population shows a significantly higher degree of durophagy (see -1) not associated with a significant morphological difference (Cutwa and Turingan, 2000).

Table 2-1: Mean percentage (± S.E.) volumetric contribution of prey categoriesconsumed by A. probatocephalus at Mosquito and Indian River lagoons. (modified fromCutwa and Turingan, 2000).

Prey Category	"Mosquito" (similar diet	Indian River
, <u> </u>	to the Port Canaveral	
	population)	
Large & broken molluscs		
Bivalves	18.40 (4.86)	
Brachidontes sp.	~ /	
Isognomon sp.		
Gastropods	0.16 (0.11)	
Small & whole molluscs		14.62 (4.73)
Bivalves		
Nucula proxima		8.91 (4.54)
Gastropods		
Nassarius ribex		
Urosalpinx cinerea		
<i>Crepidula</i> sp.		
Crustaceans		
Crabs	4.36 (1.83)	0.47 (0.20)
Broken barnacles	0.37 (0.23)	
Whole barnacles		0.38 (0.19)
Tubiculous invertebrates		
Amphipods	19.00 (5.94)	
Polychaetes	1.20 (0.97)	0.19 (0.18)
Small errant invertebrates		
Amphipods	7.93 (2.16)	2.47 (1.03)
Polychaetes	0.97 (0.73)	0.60 (0.49)
Other substrate-attached		
prey		
Ascidians	0.02 (0.01)	
Sponges	2.22 (2.22)	
Bryozoans	0.60 (0.55)	2.72 (2.41)
Other pelagic prey		
Ostracods	0.07 (0.07)	0.02 (0.01)
Eggs	0.21 (0.13)	0.06 (0.49)
Plant matter	18.31 (4.12)	35.70 (5.64)
Unidentified prey	25.98 (3.15)	26.16 (3.74)
remains		

Differences matching the dietary preferences may be recorded on the surface of their teeth, as shell-breaking and algae-browsing are likely to have a different "impact" on tooth surfaces. The results reported here and later investigations were based on data from maxillary teeth, as earlier investigations (see Appendices p. 173) found that they were exhibiting the clearest trophic signal.



Anarhichas lupus usually inhabits cold and moderately deep waters of the

northern Atlantic, though at times it approaches shallow water and tide pools or, more to the south, goes into deeper waters (Goode & Bean in Gill, 1911). This benthic species feeds mostly on hard-shelled prey, with rare occurrences of annelids and fish (references in Gill, 1911; Liao and Lucas, 2000a), and a tendency to feed on whatever prey is available (Gill, 1911), as can be seen by seasonal variability in the relative abundance of prey items (Liao and Lucas, 2000b and references therein). The sample studied here was collected from local processors in Aberdeen and sent to Dr D. Baines in 2005. Gut content data was not available but in natural conditions *Anarhichas lupus* from the North Sea always incorporates a large proportion (circa 70% or more) of crushed invertebrates in its diet (Liao and Lucas, 2000a, b). The spawning season for wolffish is followed by the loss and replacement of the whole dentition (Liao and Lucas, 2000b). As a consequence the sample includes teeth that have accumulated wear for most of a year and teeth that had been used for a limited amount of time.



Figure 2-1: Bodies (A, D, G), jaws (B, E, H) and dental surfaces (C, F, I) in compared fishes. A to C: Anarhichas lupus, dentaries (B) and dental surface (C, from individual wolffish N° 39) without clear feature but topographically complex, Z max=26.88  $\mu$ m. D to F: Archosargus probatocephalus, dentaries (E) and dental surface (F, from individual SH-PC03), note that the surface is less damaged than in the specialised shell-crusher, Z max=3.99  $\mu$ m. G to I: Astatoreochromis alluaudi, Lower pharyngeal jaws (H) and dental surface (I, from individual RMNH.PISC 37864), the surface is here similar to that of Archosargus, Z max=6.81  $\mu$ m but most of the surface is in the top 2  $\mu$ m. All surfaces: 146.07 x 110.79  $\mu$ m after spline-filtering. The colour bar is for reference when comparing the topographies, scale bars are 10 mm. A, D and G used with authorisation from Wikimedia commons; B and E from (Baines, 2010); H courtesy of Prof. Mark Purnell.

Table 2-2: Extant fish samples from which dental surfaces were compared, with their

known diet.

Taxon	Population/	Sample	Diet
	sub-group	numbers	
Archosargus	Indian River	SH-IR01 to	Generalist, mostly
probatocephalus	lagoon	06 (N=6)	herbivorous (Cutwa and
	(Florida)		Turingan, 2000)
	Port	SH-PC01 to	Generalist, significantly
	Canaveral	06 (N=6)	more durophagous than the
	lagoon		Indian River population
	(Florida)		(Cutwa and Turingan, 2000)
Anarhichas lupus	North Sea	Wolffish 21,	Specialised shell-crusher
-	(Aberdeen),	39, 45 (N=3)	(Liao and Lucas, 2000a, b)
	heavily		
	worn teeth		
	North Sea	Wolffish 4,	Specialised shell-crusher
	(Aberdeen),	36, 56, 61	(Liao and Lucas, 2000a, b)
	lesser worn	(N=4)	
	teeth		
Astatoreochromis	laboratory	RMNH.PISC	Soft food: minced heart,
alluaudi		37864,	liver vitamins and tetramin
		RMNH.PISC	flakes (Purnell et al., 2012)
		37865,	
		RMNH.PISC	
		37866 (N=3)	
	Small wild	RMNH.PISC	Molluscivorous (Purnell et
		37867,	al., 2012)
		RMNH.PISC	
		37868,	
		RMNH.PISC	
		37869 (N=3)	
	Large wild	RMNH.PISC	Molluscivorous (Purnell et
	-	37870,	al., 2012)
		RMNH.PISC	
		37871,	
		RMNH.PISC	
		37872 (N=3)	

Fishes from the family Cichlidae, in East African Great Lakes in particular, are a well-known example of explosive speciation and adaptative radiation, supposedly driven by competition for resources(Cochran-Biederman and Winemiller, 2010; Kerschbaumer et al., 2011; Liem, 1973). Recent works demonstrated that part of it is linked to the functional decoupling of oral and pharyngeal jaws (Hulsey et al., 2006). Yet specialised morphologies are acquired early in the life-history of fishes and do not always match the actual, often opportunistic, diet of the fishes (Binning and Chapman, 2010) as noticed by Liem (1980): "The greatest paradox ... is that the most specialised taxa are not only remarkable specialists in a narrow sense, but also jack-of-all-trades". Commonly referred to as "Liem's paradox", this pattern of anatomically specialised animals feeding as generalists is now considered as a common occurrence.

Three samples of Alluaud's haplo were used in this study, each one with three fishes. Pharyngeal jaws constitute the food-processing part of the feeding apparatus (prior to digestion) in Alluaud's haplo, the oral jaws are used mainly for food capture. One sample population is from a laboratory-raised controlled-feeding experiment, fed a soft diet of invertebrates (sample numbers RMNH.PISC 37864, RMNH.PISC 37865, RMNH.PISC 37866, "laboratory"). The other two "populations" were captured in Lake Victoria and are separated based on their size(s) compared to the laboratory sample: One is of individuals with a similar standard length but larger pharyngeal jaws (RMNH.PISC 37870, RMNH.PISC 37871, RMNH.PISC 37872, "large wild"), the other one of individuals with lower pharyngeal jaws of similar dimensions but smaller standard length (RMNH.PISC 37867, RMNH.PISC 37868, RMNH.PISC 37869, "small wild"). The opportunity of sampling pharyngeal jaws of different sizes comes from phenotypic plasticity in this species, where robust and gracile morphotypes are developed in response to several factors (Hulsey, 2006; Hulsey et al., 2008).

Limited sample size mimics one of the major issues in most palaeoecological studies, the scarcity of fossil material and incompleteness of both the fossil record and collections.

#### Microwear texture data: acquisition and analysis

## Preparation, imaging of casts and textural data acquisition

Surface data from *A. alluaudi* were acquired directly from gold-coated tooth surfaces (Purnell et al., 2012). All other data were acquired from casts of the original teeth, since enameloid is slightly translucent and does not allow for an accurate data acquisition and specimens are too large to fit under the microscope. Casts were prepared with epotek black epoxy (EpoTek 320 LV) poured in moulds prepared with Coltènewhaledent speedex light body polyvinylsiloxane. Both were mixed and applied following the manufacturer's instructions.

During data acquisition, care was taken to orient the casts as horizontally as possible and each analysed tooth was scanned at the highest point. These positions come in contact with food first, and the wear they exhibit is mostly an effect of abrasion (tooth-food contact) and, to a lesser extent, of attrition (tooth-tooth contact). No definite wear facet was observed on any of the dental surfaces.

3D data were obtained for all specimens using an Alicona Infinite Focus microscope G4b (IFM; software v. 2.1.2) with a x100 objective, providing high-resolution three-dimensional surfaces for a 145 x 110  $\mu$ m field of view. Exposure and contrast (gamma) settings were set for each scanning in order to optimise the quality of the 3D data.

Any errors of surface measurements were removed by manually editing the data with the "3D editor" software supplied with the microscope (InfiniteFocus 2.1.2, IFM software version 2.1.2). Fine-tuning of the settings for each scanning improves the quality of the 3D data. The approach used here was to manually delete those outliers, which were then automatically treated as defects and filled by the analysis software.

The editing of the surface does generate some potential user-induced error. Other workers on dental microtexture differ in their approach to the issue of measurement errors. Some teams do make use of tools provided by the software (Scott et al., 2006). Another approach is to select the most accurate files and make the assumption that defects will have no influence on the data (Calandra, 2011). With the exception of Purnell et al. (2012), all works applying microtextural techniques have focused on wear facets from mammals, sampling surface topographies that range only over a few microns. Mammals aside, the majority of jawed vertebrates use conical teeth or derivations of such shape, with limited occlusion and attrition (and hence faceting) involved in the food capture or processing. This dental morphology allows for dental surfaces to record a dietary signal but with a significant part of the point cloud carrying information about the tooth shape. As a range of topographic heights is scanned, errors show as outstandingly high peaks or deep valleys.

Table 2-3: Textural parameters provided by the SurfStand software (ISO 25178) and at

http://www.michmet.com/3d_	filtering.htm.

PARAMETERS	DESCRIPTION
Sq	Root-Mean-Square height of selected area
Ssk	Skewness of selected area
Sku	Kurtosis of selected area
Sp	Maximum peak height of selected area
Sv	Maximum valley depth of selected area
Sz	Maximum height of selected area
Sds	Density of peaks
Str	Texture aspect ratio (0 <= Str <= 1). Ratio from the distance with the fastest to the distance with the slowest decay of the autocorrelation function to the value 0.2. Str < 0.3: strong directional structure. Str> 0.5: uniform texture.
Sal	Auto correlation length. Horizontal distance of the auto correlation function (ACF) which has the fastest decay to the value 0.2. Large value: surface dominated by low frequencies. Small value: surface dominated by high frequencies.
Sdq	Root mean square gradient
Ssc	Mean summit curvature
Sdr	Developed interfacial area ratio. Percentage of additional surface area contributed by the texture as compared to a plane the size of the region.
Vmp	Peak material volume of the topographic surface (ml/m <sup>2</sup> )
Vmc	Core material volume of the topographic surface (ml/m <sup>2</sup> )
Vvc	Core void volume of the surface (ml/m <sup>2</sup> )
Vvv	Valley void volume of the surface (ml/m <sup>2</sup> )
Spk	Reduced peak height, mean height of the peaks above the core material
Sk	Core roughness depth, Height of the core material
Svk	Reduced valley height, mean depth of the valleys below the core material
Smr1	Peak material component, the fraction of the surface which consists of peaks above the core material
Smr2	Peak material component, the fraction of the surface which will carry the load
Std	Texture direction (°). Std = 90° means a dominant lay parallel to the y-axis.
S5z	Five point height of selected area
Sa	Average height of selected area
Vvc/Vmc	Ratio of the parameters Vvc/Vmc

The cleaned point clouds were exported as .sur files and imported in SurfStand (software version 5.0). Surfaces were then automatically treated by: 1) levelling the surface and removing gross tooth form with a 2<sup>nd</sup> order polynomial function. Since the dental surfaces are from dome-shaped teeth, this technique should provide an "SF surface" representing the raw surface of the tooth. 2) Application of a robust spline filter, based on a non-linear filter equation integrating robustness and end-effect management(which avoids reduction of the size of the surface, Blateyron, 2006). The used nesting index was 0.025 mm. 3) Calculation of standard parameters (ISO 25178) for the filtered surfaces.

#### Statistical analysis:

The analysis aimed to answer the following questions:

- Question 1: Within a species, can the dental microwear texture (or microtexture) discriminate between two populations with a different diet?
  As for example here with the different degrees of herbivory and durophagy we can observe in *Archosargus probatocephalus*.
- Question 2: Can the technique record a consistent trophic signal from teeth at different mesowear stages? In the atlantic wolffish (*Anarhichas lupus*), damages to dental surfaces are accumulated until the teeth are shed and replaced every year. The sample of wolffish includes some individuals with heavily damaged dental surfaces and others more comparable in mesowear to the sample of *Archosargus*. If differences in microtexture are associated with mesowear stages, only the lesser worn teeth will be comparable with the teeth from *Archosargus* or *Astatoreochromis*.

- Question 3: Can the technique discriminate between the surface of the teeth of a specialised shell-crusher (*Anarhichas lupus*) and that of a generalist opportunistic feeder (*Archosargus probatocephalus*)? Is there a difference in texture between such a specialist and any of our populations of generalists?
- Question 4: Can dental microwear texture be used for trophic inferences? Is it possible to compare dental surfaces from animals of different environments and sizes and still have a match between the diet and the microtextural data? A reasonable assumption based on their respective diet and the scale at which the surfaces are analysed is that the different populations of Cichlidae (*Astatoreochromis alluaudi*) will match the populations of *Archosargus probatocephalus*.

Prior to investigations, data from each individual were restricted to 5 measured surfaces (different teeth) per individual so that no single specimen would overweight the analysis. The resulting number of points per individual was then between 3 and 5, thus limiting the risk of over-dispersion.

Investigations were carried according to the following procedure:

Null hypotheses (H0) were tested at the significance level of  $\alpha$ =0.05 using Welch-Satterwaithe version of Student's t-test or Welch-Analysis of Variance (Welch-Anova) for each texture parameter. Prior to those tests, normality of the distribution for the samples was tested with a  $\alpha$ =0.05 risk using Wilks-Shapiro test. If the data failed to pass the test, values for this texture parameter were log-transformed and tested for normality again. In case of a second failure to pass the test, data were rank-transformed. As the parameter Std was found to be constant in most cases, it was discarded from the analysis. Rank transformation allows hypotheses to be tested using parametric tests despite the condition of normality of the data not being met (Conover and Iman, 1981) but it does not eliminate biases associated with heterogeneity of variance (see Zimmerman, 1999, 2000; Zimmerman, 2004). For data with non-gaussian distributions the results of parametric tests performed on rank-transformed data are consistent with those of non-parametric procedures, which perform better than parametric tests on the original data (Zimmerman, 2012).

In the statistical comparison of the data from the Cichlidae with the reference organisms, series of pairwise t-tests were carried out on the parameters displaying a significant difference for the model fishes. Tests were performed on the total dataset (Model + Cichlidae), Welch-Satterwaithe and Holm-Bonferroni corrections were applied to account for the number of tests performed.

Questions 3 and 4 were also investigated using multivariate techniques: Linear Discriminant Analysis (LDA) was applied to texture parameters from the model dataset (*Archosargus* + *Anarhichas*) using the different populations as categories. Those parameters used were the ones that displayed significant interdemic differences in the *Archosargus*. This linear discriminant function was then used as a model to infer to which dietary category individuals from the model dataset or the cichlid sample were most similar in dental microtexture.

The Principal Component Analysis was adapted from the procedure described in Ezard et al. (2010), using the packages mclust (Fraley and Raftery, 2010), mvoutlier (Gschwandtner and Filzmoser, 2009) and vegan (Oksanen et al., 2010). The texture parameters used in this PCA were those found to highlight differences between the populations of *Archosargus probatocephalus* in question 1 as well as between the three categories compared in question 3. Principal components were computed using the

function princomp (based on the correlations). The automatic clustering function Mclust was applied to the resulting projected scores and the isolated clusters compared to the known trophic categories.

All analyses were performed with R 2.15.0 (R Development Core Team, 2012), and the MASS (Venables and Ripley, 2002) and above mentioned packages.

# Results

# Between-populations comparison in Archosargus probatocephalus

Microtextural data collected from the surface of teeth of Archosargus

probatocephalus, comparing samples from the Indian River (IR) and Port Canaveral

(PC) populations, reveals that 8 textural parameters are significantly different between

populations. Different trophic preferences between otherwise anatomically similar populations of fish can as such be highlighted using dental microwear texture analysis. *Table 2-4: Summary of Welch-Satterwaithe t-tests comparing the two populations of* Archosargus probatocephalus. *Texture parameters showing a significant difference and the associated information highlighted in bold. O: test performed on the original, untransformed data; L: test performed on log-transformed data; R: test performed on rank-transformed data. df: degree of freedom.* 

Parameter	Data	t-statistic	Df	p-value
Sq	R	-1.8837	53.053	0.0651
Ssk	R	0.1388	55.842	0.8901
Sku	R	1.8498	52.779	0.0699
Sp	L	-0.9384	49.692	0.3526
Sv	R	-0.4866	54.285	0.6285
Sz	R	-0.8297	50.883	0.4106
Sds	L	-1.5503	55.421	0.1286
Str	R	-0.8376	54.319	0.4059
Sal	R	-0.2046	55.692	0.8386
Sdq	R	-2.167	54.216	0.0346
Ssc	R	0.6421	56.000	0.5234
Sdr	R	-2.6214	54.598	0.0113
Vmp	0	-0.5507	45.589	0.5846
Vmc	R	-2.3871	53.238	0.0205
Vvc	R	-1.7327	51.341	0.0891
Vvv	0	-2.5001	55.917	0.0154
Spk	0	-0.6018	46.75	0.5502
Sk	R	-2.2101	53.318	0.0314
Svk	0	-1.7045	54.673	0.0940
Smr1	0	2.9426	54.671	0.0048
Smr2	0	1.6863	55.957	0.0973
S5z	L	0.2057	38.671	0.8381
Sa	R	-2.0803	53.179	0.0423
Vvc/Vmc	0	3.0151	51.799	0.0040

# Comparison of wear stages in Anarhichas lupus

The atlantic wolffish (*Anarhichas lupus*) replaces teeth annually, with the consequence that in some individuals dental surfaces are heavily damaged (n=3), whereas others, while worn, retain a visual aspect similar to that seen on the dental surfaces from *Archosargus probatocephalus* (n=4). Is the diet (assumed as consistent

within the whole sample) the main signal recorded on the filtered surfaces or is the mesowear stage affecting the data to a greater extent? This question needs investigating in order to ensure that subsequent comparisons with other animals are reliable.

Table 2-5: Summary of Welch-Satterwaithe t-tests comparing the two mesowear categories of Anarhichas lupus. Texture parameters showing a significant difference and the associated information highlighted in bold. O: test performed on the original, untransformed data; L: test performed on log-transformed data; R: test performed on rank-transformed data. df: degree of freedom.

Parameter	Data	t-statistic	Df	p-value
Sq	L	3.7527	32.986	0.0007
Ssk	0	-1.4693	32.776	0.1513
Sku	L	-1.3402	30.588	0.1900
Sp	L	2.1600	29.904	0.0389
Sv	$\mathbf{L}$	2.4953	32.984	0.0178
Sz	$\mathbf{L}$	2.3966	32.260	0.0225
Sds	0	-1.6674	25.105	0.1079
Str	R	-0.8748	31.614	0.3883
Sal	R	-1.8895	31.152	0.0682
Sdq	R	3.9476	32.769	0.0004
Ssc	R	3.6509	31.564	0.0009
Sdr	$\mathbf{L}$	3.4207	30.915	0.0018
Vmp	$\mathbf{L}$	3.0539	32.542	0.0045
Vmc	$\mathbf{L}$	4.0134	32.960	0.0003
Vvc	$\mathbf{L}$	3.8013	32.574	0.0006
Vvv	$\mathbf{L}$	3.7777	31.709	0.0007
Spk	0	2.9995	24.293	0.0062
Sk	$\mathbf{L}$	3.973	32.872	0.0004
Svk	$\mathbf{L}$	3.4364	31.999	0.0016
Smr1	0	-1.0946	32.867	0.2816
Smr2	0	-1.8247	32.932	0.0771
S5z	$\mathbf{L}$	2.6657	32.215	0.0119
Sa	$\mathbf{L}$	3.8793	32.993	0.0005
Vvc/Vmc	0	-1.7591	32.987	0.0878

The number of textural parameters displaying a difference between the two mesowear categories in the wolffish (17) is more than twice that observed between the two populations of *Archosargus* (8). Considering the entire sample of *Anarhichas lupus* as a single trophic category could have resulted in a high proportion of type I errors (rejecting a true null hypothesis) in the following investigations. Supplementary investigations found no relation between the dimension of the jaws and microwear variables. The differences observed here are related to the accumulation of damage to dental surfaces over a period of time rather than to differences in the proportions of crushed items by individuals of different size.

#### Interspecific comparison of dental microwear texture

Can dental microtexture analysis track differences in diet in different species? Interdemic differences within *Archosargus probatocephalus* highlighted the dichotomy between hard-object and soft-object dominated diets. Whether dental microtexture records differences in a qualitative (crusher versus grazer) or quantitative (proportion of crushed material in the diet) can be tested by comparing the populations of *Archosargus* with the lightly worn individuals of wolffish (with double the proportion of crushed prey in its diet compared to the PC population)Results indicate significant differences between trophic categories for almost all the textural parameters. It is noteworthy that the parameters that do not distinguish between the trophic categories (Ssk, Sds, Str, Sal, Smr2) did not show any difference between the two populations of *Archosargus* or wear stages of *Anarhichas*.

Parameters displaying significant differences based on pairwise comparisons between trophic categories in the wolffish, *Archosargus* and populations of Cichlidae are summarized in Table 2-7. As seen in Table 2-6 a large number of differences can be highlighted between the specialist and the populations of generalists.

Table 2-6: Summary of Welch-Anovas comparing the two populations of Archosargus probatocephalus and the lesser worn Anarhichas lupus. Texture parameters showing a significant difference and the associated information highlighted in bold. O: test performed on the original, untransformed data; L: test performed on log-transformed

data; R: test performed on rank-transformed data. df: degree of freedom; num:

Parameter	Data	F-statistic	df	p-value
			(num,denom)	_
Sq	R	47.1597	2,47.449	<0.0001
Ssk	R	1.1863	2,39.136	0.3161
Sku	R	45.5724	2,46.624	<0.0001
Sp	L	13.0876	2,34.054	<0.0001
Sv	R	48.6162	2,47.615	<0.0001
Sz	R	39.9498	2,45.932	<0.0001
Sds	L	1.5595	2,44.322	0.2216
Str	R	1.6285	2,39.068	0.2093
Sal	R	1.8021	2,42.065	0.1775
Sdq	R	58.2784	2,47.820	<0.0001
Ssc	R	34.7776	2,45.957	<0.0001
Sdr	R	41.3338	2,46.689	<0.0001
Vmp	R	85.8782	2,47.413	<0.0001
Vmc	R	15.6926	2,41.106	<0.0001
Vvc	R	21.4191	2,41.484	<0.0001
Vvv	R	19.1286	2,42.899	<0.0001
Spk	R	85.1865	2,47.469	<0.0001
Ŝk	R	16.4581	2,41.207	<0.0001
Svk	R	24.2427	2,43.649	<0.0001
Smr1	0	12.6061	2,35.978	<0.0001
Smr2	R	1.2627	2,40.603	0.2938
S5z	L	20.4487	2,31.178	<0.0001
Sa	R	24.1293	2,43.178	<0.0001
Vvc/Vmc	0	10.234	2,35.586	0.0003

numerator; denom: denominator.

No differences were found between representatives of the same species, though they already were highlighted for both *Archosargus* () and *Astatoreochromis* (Purnell et al., 2012), this being in part due to corrections for heteroscedasticity and higher variability in the wolffish dataset. Nevertheless, the results indicate that the laboratoryreared cichlids are less dissimilar to the herbivorous population than to any of the shellcrushers, whereas any population of wild *Astatoreochromis* is found to be different from both generalists.<sup>2</sup>

<sup>&</sup>lt;sup>2</sup> Comparisons of the two most durophagous populations between them or of the herbivorous generalist versus the specialised shell-crusher via Welch-satterwaithe t-tests without Holm-bonferroni corrections can be found in appendix

Table 2-7: Parameters displaying a significant difference in pairwise t-tests comparing the two populations of Archosargus probatocephalus and the lesser worn Anarhichas lupus with cichlids. Bold: log-transformed data, italics: rank-transformed data. Ap:

	Ap herbivorous	Ap durophagous	Wolffish	Aa - laboratory	Aa – small wild
Ap					
	a a1	D 10 0			
Wolffish	Sq, Sku,	Sq, Sku, <b>Sp</b> ,			
	<b>Sp</b> , <i>Sv, Sz,</i>	Sv, Sz, Sdq,			
	Sdq, Ssc,	Ssc, Sdr,			
	Sdr, Vmp,	Vmp, Vmc,			
	Vmc, Vvc,	Vvc, Vvv,			
	Vvv, Spk,	Spk, Sk, Svk,			
	Sk, Svk,	Smr1, <b>S5z</b> ,			
	Smr1, <b>S5</b> z,	Sa,			
	Sa	Vvc/Vmc			
Aa -	Sv, Ssc	Sku, Sv, Ssc,	Sq, Sdq,		
laboratory		Vmc, Vvc,	Sdr;		
-		Sk, Sa	Vmc,		
			Vvc.		
			Vvv. Sk.		
			<b>S5</b> <i>z</i> , <i>Sa</i>		
Aa – small	Sku, Sv, Sz,	Sku, Sv,			
wild	Ssc	Ssc,			
Aa – large	Sku, Sv, Sz,	Sku, Sv, Sz,			
wild	Ssc, Svk	Ssc, Svk,			
		Smr1,			
		Vvc/Vmc			

Archosargus probatocephalus; Aa: Astatoreochromis alluaudi.

No differences were found between representatives of the same species, though they already were highlighted for both *Archosargus* () and *Astatoreochromis* (Purnell et al., 2012), this being in part due to corrections for heteroscedasticity and higher variability in the wolffish dataset. Nevertheless, the results indicate that the laboratoryreared cichlids are less dissimilar to the herbivorous population than to any of the shellcrushers, whereas any population of wild *Astatoreochromis* is found to be different from both generalists.<sup>3</sup>

#### Multivariate analysis

#### Linear Discriminant Analysis

As they allowed segregating between two populations of generalists, the texture parameters Sdq, Sdr, Vmc, Vvv, Sk, Smr1, Sa and Vvc/Vmc provided the data for the linear discriminant analysis. In order to limit over-imprinting as an effect of correlation, linear discriminant functions were calculated on the variables Sdr, Vmc, Smr1 and Vvc/Vmc and according to the three trophic categories: herbivorous generalist (IR), durophagous generalist (PC) and specialised durophage (Wolffish). The effect of diet was very significant (Wilks'lambda=0.3799, p<0.0001) and explained 95.69% of the between-categories variance, Vvc/Vmc and Smr1 had the highest coefficients for LD1 and LD2. Application of the linear discriminant function to the original data (for selfprediction) showed that the two categories of Archosargus (IR and PC) are similar between them, relative to the population of wolffish. For 29 teeth in each generalist populations, all were assigned to the right species though only 19 (IR) or 20 (PC) were correctly assigned to their original population. In other instances, posterior probabilities for attribution to one or the other population of generalist are similar (between 0.45 and (0.55). The 5 wolffish teeth incorrectly assigned to the IR population come from 3 different individuals (Wolffish-4, 36 and 61).

<sup>&</sup>lt;sup>3</sup> Comparisons of the two most durophagous populations between them or of the herbivorous generalist versus the specialised shell-crusher via Welch-satterwaithe t-tests without Holm-bonferroni corrections can be found in appendix



Figure 2-2: Calculated predictions of the Linear Discriminant Analysis on parameters Sdr, Vmc, Smr1, and Vvc/Vmc. Ap: Archosargus probatocephalus; Aa: Astatoreochromis alluaudi

Table 2-8: Attribution of single teeth data from the model populations (Archosargus and Anarhichas) to trophic categories in LDA performed on the parameters Sdq, Sdr, Vmc, Vvv, Sk, Smr1, Sa and Vvc/Vmc.

	IR	PC	Wolffish
Attribution to IR	19	9	5
Attribution to PC	10	20	0
Attribution to	0	0	12
Wolffish			

Predictions based on this model of trophic categories as applied to the Cichlidae show no definite match between model populations and predictions for groups. The soft-diet laboratory fish are mostly attributed to the generalist herbivorous population. Both the large and the small wild specimens are evenly spread between the specialised shell-crusher and the herbivorous generalist.

Posterior probabilities show that laboratory-reared cichlids are similar in texture to the generalist *Archosargus* and overall more similar to the herbivorous population. The two teeth attributed to the Port Canaveral population are by less than 10% probability in favour of PC over IR.

Table 2-9: Attribution of single teeth data from Cichlidae to trophic categories in LDA performed on the parameters Sdq, Sdr, Vmc, Vvv, Sk, Smr1, Sa and Vvc/Vmc. Aa:

Astatoreochromis alluaudi.

	Aa - laboratory	Aa – small wild	Aa – large wild
Attribution to	4	3	3
Archosargus			
herbivorous			
Attribution to	2	0	0
Archosargus			
durophagous			
Attribution to Wolffish	0	3	3

Table 2-10: Probability of attribution for cichlid's teeth to a trophic category in LDA.

		Probability for	Probability for
Sample number	Probability for IR	PC	wolffish
laboratory: RMNH.PISC	0.528172	0.070297	0.401531
37864	0.461989	0.536079	0.001932
laboratory: RMNH.PISC	0.560523	0.388391	0.051086
37865	0.658575	0.312682	0.028743
laboratory: RMNH.PISC	0.480726	0.519052	0.000222
37866	0.623063	0.369065	0.007872
Small wild: RMNH.PISC	0.085513	0.025457	0.889029
37867	0.608696	0.390591	0.000713
Small wild: RMNH.PISC	0.008705	0.005185	0.98611
37868	0.000019	0.000005	0.999976
Small wild: RMNH.PISC	0.524507	0.405279	0.070214
37869	0.493072	0.104129	0.402799
Large wild: RMNH.PISC	0.000111	0.000085	0.999804
37870	0.002413	0.000994	0.996593
Large wild: RMNH.PISC	0.682398	0.283018	0.034585
37871	0.600962	0.222119	0.17692
Large wild: RMNH.PISC	0.015852	0.01706	0.967088
37872	0.532464	0.409683	0.057853

Final attributions are highlighted in bold.

In the wild individuals, attributions to the specialised shell-crusher category are unambiguous (more than 90% in favour of this category). The attributions to the herbivorous population are not as strongly supported (most posterior probabilities are close to a tie with either the PC or Wolffish categories), the exception being the individual RMNH.PISC 37871, clearly attributed to the soft-objects feeding population (more than 35% probability over the next alternative for each tooth). Since scanned teeth were selected because they show wear, this result can indicate either trophic diversity within wild populations of *Astatoreochromis alluaudi* or a different microtexture being recorded as the consequence of tooth shedding and replacement.

### Principal Component Analysis

The Principal Component Analysis again highlighted the similarities between generalists relative to *Anarhichas lupus*. The textural parameters Sdr, Vmc, Smr1 and Vvc/Vmc from the model populations (*Archosargus* and *Anarhichas*) were used to calculate the projections, all parameters weighted equally on PC1 and PC2 but on the latter only Sdr and Vmc positively. A 2-dimensional ecospace with PC axes 1 and 2 explained 96.15% of the variance (respectively 63.74% for PC1 and 32.41% for PC2.

This ecospace separates both *Archosargus* populations and the wolffish along PC1, while PC2 shows limited segregation between IR and PC. The patterns found in the LDA for the attribution of the data from cichlids are found here almost identically: The laboratory-reared individuals overlap with the herbivorous population but for one ambiguous point. The wild individuals overlap between the populations but for the specimen RMNH.PISC 37871, closer to the herbivorous generalists.

The use of an automated clustering technique allows for comparison of interpretative patterns or a priori defined categories with user-independent clusters. The Mclust function applied to the first 2 PC axes identified the best model as "Ellipsoidal, unconstrained (different variances) with 4 components". These 4 clusters are of unequal sizes including between 8 (cluster 3) and 46 (cluster 4) points. They were compared to the different populations present in the dataset.



Figure 2-3: Projections of the robust Principal Components Analysis on parameters Sdr, Vmc, Smr1, and Vvc/Vmc for PC axes 1 and 2. Ap: Archosargus probatocephalus; Aa: Astatoreochromis alluaudi

Table 2-11: Comparison of automatic clusters based on dental microwear texture and populations of fishes. IR: herbivorous Archosargus; PC: durophagous Archosargus;

Clusters (and interpretation)	Cluster 1 (shell-	Cluster 2 (intermediate)	Cluster 3(PC1:	Cluster 4 (generalists)
1 /	crusher)		Wolffish,	
			PC2: IR)	
IR	1	9	1	18
PC	1	8	0	20
Wolffish	7	5	5	0
Aa –	0	1	0	5
Laboratory				
Aa – Small	2	1	2	1
wild				
Aa – Large	3	1	0	2
wild				

Aa: Astatoreochromis alluaudi.

Most of the data from generalists falls within the cluster 4, while the wolffish data is spread through clusters 1 to 3. The remainder of the *Archosargus* points share the cluster 2 with *Anarhichas*, confirming the limited similarities seen in the LDA. The data from soft-diet cichlids is found in cluster 4 with the generalists, but for one tooth in cluster 2, both clusters gather data from extant generalists. The small wild *Astatoreochromis* are spread between 4 clusters, with 2 points in clusters 1 (shell-crusher) and 3 (similar to the wolffish on PC1, to IR on PC2), and 1 tooth in each of clusters 2 (intermediate between shell-crushers and generalists) and 4 (generalists). The 2 teeth from the large wild individual RMNH.PISC 37871 are attributed to the cluster 4 (generalist), the other teeth from the large wild specimens being attributed to the clusters 1 (shell-crusher) and 2 (intermediate). The distribution of the points within the clusters confirms the earlier results without a priori defined categories.

# Discussion

The results presented here show that analysis of the microwear texture of molariform teeth has ability to track subtle diet-related differences between populations

of conspecific individuals. It can also highlight differences within a group of individuals (see the attributions of different samples from the cichlids). The morphologically identical populations of fishes studied by (Cutwa and Turingan, 2000) differed mostly in the proportions of hard prey consumed (volumetric contributions:  $42.55 \% \pm 6.05$  S.E. for the most durophagous population versus 24.58  $\% \pm 5.65$  S.E. for which most hard prey were swallowed whole) and plant matter (18.31 % ±4.12 S.E. in the durophagous population versus  $35.70 \% \pm 5.64$  S.E.). The downside of the technique they used (gut content analysis) is that it provides only a "snapshot" of the diet in the few hours or perhaps days prior to the capture of the animal and will likely provide an estimate of what was available in the environment where and when it fed last. Observed variability in diet within a species can be seasonal (Fehri-Bedoui et al., 2009; Pallaoro et al., 2006) as well as geographic (Langerhans et al., 2003; Mariani et al., 2002) or an interaction of these two factors (e.g. Chuwen et al., 2007) but also simply the result of a sampling bias depending on the used proxy. The geographic and temporal divergences were observed in the Pueblo Viejo lagoon (in the Gulf of Mexico) where differences in gut content of A. probatocephalus between two areas are directly linked to the temporal and spatial availability of trophic resources (Castillo-Rivera et al., 2007). On the contrary, since dental microwear accumulates over a period of time, it can be used to track shifts in diet or seasonal patterns (Estebaranz et al., 2009; Merceron et al., 2010), provided that microwear generated by the later diet has replaced the earlier microwear signature or teeth have been replaced and worn. Investigations in sticklebacks showed that a new microwear pattern is developed in 4 days when fishes are placed in a different environment (Baines, 2010).

