# LIFE HISTORY, ECOLOGY AND CONSERVATION OF EUROPEAN SEAHORSES

Janelle Marie Renelle Curtis

Department of Biology
McGill University
Montréal Québec

August 2004

> *"Who knows what admirable virtue of fishes may be below low-water-mark, bearing up against a hard destiny, not admired by that fellow creature who alone can appreciate it!"*
> Henry David Thoreau

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of Doctor of Philosophy

Library and
Archives Canada

Published Heritage
Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et
Archives Canada

Direction du
Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

*Your file Votre référence*
*ISBN: 0-494-12826-7*
*Our file Notre référence*
*ISBN: 0-494-12826-7*

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

# TABLE OF CONTENTS

**LIFE HISTORY OF AN UNUSUAL MARINE FISH: SURVIVAL, GROWTH AND MOVEMENT PATTERNS OF THE EUROPEAN LONG-SNOUTED SEAHORSE, *HIPPOCAMPUS GUTTULATUS* (CUVIER 1829)**

**ESTIMATING REALIZED ANNUAL FECUNDITY IN A BROODING MULTIPLE SPAWNER, *HIPPOCAMPUS GUTTULATUS***

**HABITAT USE OF SYMPATRIC SEAHORSE SPECIES ALONG A GRADIENT OF HABITAT COMPLEXITY IN A SUBTROPICAL SEAGRASS-DOMINATED COMMUNITY**

**COMMUNITY IMPACTS AND POPULATION RESPONSES OF SEAHORSES TO EXPERIMENTAL REDUCTIONS IN LOCAL SEINING EFFORT**

**PREDICTING POPULATION-LEVEL RESPONSES TO A GENERIC STRATEGY FOR SEAHORSE CONSERVATION**

# LIST OF TABLES

## Chapter 1

## Chapter 2

## Chapter 3

## Chapter 4

## Chapter 5

## Conclusions

# LIST OF FIGURES

## Chapter 1

## Chapter 2

## Chapter 3

## Chapter 4

## Chapter 5

# PREFACE

## THESIS FORMAT AND STYLE

I have chosen to submit a manuscript-based thesis as an alternative to the traditional thesis format. This is a cohesive collection of papers that report on a single program of research. In accordance with Faculty regulations I include the following text to inform the external examiners of the regulations.

1 Candidates have the option of including, as part of the thesis, the text of one or more papers submitted, or to be submitted, for publication, or the clearly duplicated text (not the reprints) of one or more published papers. These texts must conform to the "Guidelines for Thesis Preparation" with respect to font size, line spacing and margin sizes and must be bound together as an integral part of the thesis. (Reprints of published papers can be included in the appendices at the end of the thesis).

2 The thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next. In order to ensure that the thesis has continuity, connecting texts that provide logical bridges preceding and following each manuscript are mandatory.

3 The thesis must conform to all other requirements of the "Guidelines for Thesis Preparation" in addition to the manuscripts.

My manuscript-based thesis comprises the following five papers, which have been accepted by or submitted to peer-reviewed journals, or are currently in preparation for submission:

Chapter 1 Curtis JMR, Vincent ACJ. Life history of an unusual marine fish: survival, growth and movement patterns of the European long-snouted seahorse, *Hippocampus guttulatus* (Cuvier 1829). Journal of Fish Biology, submitted.

Chapter 2 Curtis JMR. Estimating realized annual fecundity in a brooding multiple spawner, *Hippocampus guttulatus*. Canadian Journal of Fisheries and Aquatic Sciences, in revision.

Chapter 3 Curtis JMR, Vincent ACJ. Habitat use of sympatric seahorse species along a gradient of habitat complexity in a subtropical seagrass-dominated community. Marine Ecology Progress Series, in press.

Chapter 4 Curtis JMR, Ribeiro J, Erzini K, Vincent ACJ. Community impacts and population responses of seahorses to experimental reductions in local seining effort. In preparation.

Chapter 5 Curtis JMR, Vincent ACJ. Predicting population-level responses to a generic conservation strategy for seahorses. In preparation.

I follow the format specified by the journal Marine Ecology Progress Series, with the following exceptions: (1) abstracts are a maximum of 350 words, (2) genus names are abbreviated except during the first mention within chapters, and at the beginning of sentences, (3) figure legends are provided on the same page as figures. References are included at the end of each chapter, followed by tables and figures.

During the course of my doctoral research (2000 – 2003), I collected a considerable amount of basic biological data from two sympatric seahorse species in the Ria Formosa lagoon, southern Portugal. The five manuscripts included in my thesis represent the greater part of my field research. However, in Chapters 1-5, I refer to analyses from two additional manuscripts (currently in preparation) that were not included in this thesis:

I A socially polygamous seahorse: greetings and courtship behaviour of *Hippocampus guttulatus*.

II Life history, ecology and conservation of the short-snouted seahorse, *Hippocampus hippocampus*.

## CONTRIBUTION OF CO-AUTHORS

With the advice and support of my thesis supervisor, Dr. Amanda Vincent, I developed a network of contacts and cooperative links in the Iberian Peninsula (Spain and Portugal), surveyed suitable sites for field research, acquired research permits and memoranda of understanding (MOUs), purchased and/or constructed all field and lab equipment, and completed a pilot study of seahorse reproductive behaviour and movement patterns during the summer of 2000. Following my pilot study, and in consultation with my supervisor, supervisory committee and fellow lab members, I conceived my doctoral research framework and sampling protocol, and then executed my studies during three field seasons (2001 – 2003).

I collected, checked and formatted all of my field data (underwater survey data, and catch data from seahorses captured in experimental seining, see below) with the tremendous assistance of 16 research assistants, work-study students, volunteers and honours students whom I gratefully acknowledge below. I carried out all statistical analyses, save one (a resampling permutation test to compare predicted and observed values from a generalized linear model, with the assistance of Denis Réale, Chapter 3), and interpreted the data. I wrote the manuscripts and created all the figures. My ideas, interpretation and the structure and writing of these manuscripts were refined with input from my supervisor, whom I am proud to include as a co-author on four of five of my manuscripts.

I have also invited two collaborators, Dr. Karim Erzini and Joaquim Ribeiro (University of Algarve, Portugal), to co-author Chapter 4 because they carried out all the experimental seining described in that chapter, preserved seahorses captured in their seines (so that I could collect detailed catch data from their experimental seining program) and assisted me with site selection for the underwater visual census (UVC) surveys I carried out. KE and JR will also assist with the final revisions of this manuscript prior to submission. I used data that I derived from these collections to address research questions in Chapters 1, 2 and 4.

## ORIGINAL CONTRIBUTIONS TO KNOWLEDGE

This thesis is the first formal study of the life history and ecology of two sympatric European seahorse species, *Hippocampus guttulatus* and *H. hippocampus*, and represents a rare fisheries-independent study of the life history of small, benthic, marine fishes. This thesis makes the following specific contributions:

1. First comprehensive study of the life history and ecology of any seahorse species, including *in situ* estimates of survival, growth and reproductive rates and direct estimates of biological reference points for population assessment and management.
2. First multivariate synthesis of seahorse life history strategy in relation to other fishes.
3. First formal study of seahorse population dynamics, including an indirect estimate of the maximum population growth rate ($r_{max}$).
4. First estimates of local abundance, distribution and habitat partitioning over multiple spatial scales for two widely distributed seahorse species (*H. guttulatus and H. hippocampus*) of conservation concern.
5. A novel validated generalized linear model that forms the basis for predicting relative abundances of *H. guttulatus*, and can be tested over greater spatial and temporal scales.
6. Evidence that seagrass-associated species with similar life histories can partition habitat according to vegetation cover, with an unusual demonstration that one species prefers sparsely vegetated substrate over landscape scales.
7. Evidence that the magnitude and direction of numerical responses of some fishes to bottom fishing depend, at least in part, on how that exploitation affects their habitats.
8. First direct estimates of seahorse catchability (for any species) in non-selective fishing gears, with an examination of the reliability of catch per unit effort data.
9. Among the few studies to apply population viability analyses to evaluate management strategies for a marine fish.
10. First quantitative evaluation of the biological implications of the first minimum size limit ever globally recommended for exploitation of an entire genus of fish.

11 Novel technical contributions such as a new statistical method for non-lethally estimating annual spawning frequency in multiple spawning fishes with parental care, and an index of realized annual fecundity.

## TAXONOMIC CLARIFICATION

I employ the taxonomy of Lourie et al. (1999), which provides detailed descriptions of most seahorse species. *Hippocampus guttulatus* (Cuvier 1829) has historically been synonymized with *H. ramulosus* (Leach 1814), but further research is required to confirm that these are indeed the same species (Lourie et al. 1999). In the meantime, I retain the name *H. guttulatus*, recognizing that future research may lead to the resurrection of the name *H. ramulosus*. Other synonyms of *H. guttulatus* include *H. hippocampus microstephanus* (Slastenenko 1937), *H. hippocampus microcoronatus* (Slastenenko 1938); and *H. guttulatus multiannularis* (Ginsburg 1937) (reviewed in Lourie et al. 1999).

The taxonomy of *H. guttulatus*' smaller sympatric congener, *Hippocampus hippocampus* L, is less uncertain (Lourie et al. 1999). Synonyms include *H. heptagonus* (Rafinesque 1810), *H. antiquorum* (Leach 1814), *H. vulgaris* (Cloquet 1821), *H. brevirostris* (Schinz 1822), *H. antiquus* (Risso 1826) and *H. europaeus* (Ginsburg 1933) (reviewed in Lourie et al. 1999).

# ACKNOWLEDGEMENTS

David O'Brien, my best friend and fiancé, has brought so much balance to my life during the last five years. I hope to support him with as much faith, and encouragement, as well as insight on multiple drafts of his own thesis. I also start by thanking 'my family and other animals' (Françoise, Philip, Joelle and Citrouille) for their unconditional love and support, unwavering optimism, and sense of humour about my 'career'.

The hard-earned data used to craft these five papers were unfailingly collected with the help of my eclectic group of research assistants, volunteers and independent studies students, many of whom I've come to love dearly over the years: Rachel Adams, Gonzalo Alatorre, Hari Balasubramanian, Joana Barosa, Kathleen Bigney, Justine d'Entremont, Belinda Gunn, Sébastien Lemieux, Claire-Marine Lesage, Eleanor Murray, Julie Nadeau, Sarah Overington, Ana Maria Santos, Sara Vanessa Santos, Mélisa Veillette, and Katherine Wieckowski. I thank them for their seahorse fanaticism, confidence in Norberto, and commitment to 'early morning dives'. I wish them the best of life in their future adventures!

My thesis supervisor, Amanda Vincent, has contributed enormously to the success of my thesis and to my intellectual and personal growth. Her guidance, continued support, and supervision has shaped my perspectives on science and applied conservation biology, as well as prepared me for research as an independent scientist. Over the years, I have developed a deep respect for Amanda, and I have drawn considerable inspiration from her own research and contributions. I am also grateful to Amanda for supporting my participation in several national and international conferences (including CCFFR, FSBI, SCB, WFC) and a workshop sponsored by the IUCN Conservation Breeding Specialist Group, trusting me with unprecedented budgets, welcoming me during many useful visits to UBC, revising multiple drafts of papers, and for imparting valuable transferable skills (budgeting, workplanning, grant writing and reporting). I wish Amanda continued courage in her life's work and look forward to developing productive collaborations with her in the future.

I feel very fortunate to have worked with the many dynamic and committed members of Project Seahorse, past and present, and thank them for having lent a helping hand along the way, especially Jonathan Anticamara, Jacqueline Blomfield, Shannon Charney, Sarah Foster, James Hrynyshyn, Heather Koldewey, Sara Lourie, Dale Marsden, Keith Martin-Smith, Jessica Meeuwig, Siân Morgan, Sue Mulligan, Jorma Nuevonen, Rhea Ravanera, Melita Samoilys, Heidi Shuter, and Alex Tewfik. I thank Jessica and Keith, in particular, for taking an active interest in the development of my thesis research.

My committee members include Donald Kramer, Joseph Rasmussen and Kevin McCann. Although only Don remains with the Department of Biology at McGill University, discussions with all of my committee members helped refine my ideas, particularly during the early stages of my thesis. I am grateful to Don for including me in his lab meetings, acting as my co-supervisor during Amanda's absence, and for reviewing my thesis prior to submission. I also thank Derek Roff and James Grant for their insightful feedback during my qualifying exam.

I am indebted to the warmth, friendship, insight and great logistical support from my many friends at the Parque Natural da Ria Formosa, Ecoteca, Universidade do Algarve, Aquario Vasco de Gama, Centro de Ciencias Vivas, Hidroespaço, Alfasub, Zoea, Escola del Mar, The Seahorse Trust, Seahorse Ireland, Weymouth Aquarium, Zoological Society of London, Caixa Geral, and Camping Olhão, including: Danylo Americano, Pedro Andrade, Enric Cahner, Juan Pablo Camblor y Luz, Domingos e Laika, Kealan Doyle, Encarnação, Paulo Encarnação, Karim Erzini and the many members of his research team, Fernanda (for her entertaining Portuguese lessons), Neil Garrick-Maidment, Fatima Gil, Robin James, Julieta Marçal de Lima (and her father, the miller), Paula Martins, Nuno Grade, Eng$^{a}$ Edite Marques, Elvio Mendonça, "Nusha" Mendonça, Fatima Noronha, Fatima Nunes, Jorge Palma, Dulce Patricio, Eunice Pereira, Angel Pérez-Ruzafa, Ana Luisa Quaresma, Antonio Quintas, Joaquim Ribeiro, Sonia Santos, Jesús Garcia Torres, os homens dos barcos (Silverio, Capela, George, Orlando and others), Vasco, José Vieira, a Capitania de Olhão, and many, many others. I am particularly grateful for my home away from home, ‘a casa do moinho’, and thank Armando Almeida for his suggestion to visit the Ria Formosa on my way to Lisbon in 2000. I thank Mario Antunes, Soraia Deus, Paulo Machado, Marta Marques, Raquel Moleiro, José Antonio Rodriguez and Pépé and for stunning photographs and/or helping to create awareness about seahorses in the Ria Formosa.

I thank my fellow graduate students (and the post-docs!) for their friendship and intellectual input, particularly Sinead Collins, Marianne Gilbert, Carolyn Hall, Lisa Jones, Ingrid Morgan, Geneviève Morinville, Rebecca Ng and many more who participated in various graduate student discussion groups and came out to Thomson House. Adrian deBruyn, Murray Humphries and Neil Rooney served as fine examiners at my mock qualifying exam; I particularly appreciated their use of a paper airplane as an analogy for a model – it looks like an airplane, but does it *fly* like one? Many other people shared their valuable insights or Endnote databases with me during the last year, including Resit Akçakaya, Natalie Ban, Andy Gonzalez, and members of the ‘Walters lab’ (Robert Ahrens, Steven Martell, Nathan Taylor, and Carl Walters).

Many people in the Department of Biology at McGill University assisted me throughout my PhD, including Susan Bocti, Ancil Gittens, Robert Lamarche, Louis Lefebvre, Robert Levine, Anna McNicoll, Linda Morai, Kathryn Peterson, Louise Sabaz Suzanne Smith and Frank Scopeletti. I also thank Argos, Pilsener and Xeres for spirited contributions and the members of the Fisheries Centre at the University of British Columbia for making me feel welcome during my visits to Project Seahorse in Vancouver.

My research was generously funded by a gift to Project Seahorse from Guylian Chocolates. My friends at Guylian in Belgium and in Portugal, especially Carl Krefting, Mieke Callebaut and João Seara, helped raise awareness about marine conservation issues in Europe. In Portugal, Guylian promoted awareness of seahorse conservation through a successful elementary school campaign as well as a press conference at the

Lisbon Oceanario. The press conference, in particular, sparked a series of television interviews, magazine articles and public interest in my research in the Ria Formosa.

I gratefully acknowledge personal financial support from the Natural Sciences and Engineering Research Council of Canada (NSERC, Postgraduate Scholarship), Fonds de Recherches sur la Nature et les Technologies Québec (FCAR, Doctoral Award), a McConnell McGill Major Fellowship, Project Seahorse, the Department of Biology at McGill University (through two teaching assistantships) and my loving parents (who, when I was a teenager, jokingly agreed to support me until I was 30). I was also fortunate to have been awarded travel grants from McGill University Alma Mater Travel Fund (twice), Department of Biology Graduate Training Committee, Clemens-Rigler Travel Fund, British Ecological Society and the Society for Conservation Biology.

*"Few people know what fish think about injustice, or anything else"*, noted Ursula Le Guin. Nevertheless, I extend my most heartfelt thanks to thousands of fish for enduring our underwater presence and voyeurism during greetings and courtship rituals.

Finally, a warm thank you to the many anonymous reviewers, editors, including Otto Kinne, and thesis examiners, including John Reynolds (external examiner), Anthony Ricciardi (internal examiner), Colin Chapman, James Grant and Graham Bell (Chair) who have helped (or will help) refine this collection of papers by sharing their insight, experience, questions and suggestions.

# THESIS ABSTRACT

My thesis examined the implications of life history and ecology for population-level responses of the European long-snouted seahorse (*Hippocampus guttulatus* Cuvier 1829) to habitat alteration, exploitation and a recommended minimum size limit for seahorse management. The research employed a mark-recapture study, underwater visual censuses (UVCs) and catch data from an unrelated experimental sampling program in the Ria Formosa Lagoon (southern Portugal). These small-bodied fish are characterized by rapid growth rate, early age at maturity, high natural mortality, short generation time, short life span and multiple spawnings per year, traits that are usually associated with resilience to exploitation. However specialized parental care, complex social interactions, small adult home ranges and benthic habit confer risk to *H. guttulatus*. Population-level responses to experimental reductions in non-selective fishing effort differed in magnitude and direction between *H. guttulatus* and its smaller congener, *Hippocampus hippocampus*: the abundance of *H. guttulatus* increased significantly while its congener decreased in abundance. *Hippocampus guttulatus* preferred more complex, vegetated habitats, while *H. hippocampus* preferred more open, sparsely vegetated habitats. Thus it seems probable that *H. guttulatus* fared better in the more complex habitats that developed when seining stopped, whereas *H. hippocampus* fared better in the less complex habitat arising from repeated seining. I used an age-structured stochastic simulation to evaluate the biological implications of a recommended generic strategy (10 cm minimum size limit) for managing the exploitation and international trade of seahorses, using *H. guttulatus* as a model. The smallest size limit that was robust (using the magnitude of population decline and probability of quasi-extinction as criteria) to a range of fishing rates, maximum population growth rates ($r_{max}$) and models of density-dependence was one that protected *H. guttulatus* until after the first full reproductive season (approximately 13 cm). In this first synthesis of the life history of a seahorse species and the first formal study of seahorse population dynamics, I identified the capacity for population recovery (e.g. migration patterns, strength and form of density-dependence), as a priority for further research to refine risk assessment models and conservation strategies.

# RÉSUMÉ

Cette thèse examine les implications du cycle vital et l'écologie d'un hippocampe européen, *Hippocampus guttulatus* (Cuvier 1829) face aux changements d'habitat, la surexploitation et une stratégie recommandée pour la gestion des hippocampes. Cette recherche a employé une étude de marquage–recapture, d'inventaires visuels sous-marins, et des captures d'échantillons expérimentaux utilisant des engins de pêche non-sélectives. Cette espèce est charactérisée par une croissance rapide, un jeune âge a la maturité, des taux de mortalité élevés, une courte durée de vie, et de multiples pontes annuelles. Ce sont des traits qui sont généralement associés aux opportunistes. Cependent, le soin paternel, les intéractions sociales complexes, le comportement sédentaire des adultes, et les habitats benthiques exploités confèrent un risque à *H. guttulatus*. La croissance des populations de *H. guttulatus* et une espèce sympatrique, *H. hippocampus*, diffère d'amplitude et de direction lorsqu'une diminution dans l'effort de pêche non-sélective est appliquée: l'abondance de *H. guttulatus* augmente significativement, tandis que l'abondance de *H. hippocampus* diminue. *Hippocampus guttulatus* préfère les habitats complexes et végétés, tandis que *H. hippocampus* préfère les habitats peu végétés. Alors les éffets differentials de pêche non-sélective semblent être reliée aux préférences d'habitat: la diminution dans l'effort de pêche a causé une augmentation de la complexité d'habitat, favorisant *H. guttulatus*. Parcontre, la pêche non-sélective maintien des habitats peu végétatifs favorisant *H. hippocampus*. En employant *H. guttulatus* comme modèle, J'ai appliqué des simulations structurées et stochastiques afin d'évaluer les implications biologiques d'une stratégie universelle (limite minimum de taille de 10 cm) pour gérer l'exploitation et le commerce international des hippocampes. La plus petite taille (13 cm) tolerante a l'incertitude de l'effort de pêche, aux taux de croissance maximale des populations, et aux éffets de la densité sur la croissance des populations ést celle qui protége *H. guttulatus* au-delà de la première saison de reproduction. Dans cette première synthèse du cycle vital d'une espèce d'hippocampe et la première étude formelle des dynamiques de populations des hippocampes, j'ai identifié la capacité de rétablissement comme priorité de recherche afin de raffiner les analyses de risque d'éxtinction et les stratégies de conservation.

# GENERAL INTRODUCTION

## THE THREATENED STATUS OF MARINE FISHES

The challenges of obtaining basic biological information for assessing the conservation status of fishes are exemplified by a high percentage (22%) of evaluated fishes listed as Data Deficient by the World Conservation Union (compared to 3.7% for birds and 5.3% for mammals, IUCN 2003). Nevertheless, fishes are of considerable conservation concern with 49% of evaluated species listed as Threatened (Vulnerable, Endangered or Critically Endangered), compared to 12% for birds and 24% for mammals (IUCN 2003).

Exploitation and habitat loss were causally related to more than 90% of historical extirpations of marine fish populations (Dulvy et al. 2003) and are considered to be the most important contemporary threats to marine ecosystem integrity (Meffe & Carroll 1997, Pauly et al. 1998, Philippart 1998, Roberts & Hawkins 1999, Turner et al. 1999, Jackson et al. 2001). Other causes of local population extirpations in the sea included species invasions (2%), as well as climate change, pollution and disease (6%) (Dulvy et al. 2003).

For many populations of fishes, the direct effects of exploitation with non-selective bottom fishing gears are inextricably linked to the indirect effects of changes in benthic community structure (Dulvy et al. 2003). Depending on the frequency and intensity of exploitation, trawling and dredging disturbance can reduce habitat complexity and alter patterns in species abundance and composition (Engel & Kvitek 1998, Kaiser et al. 1998, Tuck et al. 1998, Turner et al. 1999, Collie et al. 2000, Jennings et al. 2001, Cryer et al. 2002, Kaiser 2003, but see Drabsch et al. 2001). Many species (e.g. the endemic totoaba) are threatened by both habitat degradation and the direct effects of exploitation of vulnerable life history stages (Cisneros-Mata et al. 1995, Dulvy et al. 2003, Sadovy & Cheung 2003).