A major feature regarding the dentition of the wolffish is that it is shed and replaced annually. As a result, dental microwear is accumulated over a period of several

months, then entirely reset when new teeth erupt. The first investigations carried here showed that there was ground to separate the wolffish population in two groups based on their microtexture. Gape size as a limiting factor for access to prey thus has to be discarded as the main explanatory hypothesis. In Anarhichas lupus, the teeth are acrodont (attached to the alveolar ridge without socket, Gill, 1911) and molariform, with bundles of hydroxyapatite oriented perpendicular to the occlusal plane of the jaws. As such, the food-processing part of the jaws is mechanically adapted to cope with a shell-crushing diet over a long period of time and to efficiently discard worn teeth. Continuous wear over a dental surface is a phenomenon well documented in perissodactyls (Joomun et al., 2008; Kaiser, 2003; Schulz et al., 2010) or other hypsodont mammals with high-crowned teeth where abrasion, attrition and crown growth interact to produce a highly functional chewing apparatus and continuously imprint a microwear signal. Repeated attrition has little influence on the dental surfaces of the wolffish. The lack of wear facets or scratches on the dental surface implies that the major wear-inducing process in Anarhichas is their highly durophagous diet. Their microwear is distributed over the surface but accumulated over it rather than imprinted. This phenomenon should be taken into account whenever considering investigations into the diet of animals with a large proportion of crushed prey, in order to perform relevant comparisons, as here, where only the lesser worn teeth from Anarhichas are compared with the Archosargus or Cichlidae.

The interspecific comparison highlights the large number of differences in microtexture between the two species. The inclusion of the wolffish in the analysis confirms that differences in microwear observed between populations of *Archosargus* are quantitative (linked to the proportion of crushed material) rather than qualitative (i.e. linked to the occurrence of crushing): The proportion of crushed prey in the wolffish is

twice that of the most durophagous population of *Archosargus*. Multivariate analyses illustrate the effect of the physical properties of food on dental microwear texture: The populations of *Archosargus* being similar morphologically (Cutwa and Turingan, 2000) are able to process prey with similar mechanical properties within their respective ecosystems, resulting in a limited overlap of the data. On the other hand, *Anarhichas* crushes twice or more as much hard prey as *Archosargus* and its microwear texture is clearly segregated from the generalist populations. Dental microwear texture analysis proves itself an effective tool to separate populations of fishes based on the proportion of hard prey they effectively processed.

Alluaud's haplo is found in a variety of freshwater settings in Africa such as lakes and rivers with different degrees of turbidity and oxygenation(e.g. Binning and Chapman, 2010; Binning et al., 2010). Biotic (e.g. abundance and type of food) and abiotic (temperature, depth, salinity) factors differ from those found in lagoons of Florida or rocky marine environments of the north Atlantic, where the comparative model organisms were captured. Differences in size between the model (common TL=350 mm for *Archosargus probatocephalus*, max length=1500 mm for *Anarhichas lupus*) and the compared organism (max length=190 mm for *Astatoreochromis alluaudi*) are another source of bias if gape size leads to consumption of a type of prey that induces a significantly different mechanical stress on the dental surfaces. Despite those sources of bias, dental microwear texture data matched the samples according to their trophic preferences.

Since microwear texture analysis relies on data obtained at a very high magnification and fine scale (field of view: 146.07x110.79  $\mu$ m, lateral resolution of 0.35-0.4  $\mu$ m and vertical resolution of 0.02  $\mu$ m (Purnell et al., 2012)), compared to fibre-bundles of hydroxyapatite 2-4  $\mu$ m wide (Sasagawa, 1997), finding similarities

between the dental surfaces of fishes so different as the *Archosargus* or *Anarhichas* and Alluaud's haplo appeared as a reasonable assumption. The size of the animals is likely to have an effect on the microtexture data only if as gape-size increases (along with the volume of associated jaw-muscles) they feed on prey with a larger and thicker shell requiring more force to be crushed, but unless forced to (because of resource scarcity, competition or during a controlled feeding experiment), fishes will preferentially consume food that is easier to handle and process.

From an analytical perspective, the statistical testing of hypotheses was of limited use to attribute the populations of cichlidae to any of the model samples. Differences between the soft-objects feeders (laboratory cichlids) and the specialised shell-crusher were highlighted, as well as differences between the shell-crushing cichlids and the generalist Archosargus, but known interdemic differences between populations of Archosargus or cichlids did not appear in the results. Multivariate procedures provided several opportunities to assign single teeth from cichlids to a priori defined or user-independent categories and areas of ecospace. With the exception of specimen RMNH.PISC 37871, the expected matches between cichlids and model populations were met. The power of dental microwear texture analysis to assign individuals to model populations based on the physical properties of their prey is supported by those results. The apparent mismatch of the data from cichlid RMNH.PISC 37871 with shell-crushing populations further shows that differences at the individual level can be highlighted by this technique in fishes as they already have been in mammals (Merceron et al., 2010). The data can have come from a more recently erupted tooth with a less developed microtexture signature. Alternatively, it provides an illustration of Liem's paradox: Morphology (animal size or pharyngeal jaw size) in wild

*Astatoreochromis alluaudi* is not the sole influence on diet; prey abundance and availability are also limiting factors.

#### Conclusion

Dental microtexture analysis has been proved as a useful tool to: 1) Discriminate between two populations of *Archosargus* similar in morphology but with a marked difference in degree of durophagy. 2) Discriminate between heavily worn and worn teeth in a population of atlantic wolffish (*Anarhichas lupus*). 3) Allocate individual fishes to their trophic niche in a morphology, taxon and environment-independent way.

The way vertebrates wear their teeth provides a direct insight into their trophic ecology and a proxy which can be applied to both extant and extinct animals. A variety of techniques have thus been applied to dental surfaces in a large variety of mammals, dinosaurs, fishes and other vertebrates (Baines, 2010; Daegling, 1999; Fraser and Theodor, 2011; Goillot et al., 2009; Macho and Shimizu, 2010; Merceron, 2005; Merceron et al., 2010; Mihlbachler et al., 2012; Osi and Weishampel, 2009; Peigne et al., 2009; Purnell, 1995; Purnell et al., 2006; Ramdarshan et al., 2012; Rivals and Solounias, 2007; Rodrigues et al., 2009; Romero et al., 2009; Rots et al., 2011; Schmidt, 2010; Schubert et al., 2010; Scott et al., 2006; Scott et al., 2005; Teaford and Walker, 1983; Todd et al., 2007; Ungar et al., 2003; Walker et al., 1978; Whitlock, 2011; Williams et al., 2009). There is a rising interest in developing the available techniques to 1) bypass the traditional user-error linked to semi-automated counting of microwear features by using fully automated techniques and 2) accurately link the quantified wear and the different aspects of trophic ecology: chewing mechanisms, digestive strategies, diet. In the sample of fishes used here, chewing mechanisms are not complex when compared to mammals. Observation of the dental surfaces finds a very limited number of scratches, suggesting that prey-processing involves mostly a crushing motion,

pressing one set of teeth against another dorso-ventrally without lateral or anteroposterior movement. A digestive strategy similar to that of the ruminants (to bring the bolus back into the mouth to mechanically process it several times) is not known in fishes, which are hindgut fermenters (see Mountfort et al., 2002). Some fishes spit broken shells as they process their food, but prior to further ingestion of the material, hence no enzymes from the post-pharyngeal part of the digestive tract get in contact with teeth. Food items are thus the main influence on dental surfaces in the considered model fishes (*Archosargus probatocephalus* and *Anarhichas lupus*).

Results from *Archosargus probatocephalus* show that even a limited difference in diet can be highlighted by the dental microtexture analysis, provided investigations are carried on the most informative parts of the dentition. Investigations on teeth from *Anarhichas lupus* highlight the fact that different microtexture signatures can be obtained for a similar diet if teeth at different stages of wear are considered. This result has significant implications for investigations in animals that accumulate microwear and process a large quantity of hard prey, for comparisons should be based only on surfaces from teeth at similar mesowear stages.

The samples of Cichlidae provide an interesting example of Liem's paradox (The observation that morphologically specialised animals often behave like generalists, Liem, 1973, 1980). The a priori grouping based on the size of the wild animals provides a highly variable dataset since it mixes either different ecologies or tooth wear stages (newly erupted as opposed to worn) within one morphological class. On the other hand, focusing on the actual ecological data provides meaningful ecological classes. Groupings based on morphological dimensions then need to be checked for ecological consistency prior to extrapolation of niche occupation. Morphological groups actually

provide a good basis to check for any trophic plasticity within an otherwise homogeneous population or species.

Liem's paradox is particularly important in the context of palaeontology where observations are limited to physical features, measurements or traces of activity. Direct evidences of predation in the fossil record traditionally are as exceptional preservation of gut content (e.g. Kriwet, 2001) or marks of predation (e.g. Galle and Mikulas, 2003; Gorzelak and Salamon, 2009; Gorzelak et al., 2011), but both are rare occurrences. There also is the possibility that the fossilised gut content is evidence of what killed the animal, and evidences of predation such as bite marks could also be the effect of a failed attempt. The recent development of analytical tools applied to the food-processing apparatus has added a new source of evidence providing a direct link between a quantifiable, measurable object (microwear features and surface roughness) and the realised rather than potential diet of an organism. Moreover, the use of wild-caught populations as a reference and a size-independent technique shall overcome the limitations of artificial model and environment-induced morphologies when applied to extinct fishes. Dental microwear texture analysis has the potential to allow testing of hypotheses of trophic ecology, niche segregation and escalation in jawed vertebrates through almost 400 million years of fossil record.

# Chapter 3: Trophic ecology of late Jurassic Pycnodontiformes (Fish, Neopterygii): insights from microwear texture analysis

# Abstract

Dental microwear texture analysis is applied to teeth of pycnodontiform fishes in order to understand how morphologically specialised "shell-crushers" can coexist within an environment. In fossil ecosystems such as the Zorzino limestone, the Solnhofen Plattenkalk or the Kimmeridge Clay several species and genera of Pycnodontiformes shared an environment and must have competed for prey resources. Comparisons of textural parameters from specimens highlight niche partitioning within each considered taxonomic unit (genus, Family, sub-Order and Order). Varying conditions such as seasonality and locality, as well as competition for food resources explain trophic shifts within a fish species in modern ecosystems. The results presented here suggest that ecological plasticity is a feature that Pycnodontiformes share with modern analogues like Sparidae or Scaridae. There is no clear evidence that their apparently specialised "crushing dentition" was exclusively dedicated to shell-breaking rather than chewing of tough food. Molariform teeth in pycnodonts are interpreted as an ancestral trait that was adaptable enough to access a range of prey while preserving the option of shell-crushing as a fallback resource. Trophic diversity explains the successful survival of the Order from the Triassic to the Eocene. The associated uncertainties in identification of fragmentary remains and observed dental microtexture variability imply that future investigations regarding the diet of pycnodontiform fishes should rely on individual-level inferences and comparisons.
## Introduction

Pycnodontiformes are one of the most abundant groups of fossil fishes from the Mesozoic of the Tethyan oceans and surrounding areas. Molariform teeth and dentitionbearing bones (see Figure 3-6 for exemples) make up most of their fossil record and though of uncertain taxonomic utility (Poyato-Ariza and Wenz, 2002; Poyato-Ariza, 2003), they provide an insight into the trophic ecology of the group. Long considered as exclusively marine shell-crushers (Delsate and Kriwet, 2004; Goatley et al., 2010; Machado and Brito, 2006; Tintori, 1998), recent investigations have highlighted the potential for an unsuspectedly broad ecological spectrum (Kriwet, 2001; Kriwet and Schmitz, 2005; Poyato-Ariza, 2005; Poyato-Ariza et al., 1998).

The distribution of fishes in aquatic ecosystem is not random, but influenced by available resources and adaptations (Bellwood and Fulton, 2008; Bellwood et al., 2006; Binning and Chapman, 2010; Bonaldo and Bellwood, 2010; Choat et al., 2004; Cvitanovic and Bellwood, 2008; Ferreira et al., 2004; Hoey and Bellwood, 2009a; Mbabazi et al., 2004) and similar ecological partitioning has already been evidenced in fossil and modern fishes (Baines, 2010; Purnell et al., 2012; Purnell et al., 2006). That each species of fish would occupy a single ecological niche is extremely unlikely as abundant examples show ecological flexibility and trophic diversity among extant fish (Bellwood et al., 2006; Binning et al., 2009; Collar and Wainwright, 2006; Mbabazi et al., 2004; Svanback and Bolnick, 2007). Notably, primary consumers, one of the more recently suggested ecologies for *Gyrodus* (Baines, 2010), show weak partitioning in regards to food source (Stanley, 2008). The rule in trophic ecology is rather that a morphologically specialised animal behaves as an ecological generalist in the wild and feeds on a prey for which it is "specialised" only in times of resource scarcity, an

observation coined Liem's paradox (Binning and Chapman, 2010; Binning et al., 2009; Liem, 1973, 1980; Robinson and Wilson, 1998; Ungar et al., 2008).

In light of such complexity in modern ecosystems, that the whole group of Pycnodontiformes would be composed only of shell-crushers seems dubious, especially since several species inhabited the same environments (Delsate and Kriwet, 2004; Tintori, 1998; Vullo et al., 2009). Anatomical differences in the feeding structures, as can be seen between *Proscinetes* and *Gyrodus* for example (see Poyato-Ariza, 2005; Poyato-Ariza and Wenz, 2002), would imply at least different fallback strategies when easy to capture or process prey are less available, different trophic preferences, or even overall ecological partitioning between Pycnodontiformes in an ecosystem. On another hand, a single or several species may perform key ecological processes such as grazing on macroalgae in coral reefs (Cvitanovic and Bellwood, 2008; Hoey and Bellwood, 2009a; Mantyka and Bellwood, 2007). Investigations based on anatomical comparisons did not test whether the Pycnodontiformes shared resources or excluded each other from trophic niches, especially at the generic or specific levels.

Among the most useful tools to compare diet and trophic niches in extant and fossil vertebrates is dental microwear analysis. Prey capture and manipulation is prone to scar food-processing elements either because of contacts between teeth and exogenous materials (abrasion) or tooth-on-tooth contact during food processing (attrition). The physical properties of the ingested food (and associated particles) leave trophic-ecology-specific signatures on the surface of teeth. Application of dental microwear analysis revealed itself useful to infer the diet or feeding behaviour of many mammals, especially primates (Daegling, 1999; Merceron, 2005) and ungulates (Fraser and Theodor, 2011; Rivals and Athanassiou, 2008), but also carnivores (Goillot et al., 2009; Schubert et al., 2010), xenarthrans (Green, 2009), and recently the technique was

extended to dinosaurs (Sereno et al., 2010; Whitlock, 2011; Williams et al., 2009) and fishes (Purnell et al., 2007; Purnell et al., 2012; Purnell et al., 2006). The recent development of dental microwear texture analysis further expanded the breadth of organisms and structures on which to investigate trophic signature, as it allows for dietary comparisons between individual animals even on curved surfaces and in the absence of clear dental microwear features (pits and scratches, Purnell et al., 2012), with the added benefits of automated and observer-error free procedures for acquisition of roughness data.

Earlier investigations (presented as oral or poster communications and included in the next chapter) showed that dental microtexture of Pycnodontiformes is similar to that of fish with a generalist diet. Here I investigate aspects of trophic ecology in Pycnodontiformes so as to explain the co-occurrence of several "specialised shellcrushers" in an environment by testing for trophic specialisation or diversity. Dental microwear analysis is applied to individual pycnodonts in different genera or branches of their cladogram. I thus compare degrees of trophic diversity observed within a taxonomic ranking and whether they express differences within a taxon-specific niche or among a wider range of diets.

## Materials and methods

## Material

Analyses have been carried on the dental surfaces of 39 fossil vomers (18) or prearticulars (20) from pychodontiform fishes (Specimen P1655 preserved teeth but was too incomplete to adequately assign them to a particular tooth-bearing bone). Details of the specimens can be found in Table 3-1. Most of the fossils are incomplete and cannot be grouped according to size or age. With the exception of *Gyrodus cuvieri* and

*Gyrodus coccoderma*, all individuals in a genus had largest teeth of similar size. Specimens used are housed in the palaeontological collections of the Natural History Museum (NHM), London, United Kingdom, Muséum national d'histoire naturelle (MNHN), Paris, France, Museum für Naturkunde of Berlin, Germany, or the University of Leicester, Leicester, United Kingdom. All the specimens have been prepared according to the treatments described later with the exception of the specimens MB.F.1337, MB.F.1338 and LEIUG 76828, which had already been prepared for a previous study. Recent investigations in the taxonomy of pycnodonts (Poyato-Ariza and Wenz, 2002) suggest that some of the taxonomic information provided here could be erroneous. Following the suggestion of (Poyato-Ariza, 2003) that quantitative analysis of dental morphology could offer insight in regards to taxonomy, the outline of the main food-processing teeth was analysed in a separate study (Appendices p. 180) and found to confirm taxonomic information at the generic level for most specimen but one individual of *Proscinetes* (P6749, due to lack of ornamentation as seen in *Gyrodus*, it is considered as another *Proscinetes*).

Table 3-1: Sampled fossil specimens, indicating the tooth-bearing bone analysed, geographic origin and institution where the material is stored. NHM: Natural History Museum, London; MNHN: Muséum National d'Histoire Naturelle, Paris; MfN: Museum für Naturkunde, Berlin; UoL: Department of Geology, University of Leicester, Leicester.

<u>thi</u> thi tri tri tor gra Eor gra Eor tor tor tor tor tor	nan throd meso trigo. Tomes granu Tomes granu Tomes rugul Tomes rugul	ame don odor oniu esod ulat esod ulat esod ulat esod	sp. n cf. us lon tus lon tus lon tus		be Pr Pr Pr	earticular earticular earticular Indet. earticular	e	c origin Dives Leicester Lindnerber g Weymouth		MfN UoL NHM NHM	
thi me tri Eor gra Eor gra Eor ru Eor ru Eor ru Eor	throd trigo trigo comes granu comes granu comes rugul comes rugul	don odor oniu esod ulat ulat esod ulat esod	sp. n cf. us lon tus lon tus lon tus		P1 P1 P1 P1	earticular earticular Indet. earticular		Dives Leicester Lindnerber g Weymouth		MfN UoL NHM NHM	
ma tri Eor gra Eor gra Eor For For For For Eor	meso trigo Comes granu Comes granu Comes rugul Comes rugul	odor oniu esod ulat esod ulat esod ulat esod	n cf. 1s lon tus lon tus lon tus		P1 P1 P1	earticular Indet. earticular		Leicester Lindnerber g Weymouth		UoL NHM NHM	
tri Eor Eor Eor Eor Eor Eor Eor Eor Eor	trigo Comes granu Comes granu Comes rugul Comes rugul	oniu esod ulati esod ulati esod ulati esod	is lon tus lon tus lon tus		P1 P1	Indet. earticular		Lindnerber g Weymouth		NHM NHM	
Eor gra Eor gra Eor Eor Eor Eor Eor Eor Eor Eor	Eomes granu Eomes granu Eomes rugul Eomes rugul	esod ulati esod ulati esod ulati esod ulasi	lon tus lon tus lon tus		P1 P1	Indet. rearticular		Lindnerber g Weymouth		NHM NHM	
gra Eor Eor Eor Eor Eor Eor Eor Eor	granu Zomes granu Zomes granu Zomes rugul Zomes rugul	ulat esod ulat esod ulat esod ulosi	tus lon tus lon tus		P1 P1	earticular		g Weymouth		NHM	
Eor gra Eor gra Eor ru Eor ru Eor ru Eor	Eomes granu Eomes granu Eomes rugul Eomes rugul	esod ulati esod ulati esod ulosi	lon tus lon tus		P1 P1	earticular		Weymouth		NHM	
gra Eor Eor Eor Eor Eor Eor Eor	granu Tomes granu Tomes rugul Tomes rugul	ulati esod ulati esod ulosi	tus lon tus		Pı	earticular					
Eor gra Eor Eor Eor Eor Eor Eor	Tomes granu Tomes rugul Tomes rugul	esod ulat esod ulosi	lon tus		P	earticular					
gra Eor ru Eor Eor Eor Eor Eor	granu Iomes rugul Iomes rugul	ulat esod ılosı	tus 1			curticului		Weymouth		NHM	
Eor ru Eor ru Eor For Eor Eor	lomes rugul lomes rugul	esod 1losi	1					-			
ru Eor For Eor Eor For Eor	rugul Iomes rugul	ılosı	ion			Vomer		Stonesfield		NHM	
Eor ru Eor ru Eor For Eor	lomes rugul		us								
ruz Eor ruz Eor ruz Eor	rugul	esod	lon			Vomer		Kirtlington		NHM	
Eor rug Eor rug Eor	0	ılosı	us					C			
ruz Eor ruz Eor	lomes	esod	lon			Vomer		Stonesfield		NHM	
Eor ru <sub>z</sub> Eor	rugul	ılosı	us								
ru Zor	lomes	esod	lon		P	earticular		Stonesfield		NHM	
lor	rugul	ilosi	us								
	lomes	esod	lon		P	earticular		Peterborou		NHM	
tri	trigo	oniu	15			••••		gh			
G	Gvro	rodu.	lS			Vomer		Weymouth		NHM	
ос	occod	oderi	ma			, 011101					
G	Gvro	rodu	lS			Vomer		Weymouth		NHM	
oci	occod	nderi	ma			VOILIEI		weymouth		1 (111)1	
G	Gyro	rodu	110			Vomer		Weymouth		NHM	
oc.	occod	deri	ma			voniter		weymouth			
G	Gvro	rodu	1110 15			Vomer		Weymouth		NHM	
oc.	occod	deri	ma			voniter		weymouth			
rov	rodus		ma wi <i>o</i> ri	i	P <sub>1</sub>	earticular		Weymouth		NHM	
roc	rodus		wieri	i i	P1	earticular		Weymouth		NHM	
roc	rodus		wieri	ı i	11	Vomer		Weymouth		NHM	
	rodus		wieri	ı i		Vomer		Weymouth		NHM	
	rodus		wieri	ı i		Vomer		Weymouth		NHM	
	rodus		wieri	ı i		Vomer		Weymouth		NHM	
	rouus		wieri	ı i	D	vontigular		Weymouth		NUM	
. J.	dua r	n cu	nidar	l Na	Г	Vomor		Weymouth		MENI	
ш л.	uus p	piur	niaer	ns		Vomer		Waymouth			
	aus p	piai hia a	niaer	ns	D	voinei		Marallas	۱ ۱		
vrc	roau	us s	sp. 1		PI	earticular		Marones	ľ	VIINTIIN	
	1	,	2		р	· 1		C 11			
vrc	vrodu	us s	sp. 2		P	earticular		Coxwell		NHM	
dı	dus u	umt	bilici	US		Vomer		Boulogne-		NHM	
								sur-mer		0 W D I	
)110	rosci	cinet	etes		P	earticular	•	Cerin	Ν	MNHN	
rO	berne	nard	di					~		<b></b>	
ro be	scinet	etes	hugi	ii	P1	earticular	•	Soleure	I	MNHN	
be sci											
ro be sci			_						1	NHM	
odi vrc vrc odi	odus p vrodu vrodu odus u Prosci berna scinei	plar lus s umb cinet nard etes	nider sp. 1 sp. 2 bilicu tes di hugi	ns us ii	Pi Pi Pi Pi	Vomer earticular rearticular Vomer rearticular rearticular		Weymouth Marolles Coxwell Boulogne- sur-mer Cerin Soleure	N N N	NHM MNHI NHM MNHI MNHI	[ N [ N N

Specimen	Provided taxon	Tooth-	Geographi	Institution
number	name	bearing bone	c origin	
P1654a	Proscinetes hugii	Prearticular	Soleure	NHM
P1654b	Proscinetes hugii	Prearticular	Soleure	NHM
P2297	Proscinetes hugii	Prearticular	Hanover	NHM
P10770	Proscinetes	Vomer	Weymouth	NHM
P6170	quincucialis Proscinatas	Vomer	Weymouth	NHM
10170	quincucialis	vomer	weymouth	111111
P6749	Proscinetes	Prearticular	Weymouth	NHM
	quincucialis			
21974	Proscinetes	Vomer	Swanage	NHM
	radiatus			
P6820	Proscinetes	Vomer	Swanage	NHM
	radiatus			
K/A05/069	Pycnodus munsterii	Prearticular	Courçon	MNHN
JRE57				
K/A06/090	Pycnodus	Prearticular	Charentes	MNHN
CTE54	subclavatus			

Results of morphometric analyses support the idea that intrageneric comparison of individuals, or comparisons at the supra-generic levels occur in a taxonomically solid framework for Pycnodontiformes. The surface from specimen P6170 appeared as potentially etched, so it was only included in the last Principal Components Analysis as an example of the effect of etching on microtextural data. The classification used here is that of Poyato-Ariza and Wenz (2002).

## **Methods**

## Specimen preparation and data acquisition

Data acquisition was conducted on casts of the original teeth, since enameloid is slightly translucent and does not allow for accurate data acquisition using the methods employed here. Dental surfaces from fossil specimens were cleaned according to the protocol described in Williams and Doyle (2010). Based on the results of our comparison of several replication materials, dental surfaces were molded using a polyvinylsiloxane compound (Coltène-whaledent speedex light body). Black epoxy resin (EpoTek 320 LV) was then poured in these moulds in order to obtain highresolution replicas. Moulding media and black epoxy were mixed and applied following the manufacturers' instructions.

3D data were obtained for all specimens using an Alicona Infinite Focus microscope G4b (IFM; software v. 2.1.2) with a x100 objective, providing highresolution three-dimensional surfaces for a 145 x 110 µm field of view. Exposure and contrast (gamma) settings were set for each scan in order to optimise the quality of the 3D data. Any errors of surface measurements were removed by manually editing the data with the "3D editor" software supplied with the microscope (InfiniteFocus 2.1.2, IFM software version 2.1.2). The cleaned point clouds were exported as .sur files and imported in SurfStand (software version 5.0). Surfaces were then automatically treated by: 1) levelling the surface and removing gross tooth form with a 2<sup>nd</sup> order polynomial function. Since the dental surfaces are from dome-shaped teeth, this technique should provide an "SF surface" representing only the surface of the tooth. 2) Application of a robust spline filter, based on a non-linear filter equation integrating robustness and endeffect management (which avoids reduction of the size of the surface, Blateyron, 2006). The used nesting index was 0.025 mm. 3) Calculation of standard parameters (ISO 25178) for the filtered surfaces. Care was taken to orient the scanned surfaces as horizontally as possible prior to data acquisition.

## Data analysis

For some specimens more than 5 areas of tooth surface were sampled; the number of scans analysed was reduced according to the following procedure: deletion of data from anteriormost and posteriormost scanned teeth, limitation to one scanned area per tooth, selected from the most accurate 3D surfaces (with no or little editing required). If the sample size remained higher than 5, it was further reduced to the 5 scans providing the values for Sku that were closest to the individual median. The

parameter Sku relates to the skewness of the distribution of 3D points over the surface and is easily influenced by outliers in the point cloud, thus focusing on areas for which its values are closest to the individual median provides a sample of comparably skewed surfaces in a non-subjective way (strongly skewed surfaces had already been discarded in the selection process). As a result the complete dataset hence includes individual samples of a size between 1 and 5 data points.

The samples were compared with respect to their inferred taxonomic affinities and population of origin. The hypotheses tested were:

- All congeneric individuals have the same dental microtexture. Since roughness of teeth at the microscopic scale is linked to diet (Purnell et al., 2012; Scott et al., 2006), testing this hypothesis will highlight any trophic diversity in the considered genus. If any significant difference is found, it will be considered whether the difference can be linked to differences between tooth-bearing bones, identified species or separate geographic origins. The hypothesis will be tested in each of the genera *Proscinetes, Eomesodon* and *Gyrodus*.
- Dental microwear texture is similar between individuals (or ensemble of individuals and trophic niches identified within a genus) from the same family. According to Poyato-Ariza and Wenz (2002) reference classification, the families represented in our sample are the Gyrodontidae and Pycnodontidae. The Pycnodontidae are represented by the genera *Proscinetes* and *Pycnodus*.
- Dental microwear texture is similar between individuals (or groups of individuals) from the same sub-Order. This is equivalent to the previous hypothesis with the addition of the *Eomesodon* genus, for the sub-order Pycnodontoidei.
- Dental microwear texture is similar in all representatives of the order Pycnodontiformes. The range of fossil taxa sampled here

(Pycnodontoidei+Gyrodontidae+Athrodon) represents a fraction of the diversity in the order Pycnodontiformes but is deemed sufficient to test if all of them share a single trophic ecology.

Null hypotheses (H0) were tested at the significance level of  $\alpha$ =5% using Welch-Satterwaithe version of Student's t-test (2 samples comparisons) or Welch-Analysis of Variance (Welch-Anova, for more than 2 categories to be compared) for each texture parameter. As in the comparison of extant animals (previous chapter), the normality of the distribution for the samples was tested using Shapiro-Wilks test with or without log-transformation of the data and rank-transformation applied if necessary. Non-gaussian distribution in the data affects the results of parametric tests, but the same tests performed on rank-transformed data provide results consistent with those of nonparametric procedures (Zimmerman, 2012). Series of pairwise t-tests were carried on the parameters displaying a significant difference. Welch-Satterwaithe and Holm-Bonferroni corrections were applied to account for heteroscedasticity and the number of tests performed.

Linear Discriminant Analysis (LDA) was applied to texture parameters with a normal distribution (or normal after log-transformation) for which a significant difference was found, using one of population, species or specimen as discriminated classes. For each taxon, the number of variables incorporated was restricted to those that showed the lowest correlations. To allow for a non-categorical comparison, Principal Component Analysis was performed using the median values (for each individual specimen) of the variables used for the linear discriminant analyses. Following the procedure described in Ezard et al. (2010), clusters within the projected scores were calculated with the packages mclust (Fraley and Raftery, 2010) and vegan (Oksanen et al., 2010). All analyses were performed using the software R version 2.15.0 (R

Development Core Team, 2012), with the MASS (Venables and Ripley, 2002) and above mentioned packages.

## Results

#### Proscinetes

Welch-Anovas highlighted significant differences between individuals in *Proscinetes* for 15 texture parameters, though only 2 did not require rank-transformation prior to testing.

Linear Discriminant Analyses based on the parameters Ssc and Vvc highlighted a separation in two categories along the main discriminant axis for each explanatory factor be it individual (LD1: 79.75% of between-class variance explained, Wilks' lambda=0.1083, p<0.0001, correct assignment=61.29%), species (LD1: 79.80% of variance, Wilks' lambda=0.3831, p=0.0002, correct assignment=70.97%) or population (LD1: 83.41%, Wilks' lambda=0.3791, p=0.0012, correct assignment=67.74%). For all analyses the parameter Ssc had the highest coefficients on LD1 and LD2.

The PCA also showed the presence of 2 groups in *Proscinetes* with samples P1654, P1654a, P1654b, P10770 and P2297 (Niche.P1) separated from 21974, P6820, P6749, K/A05/035/CRN78 and K/A05/130/JRE539 (Niche.P2), essentially along PC1 (72.45% of variance explained, both parameters weight evenly on it). No species or population is clustered solely on either category, e.g. individuals from the area of Weymouth are found in both categories, as are individuals identified as *Proscinetes hugii*. Automatic clustering classified each point as a separate group, adjusting the threshold resulted in either the same output or no segregation.

Table 3-2: Results of Welch-anova for the genus Proscinetes. Tested hypothesis: all individuals have the same microwear texture. Tests performed on O: original untransformed data, L: log-transformed data, R: rank-transformed data. df: degrees of freedom, num: numerator (effect), denom: denominator (error) adjusted for

Parameter	Data	F-statistic	df	p-value
			(num,denom)	-
Sq	R	17.5132	7,7.974	0.0003
Ssk	R	8.7527	7,7.943	0.0036
Sku	R	2.1521	7,7.672	0.1568
Sp	L	0.6372	7,7.808	0.7169
Sv	L	0.7666	7,7.797	0.6309
Sz	L	0.5009	7,7.692	0.8105
Sds	R	6.2489	7,8.061	0.00946
Str	R	5.2188	7,7.899	0.0171
Sdq	R	3.3222	7,8.000	0.05708
Ssc	0	10.4256	7,7.141	0.00287
Sdr	R	2.8643	7,7.997	0.08183
Vmp	R	17.2213	7,7.796	0.0004
Vmc	R	29.2624	7,7.911	<0.0001
Vvc	0	22.8099	7,7.672	0.0001
Vvv	R	25.3897	7,7.981	<0.0001
Spk	R	3.0693	7,7.762	0.0719
Sk	R	26.1423	7,7.943	<0.0001
Svk	R	16.165	7,7.758	0.0005
Smr1	R	3.9297	7,7.742	0.0389
Smr2	R	6.0114	7,7.732	0.0119
S5z	R	0.5307	7,7.848	0.7903
Sa	R	34.2773	7,7.897	<0.0001
Vvc/Vmc	R	4.5882	7,7.804	0.02529

heteroscedasticity. Significant differences are highlighted in bold.



Figure 3-1: Multivariate analysis of microtextural parameters in Proscinetes. Convex hulls represent the two groupings separated by the analyses. A, B & C: Projections along the main linear discriminant axes for LDA with A: specimens as discriminant factor, B: species as discriminant factor, C: Population as the discriminant factor. D: Principal Components analysis. Colours for points are as indicated in D.

## **Pycnodus**

Only two individuals from *Pycnodus* are included in the dataset and will not be analysed at the generic level. (See appendix p. 179)

## Eomesodon

Almost every texture parameter showed inter-individual differences in *Eomesodon*. Linear discriminant analyses used the parameters Vmc, Sk, Smr2, and the log-transformed Sds and Vvc. As for the genus *Proscinetes*, two main trophic guilds were displayed in the linear discriminant analyses, mostly segregated along the first discriminant axis. Using any of individual sample (Wilks' lambda=0.0384, p<0.0001), species (Wilks' lambda=0.4708, p=0.0477) or population (Wilks' lambda=0.1396, p<0.0001) as an explanatory factor offered similar discriminatory power to the main linear discriminant axes: respectively 62.03, 74.00 or 66.69% of between-categories variance for LD1 and 21.41, 18.52 or 15.73% for LD2. For all LDA Sk had the highest coefficients on LD1 and LD2, followed by Vvc log-transformed.



Figure 3-2: Multivariate analysis of microtextural parameters in Eomesodon. Convex hulls represent the two groupings separated by the analyses. A, B, C: Projections along the main linear discriminant axes for LDA with A: specimens as discriminant factor, B: species as discriminant factor, C: Population as the discriminant factor. D: Principal Components analysis. Colours for points are as indicated in D.

The PCA supports the separation between samples LEIUG 76828, OR35498,

P13922, P1655, P31879, P4387 (Niche.E1) versus P1648, P40636 and P41808 (Niche.E2), the latter niche plotting exclusively on the positive side of PC1 (65.52% of explained variance, highest weights are equal for Vmc, Vvc, Sk, lesser for Smr2), whereas both groups spread along PC2 (19.90%, shaped mainly by Sds). Automatic clustering separated each individual except P41808 and P40636, both prearticulars of *Eomesodon granulatus* from the Kimmeridgian of Dorset.

Table 3-3: Results of Welch-anova for the genus Eomesodon. Tested hypothesis: all individuals have the same microwear texture. Tests performed on O: original untransformed data, L: log-transformed data, R: rank-transformed data. df: degrees of freedom, num: numerator (effect), denom: denominator (error) adjusted for

Parameter	Data	F-statistic	df	p-value
			(num,denom)	-
Sq	R	24.9778	8,11.107	<0.0001
Ssk	R	4.4411	8,11.379	0.0118
Sku	R	20.4969	8,11.784	<0.0001
Sp	R	15.4406	8,11.660	<0.0001
Sv	R	10.7353	8,11.765	0.0003
Sz	R	13.5933	8,11.875	<0.0001
Sds	L	10.8770	8,12.104	0.0002
Str	0	0.2560	8,11.135	0.9684
Sdq	R	20.2973	8,12.063	<0.0001
Ssc	R	6.6255	8,11.466	0.0023
Sdr	R	16.7228	8,12.184	<0.0001
Vmp	R	31.4127	8,11.457	<0.0001
Vmc	0	13.0430	8,11.344	0.0001
Vvc	L	11.8998	8,11.445	0.0002
Vvv	R	23.4726	8,10.887	<0.0001
Spk	R	35.3988	8,10.716	<0.0001
Ŝk	0	13.3152	8,11.299	0.0001
Svk	R	7.6838	8,10.695	0.0016
Smr1	R	2.5068	8,11.606	0.07583
Smr2	0	4.7886	8,11.019	0.00962
S5z	R	16.7217	8,11.825	<0.0001
Sa	R	21.3187	8,11.132	<0.0001
Vvc/Vmc	R	2.6483	8,11.255	0.0688

heteroscedasticity. Significant differences are highlighted in bold.

## Gyrodus

All textural parameters but Str were found to display between-individuals differences for *Gyrodus*. In order to limit the influence of correlation, LDA and PCA were performed on parameters Sk, Svk and Smr1. The first two axes of the PCA explained respectively 60.45 and 33.97% of the variance. Automatic clustering found only one all-encompassing cluster, without any form of segregation visible between groups. PC1 was shaped mostly by Sk and Svk, PC2 by Smr1.

Table 3-4: Results of Welch-anova for the genus Gyrodus. Tested hypothesis: all individuals have the same microwear texture. Tests performed on O: original untransformed data, L: log-transformed data, R: rank-transformed data. df: degrees of freedom, num: numerator (effect), denom: denominator (error) adjusted for

Parameter	Data	F-statistic	df	p-value
			(num,denom)	
Sq	R	24.9656	15,19.057	<0.0001
Ssk	R	5.1253	15,18.514	0.0006
Sku	R	60.5632	15,18.044	<0.0001
Sp	R	15.1078	15,17.996	<0.0001
Sv	R	9.1556	15,18.147	<0.0001
Sz	R	15.4158	15,18.003	<0.0001
Sds	R	7.9195	15,18.509	<0.0001
Str	R	1.4326	15,18.574	0.2292
Sdq	R	7.5069	15,18.688	<0.0001
Ssc	R	21.9186	15,18.814	<0.0001
Sdr	R	7.9324	15,18.745	<0.0001
Vmp	R	9.0602	15,18.247	<0.0001
Vmc	0	6.3570	15,18.756	0.0001
Vvc	0	7.5908	15,18.858	<0.0001
Vvv	0	8.1904	15,18.887	<0.0001
Spk	R	7.6056	15,18.245	<0.0001
Šk	0	6.7854	15,18.618	<0.0001
Svk	0	4.5100	15,18.466	0.0014
Smr1	0	4.8646	15,18.420	0.0009
Smr2	R	7.1326	15,18.998	<0.0001
S5z	R	11.8125	15,17.915	<0.0001
Sa	R	17.7244	15,18.871	<0.0001
Vvc/Vmc	R	7.3104	15,18.364	<0.0001

heteroscedasticity. Significant differences are highlighted in bold.



Figure 3-3: Multivariate analysis of microtextural parameters in Gyrodus A, B, C: Projections along the main linear discriminant axes for LDA with A: specimens as discriminant factor, B: species as discriminant factor, C: Population as the discriminant factor. D: Principal Components analysis. Colours for points are as indicated in D. Convex hulls represent the species G. planidens (yellow) and G. coccoderma (blue).

The linear discriminant analyses based on the individual samples (LD1: 50.33, LD2: 30.84% of between-class variance, Wilks'lambda=0.1216, p<0.0001) or species (LD1: 63.52, LD2: 22.86% of between-class variance Wilks'lambda=0.6593, p=0.0267) as an explanatory factor displayed no clear pattern but for a limited segregation between *G. planidens* and *G. coccoderma* (on LD2 for the sample-based LDA, on LD1 for the species-based LDA). This segregation was not apparent in the population-based LDA (the effect was not significant: Wilks'lambda=0.9183, p=0.7730) in which all samples overlap along LD1 (91.28% of variance). As almost all individuals of *Gyrodus* are from the Kimmeridgian of Dorset this last result is influenced by the large variability from the specimens of *Gyrodus cuvieri*. Overall the correct assignment rates are very low for the LDAs in *Gyrodus*, highlighting the comparatively high variability in this genus compared to *Proscinetes* or *Eomesodon*. In all LDAs Sk had higher coefficients on LD1 and Svk on LD2. Svk also had a high coefficient on LD1 for the species-based analysis.

Pycnodontiformes showed trophic diversity at the generic level as highlighted in the results presented above. As a consequence, the categories compared at the familial, sub-ordinal or ordinal level were those identified groupings rather than individuals. Comparisons will thus test whether groupings in Pycnodontiformes, interpreted as trophic niches, were exclusive or shared between taxa.

# Pycnodontidae

Results of the between-groups comparisons in Pycnodontidae (*Pycnodus+Proscinetes*) are outlined in Table 3-5.

Table 3-5: Results of Welch-anova for the family Pycnodontidae. Tested hypothesis: groupings in the family Pycnodontidae have the same microwear texture (clusters highlighted at a finer scale were restricted to this sub-sample). Tests performed on O: original untransformed data, L: log-transformed data, R: rank-transformed data. df: degrees of freedom, num: numerator (effect), denom: denominator (error) adjusted for heteroscedasticity. Significant differences are highlighted in bold.

Parameter	Data	F-statistic	df	p-value
			(num,denom)	
Sq	R	33.9641	2,17.501	<0.0001
Ssk	R	6.3426	2,20.187	0.0073
Sku	R	2.2875	2,23.175	0.1240
Sp	R	4.1768	2,19.956	0.03053
Sv	R	1.3100	2,17.007	0.2957
Sz	R	1.7954	2,17.211	0.1958
Sds	$\mathbf{L}$	5.7248	2,18.187	0.0118
Str	R	3.3208	2,17.479	0.0599
Sdq	R	36.1134	2,18.748	<0.0001
Ssc	R	9.5804	2,21.130	0.0011
Sdr	R	33.0397	2,18.678	<0.0001
Vmp	R	15.3808	2,15.449	0.0002
Vmc	R	49.8708	2,17.439	<0.0001
Vvc	0	43.4485	2,20.239	<0.0001
Vvv	R	6.8351	2,16.901	0.0067
Spk	R	14.1898	2,16.285	0.0003
Sk	R	57.1863	2,17.159	<0.0001
Svk	R	3.6011	2,18.119	0.0482
Smr1	Ο	2.0808	2,15.574	0.1582
Smr2	0	18.4164	2,19.865	<0.0001
S5z	R	1.9999	2,17.106	0.1658
Sa	R	44.9815	2,17.120	<0.0001
Vvc/Vmc	L	7.6645	2,17.857	0.0039



Figure 3-4: Multivariate analysis of microtextural parameters in Pycnodontidae (A, B, C & D) and Pycnodontoidei (E, F & G). A, B: Projections along the main linear

discriminant axes for LDA in pycnodontidae with A: specimens as discriminant factor, B: Trophic niche (P1, P2 or Py) as discriminant factor, C & D: Principal Components analysis in Pycnodontidae along PC1 & 2 (C) or PC 1 & 3 (D). E, F: Projections along the main linear discriminant axes for LDA in Pycnodontoidei with E: specimens as discriminant factor, F: Trophic niche (E1, E2, P1, or P2 + Py) as discriminant factor, G: Principal Components analysis in Pycnodontoidei along PC1 & 2. Convex hulls represent the separated trophic (A, B, E & F) niches or the computed clusters (G, clusters match the trophic niches).

Textural parameters Vvc, Smr2 and log-transformed Sds and Vvc/Vmc were included in a linear discriminant analysis with individual sample as the explanatory factor. Another analysis used the identified groupings as explanatory factor in order to compare which of individual preferences or interpretative trophic niche better explained the observed differences in microwear texture (As *Pycnodus* and *Proscinetes* were not found in the same localities, population as an explaining factor is contained in the specimen-based analysis). For both analyses Smr2 and Vvc/Vmc had the highest coefficients on LD1 and LD2.

The two main axes of the sample-based LDA explained respectively 62.59% and 17.86% of trace. Those proportions were 90.16% and 9.84% in the niche-based LDA (individual-based: Wilks' lambda=0.0143, p<0.0001; group-based: Wilks' lambda=0.1240, p<0.0001). Both discriminant analyses clearly separate the samples in two coherent ensembles along the first axis, putting together the members of the genus *Proscinetes* assigned to Niche.P2 with both representatives of *Pycnodus*. The other ensemble is composed only of individuals from Niche.P1. In the sample-based LDA, the separation between the two individuals of *Pycnodus* is similar to that observed

between members of a single trophic niche indicating a similar diet for the two specimens despite different geologic origins.

The principal components analysis run on the same parameters required 3 axes to express most of the variance (PC1: 60.39%, PC2: 20.40%, PC3: 12.50%, Sds shaped mostly PC2 while the other parameters equally weighted on PC1, Vvc weighted heavily on PC3). The same pattern observed in the LDAs was observed in the ecospace they defined, although clearer with a combination of PC1 and PC3, highlighting the presence of two groupings within the Pycnodontidae based on their dental microtexture with a convergence between the data from *Pycnodus* and the Niche.P2 category.

### **Pycnodontoidei**

Comparisons of trophic niches in the sub-order Pycnodontoidei through analysis of variance are reported in Table 3-6. The two niches found in *Eomesodon* were compared to the two niches of the family Pycnodontidae and specimen or population used as discriminatory factors for LDAs on textural parameters Smr2, Vmc, Vvc and Sk.

Both LDA had similar scores for linear discriminant axes (LD1: 64.48%, LD2: 20.92%, Wilks' lambda=0.0306, p<0.0001 for the individual-based LDA; LD1: 63.13%, LD2: 21.28%, Wilks' lambda=0.2596, p<0.0001 for the population-based LDA, in both the highest coefficients were for Sk then Smr2 for LD1 and LD2) and showed a separation into two assemblages as observed for the family-wise comparison or the comparison of samples in *Eomesodon*. The same pattern is observed in the ecospace defined by the PCA axes (PC1 expresses 83.16% of variance, PC2: 16.54%, Vvc, Vmc and Sk weighted heavily on PC1, PC2 was mostly shaped by Smr2) with overlapping niches from *Eomesodon* and the Pycnodontidae. The trophic niches "P1" and "E1" are convergent based on their dental microtexture, as are "P2", "E2" and "Py".

This convergence is further supported by the application of automatic clustering to the projected scores, with each of those two categories being assigned to two distinct clusters (Figure 3-4).

Table 3-6: Results of Welch-anova for the sub-Order Pycnodontoidei. Tested hypothesis: groupings in the sub-Order Pycnodontoidei have the same microwear texture (clusters highlighted at a finer scale were restricted to this sub-sample). Tests performed on O: original untransformed data, L: log-transformed data, R: ranktransformed data. df: degrees of freedom, num: numerator (effect), denom: denominator (error) adjusted for heteroscedasticity. Significant differences are highlighted in bold.

Parameter	Data	F-statistic	df	p-value
			(num,denom)	
Sq	R	29.9324	3,31.004	<0.0001
Ssk	R	2.6974	3,29.397	0.0639
Sku	R	2.2351	3,29.451	0.1050
Sp	R	2.5136	3,28.410	0.0785
Sv	R	6.6541	3,28.502	0.0015
Sz	R	4.7343	3,27.677	0.0086
Sds	R	0.0292	3,29.638	0.9931
Str	R	0.3947	3,29.634	0.7577
Sdq	R	24.6246	3,30.621	<0.0001
Ssc	R	13.6802	3,30.483	<0.0001
Sdr	R	18.6239	3,30.814	<0.0001
Vmp	R	16.2005	3,29.927	<0.0001
Vmc	0	41.8571	3,30.490	<0.0001
Vvc	0	43.9465	3,30.869	<0.0001
Vvv	R	8.9772	3,30.127	0.0002
Spk	R	15.5182	3,30.014	<0.0001
Sk	0	47.5205	3,30.151	<0.0001
Svk	R	7.1570	3,28.638	0.0009
Smr1	L	0.7333	3,33.289	0.5396
Smr2	0	3.8003	3,31.209	0.0197
S5z	R	8.6768	3,27.328	0.0003
Sa	R	41.6594	3,31.304	<0.0001
Vvc/Vmc	0	2.5709	3,31.489	0.07174

# **Pycnodontiformes**

Investigations in the genus *Gyrodus* only highlighted a limited separation between the two species *G. planidens* and *G. coccoderma* with *G. cuvieri* occupying the entirety of the ecospace. The *Gyrodus* dataset was thus limited to data from the two self-excluding species in order to compare them with the identified trophic niches of the Pycnodontoidei. The tested hypothesis is that *G. planidens* and *G. coccoderma* represent within the genus *Gyrodus* the same segregated groups observed between samples of Pycnodontoidei. The Genus *Athrodon* was also included in order to compare it with identified trophic categories.



Figure 3-5: Principal Components Analysis in the order Pycnodontiformes. PC1: Principal Components axis 1, PC2: Principal Components axis 2. Coloured hulls represent the computed clusters, hull in dashed lines represents the portion of ecospace empty until inclusion of Gyrodus. Specimens from the Dorset area are highlighted in bold.

Table 3-7: Results of Welch-anova for the Order Pycnodontiformes. Tested hypothesis: Trophic niches in the Pycnodontiformes have the same microwear texture (clusters highlighted at a finer scale were restricted to this sub-sample). Tests performed on O: original untransformed data, L: log-transformed data, R: rank-transformed data. df: degrees of freedom, num: numerator (effect), denom: denominator (error) adjusted for heteroscedasticity. Significant differences are highlighted in bold.

Parameter	Data	F-statistic	df	p-value
			(num,denom)	
Sq	R	30.8295	4,20.879	<0.0001
Ssk	R	5.0056	4,22.905	0.0048
Sku	R	41.2543	4,27.294	<0.0001
Sp	R	31.7288	4,24.254	<0.0001
Sv	R	15.6637	4,24.523	<0.0001
Sz	R	66.2816	4,28.779	<0.0001
Sds	R	3.2549	4,23.036	0.0296
Str	R	0.7020	4,20.688	0.5994
Sdq	R	31.3366	4,23.136	<0.0001
Ssc	R	55.3487	4,33.482	<0.0001
Sdr	R	22.4598	4,22.484	<0.0001
Vmp	R	42.3414	4,22.239	<0.0001
Vmc	0	36.0047	4,20.958	<0.0001
Vvc	$\mathbf{L}$	38.4168	4,21.051	<0.0001
Vvv	R	14.3838	4,24.830	<0.0001
Spk	R	46.8604	4,22.570	<0.0001
Ŝk	0	43.4176	4,20.726	<0.0001
Svk	R	23.3068	4,25.187	<0.0001
Smr1	0	5.6359	4,20.510	0.0032
Smr2	0	20.0904	4,21.736	<0.0001
S5z	R	62.5473	4,26.517	<0.0001
Sa	R	38.102	4,20.712	<0.0001
Vvc/Vmc	0	8.8021	4,20.224	0.0003

Comparisons of trophic niches using Welch-anovas are reported in Table 3-7. A

principal components analysis performed on the parameters Vmc, Vvc, Sk, Smr1, Smr2 and Vvc/Vmc for the individual medians expressed 52.73% of variance on PC1 and 34.70% on PC2 (Sk, Vvc and Vmc weight heavily on PC1, Smr1, Smr2 and Vvc/Vmc on PC2). Separation of the trophic niches is clearest along PC1 and highlights similarities between *G. planidens*, *Athrodon* sp. and the niche "P1+E1". *G.coccoderma* on the other hand is spread across PC1 and 2. Using the parameters from this PCA to infer the projections for other members of the genus *Gyrodus* shows a tendency for the genus to plot on the negative side of PC1. A mixture of *G.coccoderma*, *G. cuvieri*, G. sp occupies a portion of the ecospace that was left vacant by the other Pycnodontiformes.<sup>4</sup>

The projections from this PCA were clustered automatically in the same two assemblages previously found in a non-subjective way. The separation observed between the groupings of Pycnodontoidei can confidently be considered as the microtextural expression of an ecological signal.

## Discussion

#### Dental microtexture variability between congeneric individuals

Comparisons of dental microtextural data between individuals in 3 genera of pycnodontiform fishes highlighted significant differences in all of them. Data derived from microwear roughness in *Proscinetes, Eomesodon* and *Gyrodus* allows for segregation between different groups based on univariate (Anova) and multivariate (LDA, PCA) analyses. There is no strong evidence in favour of a geographic or specific signal, except in *Gyrodus*, between individuals from *G. coccoderma* and *G. planidens*. Differences in the microtexture data observed in *Gyrodus* can be explained by niche partitioning between *G. planidens* and *G. coccoderma* while *G. cuvieri* is more ecologically flexible. Individual trophic preferences persist within each of these species, as is shown by their spread and overlap along PC1 in their PCA and LD1 in their sample-based LDA. As dental microwear texture is foremost linked to diet, niche segregation at the specific level would have resulted in a specific segregation along the axis with the highest proportion of variance explained.

<sup>&</sup>lt;sup>4</sup> Specimen P6170 (acid-etched) is plotting away from any other Pycnodontoidei on PC2, within the part of ecospace unoccupied previously. Investigations regarding the effect of taphonomic and preparation processes on microtexture parameters should be considered as it has been for classical microwear in King et al., (1999) (King, T., Andrews, P., and Boz, B., 1999, Effect of taphonomic processes on dental microwear: American Journal of Physical Anthropology, v. 108, p. 369-373.).

The link between surface texture and diet at the microscopic scale has already been illustrated in extant and extinct organisms (Mainland, 2003; Merceron et al., 2009; Purnell et al., 2012; Ramdarshan et al., 2011; Scott et al., 2012) and the previous chapter, henceforth these groups can confidently be interpreted as separate trophic niches within the studied genera. Most of the parameters linked with amplitude (Sq, Sa, S5z, Spk, Sk, Svk) or volume (Vmp, Vmc, Vvc, Vvv) are related to hardness of processed food in extant fishes (see Calandra, 2011and the previous chapter; Purnell et al., 2012) and here display significant differences in the statistical comparisons, thus supporting the hypothesis of trophic diversity within the Pycnodontiformes, at least regarding the hardness of the processed food. Trophic diversity at the generic level in fishes is commonplace in modern ecosystems (Mbabazi et al., 2004; Mendes et al., 2009; Ruehl and DeWitt, 2007; Svanback and Bolnick, 2007) yet contrasts with the hypothesised order-level specialisation for Pycnodontiformes (Delsate and Kriwet, 2004; Kriwet, 2008; Nursall, 1993a; Poyato-Ariza et al., 1998; Poyato-Ariza and Bermúdez-Rochas, 2009; Tintori, 1998; Vullo et al., 2009; Walker and Brett, 2002a).

Since dental remains can rarely be identified at the specific level with confidence (Poyato-Ariza, 2003) (see Appendices p. 180) the separated trophic niches may reflect trophic specialisation for ecologically distinct species. One way to test for this would be to analyse several conspecific specimens preserved with near-complete anatomy (for identification purpose), dental surfaces exposed, from a single contemporaneous fossil deposit. Such a sample is not readily available at the moment, and if it were, curators may not allow for manipulation and risk the damage of an ensemble of exceptional specimens. The only genus supporting the hypothesis of a species-level dietary signal is *Gyrodus*, for a subset of the available data. This hypothesis cannot be rejected based on this study alone but is not strongly supported

either as *G. coccoderma* and *G. planidens* exhibit stronger differences between individuals than between species (see Figure 3-3).

Another source of variation in the dataset is the geographic origin of the fossils. Even if anatomically similar, animals from different environments would not be exposed to the same conditions with respect to available prey and concurrent predators (e.g. Cutwa and Turingan, 2000). Most of the Gyrodus from our dataset come from the Kimmeridgian of Weymouth (Dorset). This one environmental setting harbours the two specific trophic niches identified for the Gyrodontidae. Despite the lack of precise stratigraphic information, the samples must come from the Baylei, Cymodoce and/or *Mutabilis* ammonite zones (S. Etches, pers. com.). During the early to early-late Kimmeridgian, the area was a deep carbonate platform going through a transgression accompanied by at least three cycles of sea-level rise and fall (Colombié and Rameil, 2006). As a consequence, different fossil specimens may have lived in the same place but at different times and in different environments. The number of beds yielding such fossils is limited in the sequence (Dineley and Metcalf, 1999) and nearly contemporaneous individuals sharing a similar physical environment must be part of the sample. Independent of the distribution of the individuals in the stratigraphic sequence, the presence of statistically different trophic niches hints at trophic diversity within *Gyrodus*, as a result of segregation between contemporaneous individuals or an adaptation to a heterogeneous environment.

Prey availability must have shaped the diet of Pycnodontiformes as much as mechanical properties of the feeding apparatus and locomotive abilities. Predators in the wild feed on whichever available resource falls within the range of foods they can process. This range is a function of energy trade-offs balancing the costs of prey perception, capture, handling, digestion versus the nutritive qualities of the food.

Relative abundance of the prey is a major factor prone to vary quickly, e.g. spawning seasons will see a lot of eggs being laid and available to feed upon. A relatively abundant easily processed food would be preferred by Pycnodontiformes to exoskeletonized organisms. For example, the samples of *Eomesodon rugulosus* OR35498 and P4387 are separated from P1648 though all are from the Middle Jurassic Stonesfield slate. In contrast a locality-based divergence pattern is observed in *Eomesodon granulatus* samples P41808 and P40636 both from Weymouth, similar in all analyses but very different from the conspecific P1655 from Lindnerberg, that dental microtextures vary between individuals more than they do between species implies that inferences of diet within the order have to be performed for each individual separately or for samples that have been previously identified as homogeneous within a broader assemblage. The co-occurrence of samples within a single locality is no guarantee of shared ecological habits.



Figure 3-6: Body shape and dentitions in a range of Pycnodontiformes. A: Eomesodon
sp.; B: Prearticular of Eomesodon granulatus, specimen P41808; C: Gyrodus
hexagonus; D: Prearticular of Gyrodus cuvieri, specimen P41800; E Proscinetes
bernardi; F: Vomer of Proscinetes quincucialis, specimen P6170; G: Vomer of Gyrodus
cuvieri, specimen P3785. Scale bars are 1 cm for B, D, F, G and 5 cm for C. C from
(Kriwet and Schmitz, 2005), A and E courtesy of Wikimedia commons.

Having a repertoire of feeding strategies can hamper the effectiveness of biomechanical reconstructions and has lead to misconceptions about the ecology of an animal (Lauder, 1995). For predatory organisms it allows for efficient foraging despite changing environmental conditions (Binning and Chapman, 2010; Binning et al., 2009; Estebaranz et al., 2009; Mendes et al., 2009; Merceron et al., 2010). Seasonal patterns in resource availability and ecological plasticity explain divergences in diet within a taxon at the local scale. Changing settings across a coral reef or lagoonal environments account for observed trophic shifts in Scaridae (Rotjan and Lewis, 2006) or Sparidae (Castillo-Rivera et al., 2007; Cutwa and Turingan, 2000). Seasonal fluctuations have been demonstrated for the Late Jurassic (Brigaud et al., 2008) but their bearing on the composition of the ecosystem or the biology of the organisms remains unknown. Therefore, trophic diversity within the Pycnodontiformes at the genus-level was more predictable than the trophic overlap between niches across taxa.

#### Dental microtexture variability in Pycnodontiformes

As linear discriminant analysis relies on a priori defined categories for group attribution, predictions of belonging to another niche highlight clear similarities in dental microtexture. Especially in the LDA focusing on between-niche segregation, the expectation was that each niche would be isolated, with no or limited overlap. Projections in the ecospace of the PCA do not rely on a priori categories and strongly confirm the observed patterns of convergences and differences in dental texture data. Subjectivity in the definition of compared categories limits the range of results in hypothesis-testing approaches and ideally requires a blind assessment of any structure in the data. Automatic definition of clusters through a threshold-based technique (see Ezard et al., 2010) fulfils this role and supports the presence of at least 2 clusters of pycnodonts with shared dental textures and diets. Sexual information about the specimens used here (isolated dentition) is not available which makes testing for gender-based ecological partitioning impossible.

Among expectations in trophic investigations lies a limited overlap in diet between single units from a broader taxonomic sample, with the occasional ecological specialist or oddball (e.g. Panda bears among the Ursidae) standing out. Vertebrates sharing an evolutionary history also share mechanical limitations in their feeding apparatus and range of potential ecologies. Fewer limitations persist as deeperbranching taxa are included, i.e. a clear segregation between feeding strategies in taxonomic units is less likely the wider the sample is (see Anderson et al., 2011; Antonucci et al., 2009). Morphospace occupation via measurements and multivariate analysis (e.g. Anderson, 2009) captures the mechanical factor shaping the diet of vertebrates, but similarity in the feeding apparatus (or lack thereof) does not account for a shared realised ecological function and vice-versa (Alfaro and Wainwright, 2006; Wainwright et al., 2005; Wainwright, 2007). The results presented here suggest that ecological redundancy is a feature of Pycnodontiformes, and that individuals from separate taxonomic units performed similar ecological roles in their respective habitat. However Athrodon, Eomesodon, Gyrodus, Proscinetes and Pycnodus show variability in their dentition and body shape, which would hint at diverging ecological habits. A longer body shape (e.g. Gyrodus or Proscinetes compared to Eomesodon, Figure 3-6) either accounts for more cruising habits (Poyato-Ariza, 2005) or hindgut fermentation (Mountfort et al., 2002), and overall access to a different range of prey types. The same prey will not be treated and the same quantity of nutrients extracted by flat (Proscinetes) or more ornamented dental surfaces (Gyrodus, Figure 3-6). Our knowledge of jaw-motion in pychodonts is progressing but incomplete (Baines, 2010). The morphology of the coronoid process varies within the order which suggests a range of

jaw-motions (See character 47 in Poyato-Ariza and Wenz, 2002). Convergence of values in microtextural parameters can be interpreted as the effect of similar stresses on the enameloid caused by food-processing, as the result of a similar diet or different ways to process different foods with the same physical effect. Further investigations regarding jaw-motion in Pycnodontiformes along with stress diffusion in teeth and associated bones will be necessary to test either hypothesis. The "trophic niches" shared across taxa reflect first and foremost feeding habits resulting in similar dental surfaces, i.e. diets inducing similar mechanical stresses during prey-processing.

## Regarding the trophic ecology of Pycnodontiformes

The results presented here do not refute the hypothesis that pycnodonts were specialised shell-crushers. The different niches might represent different feeding strategies within an assemblage of shell-crushers or grinders. The dentition of pycnodonts has long been defined as "crushing" (even if "molariform" provides an anatomically accurate and ecologically neutral alternative Poyato-Ariza, 2005) but the presence of shells or other hard parts within the gut of some specimens of pycnodonts (Kriwet, 2001) provides limited additional support.

It was suggested above that a longer body shape, as for *Pycnodus*, could hint at herbivorous habits and hindgut fermentation. Yet, of the rare gut contents found in pycnodonts, two specimens of *Pycnodus* contain shells from a single species of bivalves. Based on feeding habits in extant fishes and the results, the interpretation of this is that bivalves were among one of the most easily available prey in the environment for those fishes in that time and place. Exceptionally preserved fossils with no preserved gut content are not accounted for in palaeoecological reconstitutions or summaries of evidence of predation. The absence of shells or hard parts within the gut of exceptionally preserved pycnodontiform fishes can be explained by any of the

following: Lack of preservation of soft prey items, starvation, spitting of the hard parts during food-processing, fine grinding of the exoskeletonized prey preserved as sediment-like gut content. To test the first two is beyond the scope of this study, though in Solnhofen for example Pycnodontiformes are preserved with other invertebrates, thus discounting the hypothesis of starvation.

Spitting or regurgitation of the shells results in accumulations of broken fragments on the site of consumption or in accumulations (Oji et al., 2003; Zatoń and Salamon, 2008) but provide limited evidence of the predator. Increases of predation on molluscs can equally be blamed on teleosts, sharks, rays or arthropods (Dietl and Vega, 2008). Tooth morphology in pychodonts lends little support to the hypothesis of shellcrushing and spitting of fragments. Their premaxillary and dentary-borne teeth display a variety of morphologies, mostly elongated or incisiform. Just as in the extant Anarhichas lupus (the wolffish, a specialised shell-crusher) or Archosargus probatocephalus (the generalist sheepshead seabream) they were used for prey grasping and manipulation, allowing access to a large range of food types (Poyato-Ariza, 2005). Crushing would have occurred on the prearticular or vomer-borne teeth, but the anatomical structure formed by these bones is comparable to a trough with lateral teeth strongly occluding, as evidenced by microwear patterns (Baines, 2010) and the development of wear facets (see examples in Kriwet, 2008; Poyato-Ariza, 2005; Poyato-Ariza and Wenz, 2002; Poyato-Ariza, 2010; Poyato-Ariza and Bermúdez-Rochas, 2009).

The alternative hypothesis of a fine grinding of the captured prey fits better with the hypotheses of limited jaw-gape and propalinal jaw-motion as evidenced by faceting of the lateral tooth rows. A grinding behaviour is not solely limited to a durophagous diet and effectively permits nutrient extraction from a variety of food types. Examples abound within the Scaridae of a variety of diets showing local scale variations and selectivity (Alwany et al., 2009; Bellwood and Choat, 1990; Bellwood et al., 2006; Bonaldo and Bellwood, 2009; Carr et al., 2006a; Carr et al., 2006b; Francini et al., 2008; Mumby, 2009; Rotjan and Lewis, 2006). Parrotfishes such as Scarus that do grind large quantities of hard material do replace their teeth regularly and show a large number of scratches on their dental surfaces (Carr et al., 2006b). There is little evidence for tooth replacement in Pycnodontiformes (Nursall, 1999), and although some specimens show teeth growing in cavities where older teeth have been lost or shed (pers. obs.), this does not appear to be the rule in the taxon. Parallel scratches on dental surfaces as a result of regular grinding of hard material have not been observed in any of the specimens considered here. Grinding or shearing habits are not disproved as such, but the ingested material was not mostly of coral, shells or coralline algae, prone to induce a scratch-rich surface (see next chapter for inferences of trophic preferencs in Pycnodontiformes). Manipulation of variedly tough foods required different amounts of chewing in the grinding mill of Pycnodontiformes as in extant mammals (Calandra, 2011; Merceron et al., 2010) thus the separation between microwear texture niches might mirror a separation between animals having processed more tough or more brittle material in their last meals. Further investigations in this field are necessary in order to assess the power of microtexture to separate fishes consuming more tough or more brittle foods.

*Gyrodus* can be found everywhere within the ordinal ecospace, but interestingly, sample OR43562 aside, *Gyrodus* individuals from Dorset are not found close to other, more derived fishes from the area. Ecological partitioning in this ecosystem is represented by the separation between Pycnodontoidei and Gyrodontoidei. One of the major differences between Gyrodontoidei and Pycnodontoidei lies in the morphology of
the prehensile teeth present on the premaxilla or dentaries, styliform in the former as opposed to incisiform (Nursall, 1993a). This led Nursall to suggest that taxa behaved rather as scrapers (Pycnodontoidei) or as pickers (Gyrodontoidei). Dental morphology in Pycnodontiformes is broader than this dichotomy (Poyato-Ariza, 2005; Poyato-Ariza and Wenz, 2002) but in effect the combination of constraints on feeding based on gape and incisiform teeth effectively restricted the range of available prey for Pycnodontoidei. Gyrodontoidei with styliform teeth would have been better equipped to e.g. pick invertebrates from the substrate. Given the variability in their data, they likely were more opportunistic feeders than the Pycnodontoidei apparently restricted to or specialised for a range of ecologies (or prey). Pycnodontoidei would have preferred grazing on algae, a resource prone to vary according to local conditions and predation (Burkepile and Hay, 2010; Cvitanovic and Bellwood, 2008; Hoey and Bellwood, 2009a; Sotka and Hay, 2009). Divergent ecologies in the Pycodontoidei can also reflect a separation between animals based on their feeding mode, even within a morphologically specialised clade as in the modern parrotfishes (Bellwood and Choat, 1990). Whether such diets are represented by the identified trophic niches is the object of the next chapter, with comparison of the dental microtextures between fossils and extant analogues with known dietary habits.

# Conclusion

This study provides the first evidence for trophic diversity within the Pycnodontiformes. Dental microwear texture analysis highlights inter-individual differences once applied to teeth of fossil fishes. Comparisons of dental microtextural data between individuals in *Proscinetes, Eomesodon* and *Gyrodus* highlights significant differences in all of them based on univariate (Anova) and multivariate (LDA, PCA) analyses. Results reject the null hypothesis that they were all specialised shell-crushers.

Recent investigations in their jaw-motion associated with the variability observed in their dental microwear texture supports the hypothesis of a group of ecologically flexible animals in regards to prey types. Neither of the considered species nor locality does provide a consistent dental microwear texture signal. As a consequence, it is strongly advised that any inference or comparison regarding the trophic ecology of Pycnodontiformes is first performed at the individual level.

Modern aquatic ecosystems teem with organisms which have to compete with other predators, conspecific or not, for access to food. Most of the time they behave as ecological generalists, feeding on the readily available and easier to process prey, independent of any special adaptation, used in times of resource scarcity (Binning et al., 2009; Robinson and Wilson, 1998). In light of our results, Pycnodontiformes are another such example of Liem's paradox, their ecological plasticity shared with modern analogues with comparable molariform or bunodont dentitions such as Sparidae and Scaridae. There is no clear evidence that the apparently specialised "crushing dentition" was in effect used to break shells. Quite the contrary, they must have regularly fed on food items that required chewing rather than breaking. The "crushing" shape of these molariform teeth is interpreted as an ancestral trait that has ensured the success of the taxon from the Triassic to the Eocene, as it could be adapted for efficient grinding and nutrient extraction while remaining useful for shell-crushing shall anything else than e.g. molluscs be available. The possibility remains that Pycnodontiformes were an order of shell-crushers but this hypothesis is weakly supported and the topic of the next chapter: Comparison of their dental textures with that of organisms with known ecologies allows for inferences of trophic ecologies in the available sample

# Chapter 4: Inferences of diet in Pycnodontiformes (Fish, Neopterygii) via dental microwear texture analysis

# Abstract

Dental microwear texture analysis of teeth in pycnodontiform fishes provides a test of the hypotheses of dietary preferences in Pycnodonts and their role as specialised shell-crushers driving the evolution of exoskeletonized animals during the Mesozoic Marine Revolution. The hypothesis of pycnodonts as specialised durophages is rejected by comparative and multivariate analyses. Results suggest similarities between the feeding habits of modern generalists and individual Pycnodontiformes whereas others had the potential to be heavy grazers on algae. Members of the Pycnodontoidei and Gyrodontoidei display different patterns of occupation of the ecospaces defined in multivariate analysis, suggesting divergent feeding behaviours potentially linked to the morphology of their incisiform teeth. The hypothesised role of pycnodonts as predators triggering the evolution of hard-shelled invertebrates during the escalation event known as the Mesozoic Marine Revolution has to be re-evaluated in the light of their more herbivorous or omnivorous habits.

# Introduction

In his 1977 study on fossil gastropods, Vermeij (1977) introduced the notion of the Mesozoic Marine Revolution (MMR), a global scale escalation event which encompasses the antagonistic evolutionary innovations from the Mesozoic to the Cenozoic. Though Vermeij's seminal work focused on gastropods, their predators and co-occurring grazers, the term MMR has been expanded to include all escalation events from the Triassic to the Late Cretaceous and beyond. The result of the revolution was the settling of novel ecological niches for which new Mesozoic predators and consumers seem to be adapted, niches still occupied today by related or morphologically similar animals. E.g.: the giant filter-feeders, today's whale sharks (Rhincodontidae), megamouth sharks (Megachasmidae), basking sharks (Cetorhinidae), manta rays (Mobulidae) and baleen whales (Mysticeti), were from the Middle Jurassic represented by the Pachycormid bony fishes until their extinction at the Cretaceous-Paleogene boundary (Friedman et al., 2010; Marx and Uhen, 2010).

Another apparently underexploited niche in the Mesozoic was that of consumers of armoured invertebrates (protected by shells or exoskeletons). Morphological innovations associated with an increase in durophagous (shell-crushing) habits are observed in arthropods at least from the Cretaceous (Dietl and Vega, 2008), in a variety of ways (See the review and study by Schweitzer and Feldmann, 2010). In what seems to be a response to increased predation, shelled organisms evolved defences such as motility in crinoids (Baumiller et al., 2010), the different versions of the alivincular ligament in bivalves (Hautmann, 2004) along with an increase in mobility or infaunalisation (Aberhan et al., 2006; McRoberts, 2001). Also increased body size in gastropods (Finnegan et al., 2011) and the development of a calcitic skeleton in algae (Aguirre et al., 2010), provide examples of this arms race (see Vermeij, 2008 for more innovations). Support for the MMR also comes in the form of fossilised traces of durophagy. Here the evidence present itself as bite marks (Martill, 1990), crushed shells (Oji et al., 2003) or drill holes and repair scars (Huntley and Kowalewski, 2007). All lines of evidence show that as a new model of marine ecologies settled, exoskeletonised organisms were under an important selective pressure.