Non-selective fishing gears incidentally capture a rich diversity of fauna and flora that vary widely in life history strategies, habitat associations, ecological interactions, behaviour, and vulnerability (Alverson et al. 1994, Stobutzki et al. 2001ab, Baum et al. 2003). While some resistant or resilient taxa (e.g. echinoderms, polychaetes) may respond positively (or neutrally) to disturbances associated with non-selective exploitation (Engel & Kvitek 1998, Kaiser et al. 1998, Kaiser 2003), less resilient species (e.g. elasmobranches, sciaenids) may become threatened by extinction or changes in population structure (Cisneros-Mata et al. 1995, Casey & Myers 1998). Variation in life history strategies and responses to disturbance, coupled with data-deficiency (Froese & Pauly 2004) makes it very challenging to predict responses of marine fishes to non-selective, multispecies fisheries as well as manage such fisheries (Stobutzki et al. 2001ab).

## FACTORS THAT CONFER RISK

For marine fishes, any biological, ecological or socio-economic feature of a species or population that increases catchability or vulnerability to exploitation and/or habitat alteration is likely to increase the risk of local extirpation (King 1995). Criteria for evaluating the risk of extinction or local extirpation usually relate to (1) population abundance, (2) the magnitude and direction of changes in population abundance (i.e. the population trajectory), (3) degree of population fluctuations, (4) the extent of endemism, (5) degree of ecological specialization, (6) degree of patchiness in distribution (7) changes in geographical distributions, (8) habitat fragmentation or degradation and (9) population productivity (e.g. Musick 1999, IUCN 2001). Recent advances in identifying correlates of vulnerability suggest that life history and behaviours that increase catchability (e.g. predictable spawning aggregations) or reduce resilience (e.g. Allee effects) should also play an important role in assessing risk and designing mitigating strategies (IUCN 1996, Vincent & Hall 1996, Vincent & Sadovy 1998, Shumway 1999, Roberts & Hawkins 1999, Dulvy et al. 2003, Hutchings & Reynolds 2004).

Declines in population sizes of species that are intentionally exploited are to be expected, thus sparking a debate about the level at which exploited marine species should be considered at risk (e.g. Musick 1999, reviewed in Dulvy et al. 2003). IUCN Red List guidelines were amended accordingly to reflect cases where the effects of exploitation were clearly reversible, understood and had ceased (IUCN 2001). Natural and/or extreme fluctuations in population size, the number of populations or the distribution of a species also confer risk (e.g. Fagan et al. 1999, 2001), although these must be distinguished from deterministic declines (IUCN 2001).

The life history rates (survival, growth, reproduction, movement) of marine fishes have important implications for their population-level responses to exploitation and habitat loss (Caswell, 1989, Koslow et al. 2000, King & McFarlane 2003). Recent comparative studies suggest that vulnerability is greater among fishes that grow more slowly, mature at an older age, and grow to larger body sizes (i.e. fishes with 'slower' life histories, Jennings et al. 1998, 1999ab, Dulvy & Reynolds 2002). Body size may be generally related to greater vulnerability because larger individuals or species (1) are often favoured (e.g. in the traditional medicine trade, Vincent 1996), (2) have greater catchability (Hilborn & Walters 1992, King 1995) and (3) have lower intrinsic rates of population increase (Frisk et al. 2001, Denney et al. 2002), which may make them less able to sustain fishing mortality than smaller individuals (Adams 1980, Pope et al. 2000, Hutchings 2001). Focus on life history parameters provides a useful starting point for assessing threats to species (Pope et al. 2000, Dulvy & Reynolds 2002, Dulvy et al. 2004), particularly when there is missing information about other important ecological correlates of extinction (e.g. ontogenetic shifts in habitat use, ecological specialization, Angermeier 1995).

Association with threatened or highly disturbed habitats confers risk, especially for species with high habitat specificity (Roberts & Hawkins 1999, Hawkins et al. 2000). Populations of shallow-water species that are relatively sedentary at one or more life history stages (e.g. echinoderms) are more vulnerable to over-exploitation, particularly where populations are easily accessible (e.g. to compressor divers) and directly targeted (King 1995, Perante et al. 1998, Roberts & Hawkins 1999). Shallow waters are more accessible to a range of marine resource users including fishers (although many deep water fisheries are also overexploited, Koslow et al. 2000). Moreover, most inshore habitats are subject to anthropogenic disturbances including increased pollution, dredging, and sea filling (Hatcher et al. 1989, Hall 1999, Pasqualini et al. 1999, Wolff 2000), which may also threaten fishes that are associated with these habitats during at least one of their life history stages (e.g. croakers, Musick et al. 2000).

One cannot ignore the role of behaviour in evaluating or predicting population-level responses to exploitation and habitat change (IUCN 1996, Vincent & Sadovy 1998, Shumway 1999). Marine organisms may either increase or decrease their vulnerability to exploitation through their behaviour and/or ecological interactions. Fishes that aggregate in predictable places to spawn may be directly targeted during aggregations (e.g. humphead wrasse), resulting in recruitment overfishing and/or the loss of important aggregation sites (Sadovy et al. 2003). By contrast, crabs may hide in crevasses or burrows during moulting, thereby temporarily reducing their vulnerability to trawls and traps (King 1995). Being able to predict the influence of behaviour on population-level responses, however, requires an understanding of the distinct ecological features of marine organisms that may not be easily extracted from the distribution and structure of catch landings (Shumway 1999).

Marine fishes that are highly valued may also be at greater risk of decline (Sadovy 2001). Historically, fisheries biologists have assumed that overexploited populations would become economically extinct, long before becoming biologically extinct (reviewed in Dulvy et al. 2003). For species of high commercial importance, however, it may be economically viable to exploit a species down to the last individual, particularly if rarity confers added economic value (Sadovy 2001, Sadovy & Vincent 2002). Even species that are not economically viable as target species may continue to be threatened by non-selective capture in multispecies fisheries (Casey & Myers 1998).

## RISK ASSESSMENTS FOR MARINE FISHES

Predicting the trajectory of populations over time and in response to alternative strategies for conservation is challenging when there is missing information about (1) life history, (2) habitat associations, (3) ecological interactions and behaviour (4) population dynamics, and/or (5) the putative agents of decline and their effects on population structure. When there is insufficient data for quantitative risk assessments (e.g. Criterion E, IUCN 2001), proactively developing means of predicting vulnerability is an important step towards setting priorities for research and management efforts, and preventing local extirpations or species extinctions (Angermeier 1995, Dulvy & Reynolds 2002, Dulvy et al. 2003, Dulvy et al. 2004).

Because size data are easy and cheap to collect (Hilborn & Walters 1992), the predictive utility of body size as an index of vulnerability (Jennings et al. 1998, 1999ab, Dulvy & Reynolds 2002) for even the most data-deficient species is fortuitous. The use of body size as a benchmark for identifying research and conservation priorities has been recently applied to skates and rays (Rajidae, Dulvy & Reynolds 2002). In this study, the smallest declining species was used as an objective means of setting priorities for research, monitoring and management of larger species (Dulvy et al. 2004).

When additional biological and ecological data are available, these can be incorporated into multivariate analyses of sustainability and risk. For example, Stobutzki et al. (2001a) used biological and ecological criteria to rank species in shrimp trawl bycatch according to their susceptibility to capture and their relative probability of recovery: life history (size, mortality rate), nature of parental care, mating structure, habitat (position in the water column, substrate preference, depth, range), diet (i.e. consumption of target species), catchability (diurnal behaviour, catch rates, vulnerable size classes) and post-discard survival probability.

Although stock assessments of exploited marine fishes are commonly carried out using a variety of robust fisheries models (Hilborn & Walters 1992, King 1995), not all of these models explicitly incorporate uncertainty, environmental variability and the risk of extinction into their design (Akçakaya & Sjorgen-Gulve 2000, Dulvy et al. 2003), which are viewed as important aspects of fisheries assessments (Reynolds & Mace 1999). However, depending on the structure and goals of the assessment, models that incorporate uncertainty and extinction risk may require more data than are currently available (Boyce 1992, Beissinger 1998, Groom & Pascual 1998).

Risk assessment models synthesize available, but often disparate information (e.g. life history, ecology, distributions, catch per unit effort data) to predict population trajectories and estimate relative extinction risks under specific hypotheses about the form and strength of disturbance regimes and alternative management strategies (Boyce 1992, Beissinger 1998, Groom & Pascual 1998). When data are available, such models may explicitly consider the demographic, genetic, and environmental uncertainty in population projection (Soulé 1987, Shaffer 1990).

Population viability analyses (PVAs), a group of risk assessment approaches, have been successfully applied to a wide range of taxa (though few to fishes, Crouse et al. 1987, Stacey & Taper 1992, Doak 1995, Wahlberg et al. 1996, Akçakaya & Sjorgen-Gulve 2000; Lindenmayer et al. 2000), and they are formally recognized as a means of carrying out species assessments (Criterion E, IUCN 2001). Despite their predictive accuracy (Brook et al. 2000) PVAs are infrequently applied for assessing conservation status (Boyce 1992), probably due to a lack of rigorous and/or quantitative biological information about life history rates, density-dependent processes, and factors conferring risk. In particular, the application of models that explicitly incorporate uncertainty and extinction risk for fish has largely been limited to freshwater fishes, including leopard darters (Williams et al. 1999), stream-dwelling salmonids (Morita & Yokota 2002) and trout cod (Todd et al. 2004), or anadromous species, including salmonids (Allendorf et al. 1997, McClure et al. 2003) and white sturgeon (Jager 2001, Jager et al. 2001). Although demographic and stochastic models have been applied to large marine fishes (e.g. sandbar shark and southern bluefin tuna, Sminkey & Musick 1996, McDonald et al. 2002), the application of PVAs to marine species has largely been to charismatic fauna including coastal cetaceans and otariids (fur seals and sea lions) (e.g. Thompson et al. 2000, Gerber & Hilborn 2001, but see Bearlin et al. 1999 for a study of seagrass dynamics). PVAs are a class of transparent risk assessment techniques that are powerful when used as a focal point for consensus building (e.g. workshops by the IUCN Conservation Breeding

Specialist Group) particularly when the aim is to develop adaptive management strategies (Ferson & Burgman 2002).

## THE THREATENED STATUS OF SEAHORSES

Species in the family Syngnathidae (seahorses, pipefishes, pipehorses, and seadragons) a group of small, benthic marine fishes, were no exceptions to the trends in threat and data-deficiency observed for other fishes: 61% of evaluated syngnathids were listed as Data Deficient and 39% were recognized as Threatened ($n = 44$, IUCN 2003). Most of these species were seahorses ($\geq 34$ *Hippocampus* spp.). Suspected and/or documented declines for 25% of seahorse species prompted their listing as Vulnerable by the World Conservation Union (IUCN), while all others save one (*Hippocampus capensis*, listed as Endangered) were listed as Data-Deficient (IUCN 2003).

Over the last decade, an apparent increase in the international trade of seahorses was coupled with growing concerns about the sustainability of their exploitation in bycatch (Vincent 1996, Baum et al. 2003) and in target fisheries (Perante et al. 1998, Martin-Smith et al. 2004). Though no long term data series of seahorse abundance (or indices of abundance) were available, semi-structured interviews with fishers suggested that many directly (by hand) and indirectly (as bycatch) exploited populations (primarily *H. comes*, some *H. histrix* and *H. kuda*) suffered declines from 1985 – 1995 (Vincent 1996, Vincent et al. unpublished data). Recently the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) decided that international trade was threatening or had the potential to threaten wild seahorse populations. As a consequence, all *Hippocampus* spp. were added to Appendix II, requiring all of the more than 165 signatory nations (Parties) to ensure that exports after 15 May 2004 did not threaten wild populations.

Broadly speaking, seahorses are characterized by small body size, paternal care, low mobility, sedentary adults and monogamous mating (reviewed in Foster & Vincent 2004). Although a dearth of basic biological information has hampered quantitative risk assessments (IUCN 2003, Foster & Vincent 2004), their life history, complex social interactions and association with shallow inshore habitats (seagrasses, coral reefs, estuaries and mangroves) were inferred to make them unsuitable for exploitation, vulnerable to habitat loss and a priority for research, monitoring and management (e.g. Foster & Vincent 2004, Martin-Smith et al. 2004). Thus, my thesis research was prompted by this first tier of risk assessment.

## SUMMARY OF RESEARCH OBJECTIVES

My thesis examines the implications of life history and ecology for the conservation of the European long-snouted seahorse *(Hippocampus guttulatus* Cuvier 1829) and its smaller congener, the short-snouted seahorse (*H. hippocampus* L). I investigate my thesis questions using data from sympatric populations of these species in the Ria Formosa lagoon, southern Portugal, where thy are locally abundant (representing >7% of fish biomass in experimental beam trawls, Erzini et al. 2002). High abundance provided an unusual opportunity to rigorously quantify seahorse life history parameters and habitat associations: most seahorse populations occur at such low densities that quantitative analyses of life history parameters are challenging. Moreover, the protected Ria Formosa lagoon allowed me to study sympatric seahorse populations subject to little or no recreational or commercial exploitation. The aims of my thesis were to:

1 Quantify patterns in the life history rates (survival, growth, reproduction and movement) of *H. guttulatus*, to fill gaps in our understanding of the biology of this species, and to provide the core elements of a risk assessment model (Chapter 1, Chapter 2).

2. Synthesize life history parameters using multivariate statistical analyses to cast seahorses in the context of general patterns in fish life history strategies. Despite a comprehensive review of seahorse life history and ecology that included univariate analyses (Foster & Vincent 2004), the implications of seahorse life history and ecology have generally been evaluated on a trait-by-trait basis (e.g. small brood size considered separately from multiple spawning behaviour). Multivariate analyses provide a conceptual framework for identifying suites of life-history correlates of

vulnerability and preliminary steps towards identifying biologically appropriate management strategies (Chapter 1, Chapter 2).

3. Investigate the implications of habitat use and preferences for population level responses of *H. guttulatus* and *H. hippocampus* to habitat degradation and exploitation. By doing so, I also aimed to quantify patterns in abundance and distribution of these sympatric species and develop regression-based habitat suitability indices to predict their relative abundances in unsurveyed habitats (Chapter 3, Chapter 4).

4. Evaluate the vulnerability (catchability) of seahorses to non-selective exploitation and predict population-level responses to a generic strategy for managing the international trade and exploitation of seahorses. I addressed the latter objective by synthesizing life history and ecological data into a structured, stochastic model that also allowed me to assess the conservation status of the population I studied and identify population parameters as priorities for further research (Chapter 4, Chapter 5).

Through my thesis research, I hope to stimulate further research and interest in (1) the life history and ecology of small, benthic marine fishes, (2) the importance of habitat use and behaviour for influencing population responses to disturbance, and (3) the application of quantitative risk assessment models that explicitly incorporate extinction risk as a plausible outcome for marine fishes.

# LITERATURE CITED

Akçakaya HR, Sjorgen-Gulve P (2000) Population viability analyses in conservation planning: an overview. Ecological Bulletins 48:9-21

Adams PB (1980) Life history patterns in marine fishes and their consequences for fisheries management. Fishery Bulletin 78:1-12

Allendorf FW, Bayles D, Bottom DL, Currens KP, Frissell CA, Hankin D, Lichatowich JA, Nehlsen W, Trotter PC, Williams TH (1997) Prioritizing Pacific salmon stocks for conservation. Conservation Biology 11:140-152

Alverson DL, Freeberg MH, Pope JG, Murawski SA (1994) A global assessment of fisheries bycatch and discards. FAO Fisheries Technical Paper No. 339. FAO, Rome

Angermeier PL (1995) Ecological Attributes of Extinction-Prone Species: Loss of Freshwater Fishes of Virginia. Conservation Biology 9:143-158

Baum JK, Meeuwig JJ, Vincent ACJ (2003) Bycatch of lined seahorses (*Hippocampus erectus*) in a Gulf of Mexico shrimp trawl fishery. Fishery Bulletin 101:721-731

Bearlin AR, Burgman MA, Regan HM (1999) A stochastic model for seagrass (*Zostera muelleri*) in Port Phillip Bay, Victoria, Australia. Ecological Modelling 118:131-148

Beissinger SR (1998) On the use of demographic models of population viability in endangered species management. Journal of Wildlife Management 62(3):821-841

Boyce M (1992) Population viability analysis. Annual Review of Ecology and Systematics 23:481-506

Brook BW, O'Grady JJ, Chapman AP, Burgman MA, Akçakaya HR, Frankham R (2000) Predictive accuracy of population viability analysis in conservation biology. Nature 404:385-387

Casey JM, Myers RA (1998) Near extinction of a large widely distributed fish. Science 281(5377):690-692

Caswell H (1989) Matrix population models: construction, analysis and interpretation. Sinauer Associates, Sunderland, Massachusetts

Cisneros-Mata MA, Montemayor-Lopez G, Roman-Rodriguez MJ (1995) Life history and conservation of *Totoaba macdonaldi*. Conservation Biology 9:806-814

Collie JS, Hall SJ, Kaiser MJ, Poiner IR (2000) A quantitative analysis of fishing impacts on shelf-sea benthos. Journal of Animal Ecology 69:785-798

Crouse D, Crowder L, Caswell H (1987) A stage-based population model for loggerhead sea-turtles and implications for conservation. Ecology 68:1412-1423

Cryer M, Hartill B, O'Shea S (2002) Modification of marine benthos by trawling: toward a generalization for the deep ocean? Ecological Applications 12(6):1824-1839

de Silva JA, Mahmoudi B, Muller RG, Murphy MD (2003) Florida's Inshore and Nearshore Species: 2002 Status and Trends Report, Appendix A. Florida Fish and Wildlife Conservation Commission - Florida Wildlife Research Institute, St. Petersburg, Florida.

Doak DF (1995) Source-sink models and the problem of habitat degradation: general models and applications to the Yellowstone grizzly. Conservation Biology 9:1370-1379

Denney NH, Jennings S, Reynolds JD (2002) Life-history correlates of maximum population growth rates in marine fishes. Proceedings of the Royal Society of London, Series B 269, 2229-2237

Drabsch, S. L., J. E. Tanner, Connell SD (2001) Limited infaunal response to experimental trawling in previously untrawled areas. ICES Journal of Marine Sciences 58: 1261-1271

Dulvy NK, Ellis JR, Goodwin NB, Grant A, Reynolds JD, Jennings S (2004) Methods of assessing extinction risk in marine fishes. Fish and Fisheries 5:255-276

Dulvy NK, Sadovy Y, Reynolds JD (2003) Extinction vulnerability in marine populations. Fish and Fisheries 4:25-64

Dulvy, NK, Reynolds JD (2002). Predicting extinction vulnerabilities in skates. Conservation Biology 16(2):440-450

Engel J, Kvitek R (1998) Effects of otter trawling on a benthic community in Monterey Bay National Marine Sanctuary. Conservation Biology 12(6):1204-1214

Erzini K, Bentes L, Coelho R, Correia C, Lino PG, Monteiro P, Ribeiro J, Goncalves JMS (2002) Recruitment of sea breams (Sparidae) and other commercially important species in the Algarve (southern Portugal), Final Report, Commission of the European Communities DG XIV/C/1: 178

Fagan WF, Meir E, Prendergast J, Folarin A, Karieva P (2001) Characterizing population vulnerability for 758 species. Ecology Letters 4:132-138

Fagan WF, Meir E, Moore JL (1999) Variation thresholds for extinction and their implications for conservation strategies. American Naturalist 154:510-520

Ferson S, Burgman M (2002) Quantitative methods for conservation biology. Springer-Verlag, New York

Foster SJ, Vincent ACJ (2004) The life history and ecology of seahorses: implications for conservation and management. Journal of Fish Biology 65:1-61

Frisk MG, Miller TJ, Fogarty MJ (2001) Estimation and analysis of biological parameters in elasmobranch fishes: a comparative life history study. Canadian Journal of Fisheries and Aquatic Sciences 58:969-981

Froese R, Pauly D (eds) (2004) FishBase. World Wide Web electronic publication. www.fishbase.org, Version 05/2004.

Gerber LR, Hilborn R (2001) Catastrophic events and recovery from low densities in populations of otariids: implications for risk of extinction. Mammal Review 31:131-150

Groom MJ, Pascual MA (1998) The analysis of population persistence: An outlook on the practice of viability analysis. In: Fieldler PL, Kareiva PM (eds) Conservation Biology For the Coming Decade (2nd ed). Chapman & Hall, New York

Hall SJ (1999) Fish Biology and Aquatic Resources Series 1: The effects of fishing on marine ecosystems and communities. Blackwell Science Ltd, Oxford

Hatcher BG, Johannes RE, Robertson AI 1989. Marine Ecosystems. Oceonog. Mar. Biol. Ann. Rev 27:337-414.

Hawkins JP, Roberts CM, Clark V (2000) The threatened status of restricted-range coral reef fish species. Animal Conservation 3:81-88

Hilborn R, Walters CJ (1992) Quantitative fisheries stock assessment: choice, dynamics and uncertainty. Kluwer Academic Publishers, London, UK

Hutchings J, Reynolds JD (2004) Marine fish population collapses: Consequences for recovery and extinction risk. BioScience 54:297-309

Hutchings J (2001) Conservation biology of marine fishes: perceptions and caveats regarding assignment of extinction risk. Canadian Journal of Fisheries and Aquatic Sciences 58:108-121

IUCN (2003) 2003 IUCN Red List of Threatened Species. <www.redlist.org>, Downloaded on 31 August 2004

IUCN (2001) IUCN red list categories and criteria: Version 3.1. In: Commission ISS (ed) IUCN, Gland, Switzerland

IUCN (1996) Threatened fish? Initial Guidelines for applying the IUCN Red List Criteria to Marine Fishes. In: Hudson E, Mace GM, Baillie J, Sobel J, Punt A, Safina C, Vincent ACJ, Roberts C, Sadovy Y, Hall H (eds) IUCN/WWF/ZSL Workshop on marine fish and the IUCN red list of threatened animals. IUCN, London, UK

Jackson JBC, Kirby MX, Berger WH, Bjorndal KA, Botsford LW, Bourque BJ, Bradbury RH, Cooke R, Erlandson J, Estes JA, Hughes TP, Kidwell S, Lange CB, Lenihan HS, Pandolfi JM, Peterson CH, Steneck RS, Tegner MJ, Warner RR (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293:629-638

Jager HI (2001) Individual variation in life history characteristics can influence extinction risk. Ecological Modelling 144:61-76

Jager HI, Chandler JA, Lepla KB, Van Winkle W (2001) A theoretical study of river fragmentation by dams and its effects on white sturgeon populations. Environmental Biology of Fishes 60:347-361

Jennings S, Reynolds JD, Mills SC (1998). Life history correlates of responses to fisheries exploitation. Proceedings of the Royal Society of London. Series B 265: 333-339

Jennings S, Pinnegar JK, Polunin NVC, Warr KJ (2001) Impacts of trawling disturbance on the trophic structure of benthic invertebrate communities. Marine Ecology Progress Series 213:127-142

Jennings S, Greenstreet SPR, Reynolds JD (1999a) Structurual change in an exploited fish community: a consequence of differential fishing effects on species with contrasting life histories. Journal of Animal Ecology 68:617-627

Jennings S, Reynolds JD, Polunin NVC (1999b) Predicting the vulnerability of tropical reef fishes to exploitation with phylogenies and life histories. Conservation Biology 13(6):1466-1475

Kaiser MJ (2003) Detecting the effects of fishing on seabed community diversity: importance of scale and sample size. Conservation Biology 17(2):512-520

Kaiser MJ, Edwards DB, Armstrong PJ, Radford K, Lough NEL, Flatt RP, Jones HD (1998) Changes in megafaunal benthic communities in different habitats after trawling disturbance. ICES Journal of Marine Sciences 55:353-361

King JR, McFarlane GA (2003) Marine fish life history strategies: applications to fishery management. Fisheries Management and Ecology 10:249-264

King M (1995) Fisheries biology, assessment and management. Fishing News Books, Oxford.