When did the revolution take place is one of the unanswered questions, although the latest consensus apparently is that of two major escalation events with a

consolidation interval. Many innovations happened in the late-Triassic/early-Jurassic and in the late Cretaceous/early-Cenozoic. The first interval for example, saw the evolution of gastropods with the emergence of Neogastropoda (Finnegan et al., 2011), and the development of escape strategies in bivalves (Aberhan et al., 2006). Such innovations apparently coincide with early occurrences of fishes with molariform teeth, such as the Pycnodontiformes, exploiting the potentially vacant niche of the shellcrushers (Tintori, 1998; Vermeij, 2008), an hypothesis supported by rare occurrences of gut content (Kriwet, 2001; Nursall, 1993a).

Pycnodonts are an extinct group of neopterygian fishes present mostly in the Tethys from the Late Triassic to the Paleocene and are among the potential triggers for the evolution of armoured prey in the MMR (Bonaldo and Bellwood, 2010). That pycnodonts were predominantly durophagous is a widespread idea, but there is little support for this hypothesis other than extrapolation from gross dental morphology. Part of the explanation for this reputation is the way their dentition is described or referred to in the literature (emphases added):

- In their review of post-Palaeozoic patterns in marine predation, Walker and Brett (2002b) write: "Jurassic Pycnodonts evolved batteries of rounded, *shell-crushing* teeth, plus specialized nipping teeth. A few Pycnodontids even developed stout pavement teeth possibly for crunching corals; rare specimens have been found with coral fragments in the gut (Viohl, 1990)" and "fish with a *durophagous* dentition, such as [...] Pycnodontidae [...]".
- Vullo et al. (2009) mention three taxa of pycnodontiformes, of which at least one "has *crushing* teeth".

- Delsate and Kriwet (2004): "Remains of Pycnodont fishes, especially their *crushing* dentition and teeth..." and "Pycnodonts are *inter alia* characterized by a highly developed and specialised heterodont *crushing* dentition".
- Speaking of functional morphology, Poyato-Ariza et al. (1998) mention: "the *crushing* teeth of Pycnodonts were obviously adapted to feeding on shelly or exoskeletonized taxa"
- Poyato-Ariza and Bermudez-Rochas (2009): "Such development of molariform teeth indicates a predominantly *durophagous* feeding strategy"
- Tintori (1998), writing about Neopterygians (including Pycnodonts): "Compared to earlier actinopterygians they developed an "underexploited" trophic adaptation: *durophagy*"
- Kriwet (2008) explains that Pycnodonts are "highly specialized with regard to their prey, which is expressed in their *durophagous* dentition."
- Nursall (1993a): "They were deep-bodied, manoeuvrable fish, restricted to a *durophagous* habit"

More recently Poyato-Ariza (2005) pointed out that a high-level of trophic specialisation was not necessarily representative of the diet of a whole group, especially when compared with the breadth of the dietary repertoire of extant fishes at a similar taxonomic level. Poyato-Ariza and Wenz (2005) suggested that the use of "crushing" (the action performed by the teeth) or "durophagous" (the diet of the animal bearing the teeth) for the description of teeth and dentition were avoided in favour of the neutral and anatomically accurate "molariform", unless direct evidence of the action is provided. Also, real trophic specialisation occurs rarely in nature (see the example of Cichlids in Binning and Chapman, 2010), and durophagy as evidenced by gut content remains in pycnodonts is supported by 16 specimens (listed in Kriwet, 2001, a 17<sup>th</sup> has actinopterygian vertebrae as gut content) out of the hundreds available. Additionally, gut remains are no proof of specialisation, but of the "last (and potentially lethal) supper" of the animal. In light of such evidence, the hypothesis that Pycnodontiformes were a driving force of the evolution of armoured invertebrates in the MMR because of a specialised durophagous diet is clearly in need of further testing.

Durophagy in extant fishes is studied through different proxies such as: handling and crushing times (Mittelbach et al., 1999), gut content (Cutwa and Turingan, 2000; Huckins, 1997), muscle mass (Cutwa and Turingan, 2000; Mittelbach et al., 1999), bite force measurements (Huber et al., 2005) and jaw morphology (Cutwa and Turingan, 2000; Wainwright and Richard, 1995), biomechanical models (Grubich, 2005; Huber et al., 2008; Huber et al., 2005; Osenberg et al., 2004) or histology (Summers, 2000; Summers et al., 2004). Most of these are inapplicable in palaeontology since preservation of soft tissues or gut content is an extremely rare occurrence in the fossil record. Moreover, remains of a durophagous diet in fossil fishes might be lacking either because the processing of the prey (crushing and milling) destroys the shell entirely (Walker et al., 2002). Direct evidence of predation by fishes on shelled preys is uncommon, and mostly a matter of interpretation of fossils (e.g. Martill, 1990).

Without any diagnostic morphological feature distinguishing them from their durophagous counterpart, marine herbivorous fishes (and particularly the fossil ones) are often considered as "durophagous" if they exhibit "crushing" teeth (e.g. Wenz, 2003). Our understanding of herbivory in extant fishes is increasing (Clements et al., 2009) yet our knowledge of occurences of herbivory in fossil fishes is very limited. Fishes are an important group of algae consumers in reefs (Cvitanovic and Bellwood, 2008; Ferreira et al., 2004; Mantyka and Bellwood, 2007), and especially in coral reefs, where they have an effect on reef resilience by removal of macroalgae or grazing of

small epilithic algae (Bonaldo and Bellwood, 2010; Christiansen et al., 2008). In such environments, some fishes also feed directly on live corals, as part of their diet or to mark the limits or their territory (Francini et al., 2008; Rotjan and Lewis, 2006). For corallivory as well as for herbivory, fishes have selective preferences (Cvitanovic and Bellwood, 2008; Rotjan and Lewis, 2006) with considerable spatial variability. Fossil evidence of gut remains of herbivory or corallivory (Kriwet, 2001, mentions a potential occurence) are very unlikely to be found and since fossil corals are often studied by observing sections, so are grazing scars. Fossilised herbivorous fishes may have been mistaken for shell-crushers for want of an efficient way of discriminating between feeding strategies.

Studies of morphological features and biomechanical models appear to be powerful tools for reconstructing the feeding behaviour of extinct vertebrates (e.g. Anderson and Westneat, 2007) and especially pycnodonts. Examples of durophagyassociated features include the following: high, broad, short jaws in Actinopterygii (But they are also associated with herbivory Bellwood, 2003); cichlid's broad, thick, lower pharyngeal jaws (see Figure 2-1 for exemples) are associated with shell-crushing (Hulsey et al., 2008); as are myliobatoid stingrays' thick, flattened, hexagonal teeth, robustly constructed jaws, stiffened by a network of trabeculae (Summers, 2000). Yet, the methodology used by Bellwood (2003) of drawing functional morphospaces in jaw closing lever ratio/relative jaw length plots, can be misleading. In labrid fishes, form maps to mechanical property in a many-to-one fashion (Wainwright et al., 2005), and in a later work, Bellwood et al. (2006) showed that several feeding types can occupy a broad expanse of morphospace with extensive overlap. i.e. Ecological functions deduced through morphology alone might prove themselves to be erroneous, since different modes of prey capture occur in a single species (Liem, 1980). Nonetheless,

species with evolved specialist traits may fall back on less-preferred food (more challenging to capture or process), for which they show a specialised morphology, only when there is a lack in more easily accessible prey (Robinson and Wilson, 1998). Such examples of Liem's paradox have been illustrated in hominins (Ungar et al., 2008), chondrichthyans (Summers et al., 2004) and abundantly in cichlids (Binning and Chapman, 2010; Binning et al., 2009; Liem, 1980). Another potential issue is that biomechanical models are inaccurate to describe complex behaviours such as limited modulated feeding kinematics in pycnodont fishes if they occur (Kriwet, 2001). Overall, functional morphology studies based on anatomical features and measurements can help us infer an organism's fundamental niche (the manifestation of the species genotype within the environment (Ferry-Graham and Wainwright, 2002) but the actual influence of an organism on other species is a matter of the realised niche (The fundamental niche narrowed by the effect of extrinsic factors such as predation and competition). In order to test the role of Pycnodonts in the Mesozoic Marine food web additional proxy data are required.

Analysis of tooth microwear texture can provide a taxon-independent marker (Bellwood, 2003) for grazing and shell-crushing in fishes, reflecting the direct functional consequences of actual diet and feeding behaviour, rather than a fundamental trophic niche. Dental microwear analysis is considered as one of the most effective methods used by researchers to infer diets of past peoples and fossil species (Ungar et al., 2003). It is now well-known that microwear in mammals is caused by a combination of the abrasives in food, forces that act on tooth surface (Walker et al., 1978), and exogenous grit and dust (Merceron, 2005; Sanson et al., 2007). The usefulness of microwear analysis for the studies of fish diet has been demonstrated on extant and fossil stickleback fish that exhibit microwear patterns according to substrate coarseness

or diet, even though their teeth are polyphiodont (Baines, 2010; Purnell et al., 2007; Purnell et al., 2006) and 3D microtexture analysis performs better than 2D operatorscoring methods as a tool to separate fishes with different diets (Purnell et al., 2012).

Since the 1990s, most dental microwear studies have relied on semi-automated image analysis procedures using scanning electron microscope (SEM) or light stereomicroscope micrographs (Daegling, 1999; Joomun et al., 2008; Merceron, 2005; Merceron et al., 2007; Merceron et al., 2005; Purnell et al., 2006; Rivals and Athanassiou, 2008; Rivals and Solounias, 2007; Semprebon et al., 2004a; Todd et al., 2007; Ungar, 1996). One of the issues with these techniques is that SEM images are 2D representations of 3D surfaces resulting from several mechanical and optical processes and depend on the settings of the instrument. The use of a standard technique for light stereomicroscopy studies allowed for comparisons between different samples (Rivals and Athanassiou, 2008; Rivals and Solounias, 2007). However the necessity for an automated technique has been highlighted by several recent studies showing statistically significant inter-observer and intra-observer error rates, part of it due to researchers' experience, for both occlusal and buccal microwear analyses, even when using semiautomated procedures (Galbany et al., 2006; Grine et al., 2002; Mihlbachler et al., 2012; Purnell et al., 2006). Three-dimensional imaging and analysis techniques, accounting for not only the patterns of microwear, but also the differences in roughness related to different diets, offer a solution. Non-contact surface metrology and focus-variation microscopy provide 3D representations of the surface of objects. It has the advantage of accurately recording the texture of curved surfaces as present on most teeth. It already proved useful to investigate dental growth patterns (Bocaege et al., 2010), cutmark micromorphology (Bello et al., 2009; Bello and Soligo, 2008) and trophic plasticity between groups of cichlid fishes (Purnell et al., 2012).

Most of the fossil material studied here comes from the late Jurassic of the Tethys, i.e. the "consolidation" time of the MMR. It would be expected under the classic escalation scenario that during that time the animals were all well settled in their respective ecological niches, that of specialised shell-crushers for the pycnodonts for example. The technique developed by Purnell et al. (2012) has been shown to allow for discrimination between soft and hard-objects feeders, as well as between specialised durophages and more generalist animals and provides an ideal tool to test this hypothesis of Pycnodontiformes as shell-crushers.

# Material and methods

# Material

The potential of dental microwear texture analysis to test hypotheses of diet in fishes has already been successfully demonstrated on small samples of cichlids (Purnell et al., 2012) and in chapter 2. Several populations of fishes with known diets but different environmental preferences (bathymetry, temperature, salinity), provide the comparative material used in this study: two populations of *Archosargus probatocephalus* (the sheepshead seabream, Teleostei, Sparidae, n=6 each), one of *Anarhichas lupus* (the atlantic wolffish, Teleostei, Anarrhichadidae, n=4).<sup>5</sup>

Analyses have been carried on the dental surfaces of 39 fossil vomers (18) or prearticulars (20) from pycnodontiform fishes. Specimen P1655 preserved teeth but was too incomplete to adequately assign them to a particular tooth-bearing bone. Details about the specimen can be found in Table 4-1. The classification used here is that of Poyato-Ariza and Wenz (2002).<sup>6</sup>

<sup>&</sup>lt;sup>5</sup> This sample is the same as used in chapter 2. Please refer to it for more details.

<sup>&</sup>lt;sup>6</sup> This sample is the same as used in chapter 3. Please refer to it for more details.

Table 4-1: Sampled fossil specimens, with geographic origin and group as described in

Specimen	Provided taxon name	Geographic	Group
number		origin	
MB.f.1337	Athrodon sp.	Dives	Athrodon
LEIUG	Eomesodon cf. trigonius	Leicester	Pycnodontoidei 1
76828			
P1655	Eomesodon granulatus	Lindnerberg	Pycnodontoidei 1
P40636	Eomesodon granulatus	Weymouth	Pycnodontoidei 2
P41808	Eomesodon granulatus	Weymouth	Pycnodontoidei 2
OR35498	Eomesodon rugulosus	Stonesfield	Pycnodontoidei 1
P13922	Eomesodon rugulosus	Kirtlington	Pycnodontoidei 1
P1648	Eomesodon rugulosus	Stonesfield	Pycnodontoidei 2
P4387	Eomesodon rugulosus	Stonesfield	Pycnodontoidei 1
P31879	Eomesodon trigonius	Peterboroug	Pycnodontoidei 1
		h	
OR41175	Gyrodus coccoderma	Weymouth	G.coccoderma
OR43562	Gvrodus coccoderma	Weymouth	G.coccoderma
P41810	Gvrodus coccoderma	Weymouth	G.coccoderma
P6747	Gvrodus coccoderma	Weymouth	G.coccoderma
OR40638	Gvrodus cuvieri	Weymouth	Other Gvrodus
OR43559	<i>Gyrodus cuvieri</i>	Weymouth	Other <i>Gyrodus</i>
OR43561	Gvrodus cuvieri	Weymouth	Other Gvrodus
OR43563	Gyrodus cuvieri	Weymouth	Other <i>Gyrodus</i>
OR44085	Gyrodus cuvieri	Weymouth	Other <i>Gyrodus</i>
P1615	Gyrodus cuvieri	Weymouth	Other <i>Gyrodus</i>
P41799	Gyrodus cuvieri	Weymouth	Other <i>Gyrodus</i>
MB f 1338	Gyrodus planidens	Weymouth	G.planidens
OR44086	Gyrodus planidens	Weymouth	G planidens
K/A05/072	Gyrodus sp 1	Marolles	Other Gyrodus
IRE360		i i i i i i i i i i i i i i i i i i i	
P9591	Gyrodus sp. 2	Coxwell	Other Gyrodus
P10423	Gyrodus umbilicus	Boulogne-	Other Gyrodus
110120		sur-mer	
K/A05/035	Proscinetes bernardi	Cerin	Pvcnodontoidei 2
CRN78	1 rosemeres sermarar	Com	5
K/A05/130	Proscinetes hugii	Soleure	Pycnodontoidei 2
IRE539	Trosenieres nugi	Solute	5
P1654	Proscinetes hugii	Soleure	Pvcnodontoidei 1
P1654a	Proscinetes hugii	Soleure	Pycnodontoidei 1
P1654b	Proscinetes hugii	Soleure	Pvcnodontoidei 1
P2297	Proscinetes hugii	Hanover	Pvcnodontoidei 1
P10770	Proscinetes auincucialis	Weymouth	Pvcnodontoidei 1
P6170	Proscinetes quincucialis	Weymouth	Pvcnodontoidei 2
P6749	Proscinetes quincucialis	Weymouth	Pvcnodontoidei 2
21974	Proscinetes radiatus	Swanaoe	Pycnodontoidei 2
P6820	Proscinetes radiatus	Swanage	Pvcnodontoidei 2
K/A05/069	Pycnodus munstorii	Courcon	Pycnodontoidei 2
11/1100/007	i yenouus munsterti	Courçon	,

chapter 3. Other details can be found in Chapter 3.

Specimen	Provided taxon name	Geographic	Group
number		origin	
JRE57 K/A06/090	Pycnodus subclavatus	Charentes	Pycnodontoidei 2
CTE54			

# Preparation of casts and data acquisition for microwear texture analysis

Data acquisition was conducted on casts of the original teeth, since enameloid is slightly translucent and does not allow for an accurate data acquisition. Dental surfaces from fossil specimens were cleaned according to the protocol described in Williams and Doyle (2010). Based on the results of our comparison of several replication materials, dental surfaces were molded using a polyvinylsiloxane compound (Coltène-whaledent speedex light body). Black epoxy resin (EpoTek 320 LV) was then poured in these formworks in order to obtain high-resolution replicas. Moulding medium and epoxy resin were mixed according to the manufacturers' instructions.

Replicated surfaces were obtained for all specimens using an Alicona Infinite Focus microscope G4b (IFM; software v. 2.1.2) with a x100 objective, providing highresolution three-dimensional surfaces for a 145 x 110  $\mu$ m field of view. Exposure and contrast (gamma) settings were set for each scan in order to optimise the quality of the 3D data. Any errors of surface measurements were removed by manually editing the data with the "3D editor" software supplied with the microscope (InfiniteFocus 2.1.2, IFM software version 2.1.2). The cleaned point clouds were exported as .sur files and imported in SurfStand (software version 5.0). Surfaces were then automatically treated by: 1) levelling the surface and removing gross tooth form with a 2<sup>nd</sup> order polynomial function. Since the dental surfaces are from dome-shaped teeth, this technique should provide an "SF surface" representing the raw surface of the tooth. 2) Application of a robust spline filter, based on a non-linear filter equation integrating robustness and endeffect management (which avoids reduction of the size of the surface, Blateyron, 2006). The used nesting index was 0.025 mm. 3) Calculation of standard parameters (ISO 25178) for the filtered surfaces. Care was taken to orient the scanned surfaces as horizontally as possible prior to data acquisition.

Datasets of textural parameters were gathered from two previous studies on extant (*Anarhichas lupus*, n=4 and 2 groups of *Archosargus probatocephalus*, n=6 each) and fossil (n=39) animals. As each individual specimen was represented by 1 to 5 measurements, the median values of textural parameters were calculated for each individual separately in order to avoid the influence of any potential outliers. The final dataset analysed here is composed of median estimates for 23 roughness parameters in 55 individuals. The use of an estimate of central tendency instead of all the available data reduces the discriminatory power of analyses (see Purnell et al., 2012) but reduces intra-individual noise.

#### Data analysis

The main objective of this investigation is to compare dental microwear texture from Pycnodontiformes with that of extant analogues in order to 1) test the hypothesis that they were shell-crushers and 2) characterise their diet by comparison with extant animals of known dietary habits. Purnell et al. (2012) showed that Welch anova, linear discriminant analysis (LDA) and principal component analysis (PCA) applied to dental microwear texture data provided efficient procedures to segregate between individuals based on their trophic niches. It has already been shown that different taxonomic units within the Pycnodontiformes display varied patterns based on their dental microtexture e.g. the genus *Gyrodus* appears to be very variable, whereas members of the Sub-Order Pycnodontoidei are structured as two trophic niches. The main hypothesis tested here is:

• H0: "Microtextures in fossil Pycnodonts are similar to that from extant shellcrushers"

It is expected that populations with different diets will exhibit different microwear textures, as has been evidenced by Purnell et al. (2012). Different textural parameters can help to discriminate between groups of fishes with shell-crushing or more generalist habits that will be used here as references.

This hypothesis (H0) was tested at the significance level of  $\alpha$ =5% using Welch-Analysis of Variance (Welch-Anova) for each texture parameter. The normality of the distribution for the samples was tested using Wilks-Shapiro test with or without log-transformation of the data and rank-transformation applied if neither original nor log-transformed data complied with the condition of normality. It has been shown that parametric tests performed on rank-transformed data provide results consistent with those of non-parametric procedures (Zimmerman, 2012). Series of pairwise t-tests were carried on the parameters displaying a significant difference. Welch-Satterwaithe and Holm-Bonferroni corrections were applied to account for heteroscedasticity and the number of tests performed respectively.

For the purpose of the Welch-Anova, the fossil samples considered were restricted to the groups identified in the Pycnodontoidei in chapter 3, or the Gyrodontoidei as a coherent assemblage. Microtexture data from the two sub-orders occupy their ecospace (Figure 3-5) in contrasting manners interpreted as different trophic ecologies. Henceforth considering those groups separately reduces the discrepancy in size between the compared classes and focuses on ecologically similar groups of individuals.

Multivariate analyses were performed on 3 alternative subsets of microtextural parameters: parameters with a normal or log-normal distribution for which a significant difference was found in extant animals (see chapter 2), parameters with a normal or lognormal distribution for which a significant difference was found in extinct animals (see

chapter 3), parameters with a normal or log-normal distribution for which a significant difference was found in the comparison of extant and extinct animals (this chapter).

For Linear Discriminant Analyses (LDA) the discriminated classes were the different groups known or identified in previous investigations. The output of the analysis was used as a model to calculate projections along linear discriminant axes and probabilities of belonging to any of the a-priori defined classes. To allow for a non-categorical comparison, Principal Component Analysis (on correlations) was performed on the same datasets and its output used to calculate projections within the described ecospace<sup>7</sup>. Outliers in the ecospace were detected using the function sign2 from the package mvoutlier (Gschwandtner and Filzmoser, 2009) and excluded from the plotted results but not excluded from analyses. All analyses were performed using the software R version 2.15.0 (R Development Core Team, 2012), with the MASS (Venables and Ripley, 2002) and above mentioned packages.

### Results

# Trophic preferences in Pycnodontoidei

The Welch-Anovas show that all the microtexture parameters allow rejection of the null hypothesis and highlight differences between dietary categories in the sample, with the exception of the textural parameters Ssk, Sds and Str. The pattern of observed differences is similar to the one in chapter 2 regarding similar investigations between *Archosargus* and *Anarhichas* although that analysis was based on several measurements per specimen.

<sup>&</sup>lt;sup>7</sup> Following the procedure described in Ezard, T. H., Pearson, P. N., and Purvis, A., 2010, Algorithmic approaches to aid species' delimitation in multidimensional morphospace: BMC Evol Biol, v. 10, p. 175., clusters within the projected scores were calculated with the packages mclust Fraley, C., and Raftery, A., 2010, mclust: Model-Based Clustering / Normal Mixture Modeling. R package version 3.4.1. and vegan Oksanen, J. F., Blanchet, G., Kindt, R., Legendre, P., O'Hara, R. G., Simpson, G. L., Solymos, P., Stevens, M. H. H., and Wagner, H., 2010, vegan:Community Ecology Package. R package version 1.17-0.. The results of this analysis can be found in Figure D-1

Table 4-2: Results of Welch-anova testing hypothesis H0 in Pycnodontoidei and extant fishes. Tests performed on O: original untransformed data, L: log-transformed data, R: rank-transformed data. df: degrees of freedom, num: numerator (effect), denom: denominator (error) adjusted for heteroscedasticity. Significant differences are

Parameter	Data	F-statistic	df	p-value
			(num,denom)	
Sq	0	54.0111	4,10.929	<0.0001
Ssk	R	2.7683	4,12.968	0.0728
Sku	R	11.1441	4,14.083	0.0003
Sp	0	4.4437	4,11.075	0.0220
Sv	R	41.6345	4,14.603	<0.0001
Sz	R	36.6452	4,14.703	<0.0001
Sds	0	1.1923	4,13.200	0.3953
Str	R	1.2215	4,11.995	0.3524
Sdq	R	57.6283	4,12.981	<0.0001
Ssc	R	13.1446	4,11.876	0.0002
Sdr	0	10.5760	4,11.324	0.0008
Vmp	0	28.0068	4,11.152	<0.0001
Vmc	0	59.6677	4,10.939	<0.0001
Vvc	0	71.2749	4,11.044	<0.0001
Vvv	0	47.1194	4,11.135	<0.0001
Spk	0	28.0866	4,11.101	<0.0001
Sk	0	67.1634	4,10.916	<0.0001
Svk	0	35.8904	4,11.344	<0.0001
Smr1	0	4.1120	4,12.396	0.0242
Smr2	0	10.7874	4,12.237	0.0006
S5z	$\mathbf{L}$	15.7049	4,11.560	0.0001
Sa	0	55.1197	4,10.981	<0.0001
Vvc/Vmc	0	7.3410	4,12.122	0.0031

highlighted in bold.

Pairwise t-tests highlight a number of differences between trophic niches. There

are a large number of significant differences between the different groups of and the extant generalists with the exception of the niches Pycnodontoidei 1 and the IR population of *Archosargus*. The small number of differences between any sample of extant animal and *Anarhichas* appears to be a consequence of corrections for heteroscedasticity, this species is more variable than any other category considered, resulting in a higher type 2 error rate. Investigations comparing populations of extant fishes highlighted differences in more textural parameters. The difference in diet

between the extant specialised shell-crusher *Anarhichas lupus* and any of the group of Pycnodontoidei is expressed on the parameters Sv, Sz, Sdq (and Sku for Pycnodontoidei 1 versus Wolffish). The same parameters contrast between the populations of extant fishes and the wolffish, which suggests that differences between Pycnodontoidei and shell-crushers are linked to the same discrepancies between dietary preferences. Further analyses of data from Pycnodontoidei relative to the populations of extant generalists all display a large number of differences, with the exception of the Pycnodontoidei 1 versus IR with only 3 parameters found to be different. The pairwise comparisons do not provide a strong support for a similar diet between groups of Pycnodontoidei and any of the considered populations of extant fishes analysed.

As segregation between groups of fishes based on microtexture is linked to the proportion of hard prey consumed (chapter 2 and Purnell et al., 2012) and a higher consumption of hard prey in pycnodonts relatively to *Anarhichas* is unlikely, the Pycnodontoidei analysed here were not specialised shell-crushers.

Table 4-3: Parameters found to display a significant difference between trophic categories in pairwise Welch-Satterwaithe t-tests for groups of Pycnodontoidei or extant fishes. Bold: log-transformed data, italics: rank -transformed data. IR: herbivorous population of Archosargus from Indian River lagoon; PC: durophagous population of Archosargus from Port Canaveral lagoon; Wolffish: specialised durophagous Anathichas from the North Atlantic. Pycnodontoidei 1 & 2 groups identified by automatic clustering based on dental microtexture data (see chapter 3).

	IR	PC	Wolffish	Pycnodontoidei
				1
PC				
Wolffish	Sv, Sz, Sdq	Sku, Sv, Sz,		
		Sdq		
Pycnodontoidei	Ssc, Vvv,	Sq, Sz, Ssc,	Sku, Sv, Sz,	
1	Svk	Vmp, Vmc,	Sdq	
		Vvc, Vvv,		
		Spk, Sk,		
		Svk, Smr2,		
		Sa,		
		Vvc/Vmc		
Pycnodontoidei	Sq, <i>Sv</i> , <i>Sz</i> ,	Sq, Sku, Sp,	Sv, Sz, Sdq	Sq, Sv, Sdq,
2	Sdr, Vmp,	Sv, Sz, Sdq,		Ssc, Sdr, Vmp,
	Vmc, Vvc,	Sdr, Vmp,		Vmc, Vvc,
	Vvv, Spk,	Vmc, Vvc,		Vvv, Spk, Sk,
	Sk, Svk,	Vvv, Spk,		Sa
	S5z, Sa	Sk, Svk,		
		<b>S5</b> z, Sa		

# Trophic preferences in Gyrodontoidei

Fewer microtexture parameters allow rejection of the null hypothesis and for most of them, application of a p-value correction results in a non-rejection of the hypothesis. At this stage, Gyrodontoidei appear as closer to the extant fishes in their microtexture as compared to Pycnodontoidei. Table 4-4: Results of Welch-anova testing hypothesis H0 in Gyrodontoidei and extant fishes. Tests performed on O: original untransformed data, L: log-transformed data, R: rank-transformed data. df: degrees of freedom, num: numerator (effect), denom: denominator (error) adjusted for heteroscedasticity. Significant differences are

Parameter	Data	F-statistic	df	p-value
			(num,denom)	
Sq	0	5.6787	3,9.006	0.0184
Ssk	R	1.2844	3,10.658	0.3295
Sku	L	6.0580	3,9.514	0.0139
Sp	0	1.5113	3,9.421	0.2744
Sv	L	3.7523	3,9.890	0.0491
Sz	0	1.8259	3,9.332	0.2103
Sds	Ο	0.7129	3,11.146	0.5642
Str	R	1.8044	3,8.999	0.2164
Sdq	R	16.3773	3,12.290	0.0001
Ssc	R	7.6616	3,10.112	0.0058
Sdr	0	1.7085	3,9.409	0.2316
Vmp	0	2.6321	3,9.529	0.1121
Vmc	0	9.3653	3,9.029	0.0039
Vvc	0	9.2715	3,8.980	0.0041
Vvv	0	5.4296	3,9.136	0.0204
Spk	0	2.0409	3,9.059	0.1782
Sk	0	10.6918	3,8.982	0.0025
Svk	L	4.8524	3,9.320	0.0269
Smr1	0	5.9639	3,9.960	0.0135
Smr2	Ο	2.0557	3,9.686	0.1719
S5z	0	1.4149	3,8.870	0.3019
Sa	0	7.7621	3,9.045	0.0072
Vvc/Vmc	0	5.0157	3,9.718	0.0233

highlighted in bold.

Pairwise t-tests highlight few differences between groups, most of them are separating the PC population from the Gyrodontoidei. Once again the small number of differences between any sample of extant animal and *Anarhichas* appears to be a consequence of type 2 errors. The only parameter found different between the wolffish and the Gyrodontoidei is Sdq which also highlights differences between the generalists from IR and PC and *Anarhichas*. Table 4-5: Parameters found to display a significant difference between trophiccategories in pairwise Welch-Satterwaithe t-tests for Gyrodontoidei or extant fishes.Bold: log-transformed data, italics: rank -transformed data. IR: herbivorous populationof Archosargus from Indian River lagoon; PC: durophagous population of Archosargusfrom Port Canaveral lagoon; Wolffish: specialised Anarhichas from the North Atlantic.

	IR	РС	Wolffish
РС			
Wolffish	Sdq, Ssc	Sdq, Ssc	
Gyrodontoidei		Sq, <b>Sku</b> , Vmc, Vvc, Vvv, Sk, Smr1, Sa, Vvc/Vmc	Sdq

#### Multivariate analyses

### Extant fishes as a model for fossils

Similarities between generalists are visible in their overlapping hulls in Figure 4-1A along the first linear discriminant axis (representing 97.71% of explained variance, Wilks'lambda=0.0342, p=0.02087). Sa, Vvc/Vmc, Sk and Sdq had the highest coefficients for the two axes. Application of the linear discriminant function attributed the specimen SH-IR01 to the Port Canaveral population while SH-PC01 and SH-PC03 are found similar to the more herbivorous *Archosargus*. The separation between generalists and the specialised shell-crusher is very clear on LD1. The linear discriminant function attributed the wolffish samples to the right category with nearly 100% probabilities. The posterior probabilities for the generalists were lower, 2 out of the 3 misattributions being between 45 and 55% for either category.

Application of this model to the Pycnodontiformes unambiguously rejects the hypothesis of pycnodonts as shell-crushers. The highest calculated probability of being similar in texture to *Anarhichas* was found in specimen OR43561 at 15.49%.

Individual attributions for *Athrodon* sp. (n=1) and *Gyrodus planidens* (n=2) are strongly in favour of them being herbivorous (IR population, probabilities>95%). *G. coccoderma* on the other hand, is similarly attributed to the more durophagous Port Canaveral population (PC), supporting the hypothesis of different feeding habits in these species. Both groups which had been identified as trophic niches in Pycnodontoidei (based on their consistent microtexture signal, see chapter 3) have a larger proportion of individuals attributed to the PC than to the IR population.

Projections along linear discriminant axes show that the data from Pycnodontiformes is not fitting within areas described by the model populations. e.g. *G. coccoderma* considered on the predictions as a generalist durophage has only 2 of its 4 representatives projected close to the PC hull. Most of the separation between PC and IR niches is expressed along LD2 which represents only 2.27% of the expressed variance. A safe interpretation is that Pycnodontiformes in this sample and generalist fishes share similarities in dental microwear texture.

In the ecospace of the Principal Components Analysis based on the extant animals, the same pattern of divergent generalist and specialist populations is seen along the first PC axis (PC1: 81.16% of expressed variance, 3 individuals from the wolffish population plotting farther to the right were discarded for clarity) whereas the divergence between herbivorous or durophagous habits in generalists is found along PC2 (16.83%). All parameters weighted equally on PC1, while Smr1 and Vvc/Vmc shaped PC2. Within this extant ecospace, the trophic niches of Pycnodontoidei are well separated along PC1, as are the two species of *G. planidens* and *G. coccoderma*, with the former closer to generalists on PC1 and more precisely, the herbivores (based on PC2).

There is little overlap between the fossil specimens and the extant populations of analogues, thus providing no direct evidence of what they were consuming. There is however no evidence in favour of the hypothesis of pycnodonts as heavy shell-crushers.

## Fossil fishes as a model for extant animals

In the fossil-based LDA, the different niches of Pycnodontoidei (1 in blue, 2 in green) are well separated along LD1 (explained variance: 63.48%) and LD2 (25.50%, Wilks'lambda=0.0643, p<0.0001). Sk and Vvc/Vmc had the highest coefficients for the first axis, the second was shaped by the latter two parameters, Smr1 and Smr2. Application of the linear discriminant function attributes every specimen in those niches correctly. *Gyrodus planidens* is equally well separated from the remainder of the fossil sample. Other groups of Pycnodontiformes show a lesser degree of identity, e.g. *G. coccoderma* is attributed to different niches: *G. coccoderma* (P41810), Pycnocdontoidei 2 (P6747, OR43562) or "other *Gyrodus*" (OR41175).

Projections for extant animals show that the wolffish is not similar to any of the groups of fossil fishes, providing further refutation of the classic interpretation of the trophic ecology in those animals. IR and PC individuals do not show any strong convergence towards any of the assemblages of fossils, rather overlapping the area where the *Gyrodus* and the individuals from the goup Pycnodontoidei 1 plot. Inferences show a mixture of attributions for the *Archosargus* but none towards the Pycnodontoidei 2 niche.

In the fossil-based PCA, differences between the niches of Pycnodontoidei are clear along PC1 (expressed variance: 52.73%), though they overlap along PC2 (34.70%). Vmc, Vvc and Sk had the largest influence on PC1 while Smr1, Smr2 and Vvc/Vmc weighted heavily on PC2. The lowest scores along both axes are observed for several individuals of *Gyrodus* and extant animals clustered together. The relative position of the Pycnodontiformes in a portion of the ecospace void of modern analogues again provides no evidence of their diet but suggests generalist or soft-objects focused dietary preferences.

# General analysis

For the Linear Discriminant Analysis the considered textural parameters were restricted to Sdr, Vvc, Smr1, Smr2 and Vvc/Vmc in order to limit the effect of correlated microtextural parameters. Highest coefficients were from parameters Smr1 and Vvc/Vmc for LD1, Sa and Vvc/Vmc for LD2. Anarhichas lupus is separated from any other niche along LD1 (63.14% of variance, Wilks'lambda=0.0412, p<0.0001), though projections attribute the sample of wolffish 4 to the herbivorous extant population. Extant generalist populations do overlap along LD1, but show a separation along LD2 as in the LDA based on extant animals. Among fossil groups the majority of individuals from Gyrodus, Athrodon and the assemblage Pycnodontoidei 1 overlap on LD1 and are separated along LD2 in a manner non dissimilar to that seen in the extant generalists. However, as one of the assemblages of Gyrodus represents samples which were not unambiguously attributed to any other category, application of a noncategorical technique is necessary before interpretation. Specimens from G. planidens, though plotting close to other specimens of *Gyrodus*, are attributed to their own niche. Only one G. coccoderma is attributed as such, 2 individuals are considered as belonging to Pycnodontoidei 2 and one to the Port Canaveral population (see Figure 4-2 C).



Figure 4-1: Linear Discriminant Analysis: projections based on the following categories A: trophic niches observed in extant fishes; B: groups of fossil individuals with consistent microwear texture signatures; C: extant and fossil groups. Hulls represent the a priori used categories. Hulls for extant animals are surrounded in dashed lines. Extant hulls: grey: specialised shell-crusher Anarhichas lupus; red: durophagous generalist Archosargus probatocephalus (PC); green: herbivorous generalist (IR). Fossil hulls: blue: Pycnodontoidei 1; green: Pycnodontoidei 2: yellow: Gyrodus coccoderma; brown: other Gyrodus.



Figure 4-2: Principal Components Analysis describing ecospaces of A: extant fishes; B: fossil fishes; C: extant and fossil groups. Hulls represent the populations of extant generalists for reference. IR and green hull: herbivorous generalist Archosargus; PC and red hull: more durophagous generalist Archosargus.

The general PCA shows a clearer separation along PC1 (77.62% of variance) and 2 (14.54%) between the herbivorous and the durophagous generalists than in the ecospace based on extant animals. All parameters weighted equally on PC1 but Smr1, Smr2 and Vvc/Vmc which shaped PC2. Similar patterns to those observed in other multivariate analyses are found in this analysis, with the exception of the individuals of *G. planidens* separated along both axes.

# Discussion

A range of hypotheses regarding the trophic ecologies of pycnodonts have recently been discussed including omnivorous suction-feeding (Kriwet, 2001), the classic specialised durophagy (Nursall, 1993a) and "potentially [...] diversified within their general manipulation strategy" (Poyato-Ariza, 2005, p. 177). It was already observed that the bauplan of Pycnodontiformes was linked to or allowed varied palaeoecologies, regarding mobility or prey-capture and processing (Baines, 2010; Kriwet, 2001; Nursall, 1999; Poyato-Ariza, 2005) and that despite a "durophagous" morphology, their molariform teeth could have processed other categories of prey.

The link between dental microwear textures and diet on molariform teeth of fishes has been illustrated in recent studies (Purnell et al., 2012, and chapter 2) and can provide a more accurate estimate of diet in an animal than the snapshot provided by gut content. Interpretations of palaeoecologies based on gut content suffer from the risk of sampling an opportunistic feeding event different from the usual diet of the animal. The results presented here provide another example of gut content as a potential source of bias in palaeoecological inferences. The presence of preserved shells or other hard parts in the gut of pycnodonts (Kriwet, 2001) does not account for the variety of foods processed by Pycnodontiformes as a whole. The variety of taxa and geographic origins of our sample avoids an ecosystem specific sampling bias.

No analytical procedure is free of any source of error. Corrections for heteroscedasticity and multiple tests result in a higher type 2 error rate. For example, Differences between the IR and PC populations in dental microtexture would be highlighted if those two samples were compared without application of Holm-Bonferroni adjustment of p-values. The use of linear discriminant analysis only provides inferences for defined categories. The parameters and loadings used above mirror the effect of the different conditions that these animals were exposed to, and may not be as relevant for inferences in fossil animals. As a consequence, in the absence of strong, unambiguous evidence, it might not be possible to unequivocally state what the diet of the pycnodonts was. However, knowing whether it was similar to extant organisms with known diet provides new hypotheses to work with.

One unambiguous result that comes from the data analysis is that the classic hypothesis of pycnodonts as specialised shell-crushers can be rejected. No analyses support this ecomorphological inference, which was based mostly on their molariform dentition (Nursall, 1993a). Earlier hypotheses of varied palaeoecologies here gain support in light of the variability observed in their dental microtexture as compared to the modern analogues sampled. Pycnodontiformes clearly had a range of trophic ecologies (see chapter 3 and discussion in Poyato-Ariza, 2005), and similarities in microtexture between some individuals from *Gyrodus* and the extant shell-crushing population of *Archosargus* from Port Canaveral (PC) suggests that this range included shell-crushing. A diet with a high proportion of soft objects appears to be the rule for a majority of Pycnodontiformes in the sample used here.

One of the diverging features between groups of pycnodonts is the morphology of their most anterior teeth (Figure 4-3). Pycnodontoidei with incisiform teeth were apparently mechanically restricted to a range of food types (see chapter3 and results

above), while Gyrodontoidei with more styliform teeth would have been capable of picking items lying on or just below the surface of the substrate (Nursall, 1993a), as seen in their wide ecospace occupation compared to other groups. Though microwear texture analysis results show that Pycnodontoidei fed on soft items, shelled prey were not inaccessible to them: *Archosargus probatocephalus* captures exoskeletonized organisms with incisiform teeth which it crushes with more posterior molariform teeth.

The different niches identified within the Pycnodontoidei hypothesised as the result of different diets (chapter 3) show a divergence in each ecospace considered here, further suggesting that their range of feeding strategies was restricted or focused as a function of anatomical limitations and environmental conditions. Their position within the ecospace suggests an herbivorous or soft-objects dietary habit for the group. Inclusion of a modern analogue exclusively herbivore or otherwise restricted to softprey could provide a better test of this hypothesis. An exemple of an extant analogue of the association of incisiform teeth and a mill-like structure as seen in Pycnodontoidei can be found in the Scaridae (parrotfishes) which use it as an efficient algae capturing and processing tool. Pycnodontiformes lack the teleosts' separation of the feeding apparatus into two independent units but have tools similar to that of extant Scaridae: anterior teeth capable of cutting or excavating food and a mill to process it (Figure 4-3 C). The propalinal motion of opposed sides of the jaw would have allowed for efficient extraction of nutrients from food items which otherwise require considerable postpharyngeal processing time and energy such as algae with tough polysaccharidic or glycoproteic cell walls as in the pharyngeal mill of Scaridae (Carr et al., 2006b).



Figure 4-3: Teeth, jaws and dental surfaces in Pycnodontiformes. A: Anterolateral view of the feeding apparatus of Gyrodus sp. showing the associated prehensile (anterior) and molariform teeth of a Gyrodontoidei. B: Lateral view of the dentition of Proscinetes sp. showing incisiform prehensile teeth (to the left) as found in Pycnodontoidei, Scale bar is 5 mm. C: Cross section of generalised pycnodont vomer (Vo) and Prearticular bones (Pra) to illustrate the occlusion of the buccal trough or mill. The star represents the likely position of the food items, the arrow points to the occluding facets of lateral

tooth rows. Not to scale. D to H: dental surfaces from molariform teeth of Gyrodontoidei (D to F) and Pycnodontoidei (G and H) all surfaces: 146.07 x 110.79  $\mu$ m after spline-filtering except F, original surface. D: Gyrodus cuvieri, note the varied, 2 to 3  $\mu$ m deep features, Z max=4.36  $\mu$ m; E: Gyrodus coccoderma, with several small pits, Z max=2.74  $\mu$ m; F: Gyrodus sp., with large pits, Z max=9.00  $\mu$ m; G: Eomesodon trigonius, with several small scratches approximately 1  $\mu$ m deep, Z max=2.76  $\mu$ m; H: Proscinetes quincucialis, note the larger but shallow scratch, Z max=2.17  $\mu$ m. The colour bar is for reference when comparing the topographies. A and B from Poyato-Ariza (2005); C modified from Baines (2010)

Scaridae are divided into two groups - scrapers and excavators - based on feeding behaviour (Bellwood and Choat, 1990) but appear to be as morphologically specialised as a group as pycnodonts. Scrapers crop epilithic algae leaving the most basal portion of the algal turf to grow again, whereas excavators take a powerful bite and remove all of the algae with a part of the substrate. The ingested particles in both groups comprise algal material, and in the latter a limited amount of harder material (sediment or coral skeleton) and this has different consequences as regards their ecosystem (Christiansen et al., 2008; Sotka and Hay, 2009). Investigations looking at the dental microtexture in those groups would provide information as to whether this divergence is of the same nature as that observed in Pycnodontoidei. Herbivores on reefs are also separated between those that browse macroscopic algae and those grazing on turfing or filamentous algae (Choat et al., 2004). In this case again a comparative study of dental microwear as the result of different diets is needed. Unlike in this study, those divergences have been observed at the specific level, but food consumed also varies according to local conditions. The propalinal jaw motion in the Pycnodontiformes as described by Baines (2010) could have induced a large degree of attrition (tooth-on-tooth contacts) and a polishing of the surfaces in the buccal mill (Figure 4-3, C). The consequence would be very low scores for any aspect of the texture associated with the vertical dimension, perhaps even in animals with durophagous habits. Tooth-on-tooth contacts as well as the processing of a large proportion of hard objects both lead to a large number of recognisable features, especially scratches as seen on the lateral dental surfaces of *Gyrodus planidens* (Baines, 2010), teeth from herbivorous dinosaurs (Williams et al., 2009), occlusal facets in mammals (Calandra, 2011; Krueger et al., 2008) and parrotfishes (Carr et al., 2006b). The antero-posterior jaw movement in the pharyngeal mill of the parrotfish *Scarus* (Carr et al., 2006b) leads to the development of wear facets and a large proportion of microwear features due to the presence of fragments of coral. Pits observed over dental surfaces in pycnodonts appear as simple circular pits (Figure 4-3 F), rarely as more elongate gouges. The latter would be expected from a hard item being crushed and carried between two strongly occluding elements.

Microwear features are generally deeper in *Gyrodus* than in Pycnodontoidei, before and after filtering of the surfaces (pers. obs.), further investigations into that aspect of microwear in pycnodonts are in progress and might provide interesting results regarding the different feeding strategies proposed by Nursall (1993a) if an effect of the substrate can be highlighted.

Faunal changes during the Mesozoic Marine Revolution, in particular for exoskeletonized organisms, cannot be blamed mostly on the Pycnodontiformes. Recent investigations abound to show that this event was temporally spread and had many origins. Pycnodontiformes as predators in Mesozoic ecosystems were affected by and part of the process of natural selection, but their role needs to be revised. As a group

they were arguably not an important driver of the evolution of shelled organisms, but *Gyrodus* might have influenced the evolution of infaunalisation or increased mobility by preying on whichever shelled prey was available. Intense grazing from the Pycnodontoidei potentially triggered the rise of coralline algae as a defense against predation. Any macro-ecological test of a scenario of co-evolution and escalation in the Mesozoic aquatic ecosystems would benefit from excluding Pycnodontoidei from any group of shell-crushers, and considering Gyrodontoidei as potential shell-crushers.

This work provides many new roads of investigation to consider so as to better understand the palaeoecologies of fossil fishes and their influence on an ecosystem. A record of dental microwear textures from a broader range of extant analogues with varied dietary habits will lead to better inferences of dietary preferences in pycnodonts and other fossil fishes. Inclusion of Pycnodontiformes spanning a larger geographic or temporal scale will provide better estimates of their range of palaeoecologies and a means to track the evolution of dietary preferences within and betwen lineages. The comparison of dental microwear patterns on premaxilla or dentary-borne teeth would allow testing of hypotheses of Pycnodontoidei as scrapers, browsers or grazers as opposed to the picking Gyrodontoidei. Different feeding strategies in fishes can be highlighted by this technique (Baines, 2010; Purnell et al., 2006), the only limitation lies in the lack of fossil dentitions of Pycnodontiformes associated with incisiform or styliform teeth.

# Conclusion

Dental microwear texture analysis provides evidence that pycnodonts were not the specialised shell-crushers that they have long been thought to be. Previous interpretations of palaeoecologies based on gut content and tooth morphology were biased towards a durophagous habit by the preservation potential of other types of prey

consumed by pycnodonts. Comparison of trophic ecologies of fossil and extant fishes through the use of dental microwear texture analysis highlights a variety of feeding habits in Pycnodontiformes as suggested by Poyato-Ariza (2005).

Different patterns of ecospace occupation are observed for the two groups of Pycnodontiformes of Nursall (1993a, b): Pycnodontoidei with incisiform teeth were potentially restricted to algae or soft-objects diets, while Gyrodontoidei with styliform teeth had access to a broader range of food types. Though perhaps not one as sophisticated as in the parrotfish Scarus (Carr et al., 2006b) the Pycnodontiformes also possessed a mill to process their food and some of them among the Pycnodontoidei may well have been grazing herbivores. The requirement to capture their prey with jaw elements anatomically linked to the food-processing apparatus may have restricted the range of ecologies accessible to them. The decoupling of food capture/food processing is considered as one of the key innovations that led to the success of teleostean fishes (Cavin et al., 2007). It has already been suggested that the lack of such a feature may have hampered the adaptability of pycnodontiformes competing with other fishes. Whether the evolution of a molariform dentition first happened in Triassic Neopterygii as an adaptation to shell-crushing before evolving to allow a more efficient processing of algal material remains an open question. Under this scenario or that of a more generalist or herbivorous diet for pycnodonts throughout all their fossil record, adaptations from exoskeletonized animals during the Mesozoic Marine Revolution were the results of more than a simple escalation event. Recent investigations in the field of dental microwear (Merceron et al., 2010; Ramdarshan et al., 2012) have highlighted the need to consider the full dietary range of fossil species in order to better understand their ecologies and place in past ecosystems. Macroecological studies looking into the

MMR need to take into account the range of ecologies of predatory animals such as Pycnodontiformes in order to better test hypotheses of co-evolution in the fossil record.

# Chapter 5: Microwear texture analysis highlights trophic diversity in early jawed vertebrates (Placodermi)

# Abstract

Placoderms are one of the earliest groups of jawed vertebrates appearing in the fossil record. As such, they are an interesting sample for investigations regarding the origin of modern feeding ecologies. Microtexture analysis, a powerful tool to compare diet in extant and fossil animals, is applied to gnathal plates of Devonian placoderms: Compagopiscis croucheri (from the Frasnian of Gogo, Australia) and Ptyctodontidae (from the Givetian of the Boulonnais, France). As enameloid in modern teleosts is significantly harder than the dentine from the jaws of placoderms, direct comparisons of microtextures is avoided. *Protopterus annectens* (Dipnoi, the african lungfish) provides a link between microtextures of petrodentine and dentine used to process the same prey. This first investigation in the microtexture of food-processing elements of placoderms provides evidence for trophic diversity in the arthrodire *Compagopiscis croucheri*. It is interpreted as an ecologically adaptable organism with shell-crushing as one of its potential fallback feeding strategies. Ptyctodontid placoderms were likely durophagous, with some variability in the hardness or proportion of the food they crushed. The ability to feed on a variety of prey allowed placoderms to adapt to heterogeneous ecosystems like Devonian reef environments.

# Introduction

The structure of food webs in modern aquatic ecosystems is of bewildering complexity (Rohr et al., 2010), the most striking examples come from coral reefs where thousands of species coexist in a limited space with few resources (Choat et al., 2004;
Opitz, 1996). Yet those macroevolutionnary scenarios often make the assumption that predatory organisms had an influence because of their occurrence in the fossil record (e.g. Goatley et al., 2010; Sallan et al., 2011). Those assumptions of predation can be derived from studies on ecomorphological comparisons (Friedman, 2009), mechanical modelling (Anderson and Westneat, 2009; Evans and Sanson, 2006; Tseng et al., 2011) or gut content (Kear, 2006; Kriwet, 2001; Nursall, 1993a). Such studies provide a potential diet as the result of investigations, rather than the realised function of an organism in its ecosystem. An accurate test of the effective range of dietary habits of fossil predators will improve macro-evolutionary studies and reconstructions of palaeoecosystems as the kind of pressure they exerted on a type of prey is known.

Estimates of the physical properties of food processed by fossil animals are of special interest since they were exposed to conditions that extant analogues may never encounter. Palaeozoic seas share little in common with modern marine ecosystems, e.g. during the Devonian aquatic gnathostomes of the present day (chondrichthyans, actinopterygians, sarcopterygians) were at the beginning of their evolutionary history (Anderson et al., 2011; Brazeau, 2009; Derycke et al., 1995; Janvier, 1996; Long et al., 2008a; Mondéjar-Fernández and Clément, 2012; Sallan and Coates, 2010; Swartz, 2009) and shared their environment with a wider range of jawless vertebrates (Blieck et al., 2010; Donoghue and Smith, 2001; Gai et al., 2011; Janvier, 1996; Janvier, 2009) and with the group of armoured jawed vertebrates known as placoderms (Carr and Hlavin, 2010; Dupret et al., 2009; Goujet and Young, 2004; Lelièvre, 2002; Long et al., 2008b; Lukševičs, 2001; Rücklin, 2011; Smith and Johanson, 2003; Vézina, 1990; Young, 2005; Zhu et al., 2012). Placoderms are an exclusively fossil group of gnathostomes known from the Silurian to the Devonian. A lot of interest has been focused on arthrodires, a group of active swimmers and predators, with iconic

representatives such as *Dunkleosteus* (Anderson, 2010; Anderson and Westneat, 2009; Anderson, 2008; Blieck et al., 1998; Carr and Hlavin, 2010; Lelièvre et al., 1981; Long et al., 2009). The evolution of those predators had the potential to lead macro-ecological events as a different type of selective pressure was introduced in Palaeozoic environments (Anderson et al., 2011; Sallan and Coates, 2010; Sallan et al., 2011).

In this study microwear texture analysis is performed on gnathal plates from 2 unidentified ptyctodontid placoderms and seven individuals of *Compagopiscis croucheri*. The latter is a small arthrodire common in the Gogo Formation (NSW, Australia) (Trinajstic, 1995; Trinajstic and Dennis-Bryan, 2009; Trinajstic and Hazelton, 2007). In the morphospace described in the study of Anderson et al. (2011), *Compagopiscis* occupies a central place, both in regards to placoderms and gnathostomes as a whole. As such, *Compagopiscis* appears as a good candidate to test whether arthrodires were specialised predators with a specific ecological function or showed variability in their diet. The Gogo Formation is the fossilised equivalent of a reef ecosystem (Long and Trinajstic, 2010) which provides an heterogeneous environment (Cvitanovic and Bellwood, 2008; Hoey and Bellwood, 2009a; Opitz, 1996) where an adaptable organism can display its full range of ecologies.

Ptyctodonts are the sister-group of arthrodires (or closely related, see Brazeau, 2009; Janvier, 1996) and appear as a morphologically specialised clade among placoderms (Denison, 1978). Their jaws had a beak-like aspect with posterior tritoral surfaces or blades (Belles-Isles et al., 1987; Lelièvre et al., 1981; Long, 1997; Trinajstic and Long, 2009) with an apparently reinforced histology (pleromin Janvier, 1996; Johanson and Smith, 2005) when compared to other placoderms. This structure was hypothetically dedicated to shell-crushing (Shin, 2007), but grazing on coral remains a possibility, making ptyctodonts the Palaeozoic equivalent of the Chaetodontidae or

Scaridae (see Bellwood et al., 2010; Francini et al., 2008). Their trophic specialisation is investigated here using samples from the Boulonnais, another reef environment with abundant brachiopods and crinoids (Brice, 1988; Mistiaen et al., 2002) and a varied fish fauna (Belles-Isles et al., 1987; Cayeux, 1888; Darras et al., 2008; Derycke et al., 1995; Dutertre, 1929a, b, 1930; Laverdière, 1929; Lelièvre et al., 1988), providing a partial test of the escalation hypothesis proposed by (Sallan et al., 2011).

#### Material and methods

The fossil material analysed here comes from the 2 localities mentioned above: *Compagopiscis croucheri* samples come from the Gogo Formation in New South Wales (Australia, Figure 5-1, A), two unidentified ptyctodonts come from Blacourt Formation from the Boulonnais (France). Gnathal plates of *Compagopiscis croucheri* were cleaned according to the protocol described in Williams and Doyle (2010), and were sputtercoated with gold (Emitech K500X, 3 minutes). All other specimens were cleaned in the same manner before moulding with Coltène-whaledent speedex light body. Black epoxy resin (EpoTek 320 LV) was used to prepare high-resolution replicas of the fossils. All manipulations were performed following the manufacturers' instructions. The use of those combined media provides impressions of dental roughness similar to that of an original gold-coated specimen (see chapter 1).

The development of wear in the gnathal plates of arthrodires leads to the development of worn areas represented by blade-like surfaces or notches. This provides efficient food-puncturing and cutting tools but results in varied tissues being used to process food, most of it dentine, remodelled dentine or bone (Johanson and Smith, 2005), (Figure 5-1, C, D). The blade-like structures have sometimes been interpreted as having a crushing function (Long and Trinajstic, 2010; Trinajstic and Hazelton, 2007).

Earlier investigations proved that dentine provides jaw-mechanics and diet-related information through microwear features analysis (Green, 2009; Williams et al., 2009)

Table 5-1: Specimens of placoderms compared with extant fishes, locality and housing institution. NHM: Natural history Museum, London (United Kingdom). MGL: Musée Gosselet de Lille (Natural history Museum), Lille, France; NSW: New South Wales, Australia. Sources for age and locality: (Lelièvre et al., 1988; Long and Trinajstic, 2010)

Specimen number	Taxon	Institution	Age and locality
P50942	Compagopiscis croucheri	NHM	Frasnian (NSW, Gogo Formation, <i>transitans</i> conodont zone)
P50947	Compagopiscis croucheri	NHM	Frasnian (NSW, Gogo Formation, <i>transitans</i> conodont zone)
P50950	Compagopiscis croucheri	NHM	Frasnian (NSW, Gogo Formation, <i>transitans</i> conodont zone)
P50954	Compagopiscis croucheri	NHM	Frasnian (NSW, Gogo Formation, <i>transitans</i> conodont zone)
P50959	Compagopiscis croucheri	NHM	Frasnian (NSW, Gogo Formation, <i>transitans</i> conodont zone)
P51007	Compagopiscis croucheri	NHM	Frasnian (NSW, Gogo Formation, <i>transitans</i> conodont zone)
P52549	Compagopiscis croucheri	NHM	Frasnian (NSW, Gogo Formation, <i>transitans</i> conodont zone)
MGL 1271	Indet. Ptyctodont	MGL	Givetian (Boulonnais, Blacourt Formation, varcus conodont zone)
MGL 1272	Indet. Ptyctodont	MGL	Givetian (Boulonnais, Blacourt Formation, <i>varcus</i> conodont zone)



Figure 5-1: Skull and trunk armour of Compagopiscis croucheri (A), Skull of Protopterus annectens (B) and scanned surfaces of gnathal plates of Compagopiscis croucheri (C and D). Scale bar is 10 mm, surfaces are 146.07 x 110.79  $\mu$ m. C: surface of a gnathal plate exposing bone, Z max=41.90  $\mu$ m (most of the surface between 26 and 36); D: surface of a gnathal plate exposing dentine, Z max=19.21  $\mu$ m (most of the surface between 12 and 14). A modified from Trinajstic and Hazelton (2007).

Whether a direct comparison of roughness data from dentine with other dental tissues as enameloid or enamel provides comparable information needs to be investigated first. In order to check that differences in microtexture between placoderms are effectively linked to changes in dietary habits, investigations regarding the consistency of dental microtexture data between dentine and harder tissues have been performed on toothplates from Protopterus annectens (Dipnoi, the African lungfish, Figure 5-1 B). Lungfish toothplates are the result of the fusion of teeth (Ahlberg et al., 2006; Smith and Johanson, 2010) following an addition pattern similar to that observed in placoderms (Smith, 2003). This feeding structure harbours 2 tissue types involved in food-processing: dentine and petrodentine (hypermineralised dentine similar in physical properties to enamel, Ishiyama and Teraki, 1990). This combination makes direct comparisons between both tissue types and associated microwear textures possible. As data can be gathered from neighbouring areas on the toothplates, there is no doubt that they have been involved in similar food-processing events. If microtextural data derived from dentine and harder tissues were to provide a significantly different signal, information gathered from *Protopterus* will provide a link between microtextural parameters from the enameloid of modern teleosts (chapter 2) and dentine of placoderms. 2 individuals of *Protopterus annectens*, made available by Prof. Jenny Clack (Cambridge university museum of zoology) had their toothplates moulded and cast as described above.

## Acquisition of microtextural data

Virtual topographic models of the replicated or gold-coated surfaces were obtained for all specimens using an Alicona Infinite Focus microscope G4b (IFM; software v. 2.1.2) with a x100 objective, providing high-resolution three-dimensional surfaces for a 145 x 110 µm field of view. Optimal quality for the 3D data was sought

by manually adjusting exposure and contrast (gamma) settings for each scan. The "3D editor" software supplied with the microscope (InfiniteFocus 2.1.2, IFM software version 2.1.2) was used to manually edit the data and remove scanning errors. The cleaned point clouds were exported as .sur files and imported in SurfStand (software version 5.0). Surfaces were automatically treated as in chapters 2 to 4 and standard parameters (ISO 25178) gathered from for the filtered surfaces. Care was taken to orient the scanned surfaces as horizontally as possible prior to data acquisition.

## Data analysis

Investigations were carried out as a series of tests of hypotheses and multivariate analyses to ensure that patterns observed in the data gathered from gnathal plates of placoderms are linked to differences in diet. The potential discrepancies in microtextural parameters between dentine and petrodentine were first investigated in *Protopterus*. The data of the harder tissue from the lungfish was then compared with extant organisms using data from a previous study (see chapter 2)<sup>8</sup> to confirm their durophagous habits. This information regarding the feeding habits of the lungfish allows using its data as a reference for comparisons of textural parameters acquired from dentine in placoderms.

Individuals of *Compagopiscis croucheri* and ptyctodontid placoderms have been compared with *Protopterus*, in the ecospace described by a PCA before comparing coherent groups of individuals. The tested hypotheses and performed investigations were:

H0-1: "Microtextural data from *Protopterus annectens* is identical on dentine and petrodentine."

<sup>&</sup>lt;sup>8</sup> 1 population of specialised shell-crushers: *Anarhichas lupus* (n=4) and 2 populations of generalists, *Archosargus probatocephalus* different in regards of the proportion of hard prey in their diet (n=6 each)

H0-2: "*Protopterus annectens* is a shell-crusher (by comparison with other extant animals)."

The textural parameters showing a normal or log-normal distribution and differences between groups of extant fishes were used to calculate a Principal Components Analysis (PCA). The data from the dentine of *Protopterus annectens* was projected in the ecospace decribed by this PCA, the projections providing a reference with which projections for fossil data were compared.

H0-3: "*Compagopiscis croucheri* was a shell-crusher (using the dentine of *Protopterus annectens* as a reference)."

H0-4: "Ptyctodontid placoderms were shell-crushers (using the petrodentine of *Protopterus annectens* as a reference)."

The hypothesis H0-1 was tested using paired t-tests with Welch-Satterwaithe correction to account for heteroscedasticity. The hypotheses H0-2 to 4 were tested at the significance level of  $\alpha$ =5% using Welch-Analysis of Variance (Welch-Anova) for each textural parameter. In each case, the normality of the distribution for the samples was tested using Shapiro- Wilks test with or without log-transformation of the data and rank-transformation applied if neither original nor log-transformed data complied with the condition of normality. Series of pairwise t-tests were carried on the parameters displaying a significant difference. Welch-Satterwaithe and Holm-Bonferroni corrections were applied to account for heteroscedasticity and the number of tests performed. All investigations were carried using R 2.15.0 (R Development Core Team, 2012) with the package MASS (Venables and Ripley, 2002).

## Results

#### Dentine versus Petrodentine in Protopterus annectens (H0-1)

Table 5-2: Summary of Welch-Satterwaithe t-tests comparing the two histologies on toothplates of Protopterus annectens. Texture parameters showing a significant difference and the associated information highlighted in bold. O: test performed on the original, untransformed data; L: test performed on log-transformed data; R: test performed on rank-transformed data. df: degree of freedom.

Parameter	Data	t-statistic	df	p-value
Sq	L	5.3479	9	0.0005
Ssk	Ο	0.5517	9	0.5946
Sku	L	-1.6651	9	0.1302
Sp	0	2.9676	9	0.0158
Sv	О	1.3411	9	0.2128
Sz	0	2.5117	9	0.0322
Sds	О	-1.2438	9	0.2450
Str	R	0.1472	9	0.8862
Sal	R	-2.2765	9	0.0488
Sdq	0	4.6065	9	0.0013
Ssc	0	3.7097	9	0.0048
Sdr	0	3.5987	9	0.0058
Vmp	L	4.4405	9	0.0016
Vmc	0	5.7835	9	0.0003
Vvc	0	5.3567	9	0.0005
Vvv	L	5.3630	9	0.0004
Spk	0	3.9512	9	0.0033
Sk	0	5.8170	9	0.0002
Svk	L	3.5724	9	0.0060
Smr1	Ο	2.2238	9	0.0532
Smr2	0	-5.5352	9	0.0004
S5z	$\mathbf{L}$	3.3204	9	0.0089
Sa	0	5.2841	9	0.0005
Vvc/Vmc	0	1.1299	9	0.2877

17 textural parameters display significant differences between the two

histologies in *Protopterus annectens*, demonstrating that the same processed food items provide different microtextures on different dental tissues. As a consequence modern animals used as reference in the earlier chapters cannot be used for direct comparisons of trophic ecologies with placoderms.

#### Protopterus as a shell-crusher (H0-2)

Table 5-3: Summary of Welch-Anovas comparing the two populations of Archosargus probatocephalus, the lesser worn Anarhichas lupus and petrodentine from Protopterus annectens. Texture parameters showing a significant difference and the associated information highlighted in bold. O: test performed on the original, untransformed data; L: test performed on log-transformed data; R: test performed on rank-transformed data. df: degree of freedom; num: numerator; denom: denominator.

Parameter	Data	F-statistic	F-statistic df	
			(num,denom)	
Sq	R	30.5329	3,25.982	<0.0001
Ssk	R	2.2832	3,25.854	0.1027
Sku	R	44.8602	3,31.710	<0.0001
Sp	L	13.0958	3,23.131	<0.0001
Sv	R	29.3128	3,26.292	<0.0001
Sz	R	26.6060	3,26.565	<0.0001
Sds	L	1.1094	3,29.452	0.3610
Str	R	4.4240	3,29.995	0.0109
Sal	R	1.4899	3,26.662	0.2399
Sdq	R	34.2720	3,25.917	<0.0001
Ssc	R	28.3194	3,29.055	<0.0001
Sdr	R	25.4195	3,25.900	<0.0001
Vmp	R	57.1227	3,25.965	<0.0001
Vmc	R	10.3340	3,25.194	0.0001
Vvc	R	14.2891	3,25.117	<0.0001
Vvv	R	12.7230	325.582	<0.0001
Spk	R	49.2136	3,25.794	<0.0001
Šk	R	10.795	3,25.172	<0.0001
Svk	R	15.5278	3,25.729	<0.0001
Smr1	0	8.6969	3,24.551	0.0004
Smr2	R	3.0599	3,26.572	0.0454
S5z	L	16.8665	3,22.183	<0.0001
Sa	R	15.5176	3,25.496	<0.0001
Vvc/Vmc	0	7.5943	3,24.236	0.0009

All of the textural parameters compared between trophic niches of extant fishes did display significant differences in the Welch-Anovas but Ssk, Sds and Sal. Pairwise comparisons of the samples highlighted no significant differences between *Protopterus annectens* and the shell-crushing specialist, confirming the durophagous habits of the lungfish.

Table 5-4: Parameters displaying a significant difference in pairwise t-tests. Bold: log-

	Ap herbivorous	Ap durophagous	Wolffish
Ap durophagous	Sdr, Smr1,		
	Vvc/Vmc		
Wolffish	Sq, Sku, <b>Sp</b> , Sv,	Sq, Sku, <b>Sp</b> , Sv,	
	Sz, Sdq, Ssc, Sdr,	Sz, Sdq, Ssc, Sdr,	
	Vmp, Vmc, Vvc,	Vmp, Vmc, Vvc,	
	Vvv, Spk, Sk, Svk,	Vvv, Spk, Sk, Svk,	
	Smr1, <b>S5z</b>	Smr1, <b>S5z</b> ,	
		Vvc/Vmc	
Protopterus	Sku, <b>Sp</b> , Sz, Ssc,	Sku, <b>Sp</b> , Sz, Str,	
	<i>Sdr</i> ; <b>S5z</b>	<i>Ssc</i> , <b>S5z</b>	

transformed data, italics: rank-transformed data. Ap: Archosargus probatocephalus.

#### **Principal Components Analysis**

The PCA was performed on the parameters displaying a significant difference which did not require rank-transformation (Smr1, Vvc/Vmc and the log-transformed Sp and S5z). The first 2 principal components axes (PC1 and 2) accounted for 95.74 % of the expressed variance (63.09% for PC1 and 32.65% for PC2, all parameters weighted equally on PC1 and PC2) and separated generalist populations of *Archosargus* and both the shell-crushers *Anarhichas* and *Protopterus* along PC1, whereas on PC2 the bulk of the more durophagous *Archosargus* displays lower scores than herbivores (Figure 5-2). This ecospace does not show a clear segregation between generalists, but the first axis is related to marked differences in feeding habits (specialised shell-crushers as opposed to generalists). The parameters (loadings) of the PCA were applied to the dentine data of *Protopterus* in order to obtain a reference area for this histology in the ecospace. The dentine scores are lower on PC1 and similar on PC2 when compared to the petrodentine.

Median values of the dentine-derived parameters for each fossil individual were similarly used to calculate projections in the ecospace. As patterns observed on the first PC axis of this ecospace are related to the proportion of crushed hard prey (approximately 35% in the generalist *Archosargus*, minimum 70% in the specialised *Anarhichas*, see chapter 2) or feeding stategies (specialist versus generalist), divergences along PC1 for fossil individuals are interpreted as the effect of different trophic ecologies.

Individuals from *Compagopiscis* or the ptyctodonts are plotting on both sides of the reference area described by the dentine of *Protopterus*. In *Compagopiscis croucheri* the individuals P50942, P50954, P50959 and P51007 (referred to as Group1) display higher scores on PC1 than P50947, P50950 and P52549 (Group2). Those latter three are plotting close to the ptyctodontid MGL1271, the other ptyctodont is projected between the first group of *Compagopiscis* and the average values for the dentine of *Protopterus*. The relative position of the projections within the ecospace are the first evidence of any potential trophic diversity in *Compagopiscis croucheri* and between ptyctodonts.

#### Compagopiscis croucheri as a shell-crusher (H0-3)

The two clusters highlighted by the PCA were considered as separate trophic niches for the subsequent comparison of *Compagopiscis* with *Protopterus annectens*. If those clusters are artificial groupings based on lucky sampling of the microtextural parameters used this test will not highlight any difference (if they were shell-crushers) or the t-tests contrasting the differences will highlight no differences between the placoderms, but with *Protopterus*. For this test, all the sampled areas representing the texture of dentine in fossils were included.



Figure 5-2: Principal Components Analysis ecospace of extant animals and projections for the dentine or pleromin of placoderms. Coloured hulls represent the reference populations of extant fishes, the dashed hull represents the area of ecospace occupied by the microtextural data from petrodentine of Protopterus. The hull in full line represents the dentine data from Protopterus. Scale bars: 1 cm. Ap: generalist Archosargus probatocephalus; wolffish: specialised shell-crusher Anarhichas lupus.

Table 5-5: Summary of Welch-Anovas comparing the two clusters of Compagopiscis croucheri with dentine from a specialised shell-crusher Protopterus annectens. Texture parameters showing a significant difference and the associated information highlighted in bold. O: test performed on the original, untransformed data; L: test performed on log-transformed data; R: test performed on rank-transformed data. df: degree of freedom; num: numerator; denom: denominator.

Parameter	Data	F-statistic df		p-value
			(num,denom)	
Sq	L	8.7765	2,11.695	0.0047
Ssk	R	8.5128	2,13.325	0.0041
Sku	R	6.2520	2,10.800	0.0157
Sp	$\mathbf{L}$	7.2648	2,7.819	0.0165
Sv	L	1.6303	2,8.417	0.2521
Sz	L	1.7885	2,7.302	0.2333
Sds	0	15.2039	2,13.697	0.0003
Str	Ο	0.5424	2,10.911	0.5962
Sal	R	2.1314	2,8.835	0.1757
Sdq	L	6.7144	2,10.287	0.0136
Ssc	R	7.3564	2,9.904	0.0109
Sdr	L	5.7491	2,9.541	0.0230
Vmp	L	5.3503	2,12.520	0.0209
Vmc	R	13.665	2,7.957	0.0027
Vvc	R	14.3205	2,8.146	0.0025
Vvv	L	7.2477	2,13.392	0.0074
Spk	Ο	5.2802	2,10.790	0.02518
Sk	R	20.0115	2,8.049	0.0007
Svk	L	4.0605	2,14.456	0.0399
Smr1	Ο	21.8577	2,15.722	<0.0001
Smr2	0	1.5266	2,9.121	0.268
S5z	L	5.6262	2,10.352	0.0222
Sa	0	9.3646	2,9.758	0.0054
Vvc/Vmc	0	4.8693	2,9.813	0.0339

Results from Welch-Anovas, highlighted significant differences for all

parameters with the exception of Sv, Sz, Str and Sal. Pairwise t-tests comparing both groups of *Compagopiscis* with the reference *Protopterus annectens* displayed a pattern similar to that observed in the ecospace, i.e. that individuals from Group2 were closer in dental microtexture to *Protopterus* than to the conspecific individuals from Group1. Putting aside the parameters on which the ecospace the two groups of *Compagopiscis*  *croucheri* are significantly different in microtexture based on the parameters Sq, Ssk, Sdq, Vmp, Vvv and Svk. This result supports the hypothesis of trophic diversity in this arthrodire.