Koslow JA, Boehlert GW, Gordon JDM, Haedrich RL, Lorance P, Parin N (2000) Conti nental slope and deep-sea fisheries: implications for a fragile ecosystem. ICES Journal of Marine Sciences 57:548-557

Lindenmayer, DB, Lacy RC Pope ML (2000) Testing a simulation model for population viability analysis. Ecological Applications 10(2):580-597

Martin-Smith KM, Samoilys MA, Meeuwig JJ, Vincent ACJ (2004) Collaborative development of management options for an artisanal fishery for seahorses in the central Philippines. Ocean and Coastal Management 47:165-193

McClure MM, Holmes EE, Sanderson BL, Jordana CE (2003) A large-scale multispecies status assessment: anadromous salmonids in the Columbia River Basin. Ecological Applications 13:964-989

McDonald AD, Sandal LK, Steinshamn SI (2002) Implications of a nested stochastic/deterministic bio-economic model for a pelagic fishery. Ecological Modelling 149:193-201

Meffe GK Carroll CR (1997) Principles of Conservation Biology (2nd ed). Sinauer Associates, Sunderland, Massachussetts

Morita K, Yokota A (2002) Population viability of stream-resident salmonids after habitat fragmentation: a case study with white-spotted charr (*Salvelinus leucomaenis*) by an individual based model. Ecological Modelling 155:85-94

Musick JA, Harbin MM, Berkeley SA, Burgess GH, Eklund AM, Findley L, Gilmore RG, Golden JT, Ha DS, Huntsman GR et al. (2000) Marine, estuarine, and diadromous fish stocks at risk of extinction in North America (Exclusive of Pacific Salmonids). Fisheries 25: 6-30

Musick J (1999) Criteria to define extinction risk in marine fishes - The American Fisheries Society initiative. Fisheries 24:6-14

Pasqualini V, Pergent-Martini C, Pergent G (1999) Environmental impact identification along the Corsican coast (Mediterranean sea) using image processing. Aquatic Botany 65: 311-320

Pauly D Christensen V, Dalsgaard J, Froese R, Torres F (1998) Fishing down marine food webs. Science 279:860-863

Perante NC, VincentACJ, Pajaro MG (1998) Demographics of the seahorse *Hippocampus comes* in the central Philippines. 3rd International Conference on the Marine Biology of the South China Sea, Hong Kong University Press, Hong Kong

Philippart CJM (1998) Long-term impact of bottom fisheries on several by-catch species of demersal fish and benthic invertebrates in the south-eastern North sea. ICES Journal of Marine Science 55: 342-352

Pope, JG, MacDonald DS, Daan N, Reynolds JD, Jennings S (2000) Gauging the impact of fishing mortality on non-target species. ICES journal of marine science 57:689-696

Reynolds JD, Mace GM (1999) Risk assessments of threatened species. Trends in Ecology & Evolution 14:215-217

Roberts CM, Hawkins JP (1999) Extinction risk in the sea. Trends in Ecology & Evolution 14(6):241-246

Sadovy Y, Kulbicki M, Labrosse P, Letourneur Y, Lokani P, Donaldson TJ (2003) The humphead wrasse, *Cheilinus undulatus*: synopsis of a threatened and poorly known giant coral reef. Reviews in Fish Biology and Fisheries 13:327-364

Sadovy Y, Cheung WL (2003) Near extinction of a highly fecund fish: the one that nearly got away. Fish and Fisheries 4:86-99

Sadovy YJ, Vincent ACJ (2002) Ecological issues and the trades in live reef fishes. In: Sale, P (ed) Coral Reef Fishes. Academic Press, San Diego, California

Sadovy Y (2001) The threat of fishing to highly fecund fishes. Journal of Fish Biology 59(Suppl. A):90-108

Shumway Caroly (1999) A neglected science: applying behavior to aquatic conservation. Environment Biology of Fishes 55:183-201

Sminkey TR, Musick JA (1996) Demographic analysis of the sandbar shark, *Carcharhinus plumbeus* in the western North Atlantic. Fishery Bulletin 94:341-347

Shaffer ML (1990) Population viability analysis. Conservation Biology 4:39-40

Soulé ME (ed) (1987) Viable Populations for Conservation. Cambridge University Press, New York

Stacey PB, Taper M (1992) Environmental variation and the persistence of small populations. Ecological Applications 2:18-29

Stobutzki I C, Miller M J, Brewer D (2001a) Sustainability of fishery bycatch: a process for assessing highly diverse and numerous bycatch. Environmental Conservation 28:167-181

Stobutzki IC, Miller MJ, Jones P, Salini JP (2001b) Bycatch diversity and variation in a tropical Australian penaeid fishery; the implications for monitoring. Fisheries Research 53:283-301

Thompson PM, Wilson B, Grellier K, Hammond PS (2000) Combining power analysis and population viability analysis to compare traditional and precautionary approaches to conservation of coastal cetaceans. Conservation Biology 14:1253-1263

Todd CR, Nicol SJ, Koehn JD (2004) Density-dependence uncertainty in population models for the conservation management of trout cod, *Maccullochella macquariensis*. Ecological Modelling 171:359-380

Tuck ID, Hall SJ, Robertson MR, Armstrong E, Basford DJ (1998) Effects of physical trawling disturbance in a previously unfished sheltered Scottish sea loch. Marine Ecology Progress Series 162: 227-242

Turner SJ, SF Thrush, JE Hewitt, VJ Cummings Funnell G (1999) Fishing impacts and the degradation or loss of habitat structure. Fisheries Management and Ecology 6: 401-420

Vincent ACJ, Sadovy Y (1998) Reproductive ecology in the conservation and management of fishes. In: Caro, Tim (ed). Behavioural Ecology and Conservation Biology. Oxford University Press, Oxford

Vincent ACJ (1996) The international trade in seahorses. TRAFFIC International, Cambridge

Vincent AC, Hall H (1996) The threatened status of marine fishes. Trends in Ecology and Evolution 11:360-361

Walberg N, Moilanen A, Hanski I (1996) Predicting the occurrences of endangered species in fragmented landscapes. Science 273: 1536-1538

Williams LR, Echelle AA, Toepfer CS, Williams MG, Fisher WL (1999) Simulation modeling of population viability for the leopard darter (Percidae : *Percina pantherina*). Southwestern Naturalist 44:470-477

Wolff WJ (2000) Causes of extirpations in the Wadden Sea, an estuarine area in The Netherlands. Conservation Biology 14: 876-885

# LINKING STATEMENT 1

The trajectory of a population over the short term is a consequence of its age structure coupled with age-specific life history rates (Caswell 1989). Yet, there are no estimates of age-specific survival, growth, or reproductive rates for any wild population of seahorse. Moreover, our understanding of movement patterns is generally limited to quantifying the home range size of reproductively active adults (up to a maximum of 16 months, Perante et al. 2002). Therefore I begin my thesis by quantifying the core elements of most population viability analyses: patterns in survival, growth, reproduction and movement of the European long-snouted seahorse, *Hippocampus guttulatus* (Cuvier 1829).

I include survival, growth and movement patterns in Chapter 1, because understanding movement patterns was central to developing an understanding of the survival and growth patterns of *H. guttulatus*. I leave the estimation of spawning frequencies and annual reproductive rates to Chapter 2.

# CHAPTER 1

# LIFE HISTORY OF AN UNUSUAL MARINE FISH: SURVIVAL, GROWTH AND MOVEMENT PATTERNS OF THE EUROPEAN LONG-SNOUTED SEAHORSE, *HIPPOCAMPUS GUTTULATUS* (CUVIER 1829)

## ABSTRACT

This paper reports the first mark-recapture estimates of life history rates (movement, growth and survival) for a wild seahorse population (European long-snouted seahorse, *Hippocampus guttulatus* Cuvier 1929). Population structure was characterized by high local densities (0.3 – 1.5 $m^{-2}$), equal sex ratios and few juveniles smaller than 70 mm. Adult *H. guttulatus* maintained small (19.9 $m^2$ ± 12.4 SD), strongly overlapping home ranges during multiple reproductive seasons. Settled (benthic) juveniles exhibited significantly lower site fidelity than adults and on average did not maintain home ranges until size at first reproduction. Fitting a von Bertalanffy growth model to annual growth increments of settled juveniles and adults led to estimates of $K = 0.571$ and $L_\infty = 197.6$ mm. However, the growth rate of planktonic juveniles (inferred from previous studies), was considerably greater than predicted by the fitted von Bertalanffy growth model. Estimates of the instantaneous rate of natural mortality, $M$, ranged from 1.15 – 1.22 $yr^{-1}$ (annual survival rate = 29.4 – 31.5 %). Sexes did not differ in movement, growth or survival patterns. On average, *H. guttulatus* measured 12.2 ± 0.8 mm at birth. Planktonic juveniles recruited to vegetated habitat at 96.0 ± 8.0 mm (0.25 years), matured at 109.4 mm (0.49 years), began maintaining home ranges and reproducing at 125 – 129 mm (0.85 – 0.94 years), and lived for 4.3 – 5.5 years. Small body size, early age at maturity, rapid growth rates, and short life spans suggest that *H. guttulatus* (a) has a high intrinsic rate of increase, (b) is likely to recover rapidly when the direct (fishing mortality) and indirect (bycatch, habitat damage) effects of exploitation cease and (c) is vulnerable to extended periods of poor recruitment.

# INTRODUCTION

The life history rates (survival, growth, reproduction, movement) of marine fishes have important implications for their population-level responses to exploitation and habitat loss (Caswell 1989, Koslow et al. 2000, King & McFarlane 2003). The risk of decline or local extirpation is greater for species that mature at an older age, grow to larger body sizes and have lower rates of population increase (Jennings et al. 1998, Jennings et al. 1999ab, Dulvy & Reynolds 2002). Marine fishes that are highly valued, part of multispecies fisheries or associated with threatened or highly disturbed habitats may also be at greater risk of decline (Sadovy 2001). Life history parameters may be used as indices to predict vulnerability (e.g. Dulvy & Reynolds 2002) or to predict life history rates (e.g. natural mortality; intrinsic rate of increase) that are difficult to estimate directly (e.g. Pauly 1980, Denney et al. 2002, Froese & Binohlan 2003). Suites of life history traits can also be used to identify general life history strategies (Winemiller & Rose 1992, McCann & Shuter 1997) as a basis for identifying potentially effective management plans (e.g. King & McFarlane 2003). However, estimates of life history parameters are lacking for most fishes (Froese & Binohlan 2003, Froese & Pauly 2004).

Despite their economic value (Vincent 1996) and concern about their conservation status (IUCN 2003), the life history parameters of most seahorse species are unknown or limited to inferences from studies of captive populations (e.g. Wilson & Vincent 1998, Woods 2000, Job et al. 2002). Of the species of seahorse (≥34 *Hippocampus* spp., Family Syngnathidae) listed by the World Conservation Union, >25% are inferred to be Vulnerable to overexploitation and/or habitat loss. With one exception (the Endangered Knysna seahorse, *Hippocampus capensis* Boulenger), the remaining species are listed as Data-Deficient (IUCN 2003). Broadly speaking, these small-bodied fishes are characterized by an unusual morphology, specialized paternal care, low mobility, small adult home ranges, and, in most studied species, mate fidelity (Breder & Rosen 1966, Boisseau 1967, Vincent & Sadler 1995, Masonjones & Lewis 1996, Lourie et al. 1999, Masonjones & Lewis, 2000, Perante et al. 2002, Vincent & Giles 2003, Moreau & Vincent 2004, reviewed in Foster & Vincent 2004). Many seahorse species are directly exploited for international trade (e.g. the tiger tail seahorse, *Hippocampus comes*

Cantor, Perante et al. 1998), but the majority of exploited seahorses are captured incidentally in multispecies fisheries (Vincent, 1996).

This paper investigates the life history of the Data-Deficient (IUCN 2003) European long-snouted seahorse, *Hippocampus guttulatus* (Cuvier 1829). One of the most comprehensive accounts of seahorse biology, a detailed study of male *H. guttulatus* physiology (Boisseau 1967), presented inferences about this species' life history, including juvenile growth rate, size and age at recruitment, size and age at first reproduction, brood sizes and longevity. These inferences were based on limited field collections and observations of captive seahorses. Previous studies have characterized the size structure (Boisseau 1967, Reina-Hervás 1989), habitat preferences (Garrick-Maidment & Jones 2004, Chapter 3) and size-specific annual reproductive output of wild *H. guttulatus* (Chapter 2). Yet despite conservation concern for this species (Foster & Vincent 2004), the population structure and life history parameters of wild *H. guttulatus* remain largely unknown. The aim of this paper was to quantify size-specific survival, growth and movement rates for future use in population dynamics models. Our specific objectives were to (a) characterize the population structure of wild *H. guttulatus,* (b) estimate natural mortality rates and longevity, (c) develop a growth model to predict age from size, (d) quantify the degree of spatial and temporal site fidelity of recruited (settled, benthic) juveniles and adults, and (e) identify potentially effective management actions based on the life history strategy of this species.

## MATERIALS AND METHODS

### Species description

This paper employs the taxonomy of Lourie et al. (1999), which provides detailed descriptions of most species in the genus *Hippocampus*. Historically, *H. guttulatus* has been synonymized with *Hippocampus ramulosus* (Leach 1814) but examination of the *H. ramulosus* holotype suggested that this might represent a different species (Lourie et al. 1999). Clarification will require further research. In the meantime the name *H. guttulatus* is retained.

Adult and settled juvenile *H. guttulatus* are associated with seagrass- and macroalgae-dominated communities (Chapter 3) in the Mediterranean Sea and northeastern Atlantic Ocean (Boisseau 1967, Reina-Hervás 1989, Lourie et al. 1999, Garrick-Maidment & Jones 2004). *Hippocampus guttulatus* spawns multiple times during extended reproductive seasons (Boisseau 1967, Reina-Hervás 1989, Chapter 2). Broods ranging from 10-581 embryos (mean = 214) develop within the male's brood pouch for ~21 days (Boisseau 1967, Chapter 2). After this brooding period, newborns are expelled from the pouch with no further parental care. Juveniles have a planktonic stage before they recruit to vegetated habitats (Boisseau 1967, Pérez-Ruzafa et al. 2004).

## Site characteristics

This study was carried out in the Ria Formosa lagoon (36°59' N, 7°51' W) in southern Portugal. The Ria Formosa is highly productive and characterized by high water turnover rates, sand flats, salt-marshes and a network of channels and tidal creeks (Sprung 1994, Machás & Santos 1999). Water temperature varied seasonally from 10-28 °C (Chapter 2). The subtidal vegetation was dominated by the seagrass *Cymodocea nodosa* and the macroalgae *Ulva lactuca* and *Codium* spp. (Alberto et al. 2001, Chapter 3). The lagoon supports two sympatric seahorse species (*H. guttulatus* and the short-snouted seahorse, *Hippocampus hippocampus* L) and five pipefish species (Erzini et al. 2002), but the syngnathid community is dominated by *H. guttulatus* (mean density is 0.07 $m^{-2}$, versus *H. hippocampus* density of 0.007 $m^{-2}$, Chapter 3).

Two focal study grids were established to monitor individually tagged *H. guttulatus* (Fig. 1.1). Grid A (10 m x 10 m, depth 6.9 – 7.2 m) was established 15 m from the intertidal zone and 50 m west of a permanent pier in the Ria Formosa Natural Park. Grid B (10 m x 10 m, depth 1.3 – 6.1 m) was established 5 m from the intertidal zone and adjacent to the east side of the pier. Both grids were marked into 1 $m^2$ cells with small, plastic, numbered flags. A 2 m wide zone around Grid B was also marked out into 1 $m^2$ cells to record the locations of tagged seahorses that moved temporarily off the grid. Mean horizontal visibility on the grids was 1.2 m, but ranged from 0.25 – 5 m depending on prevailing weather conditions and tidal phase.

### Measuring seahorses

Measuring seahorse lengths consistently is challenging because of the curvature of the trunk and tail, and because the head is held at an angle to the body (Lourie et al. 1999). All measurements used in this paper (standard length, trunk length and height) were measured to the nearest mm as in Lourie et al. (1999) with one important distinction: measurements were taken as straight lines between the appropriate reference points, rather than following the curvature of the trunk. Straight lines were used because following the curvature of the trunk was impracticable when measuring live seahorses *in situ* and time consuming when measuring large volumes of preserved specimens. Moreover, studies reporting *H. guttulatus* lengths (height and/or trunk length) used straight-line measurements (Boisseau 1967, Pérez-Ruzafa et al. 2004, A. Pérez-Ruzafa, in litt. July 2004).

Lengths reported in this paper are standard lengths, except where stated otherwise. When trunk lengths or heights were measured or reported in other studies, they were converted to standard lengths using regression equations developed for this species (see below). When measuring live seahorses *in situ*, trunk lengths were measured because of greater precision as a consequence of not needing to uncurl the tail.

### Mark-recapture study

From 4 August to 18 September 2000, 63 *H. guttulatus* were tagged and monitored on Grid A using collar tags (as in Vincent & Sadler 1995), which were removed by the end of September 2000 (Table 1.1). Collar tags were not suitable for long-term study as they had to be monitored daily to prevent fouling and injury. No further monitoring was carried out on Grid A. From 2001 to 2003, 637 *H. guttulatus* captured on Grid B were permanently and uniquely tagged using visible implant fluorescent elastomer (VIE tags, Northwest Marine Technologies, Inc.) (Table 1.1). VIE-tagged fish were monitored on Grid B from 18 July to 26 October 2001, 20 May to 12 September 2002, and 23 May to 4 June 2003. Each VIE-tagged fish received three 1-2 mm markings between the first trunk ring and dorsal fin such that tags could be discerned *in situ* without handling fish. In order to tag seahorses (using either collar or VIE tags), grid coordinates were estimated to the nearest 0.1 m, and then seahorses were gently removed from their holdfast, placed into individual ziplock bags, brought to the surface and transported to holding tanks on the pier. All seahorses were held fully submerged on site in seawater during the entire tagging process to minimize stress. While tagging, the sex, trunk length, and the developmental stage of the brood pouch (males only, see below) were recorded. All seahorses were returned to their capture location within 1-3 hours of removal. Approximately 3% of resighted fish lost part of the VIE tag (15 fish lost 1 of 3 markings, 1 fish lost 2 of 3 markings), but these fish were reliably identified throughout the study. VIE tags had no significant effect on the growth in body length of captive big-bellied seahorses, *Hippocampus abdominalis* Lesson (Woods & Martin-Smith 2004) and were assumed to have no effect on *H. guttulatus* life history rates *in situ.*

Fifty SCUBA dives (~170 person h) were carried out on Grid A in 2000 and 223 dives (~695 person h) were carried out on Grid B from 2001 to 2003 (Table 1.1). During dives, the grid coordinates, tag (if present), trunk length, and sex of seahorses were recorded. Three types of dives were carried out: (1) census dives, during which all individuals within a grid were systematically censused, and most untagged seahorses were collected for tagging, (2) survey dives, during which data were collected non-systematically from tagged individuals on the grids and up to 5 m outside the perimeter of the grids, and (3) transect dives, during which 100 – 400 $m^2$ belt transects were searched for tagged individuals (Fig. 1.1). Census and survey dives were used to detect movements on and/or close to the grids. Transect dives were used to detect emigration from Grid B (2001 – 2003 only). Belt transects (2 – 4 m wide) started on the western or eastern edge of Grid B and extended through suitable habitat up to 100 m westward or eastward, respectively. Transects could not be extended southwards because of heavy boat traffic, or northwards because of proximity to the intertidal zone. Distances from the outer perimeter of the grids were measured using measuring tapes (to the nearest 0.1 m). All SCUBA dives were carried out between sunrise and dusk, with the exception of four night dives in August 2002. The data collected on the grids are hereafter referred to as the 'mark-recapture data'.

## Experimental fishing

Seahorses were collected monthly from September 2000 to July 2002 at 53 sites throughout the western part of the Ria Formosa lagoon using either experimental beach seines or beam trawls, and then frozen. These experimental collections, henceforth referred to as the 'catch data', were part of an unrelated study of fish community structure (Erzini et al. 2002). We recorded the sex, standard length, trunk length, height, wet mass (weighed to a precision of 0.01 g), reproductive state (males: full or empty pouch; females: presence or absence of hydrated eggs, as in Vincent & Sadler 1995, Perante et al. 2002, Chapter 2) and developmental stage of the male brood pouch (see below). Signs of disease or injury were also noted. Lengths and mass were $\log_{10}$-transformed and linear regressions were used to develop equations for predicting standard length from trunk length, height and mass. The catch data were assumed to produce an unbiased sample of *H. guttulatus* population structure because no significant differences in size structure, sex ratio, reproductive state or condition (injuries) were observed in comparisons of the catch data with data collected using underwater visual census techniques at a subset of these sampling stations (Chapter 4).