Table 5-6: Parameters displaying a significant difference in pairwise t-tests comparing the two clusters of Compagopiscis croucheri with dentine from a specialised shellcrusher Protopterus annectens. Bold: log-transformed data, italics: rank-transformed data. Group1: specimens of Compagopiscis croucheri P50942, P50954, P50959 and P51007; Group2: specimens of Compagopiscis croucheri P50947, P50950 and P52549

	Group1	Group2
Group2	Sq, Ssk, Sdq, Vmp, Vvv,	
	<b>Svk</b> , Smr1, <b>S5z</b> ,	
	Vvc/Vmc	
Protopterus	Sq, Sp, Sdq, Ssc, Sdr,	Ssk, Sku, Smr1
	Vmp, Vmc, Vvc, Vvv,	
	Spk, <i>Sk</i> , <b>S5z</b> , Sa	

## Ptyctodonts as shell-crushers (H0-4)

The procedure used was similar to that for *Compagopiscis* (several measurements per individuals were used). There is no evidence that the mechanical properties of the surface of gnathal plates from ptyctodonts are similar to that of the dentine from *Protopterus*, and as pleromin is likely more similar in mechanical properties to petrodentine, the latter tissue was used in the comparison. As for *Compagopiscis*, the Welch-anovas highlighted many differences for a range of textural parameters. Subsequent pairwise t-tests confirmed the differences between the fossil specimens and showed few discrepancies between MGL 1272 and *Protopterus*.

Table 5-7: Summary of Welch-Anovas comparing the two specimens of ptyctodontid placoderms with dentine from a specialised shell-crusher Protopterus annectens. Texture parameters showing a significant difference and the associated information highlighted in bold. O: test performed on the original, untransformed data; L: test performed on log-transformed data; R: test performed on rank-transformed data. df: degree of freedom; num: numerator; denom: denominator.

Parameter	Data	F-statistic	df	p-value
			(num,denom)	-
Sq	R	25.9549	2,8.792	0.0002
Ssk	Ο	1.4533	2,7.918	0.2900
Sku	L	0.5581	2,7.637	0.5947
Sp	0	21.5757	2,9.912	0.0002
Sv	0	55.8672	2,8.001	<0.0001
Sz	0	59.2084	2,9.333	<0.0001
Sds	0	9.3824	2,9.277	0.0059
Str	R	3.9156	2,8.276	0.0636
Sdq	0	12.1752	2,6.792	0.0057
Ssc	R	16.1740	2,9.005	0.0009
Sdr	0	7.2502	2,6.729	0.0212
Vmp	R	29.2464	2,8.888	0.0001
Vmc	0	6.2087	2,6.478	0.0312
Vvc	0	6.8987	2,6.343	0.0256
Vvv	L	30.0862	2,8.426	0.0001
Spk	0	27.2158	2,7.321	0.0004
Šk	0	5.3472	2,6.457	0.0420
Svk	$\mathbf{L}$	32.3979	2,9.295	<0.0001
Smr1	0	7.2837	2,6.959	0.0196
Smr2	R	14.9838	2,9.625	0.0011
S5z	0	34.8849	2,8.687	<0.0001
Sa	0	10.6483	2,6.344	0.0094
Vvc/Vmc	Ο	3.5532	2,6.637	0.0893

Table 5-8: Parameters displaying a significant difference in pairwise t-tests. Bold: log-

transformed data, italics: rank-transformed data.

	MGL 1271	MGL 1272
MGL 1272	Sq, Sp, Sv, Sz, Sdq, Ssc,	
	Vmp, Vvv, Spk, Svk,	
	<i>Smr2</i> , S5z, Sa	
Protopterus	Sq, Sv, Sz, Sds, Sdq, Ssc,	Sv, Sz, <i>Smr2</i> , S5z
	Vmp, Vvv, Spk, Svk,	
	Smr1, <i>Smr2</i> , S5z, Sa	

#### Discussion

The main aim of this investigation was to test whether placoderms behaved as ecological specialists or showed some degree of adaptability in their environment. Statistical comparisons of textural parameters characterising the surface of gnathal plates show differences between groups of individuals in the arthrodire *Compagopiscis* croucheri from the Gogo Formation (Australia). Investigations also found differences between 2 ptyctodontid placoderms from the Blacourt Formation (France), a group of apparently specialised shell-crushers. The segregation within the ecospace of the PCA is not associated with observed wear stages: Specimens P50959 and P50947 are separated though similarly worn. A similar pattern is observed with the less worn but larger P50942 and P50950. Moreover, there was no apparent link between the size of the individuals and the distribution in the ecospace.

There is convergence in microwear texture data between the food-processing structures of *Protopterus annectens* and the group of *Compagopiscis* labelled Group2, while the remainder of them, Group1, are projected in the ecospace towards the more generalist extant fishes. The development of dental and gnathal microwear is a processus influenced by the physical properties of the processed elements and the food-processing elements (food, substrate, occluding tissues Sanson et al., 2007; Walker et al., 1978). Under the assumption that dentine in *Compagopiscis croucheri* and *Protopterus annectens* has similar physical properties, one could conclude that the food they processed were also similar. This interpretation is in agreement with the hypothesis evoked in Trinajstic and Hazelton (2007) that the development of flat areas along the jaws of arthrodires represents a paedomorphic shift to durophagy. Keeping in mind that the ability to process hard food does not involve constant durophagous habits, as for example in cichlids (Binning et al., 2009; Hulsey, 2006; Liem, 1973; Purnell et al.,

2012), the division between groups of *Compagopiscis* expresses the separation between individuals feeding at one potential extreme of their ecological spectrum: durophagy and others. This diet could represent a fallback food in times when easier to catch prey were rare in the ecosystem, while the remainder of the time *Compagopiscis* behaved as an ecological generalist. Such differences in microwear have been linked to the consumption of physically divergent resources within a species (Merceron et al., 2010; Ungar et al., 2008)

This interpretation is based on the assumption that dentine in both organisms is of similar hardness, yet dentine in mammals can vary in Vickers hardness number from circa 50 to more than 100 (Kemp, 2001). Whether this difference would lead to significant discrepancies in the microtexture recorded at the surface of the feeding apparatus needs to be evaluated. Hardness of dental tissues changes with age in the rat (Kemp, 2001), but as mentioned above, the divergences between groups in the arthrodire are not related to size or wear stage, which could be used as proxy for age. Tissues with different hardnesses provide different values of microtextural parameters in *Protopterus*, but the Vickers hardness number is 5 times higher in petrodentine (circa 250) than in dentine. In any case, microwear texture analysis evidences trophic diversity within *Compagopiscis croucheri*.

Discrepancies in the feeding habits of ptyctodonts were unexpected although there remains the possibility for specimens to represent extremes of an otherwise continuous ecological spectrum within the considered ecospace. The physical properties of the pleromin in gnathal plates of ptycodonts relative to the tissues of extant fishes are unknown. Whether the comparison with the textural parameters from the petrodentine of *Protopterus* is relevant or dentine offered a better analogue for the microtexture signature, the specimens appear separated within the ecospace described by the PCA

axes. Additional investigations carried with dentine or enameloid as references provided similar results of trophic diversity within the group. Since the histological specialisation in the gnathal plates of ptyctodonts probably provided a tissue physically closer to the enameloid of *Anarhichas lupus* than the dentine of *Protopterus* (Janvier, 1996; Smith, 2003), a varied durophagous habit remains a valid hypothesis for their diet. The fauna from the Devonian of the Boulonnais harbours an impressive amount of shelled invertebrates in a reef ecosystem (Brice, 1988) which would have provided an abundant source of food for a range of shell-crushers such as ptyctodontids (Belles-Isles et al., 1987), lungfishes (Dutertre, 1930; Laverdière, 1929; Lelièvre et al., 1988) or early holocephalans (Darras et al., 2008). The divergence we observe between the two ptyctodontid placoderms may be the result of an ecological shift as new competitors migrated within this environment.

One of the sources of variability in the diet of fishes is linked to changes in their environment either locally or seasonally (Chuwen et al., 2007; Cochran-Biederman and Winemiller, 2010; Cutwa and Turingan, 2000; Purnell et al., 2007). The specimens were sampled within the same formation but this does not entail continuity in the ecological conditions they were exposed to, as reef ecosystems are characteristically heterogeneous (Bonaldo and Bellwood, 2010; Cvitanovic and Bellwood, 2008; Opitz, 1996). The Gogo Formation is about 700 m thick and affected by sea-level changes associated with anoxic/hypoxic pulses (Long and Trinajstic, 2010). It has been suggested that trophic specialisation could in time lead to speciation (Knudsen et al., 2010). *Gogopiscis* has recently been re-assigned to *Compagopiscis* based on morphometric studies (Trinajstic and Hazelton, 2007), although the two genera previously separated could have been diverging morphotypes emerging from niche partitioning. Yet, given the thickness of the Gogo Formation (700 m) and in the absence of fine stratigraphical information, the

specimens cannot be assumed as contemporaneous, in this or the above mentioned study.Another possibility is shifts in ecology linked to gender or induced by gestation or brooding behaviour. Internal fertilization and child-bearing has been discovered in fossil arthrodires (Long et al., 2009) and ptyctodonts (Long et al., 2008b) from Gogo. The added energetic requirement of gestation would explain a shift in feeding intensity or behaviour and, perhaps, microtexture. This hypothesis could be tested in extant vivipaous sharks, but application of dental microwear and microtexture analysis in Chondrichthyans has only recently started (McLennan, Pers. comm.). Alternatively, brooding behaviour (Hoey et al., 2012) and nesting (Ota et al., 2012) involve genderbased differences in the use of jaws in fishes, with as of yet unknown consequences on dental microwear patterns.

## Conclusion

Trophic diversity within arthrodire placoderms is highlighted for the first time in *Compagopiscis* croucheri. The reef environment of the Frasnian Gogo Formation offered a variable ecosystem where adaptable organisms with a range of ecologies would have been at an advantage. Inferences of diet in this sample are not yet possible as the physical properties of their dentine need further examination. Unless those properties were highly divergent with that of the dentine in *Protopterus annectens*, a reasonable assumption of the trophic ecology of *Compagopiscis croucheri* is that of an adaptable organism with shell-crushing one of its potential feeding strategies.

Direct comparison between dentine and petrodentine in *Protopterus annectens* highlighted discrepancies in the recorded microwear texture signal associated with different histologies. Petrodentine is similar in hardness and microtexture to the foodprocessing tissues of other bony fishes. The roughness of dentine and other tissues as a result of food-processing needs to be further investigated in order to provide appropriate analogues for the variety of feeding structures in early gnathostomes. Inferences of diet in 2 individuals of ptyctodontid placoderms from the Boulonnais (northern France) for example require further investigations in the physical properties of pleromin or tubular dentine. If it were similar in hardness to dentine, durophagy in this group is a supported assumption for their diet but they could have processed softer items. If petrodentine provides a better analogue however, they crushed a variable but high proportion of hard prey. "Yet conserving living organisms is far more important than conserving fossils (and here one speaks as a lifelong palaeontologist). The Earth, in sustaining and harbouring these organisms, is by far the most intricate, the most subtle, the most complex and valuable object in space for many, many billions of miles in any direction"

Jan Zalasiewicz, "The Earth After Us – What legacy will humans leave in the rocks?"

## Conclusion

The work presented in this thesis has shown that:

- It is possible to accurately replicate the three-dimensional surface of a tooth at a microscopic scale.
- Microwear texture analysis allows us to test hypotheses of trophic ecology in extant fishes with known different trophic ecologies.
- Reliable inferences of trophic ecology in fossil fishes are possible and showed that Pycnodontiformes and Compagopiscis croucheri are ecologically diverse organism and neither were specialised shellcrushers.

Furthermore, it has demonstrated that this method provides an excellent tool for testing differing scenarios of macroevolution involving fishes with molariform dentitions or toothplates. One consequence of the investigations presented here is a reassessment of the role of pycnodontiform fishes in the Mesozoic: no longer hypothetical specialised shell-crushers, but ascertained generalists and/or grazers. Early investigations using this method also showed that trophic diversity and durophagy were present in some of the earliest jawed vertebrates.

## **Relationship between microtexture and fish trophic ecology**

Microtexture analysis performed on molariform teeth can track subtle dietrelated differences between populations of morphologically similar conspecific fishes. This has been shown on teeth from *Archosargus probatocephalus* from 2 lagoons in Florida, with differences in their diet mostly related to the proportion of ingested exoskeletonized prey (volumetric contributions of 42.55%  $\pm$  6.05 S.E. in Port Canaveral lagoon versus 24.58%  $\pm$  5.65 S.E. in Indian River lagoon, most of the prey being swallowed whole in the latter). The method has also allowed discrepancies in microtexture related to the accumulation of wear over the teeth of *Anarhichas lupus* to be tracked.

Our investigations showed that differences in microtexture between teeth at a similar wear stage were related to the proportion of crushed material. This result may in the future make possible precise inferences of the proportion of crushed invertebrates in the diet of fishes. As this proportion is linked to the recorded signal, it means that investigations can be carried out in other organisms in order to test hypotheses of trophic diversity without reference to extant analogues.

The comparison of dental microtexture of *Archosargus probatocephalus* and *Anarhichas lupus* with the cichlid *Astatoreochromis alluaudi* highlighted a major advantage of the technique: there is no link between textural parameters and the size of the animal. The most durophagous cichlids and the specialised shell-crushing wolffish provide similar microtextural parameters despite a major size discrepancy (190 mm for the cichlid versus 1500 mm for the wolffish). Since surfaces are digitized at the lateral resolution of 0.35-0.4  $\mu$ m and vertical resolution of 0.02  $\mu$ m (Purnell et al., 2012) the technique accurately records the roughness of teeth at a macromolecular level: fibre-bundles of hydroxyapatite are 2-3 $\mu$ m wide in chondrichthyans (Gillis and Donoghue, 2007) and 2-4  $\mu$ m wide in the Tilapia (Sasagawa, 1997). The orientation of those fibre-bundles at the surface of teeth may have an influence on the data, but not the size of the animal.

Direct comparison between dentine and petrodentine in *Protopterus annectens* showed that dental histologies show discrepancies in the recorded microwear texture

signal and further investigation is required to link dental histology and microtexture signature.

## Testing hypotheses regarding the trophic ecology of fossil fishes

The fact that the microtexture signal is efficiently recorded at the surface of teeth and linked to the proportion of crushed food provides a tool to test hypotheses of diet and trophic diversity in fossil fishes with molariform teeth.

Fossil fishes compared on their own did display differences in dental microtexture between individuals as well as different patterns of ecospace occupation in different taxa. The way Pycnodontiformes from the sub-orders Pycnodontoidei and Gyrodontoidei fed and behaved resulted in two patterns in multivariate analyses: an unstructured, broad and varied pattern of ecospace occupation for *Gyrodus*, interpreted as pickers capturing items from the water column or the substrate, as opposed to scrapers, grazers or excavators in the Pycnodontoidei that were restricted to more specialised trophic niches (Nursall, 1993a, b) and fed on items resulting in two divergent microtexture signatures.

Comparisons with extant analogues show that Pycnodontiformes, as a whole, did not behave as a consistent group of shell-crushers. Inferred palaeoecologies are that of a generalist or opportunist predator for *Gyrodus*. Pycnodontoidei were comparatively less diverse and restricted to a few ecologies. They are interpreted as mostly herbivorous grazers or excavators, only occasionally feeding on exoskeletonized prey. Predation on those shelled animals was present in some of the earliest gnathostomes, the ptyctodontid placoderms, and potentially in the arthrodire *Compagopiscis croucheri*.

#### **Implications for macroevolutionary hypotheses**

The first implication of this work for macroevolutionary hypotheses involving fishes as grazing or shell-crushing predators is the ability to test which of these functions they performed in their environment. One of the major findings that this allowed is that Pycnodontiformes were not specialised shell crushers. This answers the question arising from the co-occurrence of several animals sharing a similar specialised morphology within an ecosystem: it was shared by generalists, grazers and excavators.

Fishes with thick and broad jaws have the mechanical ability to behave as herbivores or durophages, without any clear diagnostic feature to separate them based on morphology. Fossils with similar feeding structures were often considered as either, depending on which aspect of fish ecology was investigated (e.g. Bellwood, 2003; Goatley et al., 2010). Dental microtexture analysis provides a complementary tool to infer their ecological function which in turn helps us to build accurate scenarios of macroevolution.

Fishes from the order Pycnodontiformes have had their palaeoecology restricted to that of shell-crushers in many recent studies. However, the results of investigations using dental microtexture analysis clearly indicate that they were an ecologically diverse group of animals, Pycnodontoidei and Gyrodontoidei showing different patterns of inter-individual separation and ecospace occupation. The dichotomy in Pycnodontoidei can be interpreted in a variety of ways in comparison with extant analogues but all the evidence points towards mostly herbivorous habits in this group.

This evidence suggests that Pycnodontiformes might have had a greater influence on algae or reef-building organisms during the Mesozoic Marine Revolution than on the evolution of shelled invertebrates. Gyrodontoidei would have driven the infaunalisation of bivalves by picking out the least buried ones but Pycnodontoidei as a major group of herbivores may have triggered the evolution of coralline algae as a defence mechanism against predation. Although the timing of such an event is highly dependent on the parameters used (Warnock et al., 2011), this group emerged, genetically speaking, during the Mesozoic (Aguirre et al., 2010). But the most important influence that they may have had on global ecosystems was to produce the conditions that allowed this major ecological innovation to flourish: by removing the macroalgal cover from around the reefs, they allowed light to reach the newly evolved symbiotic zooxanthellate corals (Stanley, 2006; Stanley and van de Schootbrugge, 2009).

Our investigations also provide evidence for trophic diversity in *Compagopiscis croucheri* from the Frasnian of Gogo (Australia) for the first time. Ptyctodontid placoderms also show trophic diversity and were an adaptable early group of shellcrushers. The ability to feed on a variety of prey allowed placoderms to adapt to heterogeneous ecosystems such as reef environments. Scenarios of macroevolution involving the earliest gnathostomes as predators can now be tested based on evidence of predator-prey interactions.

#### The future

The aims of this thesis have been achieved, in that it has demonstrated that it is possible to make reliable inferences of diet in fossil fishes with molariform dentitions and to test their role in past ecosystems. However, several questions have arisen from those investigations which suggest new directions for future research.

First, there is a need to expand the database of reference samples from modern ecosystems both in terms of diets and histologies. The former will be helpful in assessing the possibilities and limitations of dental microtexture analysis and determining how much we can know about the diet of fossil fishes with extant analogues. Investigations regarding the links between diet, microtexture and histology

will provide references for comparisons within or between groups of fishes without enameloid as part of their feeding apparatus.

Secondly, an interesting project would be to record the wear on the prehensile teeth of pycnodonts and the information provided by the microtexture from their molariform teeth. Fossils with both tooth morphologies associated are rare but combining the earlier methodologies regarding dental microwear in fishes (Baines, 2010; Purnell et al., 2007; Purnell et al., 2012; Purnell et al., 2006) with the results of this thesis would offer an almost complete picture of the feeding behaviour of pycnodonts.

Thirdly, it would also be interesting to set up controlled feeding experiments involving similar proportions of shelled prey of differing hardness or differing proportions of similarly hard prey, as processed by morphologically identical animals. It would allow us to compare the effect on tooth wear of the proportion of crushed material with that of the resilience of the prey.

Finally, can we observe similar patterns in other aquatic animals? The technique can be extended to other groups beyond actinopterygians: such investigations are only just beginning, but hold the promise of many new and exciting discoveries. For example, were transitions from water to land in early tetrapods and vice-versa in whales, sirenians or Mesozoic reptiles associated with changes in diet? Was the feeding strategy of the Triassic and Paleocene pycnodonts similar to that of the Jurassic? And since dental microtexture can be applied to curved surfaces, what can it tell us about the trophic ecology of fossil amphibians and archosaurians? Can we relate extinction and survival through extinction events such as the Permo-Triassic boundary to aspects of trophic ecology? Indeed, as nothing prevents us from searching for evidence of dental

microwear in pterosaurs, early birds and bats, one can say that not even the sky is the limit.

# Appendices:

•	Comparison of levelling and filtering techniques	164
•	Dental microwear texture within individuals of Archosargus	173
•	Supplementary t-tests in extant fishes	177
•	Supplementary t-tests in Pycnodus	179
•	Elliptic Fourier analysis of dental outlines in Pycnodontiformes	180
•	Automatic clusters in ecospaces from extant and fossil fishes	184

## **Comparison of levelling and filtering techniques**

Several of the available levelling and filtering techniques available in SurfStand were applied to a limited dataset of dental surfaces from *Archosargus probatocephalus*, including individuals from both populations, in order to find which technique, or combination of techniques, allowed for an efficient discrimination between the two populations. These techniques and combinations are listed here and will be referred to as "treatments":

"Levelling" techniques:

- Tilt: Simple levelling of the surface by automatic tilting.
- Poly2: Levelling of the surface and removal of the form with a 2<sup>nd</sup> order polynomial function. Since the dental surfaces are from dome-shaped teeth, this technique should provide an "SF surface" representing the raw surface of the tooth. As can be seen in Figure A-1 B&C, the resulting surface preserves most of the volume of pits on the surface, and the information related to scratches may be drowned in the data.
- Poly 8: Levelling of the surface and removal of the form with an 8<sup>th</sup> order polynomial function. This operation is likely to remove not only the form of the tooth but any ornamentation, ridge or irregularity of growth, along with larger-scale features such as large pits. One could consider it as a very thorough filter providing information about the surface at a small scale.

Filtering techniques: Application of these techniques provides an "SL surface" which is the original surface with both the form and the large-scale elements filtered out.<sup>9</sup>

- Gaussian: Application of a robust (insensitive to deviations due to peaks and valleys,(Blateyron, 2006)) gaussian filter, based on a linear regression function, here using a nesting index (previously known as cutoff wavelength) of 0.025 mm.
- Wavelet: Wavelet filtering automatically generates a surface containing only elements below a scale level (defined by a nesting index, here set at 0.025 mm)
- Spline: Application of a robust spline filter, based on a non-linear filter equation integrating robustness and end-effect management (which avoids reduction of the size of the surface,(Blateyron, 2006)). The used nesting index was 0.025 mm. The resulting surface excludes most of the volume of pits, focusing on the roughness of the surface alone (Figure A-1 D&E).

<sup>&</sup>lt;sup>9</sup> an S-filter excludes from the surface information at the smallest scale. No specific S-filter was applied, considering the limitations of the instrument as equivalent to it.



Figure A-1: Three-dimensional images of the surface of one tooth (A) of Archosargus probatocephalus and excluded or analysed elements of the surface for different treatments (B-E). Original tooth surface (A), second order polynomial fitting plane (B) and dental surface once removed (C), removed surface after spline-filtering (D)and remaining surface (E). For all images x: 110.79µm, y: 146.07µm. Note the different heights of the original, filtered out and preserved surfaces.

## Between-treatments comparison

Several treatments were compared with a series of paired pairwise t-tests. The samples were considered as paired since the applied filtering and/or levelling techniques are different but the surface on which they are applied remains the same. A Holm-Bonferroni correction of the p-values was used in order to limit the family-wise error rate (probability of making one or several type 1 errors, or rejecting a true null hypothesis, when performing multiple tests). The null hypothesis for each test is that

"There is no significant difference between the two surface processing techniques" and the risk taken to reject it is  $\alpha = 0.05$ .

The results clearly show that no two techniques are equivalent one to another. It is noteworthy that the treatments for which there is the lowest number of different parameters are based on the same filtering techniques. Data from surfaces on which the Gaussian filter was applied after tilting or application of a  $2^{nd}$  order polynomial exhibit significant differences for 9 parameters. Use of the Spline filter with the same levelling techniques highlights only 7 differences.

Respectively 9 and 7 different parameters over "only" 24 still is an important number, as later investigations will show. In light of these results, there is no doubt that the treatments have a major impact on the values we get from the surfaces. I can only recommend that while comparing between samples from different institutions, care is taken to exchange the files describing the original surfaces prior to application of any treatment, or that the exact same treatments are applied to the different sets of data, if possible with the same software. Table A-1: Summary of the results of pairwise t-tests between treatments. Parameters are mentioned if a significant difference was found at  $\alpha = 0.05$ .

	Poly2	Poly8	Tilt+Gaussian	Tilt+Spline	Tilt+Wavelet	Poly2+Gaussian	Poly2+Spline
Poly8	Sq, Sv, Sds, Sal, Sdq, Ssc, Sdr, Vmp, Vmc, Vvc, Vvv, Spk, Sk, Svk, S5z, Sa						
Tilt+Gaussian	Sq, Ssk, Sku, Sp, Sv, Sz, Sds, Sal, Sdq, Sdr, Vmp, Vmc, Vvc, Vvv, Spk, Sk, Svk, Smr2, S5z, Sa	Ssk, Sku, Sp, Sz, Sdq, Ssc, Sdr, Vmp, Vmc, Vvc, Vvv, Spk, Sk, Svk, Smr1, Smr2, Sa					
Tilt+Spline	Sq, Sp, Sv, Sz, Sds, Str, Sal, Sdq, Sdr, Vmp, Vmc, Vvc, Vvv, Spk, Sk, Svk, S5z, Sa	Sq, Sp, Sv, Sz, Sds, Sal, Sdq, Ssc, Sdr, Vmp, Vmc, Vvc, Vvv, Spk, Sk, Svk, S5z, Sa	Sq, Ssk, Sku, Sv, Sz, Sds, Sal, Sdq, Sdr, Vmp, Vmc, Vvc, Vvv, Sk, Svk, Smr1, Smr2, S5z, Sa				

	Poly2	Poly8	Tilt+Gaussian	Tilt+Spline	Tilt+Wavelet	Poly2+Gaussian	Poly2+Spline
Tilt+Wavelet	Ssk, Sku, Sp, Sv, Sds, Str, Sal, Vmp, Vmc, Vvc, Spk, Sk, Svk, Smr2, Sa	Sq, Ssk, Sku, Sp, Sv, Sz, Sds, Sal, Sdq, Ssc, Vmp, Vmc, Vvc, Vvv, Sk, Svk, Smr2, S5z, Sa	Sq, Ssk, Sp, Sv, Sz, Sds, Str, Sal, Sdq, Sdr, Vmc, Vvc, Vvv, Spk, Sk, Svk, Smr1, Smr2, S5z, Sa	Sq, Ssk, Sku, Sv, Sz, Sds, Str, Sal, Sdq, Sdr, Vmp, Vmc, Vvc, Vvv, Spk, Sk, Svk, Smr2, S5z, Sa			
Poly2+Gaussian	Sq, Ssk, Sku, Sp, Sv, Sz, Sds, Sal, Sdq, Sdr, Vmp, Vmc, Vvc, Vvv, Spk, Sk, Svk, Smr2, S5z, Sa	Ssk, Sku, Sp, Sz, Sal, Sdq, Ssc, Sdr, Vmc, Vvc, Vvv, Sk, Svk, Smr1, Smr2, Sa	Sp, Sz, Sdr, Vmp, Vmc, Spk, Sk, Smr1, S5z	Sq, Ssk, Sku, Sp, Sv, Sz, Sds, Str, Sal, Sdq, Sdr, Vmp, Vmc, Vvc, Vvv, Spk, Sk, Svk, Smr1, Smr2, S5z, Sa	Sq, Ssk, Sv, Sz, Sds, Str, Sal, Sdq, Sdr, Vmc, Vvc, Vvv, Sk, Svk, Smr2, S5z, Sa		
Poly2+Spline	Sq, Ssk, Sp, Sv, Sz, Sds, Str, Sal, Sdq, Sdr, Vmp, Vmc, Vvc, Vvv, Spk, Sk, Svk, S5z, Sa	Sq, Sp, Sv, Sz, Sds, Sal, Sdq, Ssc, Sdr, Vmp, Vmc, Vvc, Vvv, Spk, Sk, Svk, S5z, Sa	Sq, Ssk, Sku, Sv, Sz, Sds, Str, Sal, Sdq, Sdr, Vmc, Vvc, Vvv, Sk, Svk, Smr1, Smr2, S5z, Sa	Sdq, Sdr, Vmp, Vmc, Vvc, Vvv, Sa	Sq, Ssk, Sku, Sv, Sz, Sds, Str, Sal, Sdq, Sdr, Vvc, Vvv, Spk, Sk, Svk, Smr2, S5z, Sa	Sq, Ssk, Sku, Sp, Sv, Sz, Sds, Str, Sal, Sdq, Sdr, Vmp, Vmc, Vvc, Vvv, Spk, Sk, Svk, Smr1, Smr2, S5z, Sa	
	Poly2	Poly8	Tilt+Gaussian	Tilt+Spline	Tilt+Wavelet	Poly2+Gaussian	Poly2+Spline
--------------	---------------------------------------------------------------------------------------	-------------------------------------------------------------------------	-------------------------------------------------------------------------------------------------	---------------------------------------------------------------------------------------	-------------------------------------------------------------------------	---------------------------------------------------------------------------------------------------	-------------------------------------------------------------------------------------------
oly2+Wavelet	Sq, Ssk, Sku, Sp, Sz, Sds, Sal, Sdq, Ssc, Sdr, Vmp, Vmc, Vvc, Vvv, Sk,	Sq, Ssk, Sku, Sv, Sds, Sal, Sdq, Ssc, Vmc, Vvc, Vvv, Sk,	Sq, Sp, Sv, Sz, Sds, Sal, Sdq, Sdr, Vmc, Vvc, Vvv, Spk, Svk, Smr1, Smr2, S5z, Sa	Sq, Ssk, Sku, Sp, Sv, Sz, Sds, Str, Sal, Sdq, Sdr, Vmp, Vmc, Vvc, Vvv,	Sq, Sp, Sv, Str, Sdq, Sdr, Vmc, Vvc, Vvv, Sk, Svk, Smr2, Sa	Sq, Sp, Sv, Sz, Sds, Sal, Sdq, Sdr, Vmp, Vmc, Vvc, Vvv, Spk, Svk, Smr1, Smr2, S5z, Sa	Sq, Ssk, Sku, Sp, Sv, Sz, Sds, Str, Sal, Sdq, Sdr, Vmc, Vvc, Vvv, Spk, Sk,
Ц	Smr1, Smr2, S5z. Sa	Svk, Smr2, S5z. Sa		Spk, Sk, Svk, Smr2, S5z, Sa			Svk, Smr2, S5z, Sa

#### Between-populations comparison

Since different treatments have a major impact on the calculated roughness parameters, one of them has to be selected before application for the remaining part of this work. In order to avoid any subjectivity in this artificial selection, dental surfaces from individuals of both populations of *Archosargus probatocephalus* after application of the different treatments were compared.

Comparisons were realised with a two-sample Welch t-test testing the null hypothesis "The parameter has the same value in both populations",  $\alpha = 0.05$ .

Table A-2: Summary of the results of t-tests between populations of Archosargus probatocephalus. Parameters are checked if a significant difference was found at  $\alpha = 0.05$ ., parameters showing no differences are omitted.

		Treatment						
Paramete r	Pol y2	Pol y8	Tilt + Gaussi an	Tilt + Spli ne	Tilt + Wave let	Poly2 + Gaussi an	Poly 2 + Spli ne	Poly2 + Wave let
Sq Sku Sal Sdq	X		X X	X X X	X X X	X X	X X X	X
Ssc Sdr Vm c Vv c Vv	Х	Х	Х	X X X X		Х	X X X X	
v Sk Svk Sm			Х	X X X	Х	Х	X X X X	X X
Sm r2 S5z Sa				X X	Х		X	

By far, application of spline-filtering provides the largest number of different parameters: 11 out of 24 for both treatments (with use of a 2<sup>nd</sup> order polynomial or by tilting). The next best are both treatments using the Gaussian filter and the tilting + wavelet filter treatment with only 4 out of 24 parameters found to be different between the two populations. Out of the 11 differences highlighted by the spline-filtering treatments, 10 are common between both of them: Sq, Sdq, Ssc, Sdr, Vmc, Vvc, Vvv, Sk, Svk, and Sa. the choice to keep applying the treatment using a 2<sup>nd</sup> order polynomial along with the spline filtering was based on two reasons:

- The parameter S5z which discriminates between the two populations in the Tilt + spline treatment is calculated based on 10 points only (the 5 highest peaks and 5 deepest valleys) which makes it more error-prone if but a few outliers on the surface are present.
- As the two samples were based on dental surfaces from teeth of similar sizes, the levelling technique should not have had a major influence on the data. If comparisons are to be done with fishes exhibiting significantly more or less curved dental surfaces, either because the teeth are broader, flatter or more cone-like, application of the 2<sup>nd</sup> order polynomial will get rid of any size-related effect on the curvature of the surface data.

# Dental microwear texture within individuals of Archosargus

Prior to further between-populations comparisons, the data was investigated to highlight any significant difference in microtexture between different parts of the dentition, testing the hypotheses:

- H0A: "Dental microtexture is the same whether the analysed teeth come from the dentary or the maxilla"
- H0B: "Dental microtexture is the same whether the analysed teeth come from the labial or the lingual tooth row"
- H0C: "The location of the tooth in the jaw (dentary/maxilla, labial/lingual) has no influence on the microtexture"

The hypotheses were tested with a two-way analysis of variance on data from the specimen SH-IR01 for the Indian River (herbivorous) population and SH-PC02 for the durophagous population as they have respectively more scanned teeth than the other specimens (respectively 16 and 19).

Table A-3: Summary of the results of anova on different parts of the dentition of Archosargus probatocephalus specimens SH-IR01 and SH-PC02. Parameters are ticked if a significant difference was found at  $\alpha = 0.05$ .

Parameter	SH-	SH-	SH-	SH-	SH-	SH-
	IR01	IR01	IR01	PC02	PC02	PC02
	H0A	H0B	H0C	H0A	H0B	H0C
Ssk	Х					
Sp					Х	
Sz					Х	
Sds					Х	
Sal						Х
Vmp				Х		
Vvc				Х		
Spk				Х	Х	
Sk				Х		

SH-PC02 shows more variability in its microtexture based on the position of the sampled teeth. The parameters Sp and Sz can be influenced by a rather limited number of outstanding points over the surface, hence the rejection of H0B is not as strongly supported as that of H0A, since the volumetric parameters Vmp and Vvc, as well as the parameters Spk and Sk are based on a large part of the three-dimensional data. Differences in the volume of preserved or removed material are particularly interesting since pitting of dental surfaces is usually associated with a hard-objects diet. Teeth from the dentary of SH-PC02 have less material removed from the core section (mean=288428.6  $\mu$ m<sup>3</sup>/mm<sup>2</sup>) than those on the maxilla (mean=340166.7  $\mu$ m<sup>3</sup>/mm<sup>2</sup>), but as this is associated with a difference in height of the section (0.6141429  $\mu$ m on the dentary and 0.7261667  $\mu$ m on the maxilla) caution must be exerted when interpreting results.

The results nevertheless suggest that the origin of the teeth has to be taken into account before comparing the two populations. The same three hypotheses have been tested for each population, including all the data available, making the assumptions that inter-individual variability would have no influence on the results.

Table A-4: Summary of the results of anova on different parts of the dentition of Archosargus probatocephalus for all specimens in each population. Parameters are ticked if a significant difference was found at  $\alpha = 0.05$ .

Parameter	IR	IR	IR	PC	PC	PC
	H0A	H0B	H0C	H0A	H0B	H0C
Sq				Х		
Sp				Х		
Sds		Х			Х	
Sal						Х
Sdq			Х	Х		
Ssc					Х	
Sdr			Х	Х		
Vmc				Х		
Vvc				Х		
Vvv				Х		

Parameter	IR H0A	IR H0B	IR H0C	PC H0A	PC H0B	PC H0C
Sk				X		
SVK				Х	V	
Smri				v	Λ	
Vvc/Vmc				Λ	X	

Provided the assumptions are not false, there is a difference in the data we get from the teeth from either the dentary or the maxilla in the Port Canaveral population. The results suggest that with this sample care must be taken to focus on only one part of the jaw when carrying further investigations.

The potential effect of the position factor now assessed, it was taken into account when comparing the two populations of *Archosargus probatocephalus* IR and PC testing the hypotheses:

- H0A: Dental microtexture from the maxilla-borne teeth is the same between the two populations
- H0B: Dental microtexture from the dentary-borne teeth is the same between the two populatios"

The hypotheses were tested with a one-way analysis of variance on data restricted to one tooth-bearing bone, for all specimens from each population.

Table A-5: Summary of the results of anova comparing populations of Archosargus probatocephalus. Parameters are ticked if a significant difference was found at  $\alpha = 0.05$ .

Parameter	H0A: Maxillas	H0B: Dentaries
Sq	Х	
Sdq	Х	
Sdr	Х	
Vmc	Х	
Vvc	Х	
Vvv	Х	
Sk	Х	
Svk	Х	

Parameter	H0A: Maxillas	H0B: Dentaries
Smr1	Х	
Sa	Х	
Vvc/Vmc	Х	
01 1 11 1	1 1 1 1 1 00	1 101 1 1 1

Clearly, maxilla-borne teeth record dietary differences significantly better than

dentary-borne teeth.

## Supplementary t-tests in extant fishes

Supplementary Welch-Satterwaithe t-tests were performed to test the hypothesis "Durophages exhibit the same dental microtexture" (Table A-6) and "a specialised durophage and a heavy grazer have similar microtextures" (Table A-7).

Table A-6: Summary of Welch-Satterwaithe t-tests comparing the two populations of durophagous Archosargus probatocephalus and lesser-worn Anarhichas lupus. Texture parameters showing a significant difference and the associated information highlighted in bold. O: test performed on the original, untransformed data; L: test performed on log-transformed data; R: test performed on rank-transformed data. df: degree of

freedom.

Parameter	Data	t-statistic	df	p-value
Sq	R	7.5669	41.399	< 0.0001
Sku	R	9.5326	42.388	< 0.0001
Sp	L	4.8829	18.413	0.0001
Sv	L	5.9907	17.61	< 0.0001
Sz	R	8.0077	38.648	< 0.0001
Sdq	R	8.1141	41.297	< 0.0001
Ssc	R	7.5934	38.451	< 0.0001
Sdr	R	6.4741	37.971	< 0.0001
Vmp	L	6.3449	17.413	< 0.0001
Vmc	R	4.3230	30.487	0.0001
Vvc	R	5.5982	31.008	< 0.0001
Vvv	L	4.1250	17.842	0.0006
Spk	R	10.5166	43.722	< 0.0001
Sk	R	4.8882	30.855	< 0.0001
Svk	L	4.8704	17.731	0.0001
Smr1	Ο	4.8370	22.621	< 0.0001
S5z	L	6.4575	17.092	< 0.0001
Sa	R	5.5479	32.804	< 0.0001
Vvc/Vmc	Ο	4.2762	23.943	0.0003

Table A-7: Summary of Welch-Satterwaithe t-tests comparing the two populations of herbivorous Archosargus probatocephalus and lesser-worn Anarhichas lupus. Texture parameters showing a significant difference and the associated information highlighted in bold. O: test performed on the original, untransformed data; L: test performed on

10	a_transformed	data · R · tost	nerformed or	n rank_transformed	data df. doaroo	2 of
$\iota \upsilon$	z mansjormea	$uuuu, \pi$ . $uusi$	perjormed or	<i>i rank iransjorni</i> ca	<i>uuiu. uj. uczrcc</i>	, UJ

Parameter	Data	t-statistic	df	p-value
Sq	R	8.0592	41.614	< 0.0001
Sku	R	4.7250	41.926	< 0.0001
Sp	L	5.1254	21.116	< 0.0001
Sv	R	7.0610	41.689	< 0.0001
Sz	R	6.8319	40.486	< 0.0001
Sdq	R	9.0536	42.721	< 0.0001
Ssc	R	6.8795	36.369	< 0.0001
Sdr	R	8.1556	42.143	< 0.0001
Vmp	R	9.6688	42.899	< 0.0001
Vmc	R	5.4664	33.117	< 0.0001
Vvc	R	6.4381	32.955	< 0.0001
Vvv	R	6.1210	36.4	< 0.0001
Spk	R	9.4287	42.417	< 0.0001
Ŝk	R	5.3307	32.866	< 0.0001
Svk	R	6.7862	36.97	< 0.0001
Smr1	Ο	3.3174	20.887	0.0033
S5z	L	5.9812	21.583	< 0.0001
Sa	R	6.6345	36.575	< 0.0001
Vvc/Vmc	0	2.7318	20.425	0.0127

freedom.

# **Supplementary t-test in Pycnodus**

Only 2 individuals of *Pycnodus* are present in the fossil sample and one of them is represented by 2 points, which made testing for normality impossible. Normality has been considered as a met assumption.

Table B-1: welch t-tests for Pycnodus. Df: degrees of freedom. Parameters showing asignificant difference at 5% risk are highlighted in bold.

Parameter	t-statistic	df	p-value
Sq	-4.1266	3.201	0.0278
Ssk	-0.3738	1.058	0.7696
Sku	-2.3258	2.835	0.1076
Sp	-2.9421	4.070	0.04141
$\mathbf{Sv}$	0.2320	3.780	0.8285
Sz	-0.3475	3.254	0.7495
Sds	-0.8223	4.961	0.4486
Str	0.9585	1.036	0.5090
Sdq	-1.5947	4.443	0.1789
Ssc	1.5423	3.460	0.2087
Sdr	-1.4903	4.048	0.2096
Vmp	-1.9764	1.106	0.2791
Vmc	-1.7700	2.202	0.2071
Vvc	-3.234	4.008	0.0318
Vvv	-2.4851	1.196	0.2094
Spk	-2.3862	1.212	0.2161
Šk	-1.1510	2.070	0.3653
Svk	-1.8457	1.231	0.2786
Smr1	-3.2347	1.071	0.1775
Smr2	5.9685	2.850	0.0109
S5z	-1.1871	4.541	0.2936
Sa	-2.7172	2.425	0.0918
Vvc/Vmc	-2.0397	1.054	0.2801

Four parameters showed a significant difference in the t-test: Sq, Sp, Vvc and

Smr2. Since the samples are from different species, stratigraphic and geographic origins, whether the differences are the result of mechanical or environmental constraints cannot be demonstrated until a larger sample is analysed.

#### Elliptic Fourier analysis of dental outlines in Pycnodontiformes

"Without the wonderful element of doubt, the doorway through which truth passes would be tightly shut..." (Anton Szandor Lavey)

In Pycnodontiformes, dental characters are sometimes the only information available as the more robust tooth-bearing bones are found in isolation. It has been demonstrated that restricting the anatomical information to the dental characters resulted in phylogenetic hypotheses with lesser resolution. The phylogenetic relationships within the pycnodonts as described by are not strongly affected by the exclusion of characters based on tooth morphology.

A reasonable amount of doubt arises concerning the identification of the specimens as provided by museums. Information is often lacking whether the taxonomic status of the specimen has been reviewed in light of the latest works. In addition, the specimens may not display the diagnostic features which would allow attributing them to a species.

#### Material and methods

In order to check the provided taxonomic information, investigations were carried out on a series of photographs of available fossils of prearticular and vomer of pycnodonts labelled as *Gyrodus*, *Eomesodon* and *Microdon* (=*Proscinetes*).

Specimens were photographed using a Canon camera, ensuring that the largest tooth was oriented horizontally. For each specimen, coordinates for 256 points representing the outline of the largest tooth (on the main tooth-row) were recorded using softwares tpsDig (version 2.16) and tpsUtil (version 1.49).Outlines were drawn over digitized pictures in tpsDig with a Bamboo Wacom pen tablet. Recorded coordinates were sent to the software Past for procrustes fitting and elliptic fourier treatment (set to invariant to rotation and starting position). Outlines of teeth from the vomers (n=21) and prearticulars (n=19) were treated separately.

The results of this treatment (120 variables per tooth) were subsequently analysed in R (version 2.15.0) with the packages robustbase, pcaPP, mclust vegan and MASS. A robust Principal Components Analysis (using the method "mad": based on median average distance) was performed on each dataset separately, the number of axes necessary to express most of the variance calculated by the broken stick method (3 for vomers and 1 for prearticulars) and the scores for those principal components were used to search for structure in the data with the Mclust function. This technique does not rely on any a priori defined categories, henceforth clusters represent groups of fossils with shared morphologies unbiased by previous phylogenetic hypotheses.

#### Results

#### Prearticulars

82.00% of the variance is expressed along the first 3 axes: PC1: 59.57%, PC2: 11.84%, PC3:10.59%. There is a clear separation between Gyrodontoidei (*Gyrodus*) and Pycnodontoidei (*Eomesodon* and *Proscinetes*) with a combination of PC1 and PC2 with the exception of 2 specimens: OR41391 and P6749, the former was labelled as Gyrodus coccoderma but plots closer to the Pycnodontoidei. P6749 is labelled as *Microdon* (*=Proscinetes*). None of the specimens carry the ornamentation typical of Gyrodontoidei, so OR41391 might represent a misidentified specimen. The position of P6749 in the morphospace appears to be the result of a non-consistent orientation of the specimen. The automatic clustering technique found 8 clusters, all bar 2 of them contain only 2 individuals.

Vomers

The PCA expressed 75.15% of the variance over the first 3 axes (PC1: 46.84%, PC2: 17.97%, PC3: 10.34%). The automatic clustering highlighted 2 groups, one with most of the individuals and the other one with only *Proscinetes quincucialis*. Just as for the prearticulars, the separation between Pycnodontoidei and Gyrodontoidei is clear along PC1. Here PC3 provides a clearer separation than for prearticulars. The major discrepancy between samples of *Proscinetes* comes from the very elongated teeth in *P.radiatus* as compared to *P. quincucialis*. Overlapping *Eomesodon* and *Proscinetes* sample can easily be told apart by the very typical alternation of teeth in *Proscinetes* (1 large central, 2 small more lateral, repeated along the main tooth row)

#### **Conclusion and perspectives**

A combination of 3 principal components axes is sufficient to discriminate between individual specimens between sub-orders and even genera for prearticulars. For vomers the dental outlines combined with anatomical peculiarities of Proscinetes also allow to tell genera apart.

Elliptic fourier analysis and morphometrics sensu lato have the potential to provide a tool to assign incomplete fossils to a taxonomic category. The next step will be to re-photograph misaligned specimens, add more material and compare the results with that which can be gathered using landmarks or continuous characters with the software TNT: <u>http://www.zmuc.dk/public/phylogeny/tnt/</u>



Figure C-1: Principal components analysis based on elliptic Fourier harmonics based on outlines of teeth from prearticulars (A, B) or vomers (C, D) of pycnodonts. E: Vomer of Proscinetes quincucialis (specimen P6170); F: vomer of P.radiatus (specimen 21974) to illustrate the different dental morphologies but similar organisations. Scale bars are 1 cm.



Automatic clusters in ecospaces from extant and fossil fishes

Figure D-1: Principal Components Analysis describing ecospaces of A: extant fishes;
B: fossil fishes; C: extant and fossil groups. Hulls represent the automatically
calculated clusters. IR: herbivorous generalist sheepshead seabream; PC: durophagous
generalist sheepshead seabream.

### References

- Aberhan, M., Kiessling, W., and Fürsich, F., 2006, Testing the role of biological interactions in the evolution of mid-Mesozoic marine benthic ecosystems: Paleobiology, v. 32, no. 2, p. 259-277.
- Aguirre, J., Perfectti, F., and Braga, J., 2010, integrating phylogeny, molecular clocks, and the fossil record in the evolution of coralline algae (Corallinales and Sporolithales, Rhodophyta): Paleobiology, v. 36, no. 4, p. 519-533.
- Ahlberg, P. E., Smith, M., and Johanson, Z., 2006, Developmental plasticity and disparity in early dipnoan (lungfish) dentitions: Evolution & Development, v. 8, no. 4, p. 331-349.
- Alfaro, M. E., and Wainwright, P., 2006, Ubiquity of many-to-one mapping in functional traits: examples and evolutionary implications: Integrative and Comparative Biology, v. 46, no. Journal Article, p. E2-E2.
- Alwany, M. A., Thaler, E., and Stachowitsch, M., 2009, Parrotfish bioerosion on
  Egyptian Red Sea reefs: Journal of experimental marine biology and ecology, v.
  371, no. 2, p. 170-176.
- Anderson, P. S., 2009, Biomechanics, functional patterns, and disparity in late Devonian arthrodires: Paleobiology, v. 35, no. 3, p. 321-342.
- -, 2010, Using linkage models to explore skull kinematic diversity and functional convergence in arthrodire placoderms: J Morphol, v. 271, no. 8, p. 990-1005.
- Anderson, P. S., Friedman, M., Brazeau, M. D., and Rayfield, E. J., 2011, Initial radiation of jaws demonstrated stability despite faunal and environmental change: Nature, v. 476, no. 7359, p. 206-209.

- Anderson, P. S., and Westneat, M., 2009, A biomechanical model of feeding kinematics for Dunkleosteus terrelli (Arthrodira, Placodermi): Paleobiology, v. 35, no. 2, p. 251-269.
- Anderson, P. S. L., 2008, Shape variation between arthrodire morphotypes indicates possible feeding niches: Journal of Vertebrate Paleontology, v. 28, no. 4, p. 961-969.
- Anderson, P. S. L., and Westneat, M. W., 2007, Feeding mechanics and bite force modelling of the skull of Dunkleosteus terrelli, an ancient apex predator: Biol Lett, v. 3, no. 1, p. 77-80.
- Antonucci, F., Costa, C., Aguzzi, J., and Cataudella, S., 2009, Ecomorphology of Morpho-Functional Relationships in the Family of Sparidae: A Quantitative Statistic Approach: J Morphol, v. 270, no. 7, p. 843-855.
- Baines, D., 2010, Tooth microwear in fishes [Ph.D: University of Leicester, 219 p.
- Baumiller, T. K., Salamon, M. A., Gorzelak, P., Mooi, R., Messing, C. G., and Gahn, F.
  J., 2010, Post-Paleozoic crinoid radiation in response to benthic predation
  preceded the Mesozoic marine revolution: Proc Natl Acad Sci U S A, v. 107, no.
  13, p. 5893-5896.
- Belles-Isles, M., Vézina, D., and Vadet, A., 1987, Nouveau matériel de Placodermes (Ptyctodontidae) du Dévonien du Boulonnais (France): Annales de la Société Géologique du Nord, v. CVI, no. Journal Article, p. 275.
- Bello, S. M., Parfitt, S. A., and Stringer, C., 2009, Quantitative micromorphological analyses of cut marks produced by ancient and modern handaxes: Journal of Archaeological Science, v. 36, no. 9, p. 1869-1880.

- Bello, S. M., and Soligo, C., 2008, A new method for the quantitative analysis of cutmark micromorphology: Journal of Archaeological Science, v. 35, no. 6, p. 1542-1552.
- Bellwood, D. R., 2003, Origins and escalation of herbivory in fishes: a functional perspective: Paleobiology, v. 29, no. 1, p. 71-83.
- Bellwood, D. R., and Choat, J., 1990, A functional analysis of grazing in parrotfishes (family Scaridae): the ecological implications: Environmental Biology of Fishes, v. 28, p. 189-214.
- Bellwood, D. R., and Fulton, C., 2008, Sediment-mediated supression of herbivory on coral reefs: decreasing resilience to rising sea levels and climate change:Limnology and Oceanography, v. 53, no. 6, p. 2695-2701.
- Bellwood, D. R., Klanten, S., Cowman, P. F., Pratchett, M. S., Konow, N., and van Herwerden, L., 2010, Evolutionary history of the butterflyfishes (f: Chaetodontidae) and the rise of coral feeding fishes: J Evol Biol, v. 23, no. 2, p. 335-349.
- Bellwood, D. R., Wainwright, P. C., Fulton, C. J., and Hoey, A. S., 2006, Functional versatility supports coral reef biodiversity: Proc Biol Sci, v. 273, no. 1582, p. 101-107.
- Bigerelle, M., Van Gorp, A., and Iost, A., 2008, Multiscale roughness analysis in injection-molding process: Polymer Engineering & Science, v. 48, no. 9, p. 1725-1736.
- Binning, S. A., and Chapman, L. J., 2010, Is intraspecific variation in diet and morphology related to environmental gradients? Exploring Liem's paradox in a cichlid fish: Integrative Zoology, v. 5, no. 3, p. 241-255.

- Binning, S. A., Chapman, L. J., and Cosandey-Godin, A., 2009, Specialized morphology for a generalist diet: evidence for Liem's Paradox in a cichlid fish: J Fish Biol, v. 75, no. 7, p. 1683-1699.
- Binning, S. A., Chapman, L. J., and Dumont, J., 2010, Feeding and breathing: trait correlations in an African cichlid fish: Journal of zoology, p. no-no.
- Blateyron, F., 2006, New 3D Parameters and Filtration Techniques for Surface Metrology, Proceedings of the JSPE, March 2006 (Digital Surf, France).
- Blieck, A., Clement, G., and Streel, M., 2010, The biostratigraphical distribution of earliest tetrapods (Late Devonian): a revised version with comments on biodiversification: Geological Society, London, Special Publications, v. 339, no. 1, p. 129-138.
- Blieck, A., Lelièvre, H., Delsate, D., and Godefroid, J., 1998, Les plus anciens vertébrés du Grand-Duché de Luxembourg: des arthrodires Brachythoraci (Placodermi: Dévonien inférieur): Comptes Rendus de l'Académie des Sciences de Paris, Earth and Planetary Sciences, v. 327, p. 203-210.
- Bocaege, E., Humphrey, L. T., and Hillson, S., 2010, Technical Note: A New Three-Dimensional Technique for High Resolution Quantitative Recording of Perikymata: American Journal of Physical Anthropology, v. 141, no. 3, p. 498-503.
- Bonaldo, R. M., and Bellwood, D. R., 2009, Dynamics of parrotfish grazing scars: Marine Biology, v. 156, no. 4, p. 771-777.
- Bonaldo, R. M., and Bellwood, D. R., 2010, Spatial variation in the effects of grazing on epilithic algal turfs on the Great Barrier Reef, Australia: Coral Reefs, v. 30, no. 2, p. 381-390.

- Brazeau, M. D., 2009, The braincase and jaws of a Devonian 'acanthodian' and modern gnathostome origins: Nature, v. 457, no. 7227, p. 305-308.
- Brice, D., 1988, Le Dévonien de Ferques, Bas-Boulonnais (N. France). Paléontologie sédimentologie stratigraphie tectonique. Collection "Biostratigraphie du Paléozoïque", Brest, Université de Bretagne Occidentale, v. Book, Edited, 522 p.:
- Brigaud, B., Pucéat, E., Pellenard, P., Vincent, B., and Joachimski, M. M., 2008,
  Climatic fluctuations and seasonality during the Late Jurassic (Oxfordian–Early Kimmeridgian) inferred from δ18O of Paris Basin oyster shells: Earth and Planetary Science Letters, v. 273, no. 1-2, p. 58-67.
- Burkepile, D. E., and Hay, M. E., 2010, Impact of herbivore identity on algal succession and coral growth on a Caribbean reef: PLOS One, v. 5, no. 1, p. e8963.
- Calandra, I., 2011, Tribology of dental enamel facets of ungulates and Primates (Mammalia): Tracing tooth-food interaction through 3D enamel microtexture analyses [Ph.D: University of Hamburg, 172 p.
- Carr, A., Kemp, A., Tibbetts, I., Truss, R., and Drennan, J., 2006a, microstructure of pharyngeal tooth enameloid in the parrotfish Scarus rivulatus (Pisces: Scaridae): Journal of Microscopy, v. 221, no. 1, p. 8-16.
- Carr, A., Tibbetts, I. R., Kemp, A., Truss, R., and Drennan, J., 2006b, Inferring parrotfish (Teleostei: Scaridae) pharyngeal mill function from dental morphology, wear, and microstructure: J Morphol, v. 267, no. 10, p. 1147-1156.
- Carr, R. K., and Hlavin, W. J., 2010, Two new species of Dunkleosteus Lehman, 1956, from the Ohio Shale Formation (USA, Famennian) and the Kettle Point Formation (Canada, Upper Devonian), and a cladistic analysis of the

Eubrachythoraci (Placodermi, Arthrodira): Zoological Journal of the Linnean Society, v. 159, no. 1, p. 195-222.

- Castillo-Rivera, M., Hernández, R., and Salgado-Ugarte, I., 2007, Hábitos de alimento de juveniles y adultos de Archosargus probatocephalus (Teleostei: Sparidae) en un estuario tropical de Veracruz: Hidrobiológica, v. 17, p. 119-126.
- Cavin, L., Forey, P. L., and Lécuyer, C., 2007, Correlation between environment and Late Mesozoic ray-finned fish evolution: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 245, no. 3-4, p. 353-367.
- Cayeux, L., 1888, Excursion géologique faite dans le Boulonnais, du 21 au 25 Mai par les élèves de la faculté des sciences de Lille sous la direction de M. Gosselet, professeur: Annales de la Société Géologique du Nord, v. XV, no. Journal Article, p. 319-341.
- Choat, J. H., Robbins, W., and Clements, K., 2004, The trophic status of herbivorous fishes on coral reefs: Marine Biology, v. 145, no. 3.
- Christiansen, N. A., Ward, S., Harii, S., and Tibbetts, I. R., 2008, Grazing by a small fish affects the early stages of a post-settlement stony coral: Coral Reefs, v. 28, no. 1, p. 47-51.
- Chuwen, B. M., Platell, M. E., and Potter, I. C., 2007, Dietary compositions of the sparid Acanthopagrus butcheri in three normally closed and variably hypersaline estuaries differ markedly: Environmental Biology of Fishes, v. 80, no. 4, p. 363-376.
- Clements, K. D., Raubenheimer, D., and Choat, J. H., 2009, Nutritional ecology of marine herbivorous fishes: ten years on: Functional Ecology, v. 23, no. 1, p. 79-92.

- Cochran-Biederman, J. L., and Winemiller, K. O., 2010, Relationships among habitat, ecomorphology and diets of cichlids in the Bladen River, Belize: Environmental Biology of Fishes, v. 88, no. 2, p. 143-152.
- Collar, D., and Wainwright, P., 2006, Discordance between morphological and mechanical diversity in the feeding mechanism of centrarchid fishes: Evolution, v. 60, no. 12, p. 2575-2584.
- Colombié, C., and Rameil, N., 2006, Tethyan-to-boreal correlation in the Kimmeridgian using high-resolution sequence stratigraphy (Vocontian Basin, Swiss Jura, Boulonnais, Dorset): International Journal of Earth Sciences, v. 96, no. 3, p. 567-591.
- Conover, W., and Iman, R., 1981, Rank Transformations as a Bridge between Parametric and Nonparametric Statistics: American Statistician, v. 35, no. 3, p. 124-129.
- Cutwa, M., and Turingan, R., 2000, Intralocality variation in feeding biomechanics and prey use in Archosargus probatocephalus (Teleostei, Sparidae), with implications for the ecomorphology of fishes: Environmental Biology of Fishes, v. 59, no. 2, p. 191-198.
- Cvitanovic, C., and Bellwood, D. R., 2008, Local variation in herbivore feeding activity on an inshore reef of the Great Barrier Reef: Coral Reefs, v. 28, no. 1, p. 127-133.
- Daegling, D., 1999, Terrestrial foraging and dental microwear in Papio ursinus: Primates, v. 40, no. 4, p. 559-572.
- Darras, L. P. G., Derycke, C., Blieck, A., and Vachard, D., 2008, The oldest holocephalan (Chondrichthyes) from the Middle Devonian of the Boulonnais (Pas-de-Calais, France): Comptes Rendus Palevol, v. 7, no. 5, p. 297-304.

- Delsate, D., and Kriwet, J. r., 2004, Late Triassic pycnodont fish remains (Neopterygii, Pycnodontiformes) from the Germanic basin: Eclogae Geologicae Helvetiae, v.
  97, no. 2, p. 183-191.
- Denison, R., 1978, Placodermi. In Schultze, H.-P., ed. Handbook of Paleoichthyology, vol.2, 128 p., 94 fig: Stuttgart-New york, G. Fischer Verlag.
- Derycke, C., Brice, D., Blieck, A., and Mouravieff, N., 1995, Upper Givetian and Frasnian ichthyoliths from Bas-Boulonnais (Pas-de-Calais, France): Preliminary records: Bulletin du Museum National d'Histoire Naturelle Section C Sciences de la Terre Paleontologie Geologie Mineralogie, v. 17, no. 1-4, p. 487-511.
- Dietl, G. P., and Vega, F. J., 2008, Specialized shell-breaking crab claws in Cretaceous seas: Biol Lett, v. 4, no. 3, p. 290-293.
- Dineley, D. L., and Metcalf, S. J., 1999, Fossil fishes of Great Britain, Joint Nature Conservation Committee.
- Donoghue, P., and Smith, M., 2001, The anatomy of Turinia pagei (Powrie), and the phylogenetic status of the Thelodonti: Transactions of the Roayl Society of Edinburgh: Earth Sciences, v. 92, p. 15-37.
- Dupret, V., Zhu, M. I. N., and Wang, J.-Q., 2009, The morphology of Yujiangolepis
  liujingensis(Placodermi, Arthrodira) from the Pragian of Guangxi (south China)
  and its phylogenetic significance: Zoological Journal of the Linnean Society, v.
  157, no. 1, p. 70-82.
- Dutertre, A. P., 1929a, Découverte d'ossements de poissons dans le Dévonien du Boulonnais: Comptes-Rendus des séances de l'Académie des Sciences, v. CVIII, no. Journal Article, p. 1116.
- -, 1929b, Ossements de poissons découverts dans le Dévonien du Boulonnais: Annales de la Société Géologique du Nord, v. LIV, no. Journal Article, p. 80.

- -, 1930, Les poissons dévoniens du Boulonnais: Bulletin de la Société Géologique de France, v. XXX, no. 4, p. 571-587.
- El-Zaatari, S., 2008, Occlusal microwear texture analysis and the diets of historical/prehistoric hunter-gatherers: International Journal of Osteoarchaeology, p. n/a-n/a.
- Estebaranz, F., Martinez, L. M., Galbany, J., Turbon, D., and Perez-Perez, A., 2009, Testing hypotheses of dietary reconstruction from buccal dental microwear in Australopithecus afarensis: J Hum Evol, v. 57, no. 6, p. 739-750.
- Evans, A. R., and Sanson, G. D., 2006, Spatial and functional modeling of carnivore and insectivore molariform teeth: J Morphol, v. 267, no. 6, p. 649-662.
- Ezard, T. H., Pearson, P. N., and Purvis, A., 2010, Algorithmic approaches to aid species' delimitation in multidimensional morphospace: BMC Evol Biol, v. 10, p. 175.
- Fehri-Bedoui, R., Mokrani, E., and Ben Hassine, O. K., 2009, Feeding habits of <i>Pagellus acarne</i> (Sparidae) in the Gulf of Tunis, central Mediterranean: Scientia Marina, v. 73, no. 4, p. 667-678.
- Ferreira, C., Floeter, S., Gasparini, J., Ferreira, B., and Joyeux, J., 2004, Trophic structure patterns of Brazilian reef fishes: a latitudinal comparison: Journal of Biogeography, v. 31, p. 1093-1106.
- Ferry-Graham, L., and Wainwright, P., 2002, Using functional morphology to examine the ecology and evolution of specialization: Integrative and Comparative Biology, v. 42, no. 2, p. 265-277.
- Finnegan, S., McClain, C., Kosnik, M., and Payne, J., 2011, Escargots through time: an energetic comparison of marine gatropod assemblages before and after the Mesozoic Marine Revolution: Paleobiology, v. 37, no. 2, p. 252-269.

- Fraley, C., and Raftery, A., 2010, mclust: Model-Based Clustering / Normal Mixture Modeling. R package version 3.4.1.
- Francini, R., Ferreira, C., and Coni, E., 2008, Live coral predation by parrotfishes
  (Perciformes : Scaridae) in the Abrolhos Bank, eastern Brazil, with comments on the classification of species into functional groups: Neotropical Ichthyology, v. 6, no. 2, p. 191-200.
- Fraser, D., and Theodor, J. M., 2011, Comparing ungulate dietary proxies using discriminant function analysis: J Morphol, v. 272, no. 12, p. 1513-1526.
- Friedman, M., 2009, Ecomorphological selectivity among marine teleost fishes during the end-Cretaceous extinction: Proc Natl Acad Sci U S A, v. 106, no. 13, p. 5218-5223.
- Friedman, M., Shimada, K., Martin, L. D., Everhart, M. J., Liston, J., Maltese, A., and Triebold, M., 2010, 100-million-year dynasty of giant planktivorous bony fishes in the Mesozoic seas: Science, v. 327, no. 5968, p. 990-993.
- Gai, Z., Donoghue, P. C., Zhu, M., Janvier, P., and Stampanoni, M., 2011, Fossil jawless fish from China foreshadows early jawed vertebrate anatomy: Nature, v. 476, no. 7360, p. 324-327.
- Galbany, J., Estebaranz, F., Martinez, L. M., Romero, A., De Juan, J., Turbon, D., and Perez-Perez, A., 2006, Comparative analysis of dental enamel polyvinylsiloxane impression and polyurethane casting methods for SEM research: Microsc Res Tech, v. 69, no. 4, p. 246-252.
- Galle, A., and Mikulas, R., 2003, Evidence of predation on the rugose Coral Calceola sandalina (Devonian, Czech Republic): Ichnos, v. 10, no. 1, p. 41-45.
- Gill, t., 1911, Notes on the structure and habits of the wolffishes: Proceedings of the United States National Museum, v. 39, no. 1782, p. 157-187.

- Gillis, J. A., and Donoghue, P. C., 2007, The homology and phylogeny of chondrichthyan tooth enameloid: J Morphol, v. 268, no. 1, p. 33-49.
- Goatley, C., Bellwood, D., and Bellwood, O., 2010, Fishes on coral reefs: changing roles over the past 240 million years: Paleobiology, v. 36, no. 3, p. 415-427.
- Goillot, C., Blondel, C., and Peigné, S., 2009, Relationships between dental microwear and diet in Carnivora (Mammalia) — Implications for the reconstruction of the diet of extinct taxa: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 271, no. 1-2, p. 13-23.
- Gorzelak, P., and Salamon, M. A., 2009, Signs of Benthic Predation on Late Jurassic Stalked Crinoids, Preliminary Data: Palaios, v. 24, no. 1, p. 70-73.
- Gorzelak, P. L., Rakowicz, L., Salamon, M. A., and Szrek, P., 2011, Inferred placoderm bite marks on Devonian crinoids from Poland: Neues Jahrbuch für Geologie und Paläontologie - Abhandlungen, v. 259, no. 1, p. 105-112.
- Goujet, D., and Young, G., 2004, Placoderm anatomy and phylogeny: new insights, *in* Arratia, G., Wilmsen, M., and Cloutier, R., eds., Recent advances in the origin and early radiation of vertebrates, p. 109-126.
- Green, J. L., 2009, Dental microwear in the orthodentine of the Xenarthra (Mammalia) and its use in reconstructing the palaeodiet of extinct taxa: the case study of Nothrotheriops shastensis (Xenarthra, Tardigrada, Nothrotheriidae): Zoological Journal of the Linnean Society, v. 156, no. 1, p. 201-222.
- Grine, F., Ungar, P., and Teaford, M., 2002, Error rates in dental microwear quantification using scanning electron microscopy: Scanning, v. 24, p. 144-153.
- Grubich, J. R., 2005, Disparity between Feeding Performance and Predicted MuscleStrength in the Pharyngeal Musculature of Black Drum, Pogonias cromis(Sciaenidae): Environmental Biology of Fishes, v. 74, no. 3-4, p. 261-272.

- Gschwandtner, M., and Filzmoser, P., 2009, mvoutlier: Multivariate outlier detection based on robust methods. R package version 1.4.
- Harborne, A., Renaud, P., Tyler, E., and Mumby, P., 2009, Reduced density of the herbivorous urchin Diadema antillarum inside a Caribbean marine reserve linked to increased predation pressure by fishes: Coral Reefs, v. 28, no. 3, p. 783-791.
- Harper, E. M., 2003, Assessing the importance of drilling predation over the Palaeozoic and Mesozoic: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 201, no. 3-4, p. 185-198.
- Hautmann, M., 2004, Early Mesozoic evolution of alivincular bivalve ligaments and its implications for the timing of the 'Mesozoic marine revolution': Lethaia, v. 37, no. 2, p. 165-172.
- Hernandez, L., and Motta, P., 1997, Trophic consequences of differential performance: ontogeny of oral jaw-crushing performance in the sheepshead, Archosargus probatocephalus (Telostei, Sparidae): Journal of the Zoological Society of London, v. 243, p. 737-756.
- Hoey, A. S., and Bellwood, D. R., 2009a, Among-habitat variation in herbivory onSargassum spp. on a mid-shelf reef in the northern Great Barrier Reef: MarineBiology, v. 157, no. 1, p. 189-200.
- -, 2009b, Limited Functional Redundancy in a High Diversity System: Single Species Dominates Key Ecological Process on Coral Reefs: Ecosystems, v. 12, no. 8, p. 1316-1328.
- Hoey, A. S., Bellwood, D. R., and Barnett, A., 2012, To feed or to breed: morphological constraints of mouthbrooding in coral reef cardinalfishes: Proc Biol Sci, v. 279, no. 1737, p. 2426-2432.

- Huber, D. R., Dean, M. N., and Summers, A. P., 2008, Hard prey, soft jaws and the ontogeny of feeding mechanics in the spotted ratfish Hydrolagus colliei: J R Soc Interface, v. 5, no. 25, p. 941-952.
- Huber, D. R., Eason, T. G., Hueter, R. E., and Motta, P. J., 2005, Analysis of the bite force and mechanical design of the feeding mechanism of the durophagous horn shark Heterodontus francisci: J Exp Biol, v. 208, no. Pt 18, p. 3553-3571.
- Huckins, C., 1997, Functional linkages among morphology, feeding performance, diet, and competitive ability in molluscivorous sunfish: Ecology, v. 78, no. 8, p. 2401-2414.
- Hulsey, C., 2006, Function of a key morphological innovation: fusion of the cichlid pharyngeal jaw: Proc Biol Sci, v. 273, no. 1587, p. 669-675.
- Hulsey, C., Garcia de Leon, F., and Rodiles-Hernandez, R., 2006, Micro- and macroevolutionary decoupling of cichlid jaws: a test of Liem's key innovation hypothesis: Evolution, v. 60, no. 10, p. 2096-2109.
- Hulsey, C. D., Roberts, R. J., Lin, A. S., Guldberg, R., and Streelman, J. T., 2008, Convergence in a mechanically complex phenotype: detecting structural adaptations for crushing in cichlid fish: Evolution, v. 62, no. 7, p. 1587-1599.
- Huntley, J. W., and Kowalewski, M., 2007, Strong coupling of predation intensity and diversity in the Phanerozoic fossil record: Proc Natl Acad Sci U S A, v. 104, no. 38, p. 15006-15010.
- Ishiyama, M., and Teraki, Y., 1990, The fine structure and formation of hypermineralized petrodentine in the tooth plate of extant lungfish (Lepidosiren paradoxa and Protopterus sp.): Archives of Histology and Cytology, v. 53, no. 3, p. 307-321.

- Janvier, P., 1996, Early vertebrates, Oxford monographs on geology and geophysics, New York, Oxford university press inc., v. Book, Whole, 393 p.:
- Janvier, P., 2009, Les premiers vertébrés et les premières étapes de l'évolution du crâne: Comptes Rendus Palevol, v. 8, no. 2-3, p. 209-219.
- Johanson, Z., and Smith, M. M., 2005, Origin and evolution of gnathostome dentitions: a question of teeth and pharyngeal denticles in placoderms: Biological Reviews, v. 80, no. 2, p. 303-345.
- Jones, D., Hartley, J., Frisch, G., Purnell, M., and Darras, L., 2012, Non-destructive, safe removal of nonductivemetal coatings from fossils: a new solution: Palaeontologia electronica, v. 15, no. 2.
- Joomun, S. C., Hooker, J. J., and Collinson, M. E., 2008, Dental wear variation and implications for diet: An example from Eocene perissodactyls (Mammalia): Palaeogeography, Palaeoclimatology, Palaeoecology, v. 263, no. 3-4, p. 92-106.
- Kaiser, T. M., 2003, The dietary regimes of two contemporaneous populations of Hippotherium primigenium (Perissodactyla, Equidae) from the Vallesian (Upper Miocene) of Southern Germany: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 198, no. 3-4, p. 381-402.
- Kaiser, T. M., and Brinkmann, G., 2006, Measuring dental wear equilibriums—the use of industrial surface texture parameters to infer the diets of fossil mammals:
  Palaeogeography, Palaeoclimatology, Palaeoecology, v. 239, no. 3-4, p. 221-240.
- Kallaste, T., and Nemliher, J., 2005, Apatite varieties in extant and fossil vertebrate mineralized tissues: Journal of Applied Crystallography, v. 38, no. Journal Article, p. 587-594.