## Home range size and movement patterns

The degree of site fidelity was evaluated to characterize movement patterns and verify that a mark-recapture study was appropriate for estimating survival and growth rates in this species. Spatial and temporal site fidelity was measured as (a) home range size, (b) emigration from the grids into surrounding habitat, (c) the extent of overlap between home ranges held in different years, and (d) residency time. The size of home ranges held within years was estimated with 95% minimum convex polygons (MCP) using the Home Range Analysis Extension for Arcview$^{TM}$ (Rodgers & Carr 1998). When individuals were located multiple times within a day, only the first sighting was used as a fix (set of grid coordinates) to estimate MCP. Using a series of non-linear regressions of MCP area against the number of fixes, we found that there was no significant effect of the number of fixes on MCP area when we considered individuals with ≥15 fixes. Thefore only individuals with ≥15 fixes were included in the analyses. Therefore only individuals with ≥15 fixes were included in the analyses. As fewer than 15 dives were carried out in 2003, MCPs were only calculated within years from 2000 to 2002. MCPs were $\log_{10}$-transformed to normalize the data for statistical analyses. The percentage of overlap among home ranges held by fish in different years was also estimated using the Home Range Analysis Extension for Arcview™ (Rodgers & Carr 1998). The distance between the centers of these home ranges was estimated algebraically (Fig. 1.2). Residency time was estimated as the time elapsed between the first and last sighting on a grid, despite recognizing that some individuals were present on the grid before the study began and/or after the study ended.

### Growth

Length at age, birth, recruitment, maturity, and first reproduction were estimated. Because planktonic juveniles were not sampled in this study, only the size of newly released juveniles and the growth rates of recruited juveniles and adults were measured directly. Length at age and birth were estimated using the mark-recapture data. Length at recruitment to benthic habitat, maturity and first reproduction were estimated using the catch data.

#### *Length at age*

Conventional hard parts analysis for aging could not be used because otolith annuli from *H. guttulatus* were not clear (J. Curtis, personal observation) and ageing methods have not been validated for this species. Therefore length at age was estimated using annual length increments, assuming that the growth of recruited *H. guttulatus* conformed to the von Bertalanffy growth function (VBGF) (von Bertalanffy 1938):

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

where $L_t$ is the length at age $t$, $K$ is the instantaneous growth rate coefficient, $L_\infty$ is the theoretical maximum (asymptotic) length and $t_0$ is the theoretical age at zero length. The parameters $K$ and $L_\infty$ were estimated using a Ford-Walford plot (Walford 1946) of annual growth increments. Ford-Walford plots produced accurate estimates of $K$ and $L_\infty$ in computer simulations of mark-recapture studies (Smith et al. 1997). One annual growth increment per individual was measured from July 2001 – July 2002, September 2001 – September 2002, or May 2002 – May 2003. The third von Bertalanffy growth parameter, $t_0$, was estimated by rearranging the VBGF:

$$(2)\ t_0 = t_r + (1/K)\cdot(\ln((L_\infty - L_r)/L_\infty))$$

and substituting $L_t$ and $t$ with independent estimates of length ($L_r$) and age ($t_r$) at recruitment, respectively (see below).

### *Length at birth*

Length at birth was estimated by temporarily placing 8 brooding males in mesh cages *in situ* to capture and measure the lengths of newborn juveniles. Three juveniles per brood were measured. Brooding males were held only once, for a maximum of 48 h and were released with their newborn juveniles within 12 h of birth.

### *Length and age at recruitment*

$L_r$ and $t_r$ were estimated from the temporal lag between reproduction and juvenile recruitment. The seasonal pattern in juvenile recruitment was quantified by plotting the proportion of individuals that were juveniles (i.e. less than length at 50% maturity) over time. Similarly, the seasonal pattern of reproduction was quantified by plotting the proportion of mature males with full pouches (i.e. male spawning fraction) against time. $L_r$ was estimated as the mean size of the juveniles in the catch data during the first month of recruitment. $t_r$ was estimated as the time between the first month of the reproductive season and the first month that juveniles were captured.

### *Length and age at maturity*

Sexual maturity was inferred from the developmental stage of the brood pouch on the male's tail. Pouch development was classified into five stages (after Boisseau 1967): (1) a pigmented oval zone, (2) a slit among folds of tissue, (3) a pouch flush with the tail but too small to hold a brood, (4) empty mature pouch, and (5) full mature pouch.

Previous results suggest that the development of the pouch coincides with the development of the testes (Boisseau 1967, but see Cai et al. 1984), but because the specimens in the catch data were being used elsewhere for unrelated purposes, it was not possible to verify sexual maturity using histological techniques. Therefore males were assumed to be mature if they had mature pouches (i.e. stages 4 or 5). Length at which 50% of males were mature, $L_m$, was estimated by means of a logistic curve fitted to the proportion of mature individuals by length (binned into 5 mm length classes) using nonlinear regression techniques. Age at maturity, $t_m$, was predicted from $L_m$ using the fitted VBGF. Females and males were assumed to mature at the same length (and age).

*Length and age at first reproduction*

Mean length at first reproduction, $L_s$, was estimated as the length at which the spawning fraction (proportion of individuals that were reproducing) was 50% of the maximum value. To estimate this, the proportion of brooding males (i.e. full mature pouches) was regressed against length (binned into 5mm length classes) using logistic regression. Similarly, the proportion of females with hydrated eggs in the catch data was regressed against length. Age at first reproduction, $t_s$, was inferred from $L_s$ using the fitted VBGF.

### Survival, generation time and longevity

Survival rates were estimated using three approaches. The first method employed mark-recapture data to estimate the recapture probability, $p$, (i.e. probability of resighting tagged individuals) and apparent survival rate, $\phi$, using Program Mark Version 3.2 (White & Burnham 1999), assuming a Cormack-Jolly-Seber (CJS) model (Lebreton et al. 1992). Mark-recapture data from 9 trapping sessions (periods of high search effort that are short relative the interval of time between trapping sessions) on Grid B were used for this analysis. Trapping sessions lasted 5 – 7 days, consisted of 8 – 10 dives (3-5 census dives + 3-5 survey dives, for a total of ~32 hours of search time), and occurred monthly from August to October 2001, May to September 2002, and in May 2003. The ages of tagged individuals were predicted from lengths using the fitted VBGF and then individuals were binned into 6-month age classes. Age classes that included juveniles (i.e. <135 mm, <1 yr) were excluded from the CJS model because juveniles did not exhibit high site fidelity to home ranges (see results). Sex- and age-specific differences in $p$ and $\phi$ were evaluated by comparing increasingly complex models that included time dependence, sex structure and age structure using Akaike's information criterion (AICc).

The second method employed the empirical relationship from Pauly (1980) to estimate the instantaneous rate of natural mortality, $M$:

(3) $$\log M = 0.654 \cdot \log K - 0.28 \cdot \log L_{\infty} + 0.463 \cdot \log T$$

where $K$ and $L_{\infty}$ were the von Bertalanffy growth parameters, and $T$ was the mean annual water temperature (18.2 °C, Chapter 2). The annual survival rate, $S$ expressed as a percentage, was calculated as:

(4) $$S = 100 \cdot e^{-M}$$

The third method to estimate survival rate employed a length-converted catch curve (Ricker 1975, Pauly 1990, King 1995) to estimate the instantaneous rate of total mortality, Z (fishing + natural mortality). Z was converted to annual survival rate as above.

Generation time was calculated as the mean age of reproducing individuals in the population (IUCN 2001). This was calculated by converting the mean size of *H. guttulatus* greater than size at first reproduction in the catch data from June 2001 – August 2001 (corresponding to the seasonal peak in reproduction, Boisseau 1967, Chapter 2). Size was converted to age using the fitted von Bertalanffy growth model.

A two-pronged approach was used to estimate the longevity of wild *H. guttulatus*. First, longevity was predicted from the age corresponding to an empirical estimate of $L_{\infty}$, the mean size of the 10 largest individuals (Sparre & Venema 1992, King 1995), using the fitted VBGF. Second, the growth coefficient $K$ was used to estimate the theoretical maximum life span, $t_{max}$, using the approximation $t_{max} \approx 3/K + t_0$ (Pauly 1980).

Statistical analyses were carried out with SYSTAT 10.2 (SYSTAT Software, Inc.). All means are reported with standard deviations except where stated otherwise.

## RESULTS

On the grids, resighting rates were high. More than 76% of collar- and VIE-tagged fish were resighted at least once and 17% were resighted ≥15 times. A total of 5790 records were collected from tagged fish on both grids, for an average of 9.8 (±11.9, range 1-88) records per mature female, 6.9 (±8.9, range 1-60) records per mature male and 2.7 (±3.6, range 1-32) records per juvenile (mark-recapture data). Data were collected from 2735 *H. guttulatus* (1211 females, 1264 males and 262 juveniles) captured in the experimental seines and trawls (catch data). Not all data are available from each of the seahorses sampled in the Ria Formosa.

### Population structure

*Density*

The mean density of all tagged and untagged *H. guttulatus* encountered during censuses, $D_{census}$, from 2001 – 2003 (Grid B) was 1.02 $m^{-2}$ (± 0.40) and did not vary significantly among years (one-way ANOVA, $F = 0.922$, $p = 0.441$). Because the probability of resighting individuals during dives was <1 (see below), a correction factor was used to estimate the true density, $D_{corrected}$, of individuals on Grid B:

$$(5) \qquad D_{corrected} = \frac{D_{tagged \cdot present}}{D_{tagged \cdot observed}} \cdot D_{census}$$

where $D_{tagged \cdot present}$ was the mean density of tagged individuals whose residency period was known to encompass census dates (1.21 ± 0.23 $m^{-2}$), and $D_{tagged \cdot observed}$ was the mean density of tagged individuals encountered during censuses (0.81 ± 0.24 $m^{-2}$). Thus *H.* $D_{corrected}$ on Grid B was estimated as 1.52 $m^{-2}$. The mean $D_{census}$ on Grid A (year 2000 only), 0.22 ± 0.07 $m^{-2}$, was significantly lower than that observed on Grid B (t-test, t = -8.981, df = 75, $p < 0.001$). The $D_{corrected}$ on Grid A was 0.33 $m^{-2}$.

*Sex ratio*

The sex ratio (proportion of adults that were females) was usually unbiased on the grids during census dives from 2000 – 2003 ($\chi^2$ goodness of fit tests with Bonferroni correction, df = 1, all p > the Bonferroni-corrected p-value of 0.0006 for 68/77 census dives) with an overall sex ratio of 0.55 (± 0.09) averaged across census dives. Similarly, the mean sex ratio (0.55 ± 0.13) estimated from monthly catch data from Sept 2000 – July 2002 was not significantly female-biased ($\chi^2$ goodness of fit tests with Bonferroni correction, df = 1, all $p > 0.001$ for all 22 months).

*Size structure*

Trunk lengths were sexually dimorphic, but standard lengths were not. Trunk length, height, and mass were significantly correlated with standard length (Table 1.2). On the grids, standard length ranged from 69 – 215 mm. In the catch data, standard length ranged from 65 – 208 mm (Fig. 1.3) and mass ranged from 0.57 – 22.48 g. Females had significantly longer trunks than males when standard length was controlled (ANCOVA, multiple $r^2 = 0.244$, $F_{3,1344} = 190.02$, $p < 0.0001$). Females were also significantly heavier than males when standard length and reproductive state were controlled (ANCOVA, $F_{4,1378}$, $p < 0.0001$), but there was positive allometry in growth for both sexes (Table 1.2). The standard length frequency distributions of adult males and females did not differ significantly (Kolmogorov-Smirnov test, n = 2338, p = 0.052, individuals < 135 mm standard length excluded from analysis).

## Home range size and movement patterns

Year, sex or length did not affect the MCP area (general linear model, adjusted $r^2 = 0.017$, $F_{7,97} = 1.239$, $p = 0.290$) and only one juvenile was observed ≥15 times. Therefore home range size estimates were pooled for analyses. Mean home range size within years was 19.9 $m^2$ (± 12.4, range = 1.4 – 58.1, n = 102, including only the first home range held per individual). The mean % overlap between home ranges held by individuals in both 2001 and 2002 was 37.5 % (± 35.2, n = 47). The mean distance between the centers of home ranges held in 2001 and 2002 was 2.6 m (± 1.9, range = 0.1 – 7.4, n=47). We found no evidence of territoriality because few aggressive interactions (e.g. snapping, tail wrestling) were observed among *H. guttulatus* and home ranges held by individuals overlapped with those held by several tens of neighbours.

Juveniles and adults differed in the distances they moved from their initial capture locations. Many home ranges straddled the perimeter of a grid, with approximately 13% of all sightings of tagged *H. guttulatus* recorded between 0 and 5 m outside one of the two grids. Juveniles were significantly more likely to be encountered >5 m from the outer perimeter of a grid than adults (adults = 0.05%, juveniles = 9.4%, $\chi^2 = 6478$, df = 1, $p < 0.0001$): whereas tagged adults were only found as far as 10 m from Grid B, juveniles were encountered as far as 40 m away. The only two adults encountered 5 – 10 m from a grid were both subsequently resighted on the grid. By contrast, only 43% of juveniles encountered >5 m from the grid were later resighted. Three tagged juveniles that were found 25 – 40 m away from Grid B were encountered in their new location in both 2001 and 2002, suggesting that they had established home ranges in those locations.

Mean residency periods were at least 192.1 (± 148.5) days for females, 185.4 (± 148.1) days for males and 127.3 (± 168.7) days for juveniles. The mean residency period (linear regression, adjusted $r^2 = 0.55$, $F_{1,10} = 14.5$, $p = 0.003$), mean number of fixes (logistic regression, adjusted $r^2 = 0.81$, $F_{2,11} = 24.99$, $p = 0.0002$) and probability of resighting individuals (logistic regression, adjusted $r^2 = 0.64$, $r^2 = 0.74$; $F_{3,11} = 7.6$ $p < 0.01$) were all significantly and positively correlated with body length measured during tagging (Fig. 1.4).

## Growth

*Length at age*

Newborn juveniles trapped *in situ* in mesh cages measured 12.2 mm (± 0.8 mm, n = 24). Between July 2001 and May 2003, 1-year growth increments were collected from 83 females and 63 males (7 increments from July 2001-2002, 58 from October 2001-

2002, 82 from May 2002-2003). The significant relationship between length at time t, $L_t$, and length during the previous year, $L_{t-1}$ (linear regression, $F_{2,146} = 181.68$, $p < 0.0001$) did not differ by sex (ANCOVA, effect of sex $F_1 = 0.79$, $p = 0.376$). The regression equation fitted in the Ford-Walford plot ($r^2=0.71$, $F_{1,145} = 352.04$, $p < 0.0001$, Fig. 1.5) produced estimates of $L_\infty = 197.6$ mm and $K = 0.571$. This graphical estimate of $L_\infty$ was similar to the mean length of the ten largest individuals in the catch data, 192.4 mm standard length (± 6.9, 95% CI = 178.9 – 205.7 mm), an empirical estimate of $L_\infty$ (Sparre & Venema 1992; King 1995). $t_0$ was estimated as -0.91 yr (Fig. 1.6).

*Length and age at recruitment*

The proportion of juveniles in the catch data varied seasonally (non-linear regression, adjusted $r^2 = 0.56$, $F_{3,11} = 5.79$, $p = 0.02$). Juveniles were present in the catch data from August 2001 – May 2002 (Fig. 1.7a). The proportion of reproducing males also varied by season (non-linear regression, adjusted $r^2 = 0.92$, $F_{2,12} = 72.48$, $p<0.0001$), with all captured from May – December 2001 and from March – July 2002 (Fig. 1.7b). The recruiting juveniles appeared in the catch data three months after reproduction began in May 2001 (Figs. 1.3 & 1.7). The mean length of juveniles in the August 2001 catch data was 96.0 ± 8.0 mm (range = 80 – 109 mm, n = 16). These results were consistent with Boisseau (1967) who inferred that new recruits to seagrass habitat ranged in size from 73 – 81 mm and were three months old.

*Length and age at maturity*

The smallest male with evidence of a developing pouch was 77 mm and the largest juvenile with a flush pouch was 134.5 mm (Fig. 1.8a). The proportion of mature individuals showed a significant logistic relationship with length (non-linear regression, adjusted $r^2 = 0.97$, $F_{2,31} = 536.1$, $p <0.0001$, Fig. 1.8b). $L_m$ was 109.4 mm corresponding to a predicted age of 0.49 yr (Figs. 1.6a & 1.8b).

*Length and age at first reproduction*

The spawning fraction of males (non-linear regression, adjusted $r^2 = 0.98$; $F_{2,19} = 417.9$; $p < 0.0001$) and females (non-linear regression, adjusted $r^2 = 0.79$; $F_{2,20} = 40.0$; p <

0.0001) increased significantly with length (Figs. 1.8b & 1.8c). Mean $L_s$ for males and females was 129.1 mm (± 4.09 SE) and 125.2 mm (± 1.07 SE), respectively. By the fitted VBGF, these lengths corresponded to predicted ages of 0.94 and 0.85 yr, respectively. Boisseau's (1967) estimates of $L_s$ and $t_s$ (125.5 mm, 0.83 – 1 yr) were consistent with these results.

### Survival, generation time and longevity

The fully time-dependent CJS model provided the best fit to the observed encounter histories (Akaike information criterion, $AIC_c$, =1947.6, model deviance = 367.6, 17 parameters), suggesting that the resighting probability, $p$, and apparent survival rate, $\phi$, varied significantly over time. Models including sex and/or age had higher $AIC_c$s than the fully time-dependent CJS model, suggesting that including sex and/or age in models did not improve the overall fit when accounting for the greater number of parameters. The probability of resighting tagged individuals, $p$, during trapping sessions varied from 0.48 – 0.86. Estimates of $\phi$ varied from 0.74 to 0.96 $\text{month}^{-1}$ (Table 1.3). By multiplying estimates of $\phi$ for each monthly interval from Aug 2001 – July 2002, annual adult survival rate, $S$, was 29.4% $\text{yr}^{-1}$, corresponding to an instantaneous rate of natural mortality, $M$, of 1.224 $\text{yr}^{-1}$. This estimate of $M$ was consistent with the empirical estimate (Pauly 1980), 1.153 $\text{yr}^{-1}$, corresponding to $S$ = 31.5%. By contrast, the regression in the length-converted catch curve (linear regression, adjusted $r^2 = 0.98$; $F_{1,10} = 544.8$, $p < 0.0001$) produced an estimate of $Z = 1.789$ $\text{yr}^{-1}$, corresponding to $S$ = 16.7% (Fig. 1.9).

Generation time was considerably shorter than maximum life span. The mean size (± SD) of *Hippocampus guttulatus* that were greater than size at first reproduction was 144.4 mm (9.95 ± SD, n = 272). This corresponded to a generation time of 1.4 years (95% confidence interval = 0.84 – 2.19 years). By contrast, the age corresponding to the mean size of the 10 largest *H. guttulatus* was 5.46 yr. Using the approximation $t_{max} \approx 3/K + t_0$ (Pauly 1980), longevity was estimated as 4.29 yr.

## DISCUSSION

This study marks the first estimates of survival and growth rates for a wild seahorse population and the most comprehensive synthesis of the life history of any syngnathid (Table 1.4). Using a mark-recapture study, experimental catch data, published regression analyses, and information from previous studies, we were able to estimate and cross-validate biological reference points that are important for fisheries management and conservation.

### Population structure

The population structure of *H. guttulatus* was similar to that observed in other species including *H. breviceps* (the short-headed seahorse, Peters), *H. capensis*, *H. comes*, and *H. whitei* (White's seahorse, Bleeker) (Vincent & Sadler 1995, Perante et al. 2002, Bell et al. 2003, Moreau & Vincent 2004): equal sex ratios, similar lengths between the sexes, sexual dimorphism in body proportions, few recruited juveniles, and constant density over time. However, the local density of *H. guttulatus* on Grid B was 3 - 75 times greater than densities of other seahorse species reported on similar focal study grids in both tropical and temperate waters (Vincent & Sadler 1995, Perante et al. 2002, Bell et al. 2003, Dias & Rosa 2003, Moreau & Vincent 2004). High local densities of *H. guttulatus* in our study (Chapter 3) may reflect the unusually high productivity of the Ria Formosa lagoon (Sprung 1994).

### Home range size and movement patterns

Juvenile *H. guttulatus* exhibited an ontogenetic shift in habitat use from pelagic waters to vegetated substrate. Planktonic juveniles ranged in length from approximately 8 – 57 mm (Boisseau, 1967, Pérez-Ruzafa et al. 2004) and probably spent at least 8 weeks in the plankton (Boisseau 1967). This study supports Boisseau's inference about the duration of the planktonic stage: despite >850 hours of underwater observations and almost two years' worth of monthly sampling in the Ria Formosa, the smallest juvenile *H. guttulatus* observed in this study was 65 mm (compared with 45 mm for the sympatric *H. hippocampus*, J. Curtis, unpublished data) and juveniles were only first captured three months after the reproductive season began. High water turnover rates in the Ria Formosa lagoon (Sprung 1994, Machás & Santos 1999) mean that at least some planktonic juveniles were probably exported from the lagoon into coastal waters, a pattern observed in a population of *H. capensis* in South Africa (Whitfield 1989). However, the location and dispersal rate of planktonic juvenile *H. guttulatus* born in the Ria Formosa are not known, nor is the degree of philopatry. Quantifying the spatial extent of juvenile movements should be a priority for further research as dispersal rates and distances influence population-level responses to localized disturbances and/or management strategies.

*Hippocampus guttulatus* recruitment to benthic habitat in the Ria Formosa lagoon coincided with the onset of maturity. The shape of the catch curve suggests that recruitment occurred from 0.8 – 1.8 relative years. These relative (but not true) ages corresponded to length classes ranging from 70 – 130 mm, which corresponds closely to the range of sizes of juvenile males with immature pouches. Relatively few juvenile seahorses of any species have been encountered in random underwater visual census surveys (Bell et al. 2003, Martin-Smith & Vincent in prep, Chapter 3) or in focal populations where reproductively active adults were present (Perante et al. 2002, Bell et al. 2003, Moreau & Vincent 2004). These observations suggest that juvenile seahorses delay recruitment to adult habitat until they are on the threshold of maturity.

Adult *H. guttulatus* maintained small home ranges over multiple years and probably had low emigration rates. The average home range size of adult *H. guttulatus* was larger than observed in other seahorse species (Vincent & Sadler 1995, Perante et al. 2002, Bell et al. 2003, Moreau & Vincent, 2004, although not for *H. abdominalis*, Martin-Smith & Vincent in prep), but home ranges were displaced by only a few metres between years. Although both grids were surrounded by contiguous seagrass and macroalgae habitat (preferred by *H. guttulatus*, Chapter 3), tagged adults were rarely encountered farther than a few metres from the grid within which they were tagged. Boisseau (1967) inferred that *H. guttulatus* migrated from Arcachon Basin, France, to deeper, warmer waters from November – March because he rarely captured them during this time period. However, relatively constant monthly catches in the Ria Formosa lagoon suggested that adult *H. guttulatus* remained within their home ranges year-round.