- Kear, B. P., 2006, First gut contents in a Cretaceous sea turtle: Biol Lett, v. 2, no. 1, p. 113-115.
- Kemp, A., 2001, Petrodentine in derived dipnoan tooth plates: Journal of Vertebrate Paleontology, v. 21, no. 3, p. 422-437.

Kerschbaumer, M., Postl, L., Koch, M., Wiedl, T., and Sturmbauer, C., 2011,
Morphological distinctness despite large-scale phenotypic plasticity--analysis of wild and pond-bred juveniles of allopatric populations of Tropheus moorii:
Naturwissenschaften, v. 98, no. 2, p. 125-134.

- King, T., Andrews, P., and Boz, B., 1999, Effect of taphonomic processes on dental microwear: American Journal of Physical Anthropology, v. 108, p. 369-373.
- Knudsen, R., Primicerio, R., Amundsen, P. A., and Klemetsen, A., 2010, Temporal stability of individual feeding specialization may promote speciation: J Anim Ecol, v. 79, no. 1, p. 161-168.
- Kolmann, M. A., and Huber, D. R., 2009, Scaling of feeding biomechanics in the horn shark Heterodontus francisci: ontogenetic constraints on durophagy: Zoology (Jena), v. 112, no. 5, p. 351-361.
- Komiya, T., Fujita, S., and Watanabe, K., 2011, A novel resource polymorphism in fish, driven by differential bottom environments: An example from an ancient lake in Japan: PLOS One, v. 6, no. 2.
- Kriwet, J., 2001, Feeding mechanisms and ecology of pycnodont fishes (Neopterygii, Pycnodontiformes): Fossil Record, v. 4, no. 1, p. 139-165.
- Kriwet, J., 2008, The dentition of the enigmatic pycnodont fish, Athrodon wittei(Fricke, 1876) (Neopterygii, Pycnodontiformes; Late Jurassic; NW Germany):Fossil Record, v. 11, no. 2, p. 61-66.

- Kriwet, J., and Schmitz, L., 2005, New insight into the distribution and palaeobiology of the pycnodont fish Gyrodus: Acta Palaeontologica Polonica, v. 50, no. 1, p. 49-56.
- Krueger, K. L., Scott, J. R., Kay, R. F., and Ungar, P. S., 2008, Technical note: Dental microwear textures of "Phase I" and "Phase II" facets: American Journal of Physical Anthropology, v. 137, no. 4, p. 485-490.
- Langerhans, R., Chapman, L., and Dewitt, T., 2007, Complex phenotype-environment associations revealed in an East African cyprinid: Journal of Evolutionary Biology, v. 20, no. 3, p. 1171-1181.
- Langerhans, R., Layman, C., Langerhans, A., and DeWitt, T., 2003, Habitat-associated morphological divergence in two neotropical fish species: Biological Journal of the Linnean Society, v. 80, p. 689-698.
- Las Casas, E., Bastos, F., Godoy, G., and Buono, V., 2008, Enamel wear and surface roughness characterization using 3D profilometry: Tribology International, v. 41, no. 12, p. 1232-1236.
- Lauder, G. V., 1995, On the inference of function from structure
- *in* Thomason, J. J., ed., Functional Morphology in Vertebrate Paleontology: Cambridge, Cambridge University Press p. 1-18.
- Laverdière, J. W., 1929, Description d'une plaque dentaire de Dipneuste Palaedaphus ferquensis nov.sp. du Dévonien supérieur du Boulonnais: Annales de la Société Géologique du Nord, v. LIV, no. Journal Article, p. 94.
- Lelièvre, H., 2002, Phylogénie des Brachythoraci (Vertebrata, Placodermi) et ajustement de la phylogénie à la stratigraphie. Les sites du Dévonien terminal, la caratérisation de leur milieu de dépôt par analyse de similitude de leur

ichthyofaune [Thèse d'habilitation à diriger des recherches de l'Université des Sciences et Technologies de Lille, spécialité: Sciences naturelles, 1-188 p.

- Lelièvre, H., Goujet, D., Blieck, A., and Janvier, P., 1988, Poissons du Dévonien du
  Boulonnais (France), *in* Brice, D., ed., Le Dévonien de Ferques, Bas-Boulonnais
  (N. France) Paléontologie sédimentologie stratigraphie tectonique.
  Collection "Biostratigraphie du paléozoïque" VII, p. 503-522.
- Lelièvre, H., Janvier, P., and Goujet, D., 1981, Devonian Vertebrates of Central Iran 4. Arthrodires and Ptyctodonts: Geobios, v. 14, no. 6, p. 677-710.
- Liao, Y., and Lucas, M., 2000a, Diet of the common wolffish Anarhichas lupus in the North Sea: Journal of the Marine Biological Association of the United Kingdom, v. 80, p. 181-182.
- -, 2000b, Growth, diet and metabolism of common wolf-fish in the North Sea, a fastgrowing population: Journal of fish biology, v. 56, no. 4, p. 810-825.
- Liem, K., 1973, Evolutionary strategies and morphological innovations: Cichlid pharyngeal jaws: Systematic Zoology, v. 22, no. 4, p. 425-441.
- -, 1980, Adaptive significance of intra- and interspecific differences in the feeding repertoires of cichlid fishes: American Zoologist, v. 20, no. 295-314.
- Long, J., 1997, Ptyctodontid fishes (Vertebrata, Placodermi) from the Late Devonian Gogo formation, Western Australia, with a revision of the European genus Ctenurella Ørvig, 1960: Geodiversitas, v. 19, no. 3, p. 515-555.
- Long, J. A., Choo, B., and Young, G. C., 2008a, A new basal actinopterygian fish from the Middle Devonian Aztec Siltstone of Antarctica: Antarctic Science, v. 20, no. 4, p. 393-412.

- Long, J. A., and Trinajstic, K., 2010, The Late Devonian Gogo Formation Lägerstatte of Western Australia: Exceptional Early Vertebrate Preservation and Diversity:
   Annual Review of Earth and Planetary Sciences, v. 38, no. 1, p. 255-279.
- Long, J. A., Trinajstic, K., and Johanson, Z., 2009, Devonian arthrodire embryos and the origin of internal fertilization in vertebrates: Nature, v. 457, no. 7233, p. 1124-1127.
- Long, J. A., Trinajstic, K., Young, G. C., and Senden, T., 2008b, Live birth in the Devonian period: Nature, v. 453, no. 7195, p. 650-652.
- Lukševičs, E., 2001, Bothriolepid antiarchs (Vertebrata, Placodermi) from the Devonian of the noth-western part of the East European Platform.: Geodiversitas, v. 23, no. 4, p. 489-609.
- Machado, L. P. C., and Brito, P. M., 2006, The new genus Potiguara (Actinopterygii: Pycnodontiformes) from the Upper Cretaceous of northeast Brazil: Journal of Vertebrate Paleontology, v. 26, no. 1, p. 1-6.
- Macho, G. A., and Shimizu, D., 2010, Kinematic parameters inferred from enamel microstructure: new insights into the diet of Australopithecus anamensis: J Hum Evol, v. 58, no. 1, p. 23-32.
- Mainland, I., 2006, Pastures lost? A dental microwear study of ovicaprine diet and management in Norse Greenland: Journal of Archaeological Science, v. 33, no.
  2, p. 238-252.
- Mainland, I. L., 2003, Dental microwear in grazing and browsing Gotland sheep (Ovis aries) and its implications for dietary reconstruction: Journal of Archaeological Science, v. 30, no. 11, p. 1513-1527.
- Mantyka, C. S., and Bellwood, D. R., 2007, Direct evaluation of macroalgal removal by herbivorous coral reef fishes: Coral Reefs, v. 26, no. 2, p. 435-442.

- Mariani, S., Maccaroni, A., Massa, F., Rampacci, M., and Tancioni, L., 2002, Lack of consistency between the trophic interrelationships of five sparid species in two adjacent central Mediterranean coastal lagoons: Journal of fish biology, v. 61, no. sa, p. 138-147.
- Martill, D., 1990, Predation on Kosmoceras by semionotid fish in the Middle jurassic Lower Oxford Clay of England: Palaeontology, v. 33, no. 3, p. 739-742.
- Marx, F. G., and Uhen, M. D., 2010, Climate, critters, and cetaceans: Cenozoic drivers of the evolution of modern whales: Science, v. 327, no. 5968, p. 993-996.
- Mbabazi, D., Ogutu-Ohwayo, R., Wandera, S., and Kiziito, Y., 2004, Fish species and trophic diversity of haplochromine cichlids in the Kyoga satellite lakes (Uganda): African Journal of Ecology, v. 42, p. 59-68.
- McRoberts, C., 2001, Triassic bivalves and the initial marine Mesozoic revolution: a role for predators?: Geology, v. 29, no. 4, p. 359-362.
- Mendes, T. C., Villaca, R. C., and Ferreira, C. E., 2009, Diet and trophic plasticity of an herbivorous blenny Scartella cristata of subtropical rocky shores: J Fish Biol, v. 75, no. 7, p. 1816-1830.
- Merceron, G., 2005, A New Method of Dental Microwear Analysis: Application toExtant Primates and Ouranopithecus macedoniensis (Late Miocene of Greece):Palaios, v. 20, no. 6, p. 551-561.
- Merceron, G., Blondel, C., Brunet, M., Sen, S., Solounias, N., Viriot, L., and Heintz, E., 2004, The Late Miocene paleoenvironment of Afghanistan as inferred from dental microwear in artiodactyls: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 207, no. 1-2, p. 143-163.

- Merceron, G., Escarguel, G., Angibault, J. M., and Verheyden-Tixier, H., 2010, Can dental microwear textures record inter-individual dietary variations?: PLOS One, v. 5, no. 3, p. e9542.
- Merceron, G., Schulz, E., Kordos, L., and Kaiser, T. M., 2007, Paleoenvironment of Dryopithecus brancoi at Rudabanya, Hungary: evidence from dental meso- and micro-wear analyses of large vegetarian mammals: J Hum Evol, v. 53, no. 4, p. 331-349.
- Merceron, G., Scott, J., Scott, R. S., Geraads, D., Spassov, N., and Ungar, P. S., 2009, Folivory or fruit/seed predation for Mesopithecus, an earliest colobine from the late Miocene of Eurasia?: Journal of human evolution, v. 57, no. 6, p. 732-738.
- Merceron, G., Viriot, L., and Blondel, C., 2005, Dental microwear of the late Miocene bovids of northern Greece: Vallesian/Turolian environmental changes and disappearance of Ouranopithecus macedoniensis?: Bulletin de la Societe Geologique de France, v. 176, no. 5, p. 475-484.
- Mihlbachler, M., Beatty, B., Caldera-Siu, A., Chan, D., and Lee, R., 2012, Error rates and observer bias in dental microwear analysis using light microscopy: Palaeontologia electronica, v. 15, no. 1, p. 22.
- Mistiaen, B., Becker, R., Brice, D., Degardin, J. M., Derycke, C., Loonès, C., and
  Rohart, J. C., 2002, Données nouvelles sur la partie supérieure de la Formation
  de Beaulieu (Frasnien de Ferques, Boulonnais, France): Annales de la Société
  Géologique du Nord, v. IX, series 2, no. Journal Article, p. 75-84.
- Mittelbach, G., Osenberg, C., and Wainwright, P., 1999, Variation in feeding morphology between pumpkinseed populations: phenotypic plasticity or evolution?: Evolutionary Ecology Research, v. 1, p. 111-128.

- Mondéjar-Fernández, J., and Clément, G., 2012, Squamation and scale microstructure evolution in the Porolepiformes (Sarcopterygii, Dipnomorpha) based on *Heimenia ensis* from the Devonian of Spitsbergen: Journal of Vertebrate Paleontology, v. 32, no. 2, p. 267-284.
- Mountfort, D. O., Campbell, J., and Clements, K. D., 2002, Hindgut Fermentation in Three Species of Marine Herbivorous Fish: Applied and Environmental Microbiology, v. 68, no. 3, p. 1374-1380.
- Mumby, P. J., 2009, Herbivory versus corallivory: are parrotfish good or bad for Caribbean coral reefs?: Coral Reefs, v. 28, no. 3, p. 683-690.
- Nursall, J. R., 1993a, Distribution and ecology of pycnodont fishes, *in* Arratia, G., and Viohl, G., eds., Mesozoic Fishes - Systematics and Paleoecology: München, Germany, Verlag Dr. Friedrich Pfeil, p. 115-124.
- -, 1993b, The phylogeny of pycnodont fishes, *in* Arratia, G., and Viohl, G., eds., Mesozoic Fishes - Systematics and Paleoecology: München, Germany, Verlag Dr. Friedrich Pfeil, p. 125-152.
- -, 1999, The pycnodontiform bauplan: The morphology of a successful taxon, *in* Arratia, G., and Cloutier, R., eds., Mesozoic Fishes 2. Systematics and Fossil Record.: München, Germany, Verlag Dr. Friedrich Pfeil, p. 189-214.
- Oji, T., Ogaya, C., and Sato, T., 2003, Increase of shell-crushing predation recorded in fossil shell fragmentation: Paleobiology, v. 29, no. 4, p. 520-526.
- Oksanen, J. F., Blanchet, G., Kindt, R., Legendre, P., O'Hara, R. G., Simpson, G. L., Solymos, P., Stevens, M. H. H., and Wagner, H., 2010, vegan:Community Ecology Package. R package version 1.17-0.
- Opitz, S., 1996, Trophic Interactions in Caribbean Coral Reefs, International Center for Living Aquatic Resources Management.
- Osenberg, C. W., Huckins, C. J., Kaltenberg, A., and Martinez, A., 2004, Resolving within- and between-population variation in feeding ecology with a biomechanical model: Oecologia, v. 141, no. 1, p. 57-65.
- Osi, A., and Weishampel, D. B., 2009, Jaw mechanism and dental function in the late cretaceous basal eusuchian Iharkutosuchus: J Morphol, v. 270, no. 8, p. 903-920.
- Ota, K., Hori, M., and Kohda, M., 2012, Changes in reproductive life-history strategies in response to nest density in a shell-brooding cichlid, Telmatochromis vittatus: NATURWISSENSCHAFTEN, v. 99, no. 1, p. 23-31.
- Pallaoro, A., Šantić, M., and Jardas, I., 2006, Feeding habits of the common two-banded sea bream, Diplodus vulgaris (Sparidae), in the eastern Adriatic Sea: Cybium, v. 30, no. 1, p. 19-25.
- Pedreschi, F., and Aguilera, J., 1999, Characterization of food surfaces using scalesensitive fractal analysis: Journal of Food Process Engineering, v. 23, p. 127-143.
- Pedreschi, F., Aguilera, J., and Brown, C., 2002, Characterization of the Surface Properties of Chocolate Using Scale-Sensitive Fractal Analysis: International Journal of Food Properties, v. 5, no. 3, p. 523-535.
- Peigne, S., Goillot, C., Germonpre, M., Blondel, C., Bignon, O., and Merceron, G., 2009, Predormancy omnivory in European cave bears evidenced by a dental microwear analysis of Ursus spelaeus from Goyet, Belgium: Proc Natl Acad Sci U S A, v. 106, no. 36, p. 15390-15393.
- Poyato-Ariza, F., 2005, Pycnodont fishes: morphologic variation, ecomorphologic plasticity, and a new interpretation of their evolutionary history: Bulletin of the KitakyushuMuseum of Natural History and Human History Series A (Natural History), v. 3, p. 169-184.

- Poyato-Ariza, F., Talbot, M., Fregenal-Martinez, M., Melendez, N., and Wenz, S., 1998, First isotopic and multidisciplinary evidence for nonmarine coelacanths and pycnodontiform fishes: palaeoenvironmental implications: Palaeogeography Palaeoclimatology Palaeoecology, v. 144, p. 65-84.
- Poyato-Ariza, F., and Wenz, S., 2002, A new insight into pycnodontiform fishes: Geodiversitas, v. 24, no. 1, p. 139-248.
- Poyato-Ariza, F. J., 2003, Dental characters and phylogeny of pycnodontiform fishes: Journal of Vertebrate Paleontology, v. 23, no. 4, p. 937-940.
- -, 2010, Polazzodus, gen. nov., a new pycnodont fish from the Late Cretaceous of northeastern Italy: Journal of Vertebrate Paleontology, v. 30, no. 3, p. 650-664.
- Poyato-Ariza, F. J., and Bermudez-Rochas, D. D., 2009, New Pycnodont fish
  (Arcodonishthys pasiegae gen. et sp. nov.) from the early Cretaceous of the
  basque-cantabrian basin, northern Spain: Journal of Vertebrate Paleontology, v.
  29, no. 1, p. 271-275.
- Poyato-Ariza, F. J., and Bermúdez-Rochas, D. D., 2009, New pycnodont fish (Arcodonichthys pasiegaegen. et sp. nov.) from the Early Cretaceous of the Basque-Cantabrian Basin, northern Spain: Journal of Vertebrate Paleontology, v. 29, no. 1, p. 271-275.
- Poyato-Ariza, F. J., and Wenz, S., 2005, Akromystax tilmachitongen. et sp. nov., a new pycnodontid fish from the Lebanese Late Cretaceous of Haqel and En Nammoura: Journal of Vertebrate Paleontology, v. 25, no. 1, p. 27-45.
- Purnell, M., 1995, Microwear on conodont elements and macrophagy in the first vertebrates: Nature, v. 374, p. 798-800.
- Purnell, M., Bell, M., Baines, D., Hart, P., and Travis, M., 2007, Correlated evolution and dietary change in fossil stickleback: Science, v. 317, p. 1887.

- Purnell, M., Seehausen, O., and Galis, F., 2012, Quantitative three-dimensional microtextural analyses of tooth wear as a tool for dietary discrimination in fishes: Journal of the Royal Society Interface.
- Purnell, M. A., Hart, P. J. B., Baines, D. C., and Bell, M. A., 2006, Quantitative analysis of dental microwear in threespine stickleback: a new approach to analysis of trophic ecology in aquatic vertebrates: Journal of Animal Ecology, v. 75, no. 4, p. 967-977.
- R Development Core Team, 2012, R: A language and environment for statistical computing., *in* R Foundation for Statistical Computing, V., Austria, ed.
- Ramdarshan, A., Alloing-Seguier, T., Merceron, G., and Marivaux, L., 2011, The primate community of Cachoeira (Brazilian Amazonia): a model to decipher ecological partitioning among extinct species: PLOS One, v. 6, no. 11, p. e27392.
- Ramdarshan, A., Merceron, G., and Marivaux, L., 2012, Spatial and temporal ecological diversity amongst eocene primates of france: Evidence from teeth: American Journal of Physical Anthropology, v. 147, no. 2, p. 201-216.
- Rivals, F., and Athanassiou, A., 2008, Dietary adaptations in an ungulate community from the late Pliocene of Greece: Palaeogeography Palaeoclimatology Palaeoecology, v. 265, no. 1-2, p. 134-139.
- Rivals, F., and Solounias, N., 2007, Differences in tooth microwear of populations of caribou (Rangifer tarandus, Ruminantia, Mammalia) and implications to ecology, migration, glaciations and dental evolution: Journal of Mammalian Evolution, v. 14, no. 3, p. 182-192.
- Robinson, B., and Wilson, D., 1998, optimal foraging, specialization, and a solution to Liem's paradox: American Naturalist, v. 151, no. 3, p. 223-235.

- Rodrigues, H. G., Merceron, G., and Viriot, L., 2009, Dental microwear patterns of extant and extinct Muridae (Rodentia, Mammalia): ecological implications: Naturwissenschaften, v. 96, no. 4, p. 537-542.
- Rodriguez, J. M., Curtis, R. V., and Bartlett, D. W., 2009, Surface roughness of impression materials and dental stones scanned by non-contacting laser profilometry: Dent Mater, v. 25, no. 4, p. 500-505.
- Rohr, R. P., Scherer, H., Kehrli, P., Mazza, C., and Bersier, L. F., 2010, Modeling food webs: exploring unexplained structure using latent traits: Am Nat, v. 176, no. 2, p. 170-177.
- Romero, A., Galbany, J., Martinez-Ruiz, N., and De Juan, J., 2009, In vivo turnover rates in human buccal dental-microwear: 78th Annual meeting of the American Association of Physical Anthropologists.
- Rotjan, R. D., and Lewis, S. M., 2006, Parrotfish abundance and selective corallivory on a Belizean coral reef: Journal of experimental marine biology and ecology, v. 335, no. 2, p. 292-301.
- Rots, V., Van Peer, P., and Vermeersch, P. M., 2011, Aspects of tool production, use, and hafting in Palaeolithic assemblages from Northeast Africa: J Hum Evol, v. 60, no. 5, p. 637-664.
- Rücklin, M., 2011, First selenosteid placoderms from the eastern Anti-Atlas of Morocco; osteology, phylogeny and palaeogeographical implications:
  Palaeontology, v. 54, no. 1, p. 25-62.
- Ruehl, C. B., and DeWitt, T. J., 2007, Trophic plasticity and foraging performance in red drum, Sciaenops ocellatus (Linnaeus): Journal of experimental marine biology and ecology, v. 349, no. 2, p. 284-294.

- Sallan, L. C., and Coates, M. I., 2010, End-Devonian extinction and a bottleneck in the early evolution of modern jawed vertebrates: Proc Natl Acad Sci U S A, v. 107, no. 22, p. 10131-10135.
- Sallan, L. C., Kammer, T. W., Ausich, W. I., and Cook, L. A., 2011, Persistent predator-prey dynamics revealed by mass extinction: Proc Natl Acad Sci U S A, v. 108, no. 20, p. 8335-8338.
- Sanson, G. D., Kerr, S. A., and Gross, K. A., 2007, Do silica phytoliths really wear mammalian teeth?: Journal of Archaeological Science, v. 34, no. 4, p. 526-531.
- Sasagawa, I., 1997, Fine structure of the cap enameloid and of the dental epithelial cells during enameloid mineralisation and early maturation stages in the tilapia, a teleost: Journal of anatomy, v. 190, p. 589-600.
- Schaack, S., and Chapman, L., 2004, Interdemic variation in the foraging ecology of the African cyprinid, Barbus neumayeri: Environmental Biology of Fishes, v. 70, p. 95-105.
- Schmidt, C. W., 2010, On the relationship of dental microwear to dental macrowear: Am J Phys Anthropol, v. 142, no. 1, p. 67-73.
- Schubert, B. W., Ungar, P. S., and DeSantis, L. R. G., 2010, Carnassial microwear and dietary behaviour in large carnivorans: Journal of zoology, v. 280, no. 3, p. 257-263.
- Schulz, E., Calandra, I., and Kaiser, T. M., 2010, Applying tribology to teeth of hoofed mammals: Scanning, v. 32, no. 4, p. 162-182.
- Schweitzer, C. E., and Feldmann, R. M., 2010, The Decapoda (Crustacea) as Predators on Mollusca through Geologic Time: Palaios, v. 25, no. 3, p. 167-182.
- Scott, J. R., Godfrey, L. R., Jungers, W. L., Scott, R. S., Simons, E. L., Teaford, M. F., Ungar, P. S., and Walker, A., 2009, Dental microwear texture analysis of two

families of subfossil lemurs from Madagascar: Journal of human evolution, v. 56, no. 4, p. 405-416.

- Scott, R. S., Teaford, M. F., and Ungar, P. S., 2012, Dental microwear texture and anthropoid diets: Am J Phys Anthropol, v. 147, no. 4, p. 551-579.
- Scott, R. S., Ungar, P. S., Bergstrom, T. S., Brown, C. A., Childs, B. E., Teaford, M. F., and Walker, A., 2006, Dental microwear texture analysis: technical considerations: Journal of human evolution, v. 51, no. 4, p. 339-349.
- Scott, R. S., Ungar, P. S., Bergstrom, T. S., Brown, C. A., Grine, F. E., Teaford, M. F., and Walker, A., 2005, Dental microwear texture analysis shows within-species diet variability in fossil hominins: Nature, v. 436, no. 7051, p. 693-695.
- Semprebon, G., Janis, C., and Solounias, N., 2004a, The diets of the dromomerycidae (Mammalia : Artiodactyla) and their response to miocene vegetational change: Journal of Vertebrate Paleontology, v. 24, no. 2, p. 427-444.
- Semprebon, G. M., Godfrey, L. R., Solounias, N., Sutherland, M. R., and Jungers, W. L., 2004b, Can low-magnification stereomicroscopy reveal diet?: J Hum Evol, v. 47, no. 3, p. 115-144.
- Sereno, P. C., Xijin, Z., and Lin, T., 2010, A new psittacosaur from Inner Mongolia and the parrot-like structure and function of the psittacosaur skull: Proc Biol Sci, v. 277, no. 1679, p. 199-209.
- Shin, J., 2007, Bite force analysis of the Ptyctodont placoderms, the earliest vertebrate durophage: Journal of Vertebrate Paleontology, v. 27, no. 3, p. 145A-145A.
- Silverstein, R. N., Correa, A. M., and Baker, A. C., 2012, Specificity is rarely absolute in coral-algal symbiosis: implications for coral response to climate change: Proc Biol Sci, v. 279, no. 1738, p. 2609-2618.

- Smith, M., 2003, Vertebrate dentitions at the origin of jaws: when and how pattern evolved: Evolution, v. 5, no. 4, p. 394-413.
- Smith, M., and Johanson, Z., 2010, The dipnoan dentition: a unique adaptation with a longstanding evolutionary record, *in* Jørgensen, J. M., and Joss, J., eds., The Biology of Lungfishes, Science Publisher, p. 530.
- Smith, M. M., and Johanson, Z., 2003, Separate evolutionary origins of teeth from evidence in fossil jawed vertebrates: Science, v. 299, no. 5610, p. 1235-1236.
- Sotka, E. E., and Hay, M. E., 2009, Effects of herbivores, nutrient enrichment, and their interactions on macroalgal proliferation and coral growth: Coral Reefs, v. 28, no. 3, p. 555-568.
- Stahl, B., 1999, Chondrichthyes III: Holocephali. In Schultze, H.-P., ed. Handbook of Paleoichthyology, vol.2, 128 p., 94 fig, Volume 4: München, Dr. Friedrich Pfeil Verlag, p. 1-164.
- Stanley, G., 2006, Photosymbiosis and the evolution of modern coral reefs: Science, v. 312, p. 857-858.
- Stanley, G., and van de Schootbrugge, B., 2009, The evolution of the coral-algal symbiosis, *in* van Oppen, M., and Lough, J., eds., Coral bleaching: Patterns, processes, causes and consequences: Berlin, Springer-Verlag.
- Stanley, S. M., 2008, Predation defeats competition on the seafloor: Paleobiology, v. 34, no. 1, p. 1-21.
- Stevens, N. E., Harro, D. R., and Hicklin, A., 2010, Practical quantitative lithic usewear analysis using multiple classifiers: Journal of Archaeological Science, v. 37, no. 10, p. 2671-2678.

- Summers, A., 2000, Stiffening the stingray skeleton An investigation of durophagy in Myliobatid stingrays (Chondrichthyes, Batoidea, Myliobatidae): Journal of Morphology, v. 243, p. 113-126.
- Summers, A. P., Ketcham, R. A., and Rowe, T., 2004, Structure and function of the horn shark (Heterodontus francisci) cranium through ontogeny: development of a hard prey specialist: J Morphol, v. 260, no. 1, p. 1-12.
- Svanback, R., and Bolnick, D. I., 2007, Intraspecific competition drives increased resource use diversity within a natural population: Proc Biol Sci, v. 274, no. 1611, p. 839-844.
- Swanson, B., Gibb, A., Marks, J., and Hendrickson, D., 2003, Trophic polymorphism and behavioral differences decrease intraspecific competition in a cichlid, Herichthys minckleyi: Ecology, v. 84, no. 6, p. 1441-1446.
- Swartz, B. A., 2009, Devonian actinopterygian phylogeny and evolution based on a redescription of Stegotrachelus finlayi: Zoological Journal of the Linnean Society, v. 156, no. 4, p. 750-784.
- Teaford, M., and Walker, A., 1983, Dental microwear in adult and still-born guinea pigs (Cavia porcellus): Archives of Oral Biology, v. 28, no. 11, p. 1077-1081.
- Tintori, A., 1998, Fish biodiversity in the marine Norian (Late Triassic) of northern Italy: The first Neopterygian radiation: Italian Journal of Zoology, v. 65, no. sup1, p. 193-198.
- Todd, N., Falco, N., Silva, N., and Sanchez, C., 2007, Dental microwear variation in complete molars of Loxodonta africana and Elephas maximus: Quaternary International, v. 169-170, p. 192-202.
- Trinajstic, K., 1995, The role of heterochrony in the evolution of eubrachythoracid arthrodires with special reference to Compagopiscis croucheri and Incisoscutum

ritchei from the Late Devonian Gogo Formation, Western Australia: Geobios, v. M.S. 19, p. 125-128.

- Trinajstic, K., and Dennis-Bryan, K., 2009, Phenotypic plasticity, polymorphism and phylogeny within placoderms: Acta Zoologica, v. 90, p. 83-102.
- Trinajstic, K., and Hazelton, M., 2007, Ontogeny, phenotypic variation and phylogenetic implications of arthrodires from the Gogo Formation, Western Australia: Journal of Vertebrate Paleontology, v. 27, no. 3, p. 571-583.
- Trinajstic, K., and Long, J. A., 2009, A new genus and species of Ptyctodont (Placodermi) from the Late Devonian Gneudna Formation, Western Australia, and an analysis of Ptyctodont phylogeny: Geological Magazine, v. 146, no. 05, p. 743.
- Tseng, Z., Antón, M., and Salesa, M., 2011, The evolution of the bone-cracking model in carnivorans: cranial functional morphology of the plio-Pleistocene cursorial hyaenid Chasmaporthetes lunensis (mammalia, carnivora): Paleobiology, v. 37, no. 1, p. 140-156.
- Turner, S., Wang, S.-T., and Young, G., 1995, Lower Devonian microvertebrates from Longmenshan, Sichuan, China: preliminary report: Geobios, v. M.S. 19, p. 383-387.
- Ungar, P., 1996, Dental microwear of European Miocene catarrhines: evidence for diets and tooth use: Journal of human evolution, v. 31, p. 335-366.
- Ungar, P., Brown, C., Bergstrom, T., and Walker, A., 2003, Quantification of dental microwear by tandem scanning confocal microscopy and scale-sensitive fractal analyses: Scanning, v. 25, p. 185-193.
- Ungar, P. S., Grine, F. E., and Teaford, M. F., 2008, Dental microwear and diet of the Plio-Pleistocene hominin Paranthropus boisei: PLOS One, v. 3, no. 4, p. e2044.

- Ungar, P. S., Merceron, G., and Scott, R. S., 2007, Dental Microwear Texture Analysis of Varswater Bovids and Early Pliocene Paleoenvironments of Langebaanweg, Western Cape Province, South Africa: Journal of Mammalian Evolution, v. 14, no. 3, p. 163-181.
- Ungar, P. S., Scott, R. S., Grine, F. E., and Teaford, M. F., 2010, Molar microwear textures and the diets of Australopithecus anamensis and Australopithecus afarensis: Philos Trans R Soc Lond B Biol Sci, v. 365, no. 1556, p. 3345-3354.
- Venables, W. N., and Ripley, B. D., 2002, Modern Applied Statistics with S, New York, Springer.
- Vermeij, G. J., 1977, The Mesozoic Marine Revolution. Evidence from snails, predators and grazers: Paleobiology, v. 3, no. 3, p. 245-258.
- Vermeij, G. J., 2008, Escalation and its role in Jurassic biotic history: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 263, no. 1-2, p. 3-8.
- Vézina, D., 1990, Plourdosteidae Fam-Nov (Placodermi, Arthrodira) and their
  Phylogenic Relation to Brachythoraci: Canadian Journal of Earth Sciences, v.
  27, no. 5, p. 677-683.
- Vullo, R., Bernárdez, E., and Buscalioni, A. D., 2009, Vertebrates from the middle?– late Cenomanian La Cabaña Formation (Asturias, northern Spain):
  Palaeoenvironmental and palaeobiogeographic implications: Palaeogeography, Palaeoclimatology, Palaeoecology, v. 276, no. 1-4, p. 120-129.
- Wainwright, P., Alfaro, M., Bolnick, D., and Hulsey, C., 2005, Many-to-one mapping of form to function: a general principle in organismal design?: Integrative and Comparative Biology, v. 45, p. 256-262.
- Wainwright, P., and Richard, B., 1995, Predicting patterns of prey use from morphology of fishes: Environmental Biology of Fishes, v. 44, p. 97-113.

- Wainwright, P. C., 2007, Functional Versus Morphological Diversity in
  Macroevolution: Annual Review of Ecology, Evolution, and Systematics, v. 38, no. 1, p. 381-401.
- Walker, A., Hoeck, H., and Perez, L., 1978, Microwear of mammalian teeth as an indicator of diet: Science, v. 201, p. 908-910.
- Walker, S., and Brett, C., 2002a, Post-Palaeozoic patterns in marine predation: was there a Mesozoic and Cenozoic predatory revolution?: Paleontological Society Papers, v. 8.
- Walker, S., Parsons-Hubbard, K., Powell, E., and Brett, C., 2002, Predation on Experimentally deployed molluscan shells from shelf to slope depths in a tropical carbonate environment: Palaios, v. 17, p. 147-170.
- Walker, S. E., and Brett, C. E., 2002b, Post-Paleozoic patterns in marine predation: Was there a Mesozoic and Cenozoic marine predatory revolution?: Paleontological Society Papers, v. 8, p. 119-193.
- Warnock, R. C., Yang, Z., and Donoghue, P. C., 2011, Exploring uncertainty in the calibration of the molecular clock: Biol Lett.
- Wenz, S., 2003, Les Lepidotes (Actinopterygii, Semionotiformes) du Crétacé inférieur (Barrémien) de Las Hoyas (Province de Cuenca, Espagne): Geodiversitas, v. 25, no. 3.
- Whitlock, J. A., 2011, Inferences of diplodocoid (Sauropoda: Dinosauria) feeding behavior from snout shape and microwear analyses: PLOS One, v. 6, no. 4, p. e18304.
- Williams, V., and Doyle, A., 2010, Cleaning fossil tooth surfaces for microwear analysis: use of solvent gel to remove consolidant: Palaeontologia electronica, v. 13, no. 2.

- Williams, V., Purnell, M., and Gabbott, S., 2006, Dental microwear in dinosaurs: a comparative analysis of polysiloxane replication: Dental Practice.
- Williams, V. S., Barrett, P. M., and Purnell, M. A., 2009, Quantitative analysis of dental microwear in hadrosaurid dinosaurs, and the implications for hypotheses of jaw mechanics and feeding: Proc Natl Acad Sci U S A, v. 106, no. 27, p. 11194-11199.
- Young, G. C., 2005, An articulated phyllolepid fish (Placodermi) from the Devonian of central Australia: implications for non-marine connections with the Old Red Sandstone continent: Geological Magazine, v. 142, no. 2, p. 173-186.
- Zatoń, M., and Salamon, M. A., 2008, Durophagous Predation on Middle Jurassic Molluscs, as Evidenced from Shell Fragmentation: Palaeontology, v. 51, no. 1, p. 63-70.
- Zhu, M., Yu, X., Choo, B., Wang, J., and Jia, L., 2012, An antiarch placoderm shows that pelvic girdles arose at the root of jawed vertebrates: Biol Lett, v. 8, no. 3, p. 453-456.
- Zimmerman, D. W., 1999, Type I error probabilities of the Wilcoxon-mann-Whitney test and Student T test altered by heterogeneous variances and equal sample sizes: Perceptual and Motor Skills, v. 88, no. 556-558.
- -, 2000, Statistical significance levels of nonparametric tests biased by heterogeneous variances of treatment groups: Journal of General Psychology, v. 127, no. 4, p. 354-364.
- Zimmerman, D. W., 2004, Conditional Probabilities of RejectingH0by Pooled and Separate-VariancestTests Given Heterogeneity of Sample Variances:
  Communications in Statistics - Simulation and Computation, v. 33, no. 1, p. 69-81.

Zimmerman, D. W., 2012, A note on consistency of non-parametric rank tests and related rank transformations: Br J Math Stat Psychol, v. 65, no. 1, p. 122-144.