Strong site fidelity over space and time was associated with the onset of reproduction in *H. guttulatus*. The lengths at which the mean number of fixes and probability of resighting increased most rapidly corresponded closely with the length at first reproduction for males and females. The maintenance of home ranges has been associated with reproductive behaviour in other seahorse species. For example, monogamous pairs of *H. comes* and *H. whitei* were more faithful to home ranges than juveniles or unpaired adults (Perante et al. 2002, Vincent et al. in press).

## Growth

The growth rate of *H. guttulatus* appeared to change abruptly in association with the ontogenetic shift from pelagic to benthic habitat use. The von Bertalanffy growth model fitted yearly growth increments of benthic juvenile and adult *H. guttulatus* well, thus providing a useful model for predicting relative ages for individuals ranging in size from ~105 – 195 mm. This is an important result because the von Bertalanffy growth model forms the basis of many more complex models in fisheries stock assessment (Hilborn & Walters 1992, King 1995). The large negative value of $t_0$ indicated that the growth rate of young juveniles was probably faster than that predicted by the fitted VBGF (King 1995). Indeed, the inferred and observed growth rates of planktonic juveniles were much faster than predicted by the model (Fig. 1.6a,b). Rapid growth of captive-reared juveniles has also been reported for the oceanic seahorse, *Hippocampus kuda* Bleeker (Job et al. 2002) and *H. abdominalis* (Woods 2000). A slowing of growth rate with age occurs in other marine fishes (e.g. red drum, *Sciaenops ocellatus* L, Ross et al. 1995). Several modified von Bertalanffy growth models account for such ontogenetic changes in growth rate (Laslett et al. 2002, Porch et al. 2002). More data are required to validate length at age estimates (Campana 2001) and to fit such models (Laslett et al. 2002).

### Survival and longevity

Adults had high site fidelity to their home ranges over multiple reproductive seasons; thus it is reasonable to assume that the apparent survival rate, $\phi$, is a good approximation for adult survival rate, $S$. This assumption is supported by a strong concordance between $S$ estimated using mark-recapture data and $M$ estimated using the regression equation for predicting natural mortality, $M$, with estimates of $L_\infty$, $K$ and $T$ (Pauly 1980). Natural mortality rates are generally assumed to be the same for all recruited individuals (King 1995), but because settled juveniles had lower site fidelity, it was not possible to verify this assumption. The estimate of total mortality, $Z$, included a component of fishing mortality due in part to repeated experimental sampling within sites (Erzini et al. 2002) and possibly to exploitation by means of illegal, bottom-dragged fishing gears that are occasionally employed in the Ria Formosa lagoon (Monteiro 1989, Erzini et al. 2002). Nevertheless, this estimate of $Z$ provides an upper bound for estimates of the rate of natural mortality, and thus a lower bound for survival rate.

The primary sources of natural mortality are unknown for settled *H. guttulatus*. Juvenile cuttlefish preyed on seahorses in France (Blanc et al. 1998) and were common in the Ria Formosa (Erzini et al. 2002, J. Curtis, personal observation). A small fraction (3%) of the population with injuries (e.g. punctured brood pouch, damaged tail) probably indicated feeding attempts by predators. A higher proportion (7%) of individuals were also observed with white skin lesions (often accompanied by several small black dots) indicating the prevalence of an undetermined disease in this population.

Estimates of the longevity of wild *H. guttulatus* (4.3 – 5.5 years), were consistent with that inferred by Boisseau (1967). Boisseau counted 3 – 4 stages of atretic follicles that had accumulated in the ovaries of large females, which probably corresponded to different reproductive seasons. Thus he concluded that longevity was on average four years, and commented that it was not uncommon to find large individuals that were probably older than this. In captivity, *H. guttulatus* are known to survive as long as 7.25 years (Neil Garrick-Maidment, personal communication).

## Life history strategy

In data-poor situations, empirical frameworks can be used to predict the relative risk of disturbance to species with different life history traits (e.g. Dulvy & Reynolds 2002). Three to five generalized life history strategies for marine and freshwater fishes have been characterized by means of multivariate or regression analyses (e.g. Winemiller & Rose 1992, McCann & Shuter 1997). The results of these analyses provide a conceptual basis for developing and implementing appropriate management actions (e.g. King & McFarlane 2003). The life history groupings included equilibrium, opportunistic, periodic, salmonic and intermediate strategists, which were distinguished primarily on the basis of age at maturity, fecundity and parental care (Winemiller & Rose 1992, McCann & Shuter 1997) as well as length at maturity, asymptotic length, growth rate, longevity, and egg size (King & McFarlane 2003).

We characterized the life history strategy of *H. guttulatus* in relation to other marine fishes by means of a principal components analysis (PCA), as carried out in King and McFarlane (2003). Relative to 42 other marine fishes that varied widely in habitat associations (pelagic to benthic habitats from near-shore to continental slope), trophic level (detritivores to piscivores) and parental investment (broadcast spawners to elasmobranches with lengthy gestation periods), *H. guttulatus* matured early, had low fecundity and was small-bodied, short-lived, and fast-growing. Six life history traits ($L_m$, $L_\infty$, $K$, fecundity, $t_{max}$ and egg size) of *H. guttulatus* were shared by opportunistic strategists including herring (*Clupea harengus pallasi* Valenciennes), sardine (*Sardinops sagax* Jenyns), Pacific sandlance (*Ammodytes hexapterus* Pallas), eulachon (*Thaleichthys pacificus* Richardson) and Northern lampfish (*Stenobrachius leucopsarus* Eigenmann and Eigenmann) (Fig. 1.10a; King & McFarlane 2003). Other shared characteristics included short life span, multiple spawning behaviour (McCann & Shuter 1997, Chapter 2) and trophic level (King & McFarlane 2003), as *H. guttulatus* feeds mainly on zooplankton and small benthic crustaceans, (d'Entremont 2002).

One question raised by the multivariate analysis was whether *H. guttulatus* was grouped with opportunistic species simply by virtue of its small body size, which is correlated with most other life history traits included in the analysis (e.g. length and age at maturity, maximum theoretical length, maximum life span, growth coefficient, fecundity, Froese & Binohlan 2000, Froese & Binohlan 2003). Indeed, the considerable parental investment of seahorses is characteristic of equilibrium strategists (McCann & Shuter 1997). If *H. guttulatus* were an equilibrium strategist, however, one would have expected this species to mature at a significantly later age given its body size at maturity: the natural log of age at maturity (-0.67) and mass at maturity (0.92) of *H. guttulatus* fall on the allometric relationship for opportunistic species of fish (Fig. 1.10b, after McCann & Shuter 1997). Thus, further investigation is required to clarify the life history strategy of *H. guttulatus* relative to other small marine fishes. Nevertheless, the suite of life history traits of *H. guttulatus* can be used as a basis for inferring population-level responses to disturbance and identifying biologically appropriate management strategies.

### Implications for management and conservation

Inferences about responses to disturbance will need to balance assessments of life history data with an understanding about the ecology and behaviour of *H. guttulatus*. The rapid growth, early age at maturity and short generation time of *H. guttulatus* mean that they likely have high intrinsic rates of population increase and relatively greater probabilities of recovery, despite having relatively low fecundity (Myers et al. 1997, Hutchings 2001, Denney et al. 2002, King & McFarlane 2003, Hutchings & Reynolds 2004). A high intrinsic rate of population increase might suggest that as long as a critical spawning biomass were maintained, *H. guttulatus* could have high resilience to exploitation (Jennings et al. 1998) and recover rapidly when the direct (fishing mortality) and indirect (bycatch, habitat damage) effects of exploitation cease (Hutchings 2001). Multiple spawning behaviour (Chapter 2) also suggests that *H. guttulatus* is more resilient to environmental stochasticity and catastrophic loss of eggs or larvae (McEvoy & McEvoy 1992) than fishes that only breed one per year. However a short life span means that *H. guttulatus* is less resilient to extended periods of poor recruitment than fishes with longer life spans.

Short generation times mean that the abundance of opportunistic strategists can fluctuate in response to environmental conditions, making them susceptible to population declines that could be exacerbated by high levels of exploitation (King & McFarlane 2003). Too few long term studies report indices of seahorse population abundance. However fisheries-independent surveys from the Atlantic and Gulf coasts of Florida (de Silva et al. 2003) and north-eastern Atlantic Ocean (Monteiro 1989) suggest that seahorse populations may fluctuate by as much as one order of magnitude between years (Monteiro 1989, de Silva et al. 2003, Chapter 5). Flexible management plans that incorporate frequent monitoring on the scale of seahorse life spans could be effective at responding rapidly to changes in population abundance and preventing recruitment overfishing in exploited seahorse populations.

Determining what constitutes a minimum viable population size for successful recruitment will require information that is currently unavailable for any seahorse species (and not usually considered for marine fishes, Dulvy et al. 2004), including an understanding of the patterns of juvenile dispersal and recruitment as well as the relationship between spawning biomass and recruitment success. In the absence of such information, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) has recently recommended the implementation of a minimum size limit as an interim measure for helping to secure the sustainability of populations exploited for the international trade of seahorses. Adopting a minimum size limit that is greater than the average size at first reproduction (i.e. ~130 mm for *H. guttulatus*) could help ensure that at least some adults spawn before becoming vulnerable to exploitation (Chapter 5).

Site fidelity to small, overlapping home ranges by adults means that marine protected areas may be effective tools for protecting critical spawning biomasses for *H. guttulatus* populations (Kramer & Chapman 1999). The establishment of marine protected areas may also protect benthic habitats used by settled juvenile and adult *H. guttulatus*. Although the life history traits of *H. guttulatus* may confer resilience to overexploitation, a preference for vegetated habitats with suitable holdfasts (Chapter 3) means that this species is not likely to be resilient to the loss of seagrass-dominated habitats.

## CONCLUSIONS

The results presented in this study were strongly concordant with inferences based on captive populations and limited field sampling. This suggests that where field data are lacking, unpublished or *ex situ* studies may be valuable sources of life history information. *Hippocampus guttulatus* life history parameters, including age at maturity and growth coefficient, were also within the ranges ($t_m$ = 1-3 yr, $K$ = 0.3-0.79 $yr^{-1}$) predicted using empirical relationships for small temperate fishes (Froese & Binohlan 2003). Thus simple empirical relationships based on life history parameters that are easily obtained (e.g. maximum body length) are another valuable source of life history information. In cases where there are more life history data available, multivariate analyses (e.g. PCA) provide a useful starting point for identifying general life history strategies and developing successful monitoring and management programs. The predictive accuracy of such multivariate analyses would likely be improved by considering not only the life history traits of fishes, but also the implications of ecology and behaviour (e.g. movement patterns, reproductive behaviour, ecology) when evaluating the dynamics of marine fish populations in response to disturbance (Vincent & Sadovy 1998, Kramer & Chapman 1999, Shumway 1999; Stobutzki et al. 2001).

*Acknowledgements.* This is a contribution from Project Seahorse. We gratefully acknowledge K. Erzini, J. Ribeira and members of their research team for providing seahorses captured during their experimental sampling from 2000-2002 and for sharing their lab space and insight. Many thanks to our capable research assistants, R. Adams, G. Alatorre, H. Balasubramanian, K. Bigney, J. d'Entremont, B. Gunn, S. Lemieux, C.-M. Lesage, E. Murray, J. Nadeau, S. Overington, A. M. Santos, S.V. Santos, M. Veillette, and K. Wieckowski. Data for captive-reared juvenile growth rates were kindly provided by Robin James. We thank all members of the Parque Natural da Ria Formosa for their kind logistical support, Siân Morgan for examining otoliths and D. Americano for useful discussions. We appreciated the enthusiasm and curiosity of 'os rapazes do cais'. Many thanks to R. Ahrens, K. Martin-Smith and I. Morgan for helpful comments on a previous draft of this manuscript. Guylian Chocolates, Belgium, generously supported this study. J.M.C. was supported by McGill Major, Natural Sciences and Engineering Research Council of Canada (NSERC) and Fonds de Recherches sur la Nature et les Technologies Québec (FCAR) doctoral awards. The John G. Shedd Aquarium in Chicago generously supported AV's operating costs, through its partnership with Project Seahorse.

## LITERATURE CITED

Alberto F, Mata L, Santos R (2001) Genetic homogeneity in the seagrass *Cymodocea nodosa* at its northern Atlantic limit revealed through RAPD. Marine Ecology Progress Series 221:299-301

Bell E, Lockyear J, McPherson J, Marsden AD, Vincent ACJ (2003) First field studies of an Endangered South African seahorse, *Hippocampus capensis*. Environmental Biology of Fishes 67(1):35-46

Blanc A, Pinczon du Sel G, Daguzan J (1998) Habitat and diet of early stages of *Sepia officinalis* L (Cephalopoda) in Morbihan Bay, France. Journal of Molluscan Studies 64:263-274

Boisseau J (1967) Régulations hormonales de l'incubation chez un vertébré mâle: recherches sur la reproduction de l'Hippocampe. PhD thesis, University of Bordeaux, Bordeaux, France

Breder CM, Rosen DE (1966) Suborder Syngnathoidei. Modes of Reproduction in Fishes. Natural History Press, New York

Cai N, Xu Q, Yu F, Wu X, Sun G (1984) Studies on the reproduction of the seahorse *Hippocampus trimaculatus*. Studia Marina Sinica 23:83-93

Campana SE (2001) Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology 59:197-242

Caswell H (1989) Matrix population models: construction, analysis and interpretation. Sinauer Associates, Sunderland Massachusetts

Denney NH, Jennings S, Reynolds JD (2002) Life-history correlates of maximum population growth rates in marine fishes. Proceedings of the Royal Society of London, Series B 269:2229-2237 d'Entremont J (2002) Sex-related differences in feeding behaviour and diet in *Hippocampus guttulatus*. BSc thesis, McGill University, Montreal

de Silva JA, Mahmoudi B, Muller RG, Murphy MD (2003) Florida's inshore and nearshore species: 2002 status and trends report, Appendix A. Florida Fish and Wildlife Conservation Commission, Florida Wildlife Research Institute, St. Petersburg, Florida

Dias TLP, Rosa IL (2003) Habitat preferences of a seahorse species, *Hippocampus reidi* (Teleostei: Syngnathidae) in Brazil. Aqua, Journal of Ichthyology and Aquatic Biology 6(4):165-176

Dulvy NK, Ellis JR, Goodwin NB, Grant A, Reynolds JD, Jennings S (2004) Methods of assessing extinction risk in marine fishes. Fish and Fisheries 5:255-276

Dulvy NK, Reynolds JD (2002) Predicting extinction vulnerabilities in skates. Conservation Biology 16(2):440-450

Erzini K, Bentes L, Coelho R, Correia C, Lino PG, Monteiro P, Ribeiro J, Goncalves JMS (2002) Recruitment of sea breams (Sparidae) and other commercially important species in the Algarve (southern Portugal), Final Report., Commission of the European Communities DG XIV/C/1: 178

Foster S, Vincent, ACJ (2004) The life history and ecology of seahorses, *Hippocampus* spp.: implications for conservation and management. Journal of Fish Biology 65:1-61

Froese R, Pauly D (eds) (2004) FishBase. World Wide Web electronic publication. www.fishbase.org, Version 05/2004.

Froese R, Binohlan C (2003) Simple methods to obtain preliminary growth estimates for fishes. Journal of Applied Ichthyology 19:376-379

Froese R, Binohlan C (2000) Empirical relationships to estimate asymptotic length, length at first maturity and length at maximum yield per recruit in fishes, with a simple method to evaluate length frequency data. Journal of Fish Biology 56:768-773

Garrick-Maidment N, Jones L (2004) British Seahorse Survey Report. The Seahorse Trust, Topsham, UK

Hilborn R, Walters CJ (1992) Quantitative fisheries stock assessment: choice, dynamics and uncertainty. Kluwer Academic Publishers, London, UK

Hutchings J, Reynolds JD (2004) Marine fish population collapses: Consequences for recovery and extinction risk. BioScience 54:297-309

Hutchings J (2001) Conservation biology of marine fishes: perceptions and caveats regarding assignment of extinction risk. Canadian Journal of Fisheries and Aquatic Sciences 58:108-121

IUCN (2003) 2003 IUCN Red List of Threatened Species. <www.redlist.org> Downloaded on 31 August 2004

IUCN (2001) IUCN red list categories and criteria: Version 3.1. In: Commission ISS (ed) IUCN, Gland, Switzerland

Jennings S, Greenstreet SPR, Reynolds JD (1999a) Structurual change in an exploited fish community: a consequence of differential fishing effects on species with contrasting life histories. Journal of Animal Ecology 68: 617-627

Jennings S, Reynolds JD, Polunin NVC (1999b) Predicting the vulnerability of tropical reef fishes to exploitation with phylogenies and life histories. Conservation Biology 13(6):1466-1475

Jennings S, Reynolds JD, Mills SC (1998) Life history correlates of responses to fisheries exploitation. Proceedings of the Royal Society of London Series B 265:333-339

Job SD, Do HH, Meeuwig JJ, Hall HJ (2002) Culturing the oceanic seahorse, *Hippocampus kuda*. Aquaculture 214:333-341

King JR, McFarlane GA (2003) Marine fish life history strategies: applications to fishery management. Fisheries Management and Ecology 10:249-264

King M (1995) Fisheries biology, assessment and management. Fishing News Books, Oxford

Koslow JA, Boehlert GW, Gordon JDM, Haedrich RL, Lorance P, Parin N (2000) Continental slope and deep-sea fisheries: implications for a fragile ecosystem. ICES Journal of Marine Sciences 57:548-557

Kramer DL, Chapman MR (1999) Implications of fish home range size and relocation for marine reserve function. Environmental Biology of Fishes 55:65-79

Laslett GM, Eveson JP, Polacheck T (2002) A flexible maximum likelihood approach for fitting growth curves to tag-recapture data. Canadian Journal of Fisheries and Aquatic Sciences 59:976-986

Lebreton J-D, Burnham KP, Clobert J, Anderson DR (1992) Modeling survival and testing biological hypotheses using marked animals: case studies and recent advances. Ecological Monographs 62:67-118

Lourie SA, Vincent ACJ, Hall HJ (1999) Seahorses: An identification guide to the world's species and their conservation. Project Seahorse, London UK

Machás R, Santos R (1999) Sources of organic matter in Ria Formosa revealed by stable isotope analysis. Acta Oecologica 20(4):463-469

Masonjones HD, Lewis SM (2000) Differences in potential reproductive rates of male and female seahorses related to courtship roles. Animal Behaviour 59(1):11-20

Masonjones HD, Lewis SM (1996) Courtship behavior in the dwarf seahorse, *Hippocampus zosterae*. Copeia 1996(3):634-640

McCann K, Shuter B (1997) Bioenergetics of life history strategies and the comparative allometry of reproduction. Canadian Journal of Fisheries and Aquatic Sciences 54:1289-1298

McEvoy LA, McEvoy J (1992) Multiple spawning in several commercial fish species and its consequences for fisheries management, cultivation and experimentation. Journal of Fish Biology 41(Suppl. B): 125-136

Monteiro CC (1989) La faune ichthyologique de la Ria Formosa: Répartition et organisation spatio-temporelle des communautés. PhD Dissertation, Université des Sciences et Techniques du Languedoc, Montpellier, France

Moreau MA, Vincent ACJ (2004) Social structure and space use in a wild population of the Australian short-headed seahorse, *Hippocampus breviceps* Peters, 1869. Marine and Freshwater Research 55(3): 231-239

Myers RA, Mertz G, Fowlow RS (1997) Maximum population growth rates and recovery times for Atlantic cod, *Gadus morhua*. Fishery Bulletin 95:762-772

Pauly D (1990) Length-converted catch curves and the seasonal growth of fishes. Fishbyte 8(3):33-38

Pauly D (1980) On the interrelationships between natural mortality, growth-parameters, and mean environmental-temperature in 175 fish stocks. Journal du Conseil 39(2):175-192

Perante NC, Pajaro MG, Meeuwig JJ, Vincent ACJ (2002) Biology of a seahorse species *Hippocampus comes* in the central Philippines. Journal of Fish Biology 60:821-837

Perante NC, Vincent ACJ, Pajaro MG (1998) Demographics of the seahorse *Hippocampus comes* in the central Philippines. 3rd International Conference on the Marine Biology of the South China Sea, Hong Kong, Hong Kong University Press.

Pérez-Ruzafa A, Quispe-Becerra JI, García-Charton JA, Marcos C (2004) Composition, structure and distribution of the ichthyoplankton in a Mediterranean coastal lagoon. Journal of Fish Biology 64:202-218

Porch CE, Wilson CA, Nieland DL (2002) A new growth model for red drum (*Sciaenops ocellatus*) that accommodates seasonal and ontogenetic changes in growth rates. Fishery Bulletin 100:149-152

Reina-Hervás JA (1989) Contribucion al estudio de la F. Syngnathidae (Pisces) en Las Costas del Sureste de España. Arquivos do Museu Bocage 1(21):325-334

Ricker WE (1975) Computation and interpretation of biological statistics for fish populations. Fisheries Research Board of Canada Bulletin

Rodgers AR, Carr AP (1998) HRE: the home range extension for ArcView. Users Manual. Ontario Ministry of Natural Resources, Thunder Bay, Ontario. Available at: http://blue.lakeheadu.ca/hre

Ross J, Stevens T, Vaughan DS (1995) Age, growth, mortality, and reproductive-biology of red drums in North-Carolina waters. Transactions of the American Fisheries Society 124(1):37-54

Sadovy Y (2001) The threat of fishing to highly fecund fishes. Journal of Fish Biology 59(Suppl. A):90-108

Santos MN, Gaspar MB, Vasconcelos P (2002) Weight-length relationships for 50 selected fish species of the Algarve coast (southern Portugal). Fisheries Research 59:289-295

Shumway Caroly (1999) A neglected science: applying behavior to aquatic conservation. Environment Biology of Fishes 55:183-201

Smith EB, Williams FM, Fisher CR (1997) Effects of intrapopulation variability on von Bertalanffy growth parameter estimates from equal mark-recapture intervals. Canadian Journal of Fisheries and Aquatic Sciences 54:2025-2032

Sparre P, Venema SC (1992) Introduction to tropical fish stock assessment. FAO Fisheries Technical Paper 306(1)

Sprung M (1994) Macrobenthic secondary production in the intertidal zone of the Ria Formosa - A lagoon in Southern Portugal. Estuarine Coastal and Shelf Science 38(6):539-558

Stobutzki IC, Miller MJ, Brewer D (2001a) Sustainability of fishery bycatch: a process for assessing highly diverse and numerous bycatch. Environmental Conservation 28:167-181

Vincent ACJ, Evans KL, Marsden AD. in press. Home range behaviour of the monogamous Australian seahorse, *Hippocampus whitei*. Environmental Biology of Fishes [ms 3524-02].

Vincent ACJ, Giles BG (2003) Correlates of reproductive success in a wild population of *Hippocampus whitei*. Journal of Fish Biology 63(2):344-355

Vincent ACJ, Sadovy Y (1998) Reproductive ecology in the conservation and management of fishes. In: Caro, Tim (ed). Behavioural Ecology and Conservation Biology. Oxford University Press, Oxford

Vincent ACJ (1996) The International Trade in Seahorses. TRAFFIC International, Cambridge

Vincent AC, Sadler LM (1995) Faithful pairbonds in wild seahorses, *Hippocampus whitei*. Animal Behaviour 50:1557-1569

von Bertalanffy L (1938) A quantitative theory of organic growth. Human Biology 10:181-213

Walford L (1946) A new graphid method of describing the growth of animals. Biol. Bull. 90:141-147

Whitfield AK (1989) Ichthyoplankton interchange in the mouth region of a southern African estuary. Marine Ecology Progress Series 54:25-33

White GC, Burnham KP (1999) Program MARK: Survival estimation from populations of marked animals. Bird Study 46 (Supplement): 120-138 http://www.cnr.colostate.edu/~gwhite/mark/mark.htm

Wilson MJ, Vincent ACJ (1998) Preliminary success in closing the life cycle of exploited seahorse species, *Hippocampus* spp., in captivity. Aquarium Sciences and Conservation 2:179-196

Winemiller KO, Rose KA (1992) Patterns of life-history diversification in North American fishes: Implications for population regulation. Canadian Journal of Fisheries and Aquatic Sciences 49:2196-2218

Woods CMC, Martin-Smith K (2004) Visible implant fluorescent elastomer tagging of the big-bellied seahorse, *Hippocampus abdominalis*. Fisheries Research 66:363-371

Woods CMC (2000) Preliminary observations on breeding and rearing the seahorse *Hippocampus abdominalis* (teleostei: Syngnathidae) in captivity. New Zealand Journal of Marine and Freshwater Research 34:475-485

**Table 1.1** Number of female (F), male (M) and juvenile (J) *H. guttulatus* tagged, and the number of underwater dives carried out each year.

| YEAR | F | M | J | CENSUS DIVES | SURVEY DIVES | TRANSECT DIVES | CUMULATIVE SEARCH TIME (HOURS) |
|---|---|---|---|---|---|---|---|
| 2000[A] | 12 | 15 | 36 | 21 | 29 | 0 | 170 |
| 2001[B] | 143 | 123 | 37 | 23 | 58 | 8 | 273 |
| 2002[B] | 111 | 118 | 21 | 28 | 78 | 15 | 375 |
| 2003[B] | 38 | 10 | 36 | 5 | 7 | 1 | 46.5 |

[A] Collar tags, Grid A

[B] VIE tags, Grid B

**Table 1.2** Length-mass and length-length conversions for male (M) and female (F) *H. guttulatus*, and for pooled (P) sexes (DF = degrees of freedom, $M_{wet}$ = wet mass, $L$ = standard length, $L_{tr}$ = trunk length, $L_h$ = height, see text for more details). Standard errors (SE) of the regression intercept (A) and slope (B) are given. Regressions for pooled sexes were required to convert between lengths for juveniles of unknown sex.

| SEX | DF | EQUATION | P | $R^2$ | A<br>SE | B<br>SE |
|---|---|---|---|---|---|---|
| F | 977 | $\log M_{wet} = 3.276 \cdot \log L - 6.283^a$ | <0.0001 | 0.820 | 0.104 | 0.049 |
| M | 1097 | $\log M_{wet} = 3.423 \cdot \log L - 6.579^a$ | <0.0001 | 0.852 | 0.091 | 0.043 |
| P | 2120 | $\log M_{wet} = 3.370 \cdot \log L - 6.474$ | <0.0001 | 0.849 | 0.065 | 0.031 |
| F | 637 | $\log L_{tr} = 0.950 \cdot \log L - 0.474$ | <0.0001 | 0.847 | 0.034 | 0.016 |
| M | 703 | $\log L_{tr} = 0.872 \cdot \log L - 0.325$ | <0.0001 | 0.856 | 0.029 | 0.013 |
| P | 1391 | $\log L_{tr} = 0.903 \cdot \log L - 0.383$ | <0.0001 | 0.852 | 0.021 | 0.010 |
| F | 985 | $\log L_h = 1.001 \cdot \log L - 0.057$ | <0.0001 | 0.980 | 0.010 | 0.005 |
| M | 1109 | $\log L_h = 0.999 \cdot \log L - 0.052$ | <0.0001 | 0.983 | 0.008 | 0.004 |
| P | 2144 | $\mathrm{Log} L_h = 0.999 \cdot \log L - 0.0526$ | <0.0001 | 0.983 | 0.006 | 0.003 |

[a] Positive allometry (Females: p = 0.004, Males: p<0.0001, as in Santos et al. 2002).

**Table 1.3** Estimates of recapture probabilities, $p$ (± SE), by trapping session and apparent monthly survival rates, $\phi$ (± SE), by trapping interval.

| TRAPPING SESSION | $p$ | TRAPPING INTERVAL | $\phi$ |
|---|---|---|---|
| August 2001 | 0.825 (±0.052) | July – August 2001 | 0.888 (±0.044) |
| September 2001 | 0.611 (±0.059) | August – September 2001 | 0.884 (±0.059) |
| October 2001 | 0.754 (±0.062) | September - October 2001 | 0.744 (±0.070) |
| May 2002 | 0.484 (±0.054) | October 2001 – May 2002 | 0.934 (±0.009) |
| June 2002 | 0.861 (±0.029) | May – June 2002 | 0.964 (±0.022) |
| July 2002 | 0.829 (±0.032) | June – July 2002 | 0.835 (±0.031) |
| August 2002 | 0.678 (±0.043) | July – August 2002 | 0.870 (±0.040) |
| September 2002 | 0.693 (±0.056) | August – September 2002 | 0.925 (±0.064) |

**Table 1.4** Biological points of reference from five sources of data: A = this study; B = Boisseau (1967); C = Neil Garrick-Maidment (in litt. July 2004); D = Robin James (unpublished data); E = Pérez-Ruzafa et al. (2004). Ranges are given in parentheses.

| PARAMETER | SOURCE | L (MM) | AGE (YRS) |
|---|---|---|---|
| Birth | A | 12.2 ± 0.8 SD | 0 |
| | B | 12.3[1] | 0 |
| Sexual differentiation | B | 25[1] | |
| Recruitment | | | |
| Juveniles in plankton tows | B | 12 – 57[1] | |
| | E | 9 – 38[1] | |
| Smallest recruited juvenile | A | 65 | |
| Mean length at recruitment, $L_r$ | A | 96 ± 8.0 SD<br>(80 – 109) | 0.25 |
| | B | 77[1]<br>(73 –81)[1] | 0.25 |
| Maturation | | | |
| Smallest male with developing pouch | A | 77 | |
| | B | 65 – 74[1] | |
| Length at 50% maturity, $L_m$ | A | 109.4 ± 1.36 SE | 0.5[2] |
| Largest male with immature pouch | A | 135 | 1.08[2] |
| Age at maturity *ex situ* | C | | 0.41 – 0.58 |
| Reproduction | | | |
| Smallest male with full pouch | A | 108 | 0.5[2] |
| | B | 86 – 90[1] | |
| Length at first reproduction, $L_s$ | A | 129.1 ± 1.07 SE (males) | 0.94[2] |
| | | 125.2 ± 4.09 SE (females) | 0.85[2] |
| | B | 125.5[1] | 0.83 – 1 |
| Longevity and maximum length | A | 192. 4 ± 6.9 SD<br>(178.9 – 205.7) | 5.46[2]<br>(4.06 – ∞) |
| | A | | 4.23 |
| | B | | 4 – 5 |
| | C | | 7.25 |
| Growth | | | |
| Recruits > 105 mm | | | |
| $L_\infty$ | A | 197.6 | |
| K | A | 0.571 | |
| $t_0$ | A | -0.91 | |
| Juveniles < 70 mm | | | |
| Length at age (*in situ*) | B, D | 22.9 – 26.5[1] | 0.08 |
| | B, D | 49.3 – 57.2[1] | 0.17 |
| | B, D | 73.2 – 81.4[1] | 0.25 |

[1] Estimated by converting average trunk length or height to standard length using regression equations in Table 1.2

[2] Estimated from fitted VBGF for lengths > 105 mm.

**Figure 1.1** Representation of focal study grid locations (A and B), census and tagging dive areas (inner squares), survey areas (inner and outer squares), and transect dives (shaded areas). The starting points for transect dives were selected haphazardly from the western and eastern edges of Grid B.

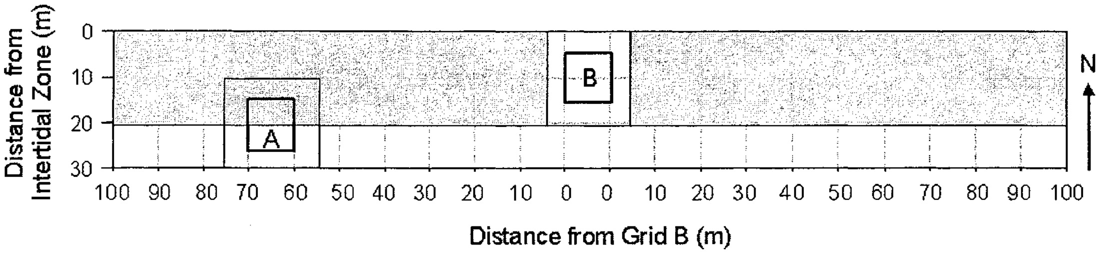


**Figure 1.2** The location of fixes and 95% minimum convex polygons (MCPs) for one VIE-tagged male *H. guttulatus* on Grid B from 2001 – 2003. Large open circles indicate the centers of home ranges occupied in 2001 and 2002.

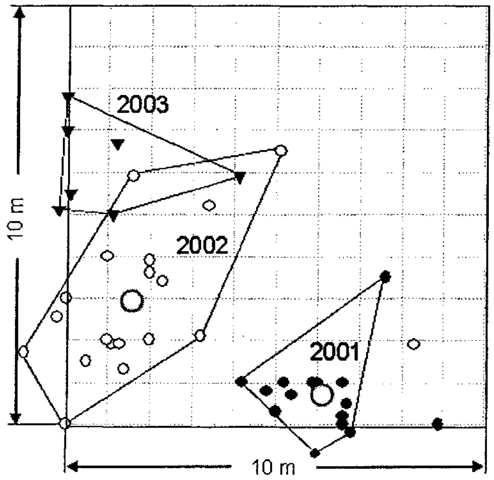


**Figure 1.3** Standard length frequencies of monthly catches of *H. guttulatus* from October 2000 to March 2002 (relative frequencies on y axes, lengths binned into 5mm size classes on x axes). The timing of recruitment is evident with the first appearance of 80 – 109 mm juveniles in the catch data in August 2001.

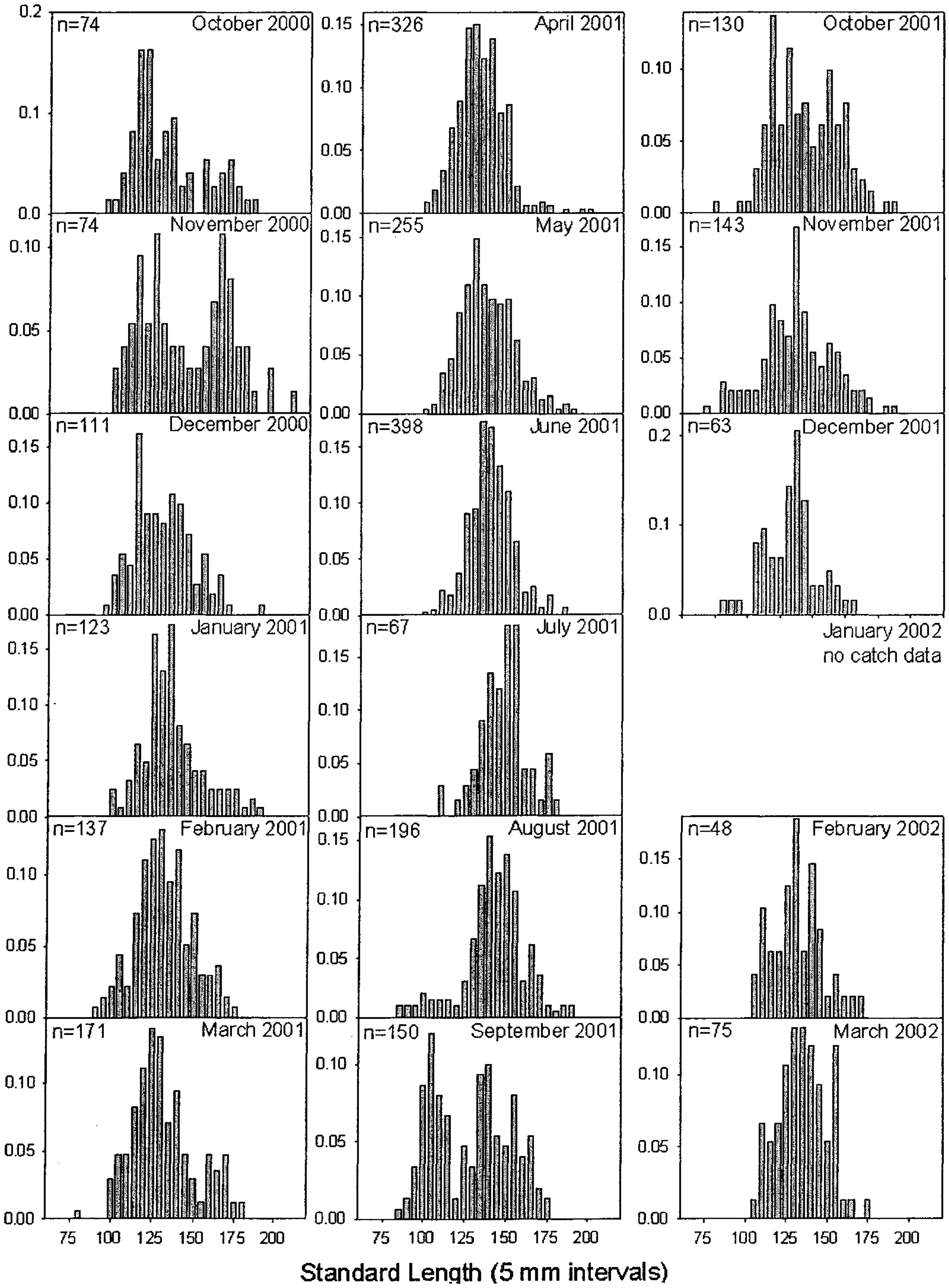


**Figure 1.4** Mean number of fixes (top) and the proportion of VIE-tagged individuals that were resighted at least once (bottom) as a function of body length. Sample sizes are given for each length class.

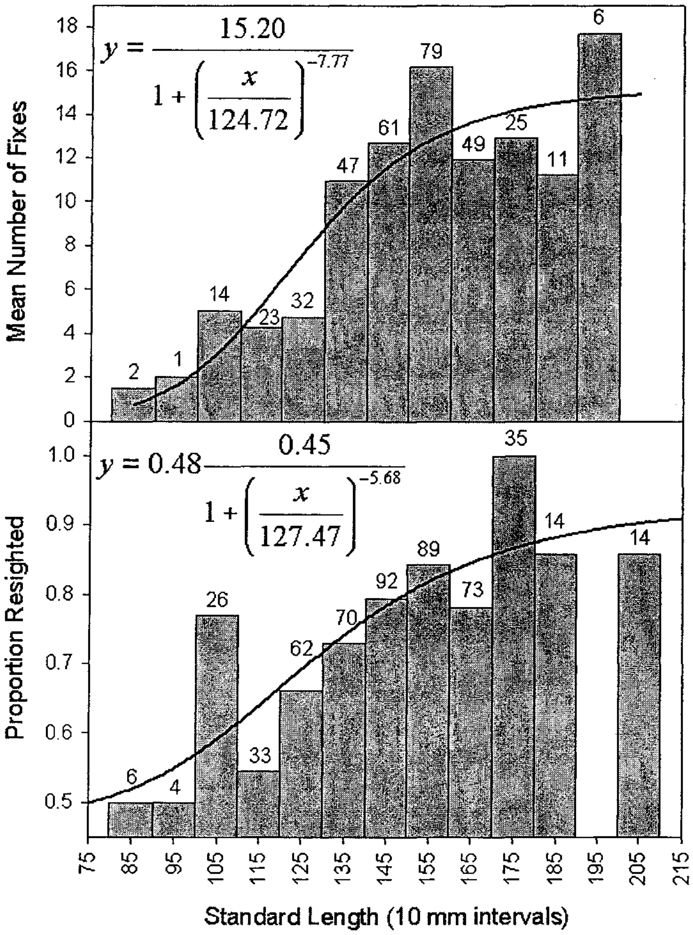


**Figure 1.5** Ford-Walford plot of standard lengths at time t, $L_t$, and $L_{t-1}$ for male (solid circles) and female (open circles) *H. guttulatus* (n=147 increments, each from a different individual). An estimate of $L_\infty$ (197.6 mm) is given by the intersection between the fitted line (equation give) and a 1:1 line.

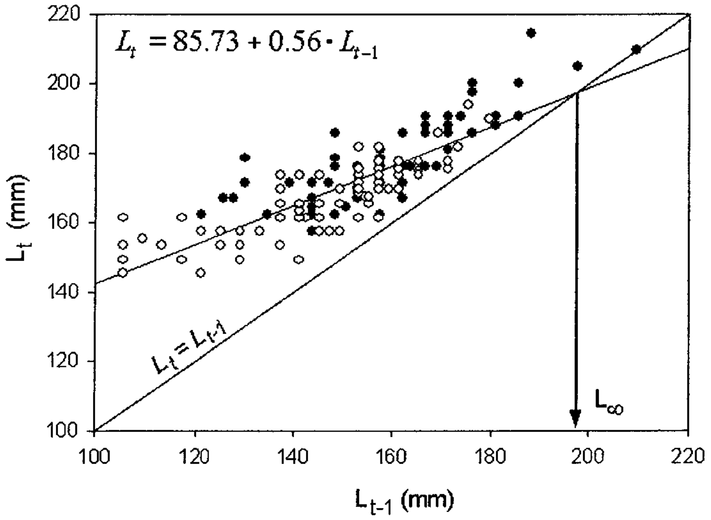


**Figure 1.6** *Hippocampus guttulatus* growth trajectories. (A) Predicted von Bertalanffy growth curve (solid and dashed line) for wild *H. guttulatus*. The dashed part of the curve represents the range of sizes for which there were no mark-recapture data available. Life history reference points include length at birth and length and age at recruitment, maturity and first reproduction, as well as mean maximum observed length (solid circles). The size ranges of a cohort of juveniles at 1, 2 and 3 months of age (represented by paired, open circles) are plotted for comparison (data from Boisseau 1967). Boisseau inferred that planktonic juveniles settled into seagrass habitat when 3 months old. (B) The logistic growth of captive-reared juveniles (solid circles, adjusted $r^2 = 0.988$, $F_{3,25} = 676.5$, $p < 0.0001$) was similar to the *in situ* growth pattern of planktonic juveniles (paired, open circles) inferred by Boisseau (1967), suggesting that *ex situ* growth data may be useful for predicting the growth trajectories of wild juvenile seahorses. Solid circles represent the mean size for each of two captive-reared broods measured at 1-week intervals for three months (R. James, unpublished data).

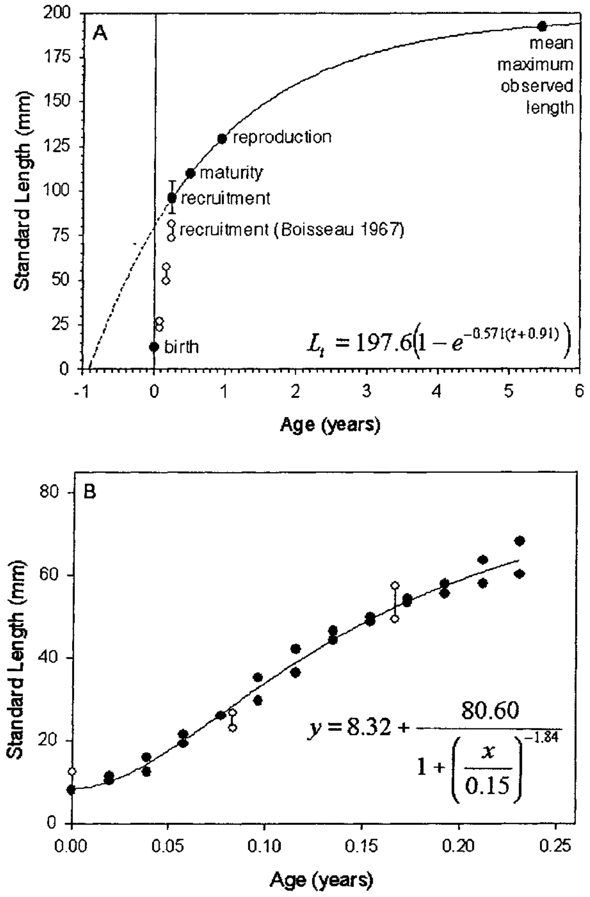


**Figure 1.7** The timing of juvenile recruitment inferred from the seasonal pattern in the proportion of juveniles in the catch data (top) and the seasonal peak in reproductive activity (bottom). The log-normal shape of the proportion of juveniles in the catch data against time reflects both recruitment and growth through the juvenile phase. Open circles represent reproductive activity during the 2002 reproductive season (not included in the non-linear regression). Age at recruitment (0.25 years) was inferred from the temporal lag between the first month during which reproducing males were captured (May 2001) and the first month during which juveniles were captured (August 2001).

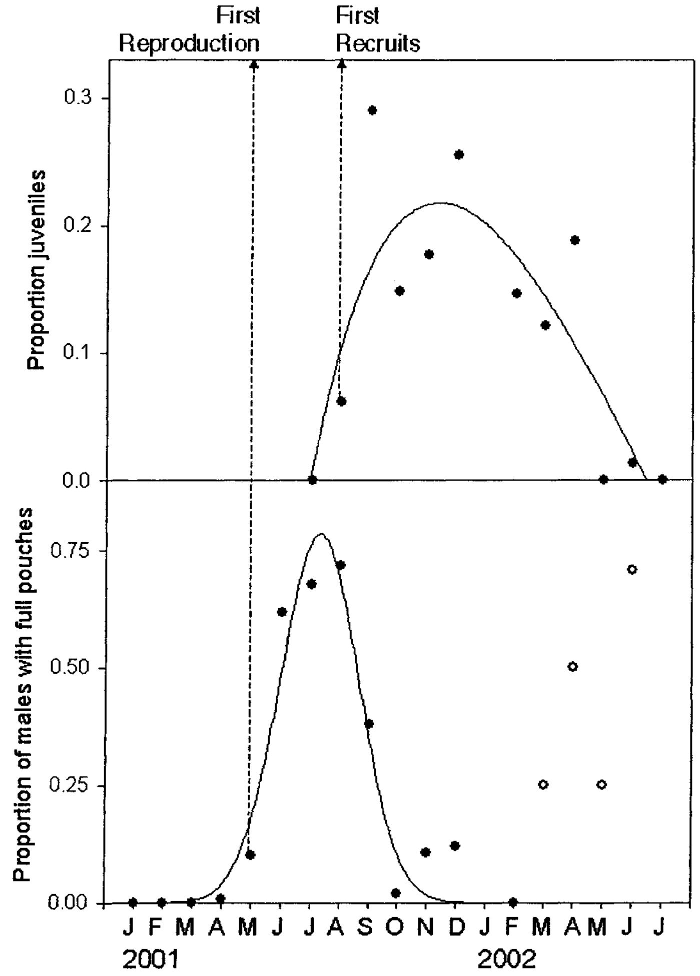


**Figure 1.8** Length at maturation and reproduction. (A) A total of 774 males showed stages 1 – 5 of pouch development in the catch data (samples sizes given in parentheses, open triangles represent mean size within stages). (B) The proportion of mature males (solid circles) and proportion of males with full pouches (spawning fraction, open circles) in each 5 mm length class (n = 774). (C) The proportion of individuals that were females with hydrated eggs in each 5mm length class (n = 2187).

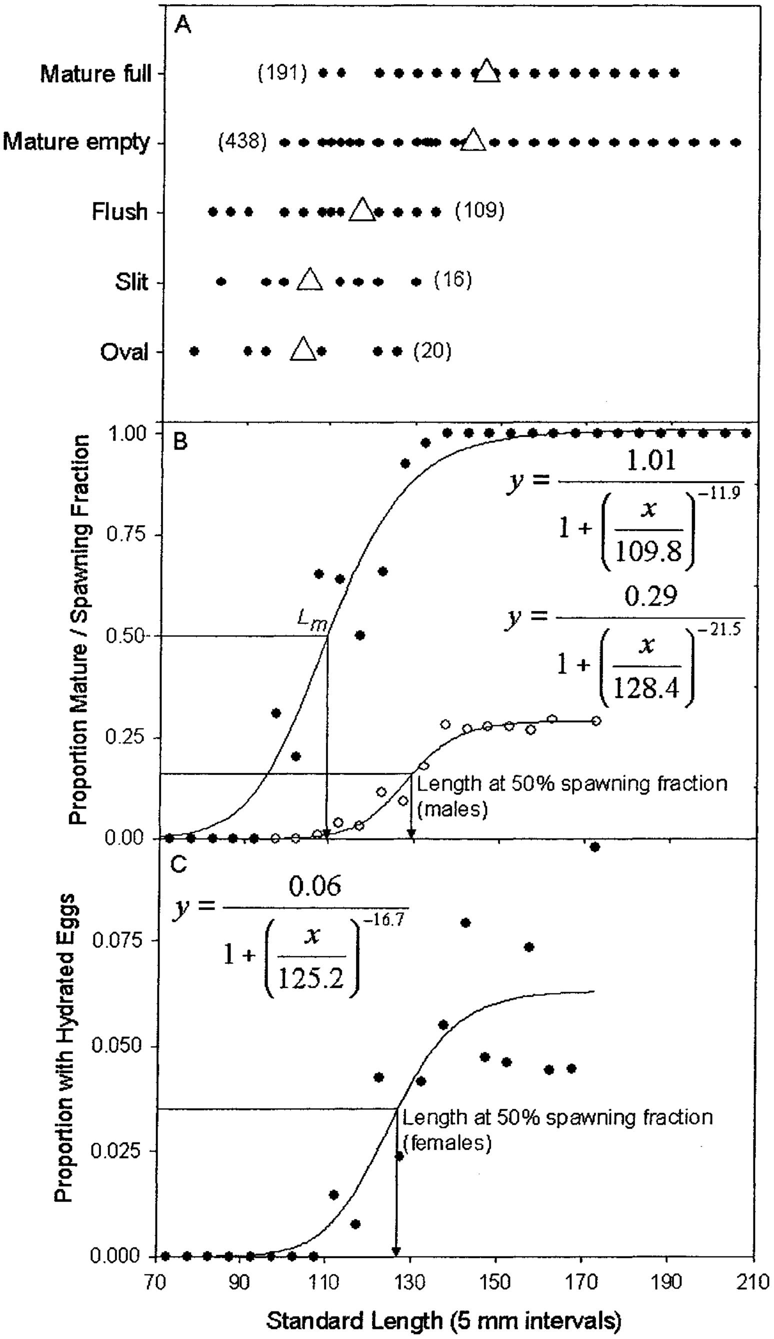


**Figure 1.9** Length-converted catch curve for *H. guttulatus* captured from October 2000 – July 2002. Data represented by solid circles were used in the regression analysis to estimate Z. Note that these relative ages are not true ages: because the fitted VBGF underestimated the growth rate of planktonic juveniles (see text and Fig. 1.6), the growth parameters used to predict the relative ages of different length classes overestimated the ages of smaller length classes.

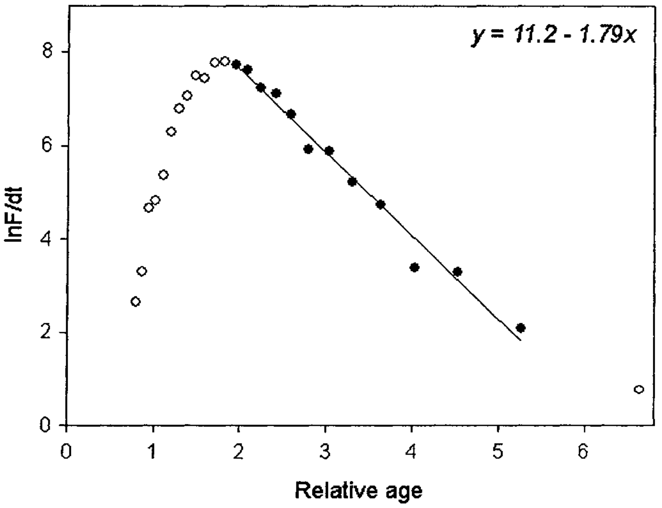
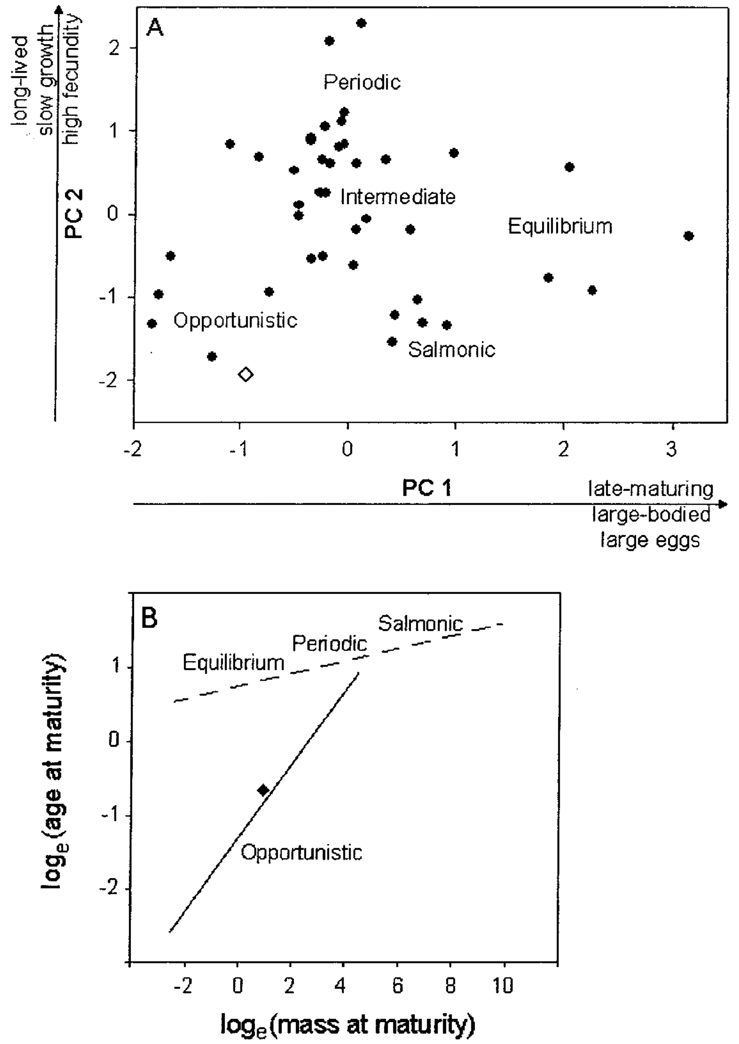


**Figure 1.10** (A) Standardized scores from the first two components of a principle components analysis of six life history traits ($L_m$, $L_\infty$, $K$, fecundity, $t_{max}$ and egg size) from *H. guttulatus* (open diamond) and 42 other marine fishes (solid circles). Life history values employed for *H. guttulatus* were: $L_m$ = 109.4 mm, $L_\infty$ = 197.6 mm, $K$ = 0.571, fecundity = 214 (Chapter 2), $t_{max}$ = 5 years and egg size = 2.0 mm (as reported in Foster & Vincent 2004). Data for the remaining 42 species were from King & McFarlane (2003) (after King & McFarlane 2003). (B) Allometric relationships between age at maturity and mass at maturity for opportunistic strategists (solid line) and periodic, equilibrium and salmonic strategists (dashed line). *Hippocampus guttulatus* (closed diamond) falls close to the relationship for opportunistic strategists (after McCann & Shuter, 1997).



# LINKING STATEMENT 2

In chapter 1, I used multiple approaches (direct estimates, mark-recapture statistics, regression analyses) to estimate the survival, growth and movement patterns of *Hippocampus guttulatus*. I then synthesized these life history traits using a multivariate approach to set this species in the context of general fish life history strategies. To further the analysis of core life history rates for this species in Chapter 2, I estimate annual reproductive output by expanding and validating a model developed to estimate the spawning frequency of multiple spawners (Hunter & Leong 1981).

Although brood size is among the easiest life history parameters to quantify for seahorses (Foster & Vincent 2004), estimating annual fecundity is challenging because the annual spawning frequency of seahorses is not known. Previous studies have used estimates of the duration of the reproductive season and the duration of the time required to brood young to predict spawning frequency and thus annual fecundity (Vincent & Giles 2003, Foster & Vincent 2004). However, this method assumes that individuals reproduce during the entire reproductive season and that the time between broods is negligible, which may not be true for all species. In Chapter 2, I advance our understanding of seahorse spawning frequency and annual fecundity by evaluating the implications of these assumptions for estimating annual reproductive output for data-deficient species. I also propose a non-lethal method of estimating the mean spawning frequency for species that exhibit parental care using underwater surveys.

# CHAPTER 2

# ESTIMATING REALIZED ANNUAL FECUNDITY IN A BROODING MULTIPLE SPAWNER, *HIPPOCAMPUS GUTTULATUS*

# ABSTRACT

This study used a multiple spawner with obligate paternal care, *Hippocampus guttulatus*, as a model to validate the predictive accuracy of three progressively more realistic models for estimating realized annual fecundity. Underwater surveys and fisheries-independent catch data were used to estimate the duration of the reproductive season, female spawning frequency, male brooding frequency and batch fecundity (young brooded male$^{-1}$ mating$^{-1}$). The most realistic model, which is an extension of the 'spawning fraction' method, was parameterized with catch data and validated with underwater survey data. This model produced unbiased estimates of male brooding frequency (4.2 ±1.6 broods yr$^{-1}$). Mean batch fecundity was 213.9 (±110.9) giving an estimate of realized annual fecundity of 903.6 (±574.7) offspring yr$^{-1}$. The mean number of clutches prepared by females was 1.2 – 1.7 times greater than the mean number of broods produced by males. Thus methods inferring spawning frequency from female egg production (e.g. histological assessments) may lead to significant overestimates of realized annual fecundity. The spawning fraction method is broadly applicable to many fishes with parental care and can be applied non-lethally to fishes of conservation concern.

# INTRODUCTION

Age- or size-specific variation in life history rates (survival, growth, reproduction, dispersal) has important implications for population-level responses to alternative management scenarios (Parrish et al. 1986; Rothschild 1986; Lowerre-Barbieri 1996; Myers et al. 1999). Although fecundity *per se* is a poor predictor of resilience to exploitation of fishes (Hutchings 2001, Sadovy 2001), demographic changes driven by exploitation may lead to reductions in average fecundity per unit biomass, shortened spawning seasons and changes in mating success, possibly resulting in reduced recruitment (Parrish et al. 1986, Rothschild 1986, Tegner et al. 1996). Obtaining reliable estimates of realized annual fecundity and an understanding of the factors that affect reproductive rates is therefore an important step towards understanding and predicting population-level responses to disturbance.

Estimating annual fecundity in multiple spawning fishes is difficult because of the practical challenges of tracking mature individuals through time: estimates require both annual spawning frequency and batch fecundity (Hunter et al. 1986, Lowerre-Barbieri et al. 1996). Because there is considerable inter- and intra-annual plasticity in spawning frequency and batch fecundity in many fishes (Lasker 1985, Gale 1986, Hunter & Leong 1981), accurate estimates of annual fecundity also require an understanding of the factors that drive this variation (Lowerre-Barbieri et al. 1996). In this paper, I define realized annual fecundity as the sum of batch fecundities tallied over one year, and batch fecundity as the number of offspring produced per spawning.

Histological methods for inferring annual fecundity vary among species according to the reproductive strategies of females (reviewed in Murua & Saborido-Rey 2003). Most fishes are characterized by group-synchronous ovarian structure and determinate fecundity (e.g. *Gadus morhua*, *Salmo salar*). Whether these fishes are total or batch spawners, the size structure of oocytes within the ovary can be used to infer realized annual fecundity because all oocytes spawned within a year mature as a distinct cohort. The reliability of histological assessments is limited, however, for estimating the realized annual fecundity of fishes with asynchronous oocyte development and indeterminate recruitment (e.g. *Engraulis* spp.) (Brown-Peterson et al. 1988, Murua & Saborido-Rey 2003). In these fishes, oocytes of all stages are present without any one cohort dominating the distribution of oocytes within the ovary, and yolked oocytes forming new clutches are recruited from previtellogenic stages on a continual basis throughout the reproductive season (Wallace & Selman 1981).

The spawning frequency of asynchronous, indeterminate fishes has been inferred using the fraction of females that are spawning (indicated by the presence of hydrated eggs or new postovulatory follicles) plotted as a function of sampling date, or the 'spawning fraction' method (Hunter & Leong 1981, Murua & Saborido-Rey 2003). The batch fecundity of asynchronous, indeterminate spawners can be inferred from the number of eggs released or number of hydrated eggs in the ovary. The inferred spawning frequency multiplied by inferred batch fecundity gives an estimate of annual fecundity.

Inferences based on histological approaches are useful provided that populations are sampled adequately and appropriately (DeMartini & Fountain 1981, Hunter & Leong 1981, Clarke 1987) and that inferences are reliable proxies for the actual number of offspring produced (Weddle & Burr 1991). Unfortunately, because of the practical challenges of tracking early life history stages (e.g. eggs, embryos, larvae) over time and space, these proxies are difficult to validate. Histological methods for estimating annual fecundity may overestimate the actual number of young produced (Weddle & Burr 1991), especially if estimates do not correct for losses due to atresia, inability of females to secure mates, low fertilization efficiency or clutch predation (Weddle & Burr 1991, Cole & Sadovy 1995). For broadcast spawners, histological assessments may yet be the best proxy for estimating annual fecundity (Weddle & Burr 1991, Murua & Saborido-Rey 2003), even if it is not feasible to validate the method(s) employed. In many species with parental care, however, both mating success and brood success may be directly observed (e.g Vincent & Giles 2003), providing an opportunity to validate indirect inferences based on egg production and obtain more accurate estimates of the actual number of young produced (e.g. Cole & Sadovy 1995).

Seahorses (genus *Hippocampus*, Family Syngnathidae) are asynchronous, indeterminate, multiple spawners that provide obligate paternal care in the form of a sealed brood pouch (Boisseau 1967, Wallace & Selman 1981, Vincent & Sadler 1995, Masonjones & Lewis 2000, Perante et al. 2002, Poortenaar et al. 2004). All seahorses (≥34 *Hippocampus* spp.) are of international conservation concern because of suspected or documented declines in population size associated with overexploitation, as well as their association with threatened habitats (Vincent 1996, IUCN 2003, Foster & Vincent 2004). Because of a paucity of basic biological information, there is need for developing methods for estimating or predicting life history parameters in wild seahorse populations so that population assessments and management strategies may be refined (Foster & Vincent 2004).

Although numerous studies have investigated aspects of seahorse reproductive behaviour (e.g. Vincent & Sadler 1995, Masonjones & Lewis 2000, Perante et al. 2002), mean annual fecundity has been estimated for only one wild seahorse population (*H. whitei*, Vincent & Giles 2003) by first dividing the duration of the reproductive season by the brooding period (time required to brood a clutch of eggs), and then multiplying this estimate of spawning frequency by the average brood size. Vincent & Giles (2003) assumed that (a) the time elapsed between release of young and remating was negligible, an assumption that was supported by observations of rapid remating in *H. whitei* (Vincent & Sadler 1995), (b) individuals began mating at the beginning of the reproductive season and continued until the end of the reproductive season, and (c) there was no seasonal variation in brood size (a potentially unrealistic assumption, Vincent & Giles 2003). If validated, this method could be used to estimate realized annual fecundity for several *Hippocampus* spp. using existing data (reproductive season, brooding period and brood sizes, summarized in Foster & Vincent 2004).

In this paper, I use the European long-snouted seahorse, *Hippocampus guttulatus* Cuvier 1829, as a model for developing and validating models for estimating realized annual fecundity for seahorses. The objectives of this study were to (a) quantify the spawning frequency and batch fecundity of *H. guttulatus*, (b) identify correlates of realized annual and batch fecundities, and (c) evaluate the predictive accuracy of three progressively more realistic (and data intensive) models for estimating realized annual fecundity in multiple spawners. The simplest model is the 'back of the envelope' calculation employed by Vincent & Giles (2003). The most realistic model, requiring data that are currently not available for most seahorse species, is a generalization of the 'spawning fraction' method (Hunter & Leong 1981) that could be applied non-lethally to estimate realized annual fecundity in species of conservation concern. I address these objectives using *in situ* behavioural observations and fishery-independent collections from a locally abundant and unexploited population of *Hippocampus guttulatus*.

## METHODS

### Species description

*Hippocampus guttulatus* is a cryptic canopy dweller in seagrass- and macroalgae-dominated communities and is distributed throughout the Mediterranean Sea and the Northeastern Atlantic Ocean (Boisseau 1967, Reina-Hervás 1989, Lourie et al. 1999, Chapter 3). Individuals mature at approximately 1 year of age and live for 4 – 5.5 years (Boisseau 1967, Chapter 1). Adults range in size from 108 – 210 mm standard length (sum of head, trunk and tail lengths, Lourie et al. 1999) and 2.4 – 22.5 g (Chapter 1). Males and females mate monogamously within reproductive cycles: the female deposits her entire clutch into the male's brood pouch (Jones et al. 1998), where embryos are brooded for approximately three weeks (in captivity, Boisseau 1967). Males with full pouches have been captured from March to October (Boisseau 1967, Reina-Hervás 1989) and in January (Lo Bianco 1888). Although no previous studies have estimated spawning frequency *in situ*, the ovarian structure of *H. guttulatus* suggests that females produce more than one clutch per year (Boisseau 1967).

### Site description

This study was carried out in the Ria Formosa lagoon in southern Portugal (Fig. 2.1). The Ria Formosa is a shallow, productive coastal lagoon characterized by high water turnover rates, seagrass beds sand flats, salt marshes and a network of channels and tidal creeks (Machás & Santos 1999, Chapter 3). In July 2001, a 100 m$^2$ focal study grid was established 5 m from the intertidal zone next to a permanent pier located within the Ria Formosa Natural Park.

### Underwater surveys

In order to track changes in reproductive activity, 553 *H. guttulatus* (264 males, 254 females, 35 juveniles) were individually tagged using visible implant fluorescent elastomer (VIE tags, Northwest Marine Technologies, Inc.) from May – October in 2001 and 2002 (as in Chapter 1). During tagging, trunk length, (measured as a straight line from the cleithral ring to the last trunk ring, Lourie et al. 1999), life history stage (juvenile, adult), sex and reproductive state were recorded. Individuals were classified as adults if they were larger than size at 50% maturity (corresponding to 28 mm trunk length, Chapter 1) or if, in the case of small males, they had mature brood pouches. Male reproductive state (full or empty brood pouch) was determined by visual (and in ambiguous cases, manual) inspection. Female trunk girth was used as an indicator of preparedness for mating (Vincent & Sadler 1995, Perante et al. 2002). Females whose trunks were bulging between trunk rings (with occasionally visible eggs) were assumed to be preparing a clutch of eggs for mating. Within monogamous pairs of *H. comes* (Perante et al. 2002) and *H. whitei* (Vincent & Sadler 1995), changes in female trunk girth were significantly correlated with changes in the reproductive state of their partner. Therefore I assumed that trunk girth was a reliable proxy for clutch preparation in *H. guttulatus*.

A total of 163 SCUBA dives (2-5 person-hours per dive) were carried out on the grid during two underwater observation periods: 17 July to 26 October 2001 and 23 May to 12 September 2002 (i.e. on average, one dive every 1-2 days). During dives, observers swam ~1m above the substrate and searched for tagged seahorses. The date, tag, life history stage, sex and reproductive state of all marked seahorses were noted (as above). Trunk length was measured *in situ* at least twice per observation period for most tagged individuals and converted to standard length using equations developed for *H. guttulatus* (Chapter 1). The data collected on this grid are hereafter referred to as the underwater survey data.

Between 18 July and 16 August 2002, 8 brooding males were temporarily placed in cages (lined with no-seeum mesh) *in situ* to capture and count the total number of juveniles produced per brood (i.e. brood size), $f_b$ (Table 2.1), (as in Vincent & Giles 2003). Each male was held once for a maximum of 48 h, and both males and their juveniles were released to their original capture site within 12 hours of birth. Daily and mean monthly water temperatures were recorded using an Onset HOBO Temp logger placed 0.3 m above the substrate on the grid from June 2001 to September 2002.

### Experimental fishing

Seahorses were collected monthly from September 2000 to July 2002 (except January 2002) at 53 stations throughout the western part of the Ria Formosa lagoon using either experimental beach seines or beam trawls, and then frozen. These collections, hereafter referred to as the catch data, were part of an unrelated study of fish community structure in the lagoon (Erzini et al. 2002). The standard length, life history stage, sex, reproductive state, mass and brood size (estimated from number of embryos dissected from male pouches) of seahorses captured during the experimental fishing program were recorded. The developmental stages of embryos were estimated *sensu* Boisseau (1967). Broods with embryos at stages <10 (corresponding to ~1 – 10 days of development) were discarded because they were in poor condition due to freezing and were thus difficult to count. It was not possible to assess the females' ovaries histologically as these specimens were used for other purposes. The catch data were an unbiased sample of the lagoon's seahorse population: there were no significant differences in size structure, sex ratio, reproductive state or condition observed in comparisons of the catch data with data collected using underwater visual census techniques at a subset of these sampling stations (Chapter 4).

### Spawning frequency

The brooding period, $t_b$, was estimated using the underwater survey data. Changes in reproductive state were plotted as a function of sampling date for each male. Because of high seahorse density on the grid (~1 $m^{-2}$), it was not feasible to track all individuals each day; therefore, $t_b$ was estimated by recording the minimum and maximum possible duration of each brooding period, as graphically depicted in Figure 2.2. Unmated male *H. guttulatus* occasionally mimic mated males by filling their pouches with water during displays to females (personal observation), therefore putative brooding periods were only considered when a male was observed $\geq 3$ times with a full pouch during a period bracketed by observations of an empty pouch. The minimum and maximum intervals were averaged within and across all males. A similar approach was used to estimate the interbrood interval, $t_{ib}$ (time elapsed between release of young and remating in males), the time required by females to prepare eggs, $t_c$, and the interval between inferred clutches, $t_{ic}$. Because female *H. fuscus* prepared and released their eggs within approximately 3 days (Vincent 1990), $t_{ic}$ was only estimated during periods when female *H. guttulatus* were observed, on average, at least once every 2-3 days.

### Predictors of batch fecundity and spawning frequency

The underwater survey data were used to test for correlations between male standard length and the number of broods produced, as well as for correlations between $t_b$, $t_{ib}$, or $t_{ic}$ and temperature, which was averaged over the corresponding time period. The catch data were used to test whether there were effects of season or mean monthly temperature on reproductive activity (defined here as the proportion of mature males with full pouches or proportion of mature females preparing eggs), as well as effects of season, temperature or male size on brood size. Periodic regression was used to test for temporal effects of lunar phase and season (deBruyn & Meeuwig 2001), otherwise linear regression was used.

### Models for estimating realized annual fecundity

The assumptions of rapid remating and continuous reproduction during the entire reproductive season were examined using three progressively more realistic models to estimate realized annual fecundity, $f_a$.

*Continuous Reproduction (CR) Model*: In the simplest model, individuals were assumed to reproduce continuously from the beginning to the end of the reproductive season, from

March – October (Reina-Hervás 1989). Using mean estimates of brooding period, $t_b$, and brood size, $f_b$:

(1) $f_a = f_b \cdot t_s / t_b$

Where $f_a$ was the number of young produced $\text{male}^{-1}\ \text{yr}^{-1}$, $t_s$ was the duration of the reproductive season, $t_b$ and $f_b$ were assumed to be constant throughout $t_s$, and the interbrood interval, $t_{ib}$, was assumed to be 0.

*Intermittent Reproduction (IR) Model*: This model expanded the CR Model by incorporating a mean estimate of interbrood interval, $t_{ib}$, which was assumed to be constant throughout the reproductive season:

(2) $f_a = f_b \cdot t_s / (t_b + t_{ib})$

*Intermittent and Seasonal Reproduction (ISR) Model:* This model expanded (1) and (2) by incorporating information about seasonal trends in reproductive activity, rather than assuming constant reproduction throughout the reproductive season. This model was adapted from the 'spawning fraction' method used to estimate the average number of spawnings $\text{yr}^{-1}$ of female Northern anchovy (Hunter & Leong 1981). A normal curve was fitted to the fraction of mature males with full pouches in the catch data plotted against sampling month. By integration, the area beneath this curve produced an estimate of the total number of days that the average adult male brooded embryos per year, $t_{by}$. The mean number of broods produced $\text{male}^{-1}\ \text{yr}^{-1}$, $s_b^{yr}$, was estimated as $t_{by}/t_b$. It was assumed that $f_a = f_b \cdot s_b^{yr}$. Similarly, the ISR model was applied to the proportion of females preparing eggs in the catch data, where the number of clutches produced by females each year, $s_c^{yr}$ was estimated as $t_{cy}/t_c$ (Table 2.1). In order to characterize among-population differences in *H. guttulatus* spawning frequency, this method was also applied to male catch data (Boisseau 1967) from the Arcachon Basin, France.

Because the ISR Model only provides mean estimates of spawning frequency and thus realized annual fecundity, a simple individual based simulation was constructed to characterize variance in realized fecundity among males of different sizes. The individual based model tracked the spawning frequency of 1000 males throughout one reproductive season (assumed to be from March to November based on the catch data). Male standard length was randomly drawn from the size distribution of mature males in the catch data. During the first step in the loop, males were assigned a brooding period, $t_b$, (in days), sampled randomly from a normal distribution of $t_b$ observed in the underwater survey data. Next, males were assigned a probability of breeding, (0 or 1), which was sampled from a binomial distribution, where $p_i$ was equal to the predicted fraction of males brooding using the ISR Model. The fraction of mature males with empty pouches (i.e. $1 - p_i$) was equal to the probability of a mature male being captured during an interbrood interval, $t_{ib}$, or when not reproductively active, therefore $t_{ib}$ was not explicitly modeled. If a male successfully bred during a cycle (i.e. had a full pouch), the spawning frequency was augmented by 1, and brood size ($f_b$) was predicted using a regression equation of brood size against standard length (see results). Then the male returned to step one at the end of the brooding period, at which point a new brooding period, $t_b$, was randomly selected for the next reproductive cycle. If a male did not breed successfully during a cycle, he remained inactive until $t_b$ had elapsed, before entering back into the loop. All males entered the simulation at the beginning of the reproductive season and their reproductive activity was tracked until the end of the reproductive season. For each male, realized annual fecundity, $f_a$, was calculated as the spawning frequency times the brood size, $s_b^{yr} \cdot f_b$.

## Model validation

An independent estimate of the brooding period (21 days, Boisseau 1967) was used to evaluate the predictive accuracy of the CR and ISR Models. The CR Model was used to predict the expected number of broods produced by males, $s_b^{obs}$, during the observation periods from 18 July to 26 October (2001, 112 days) and 23 May to 12 September (2002, 108 days). Similarly, the curve fitted to the male catch data using the ISR Model was used to predict $s_b^{obs}$. Because the only estimate of the time females take to prepare eggs, $t_c$ (used to estimate the number of clutches produced by females) was based on the underwater survey data, the number of clutches produced by females that was predicted by the ISR Model for each observation period could not be validated. Therefore the ISR Model was used to predict the total number of days females spent preparing eggs during the observation periods, $t_c^{obs}$. Expected values of $s_b^{obs}$ and $t_c^{obs}$ based on the CR and ISR Models were compared to the mean values of $s_b^{obs}$ and $t_c^{obs}$ directly observed on the grid during the corresponding observation periods in 2001 and 2002. The IR Model could not be validated because there was no independent estimate of interbrood intervals, $t_{ib}$. All means are reported with standard deviations or 95% confidence intervals (95% CI).

## RESULTS

### Seasonal trends in reproductive activity

Data were recorded from 1264 males and 1211 females in the catch data. There was significant within-year variation in male reproductive activity (adjusted $r^2$=0.78, $F_{[2,19]}$=39.28, $p$<0.0001, Fig. 2.3a). *Hippocampus guttulatus* males reproduced during most of the year with a peak in activity from June – August. No reproductively active males were captured from December 2000 – March 2001, or in February 2002. Male reproductive activity was also significantly and positively correlated with water temperature ($r^2$=0.55, $F_{[1,12]}$ = 13.32, $p$=0.004), which varied seasonally from 10 – 28 °C with a mean annual temperature of 18.2 °C. There was no effect of lunar phase on male reproductive activity ($r^2$=0.01, $F_{[2,50]}$=0.256, $p$=0.775). The fraction of mature females that were inferred to be preparing eggs was also seasonal (adjusted $r^2$=0.21, $F_{[2,17]}$ = 3.59, $p$=0.05, Fig. 2.3b). Temperature was weakly correlated with female reproductive activity ($r^2$=0.30, $F_{[1,12]}$=4.80, $p$=0.051) but lunar phase was not ($r^2$=0.04, $F_{[2,50]}$=1.149, $p$=0.326).

### Spawning frequency

A total of 4550 records were collected from the 553 individually tagged *H. guttulatus* in 2001 and 2002 for an average of 8.2 records per individual (maximum 87). On the grid, 94% of resighted, mature males mated at least once within an observation period. There was no effect of male size on the number of broods produced during the observation period, $s_b^{obs}$ (Kruskal-Wallis test, $\chi^2 = 3.003$, n=73, df=5, p=0.699), which was 2.07 (± 1.10) and 2.72 (± 0.86) during the observation periods in 2001 and 2002, respectively (Table 2.2). Mean brooding period, $t_b$, was 21.4 (± 5.5) days (n=80 intervals from 73 males, median = 20.0 days) and the interbrood interval, $t_{ib}$, ranged from 0-61 days with a mean of 12.9 (± 8.2) days (n=98 intervals from 58 males; median = 10.5 days) (Table 2.3). All resighted, mature females prepared at least one clutch of eggs within a reproductive season. The mean number of days females were inferred to have hydrated eggs, $t_c$, was 2.58 (± 2.66) days (n=72 inferred clutches), suggesting that females required 2.5 days to prepare eggs for mating. The mean interclutch interval, $t_{ic}$, was 21.06 (± 6.6) days (n=102 intervals from 26 females; median = 20 days). Although there was no significant difference between $t_b$ (males) and $t_{ic}$ (females) (t-test, $df$=180, $t$=-0.36, $p$=0.720), females, on average, produced significantly more clutches than males brooded during both observation periods (2001: df=37, t=3.318, p=0.002; 2002: df=62, t=7168, p<0.0001, Table 2.2). Temperature was not a significant predictor of $t_b$ ($r^2$= 0.004, $F_{[1,55]}$ = 0.218, $p$=0.643), $t_{ib}$ ($r^2$= 0.001, $F_{[1,97]}$=0.071, $p$=0.790) or $t_{ic}$ ($r^2$= 0.016, $F_{[1,101]}$=1.663, $p$=0.200).

The ISR Model predicted that the total number of days that an average male *H. guttulatus* spent brooding embryos per year in the Ria Formosa was 90.4, giving an estimate of male annual spawning frequency, $s_b^{yr}$, of 4.22 broods $yr^{-1}$. Similarly, the total number of days that the average female spent preparing eggs per year was 13.51, giving an estimate of female annual spawning frequency, $s_c^{yr}$, of 5.23 clutches $yr^{-1}$. These estimates of annual spawning frequency were four times greater than estimates from the Arcachon Basin population. During the reproductive season, which lasted 3.5 months (Fig. 2.4), the average male had a full pouch for 20.7 days, suggesting that on average, males in the Arcachon Basin population only bred once per year (Table 2.3).

### Batch fecundity

The size of male *H. guttulatus* was a significant predictor of brood size, $t_b$, which varied from 10 – 567 embryos, with a mean of 213.9 ± 110.9 (n=117, estimates of brood size from caged and dissected males pooled). Both standard length ($r^2$=0.29,

$F_{[1,116]}$=46.98, $p<0.0001$) and mass ($r^2$=0.20, $F_{[1,39]}$=9.69, $p<0.003$) significantly predicted $t_b$ (Fig. 2.5). $t_b$ was not correlated with mean monthly temperature ($r^2$=0.00, $F_{[1,98]}$=0.014, $p$=0.907) but was correlated with Julian day in 2001 ($r^2$= 0.06, $F_{[1,106]}$=6.161, $p$=0.015).

## Model predictions

The simplest model (CR Model) gave the highest estimates of annual spawning frequency, $s_b^{yr}$, and realized annual fecundity, $f_a$, and as model complexity increased, the predicted values of $s_b^{yr}$ and $f_a$ decreased (Table 2.3). Incorporation of the interbrood interval, $t_{ib}$, and seasonal variation in reproductive activity in the ISR Model resulted in estimates of $s_b^{yr}$ and $f_a$ that were 63.1% smaller than those predicted by the CR Model. Although the individual based model suggests that there was considerable variance in $s_b^{yr}$ (4.22 ± 1.57) and $f_a$ (889.6 ± 377.3) among males of different sizes (Fig. 2.6), no males simulated in the individual based model had an $s_b^{yr}$ or $f_a$ that were as high as estimates predicted by the CR Model, and only 10% of simulated males had an $s_b^{yr}$ and $f_a$ that exceeded estimates given by the IR Model.

## Model validation

The CR Model produced expected values of the number of broods produced during observation periods, $s_b^{obs}$, that were significantly greater than the observed values (Table 2.2), suggesting that this model produces upwardly biased estimates of spawning frequency for *H. guttulatus*. Conversely, the more realistic ISR Model produced expected values of $s_b^{obs}$ and the total number of days female spent with hydrated eggs, $t_c^{obs}$, which were well within the 95% confidence intervals for the observed mean values on the grid in both 2001 and 2002. Thus the ISR Model is appropriate for indirectly estimating annual spawning frequency in this species. This is supported by the fact that observed male and female reproductive activity plotted as a function of underwater survey date on the grid closely matched the predicted values based on the ISR Model (Fig. 2.3).

## DISCUSSION

This paper presents the first estimates of *in situ* reproductive rates for the European long-snouted seahorse, *H. guttulatus* and validates an application of the 'spawning fraction' method (Hunter & Leong 1981) for estimating spawning frequency. These results underscore the importance of testing assumptions when using proxies for estimating spawning frequency in iteroparous fishes. In particular, this study suggests that inferences of spawning frequency based on egg production alone may lead to significant overestimates of spawning success and realized annual fecundity.

### Temporal trends in reproductive activity

Reproduction of *H. guttulatus* is seasonal and the duration of the reproductive season varies among populations (Boisseau 1967, Reina-Hervás 1989). Peaks in the reproductive activity of male and female *H. guttulatus* in the Ria Formosa lagoon, corresponded to months with warmer water temperatures and higher primary and secondary production (Sprung 1994ab), as observed in other fishes (Bye 1984, Milton & Blaber 1990). The duration of the reproductive season in the Ria Formosa lagoon (March – November) was very similar to that reported for populations in southern Spain at a similar latitude (March – October, 36.7 °N, Reina-Hervás 1989), but almost twice as long as in the higher latitude Arcachon Basin population (May – September, 44.7 °N, Boisseau 1967). Among-population differences in the duration of the reproductive season may be attributable to cooler water temperatures (Robert et al. 1993) and shorter photoperiods at higher latitudes. Although Boisseau (1967) suggested that peaks in *H. guttulatus* reproductive activity occurred during full moons in the Arcachon Basin, there was no evidence of a correlation between lunar phase and reproduction in the Ria Formosa.

### Spawning frequency

A four-fold difference in estimated spawning frequency between the Arcachon Basin (1 $yr^{-1}$) and the Ria Formosa (4.2 $yr^{-1}$) suggests that realized annual fecundity varies considerably among *H. guttulatus* populations. Spawning frequencies are positively correlated with temperature in many marine fishes (Bone et al. 1995), including darters (Gale & Deutsch 1985). Variation in spawning frequency among northern anchovy populations was also linked to energetic constraints (4 –20 spawnings $yr^{-1}$, Hunter & Leong 1981). With an estimated mean spawning frequency of 1 $yr^{-1}$ and a shorter reproductive season, *H. guttulatus* in the Arcachon Basin may be more vulnerable to environmental stochasticity than the Ria Formosa population. This is because spawning multiple times within a reproductive season confers fitness benefits, particularly for small-bodied fishes inhabiting variable environments. By spawning fewer times in variable environments, the risk of catastrophic losses of eggs and larvae due to temporarily poor environmental conditions are increased (Nikolsky 1963, McEvoy & McEvoy 1992).

The underwater survey data suggest that on average, female *H. guttulatus* prepared significantly more clutches of eggs (1.2 – 1.7 times as many) than males brooded in the Ria Formosa in 2001 and 2002. This means that estimates of spawning frequency and batch fecundity based on female egg production (e.g. using histological assessments) may significantly overestimate the actual number of young produced. The mean number of broods produced by males was probably not limited by availability of mature females because there was a slightly female-biased sex ratio (~55% females, including all tagged and untagged individuals on the grid) in both 2001 and 2002 and the interclutch times of females were equal to the brood times of males. Alternatively, longer interbrood intervals for this population of *H. guttulatus* may have derived in part from fishes depredating eggs as these were being transferred to males during mating. This occurred during one of three matings witnessed on the grid. Approximately 1% of male *H. guttulatus* in the Ria Formosa had gaping holes in their pouch suggesting that predators may also depredate eggs or developing embryos directly from the pouch. A wounded wild *H. whitei* male had a hole in his pouch that precluded mating for nearly three reproductive cycles (~60 days, Vincent & Sadler 1995; A. Vincent, pers comm). The degree to which predation affects reproductive success in *H. guttulatus* is unknown, but reproductive success in other fishes with male parental care is strongly affected by interspecific predation (e.g. Cole & Sadovy 1995).

## Batch fecundity

A significant relationship between *H. guttulatus* male size and brood size means that male length or mass is a reasonable predictor of brood size in this species. Among fishes, fecundity is strongly size- and/or age-dependent (Bagenal 1978, Davis & West

1993, Lowerre-Barbieri et al. 1996). Female size typically predicts clutch size in fishes (Bagenal 1978), including syngnathids (Vincent 1990, Teixeira & Musick 2001, Vincent & Giles 2003), but male dimensions also predict brood size in fishes with paternal care, including syngnathids (Strawn 1958, Masonjones 1997, Teixeira & Musick 2001) and mouthbrooding cardinalfishes (Okuda et al. 1998, Kolm 2002). Because both the volume of the female's abdominal cavity and volume of the male's sealed brood pouch potentially limit the number and/or size of embryos that can be successfully produced by seahorses (Boisseau 1967, Masonjones 1997), correlations between the dimensions of both parents and brood size likely reflect mutual mate selection for size (Vincent & Sadler 1995, Teixeira & Musick 2001, Vincent & Giles 2003). A positive correlation between the standard lengths of *H. guttulatus* males and females engaged in courtship behaviour on the grid (unpublished data), suggests that there is size-assortative mating in this population. At the population level, *H. guttulatus* brood size increased during the reproductive season in 2001 (although little variance in brood size was explained by the relationship and, brood size did not vary in 2002). Because the brood sizes of individuals were not tracked through time, it was not possible to test whether the number of juveniles per brood declined over time within individuals (as observed in Vincent & Giles 2003), nor was it possible to control for the effects of male and female growth on brood size.

### Annual realized fecundity: model comparisons

Neither assumption of the CR Model - negligible interbrood intervals and continuous reproduction during the entire reproductive season - was met empirically for *H. guttulatus* (Table 2.3, Fig. 2.3). This means that accurate estimates of annual realized fecundity in other seahorse species of conservation concern (IUCN 2003, Foster & Vincent 2004), may require further sampling. Incorporating an estimate of interbrood interval into the IR Model produced intermediate estimates of annual spawning frequency, however, estimating interbrood intervals required considerable effort to monitor changes in the reproductive activity of individually tagged seahorses. Use of the ISR Model circumvented the need to directly estimate interbrood intervals (see methods).

The ISR Model, parameterized by the catch data, produced indirect estimates of the expected number of broods produced by males and number of days spent preparing eggs by females that closely matched values directly observed among tagged individuals on the grid. This suggests that the ISR Model produced unbiased estimates of the mean annual frequencies of male brood and female clutch production in *H. guttulatus*. Thus annual spawning frequency and realized annual fecundity may be overestimated by as much as 270% when the assumptions of rapid remating and/or continuous reproduction have not been met. Assumptions of the CR Model may be more likely to hold for monogamous species with low interbrood intervals (Vincent & Sadler 1995) that breed year round in tropical environments (e.g. *H. comes*, Perante et al. 2002).

Although the ISR model only provides mean estimates of spawning frequency, simulations that incorporate parameters including male size, brood size, brooding period and duration of the reproductive season (data that are available for many species, Foster & Vincent 2004) can be used to predict variance in size-specific annual spawning frequencies and realized annual fecundities.

## CONCLUSIONS

Histological methods used to infer spawning frequency and fecundity (Hunter & Goldberg 1980, DeMartini & Fountain 1981, Hunter & Leong 1981, Murua & Saborido-Rey 2003) are probably less costly and more feasible than directly measuring the spawning frequency and batch fecundity of most fishes. As shown here, however, inferring spawning frequency and batch fecundity from egg production alone may lead to significant overestimates of spawning frequency and hence realized annual fecundity. Fishes that provide parental care offer an opportunity to validate indirect estimates of spawning frequency (e.g. Cole & Sadovy 1995). Parental care, which is common among marine teleosts (Baylis 1981), is often in the form of nest guarding (e.g. common goby, Kvarnemo et al. 1998) or brood bearing (e.g. mouthbrooding Bengaii cardinalfishes, Kolm 2002). The brood size and rearing success of such species can be directly quantified and provide a more accurate estimate of fecundity than characterizing egg production or mating success alone (Cole & Sadovy 1995).

*In situ* behavioural observations provided an opportunity to validate inferences from the catch data (ISR Model) and ultimately enhanced understanding of this species' reproductive ecology, which could not have been inferred from fisheries-dependent sampling alone (Shumway 1999). The effort associated with testing the assumption of rapid remating in *H. guttulatus* using underwater surveys greatly improved estimates of spawning frequency in the IR Model and underscored the importance of testing model assumptions for data-deficient species.

The success of the ISR model in predicting observed estimates of male spawning frequency and the number of days females spent preparing eggs suggests that this approach, based on the 'spawning fraction' method developed by Hunter & Leong (1981), may be broadly applicable to all organisms for which batch fecundity and the time to prepare or brood a batch of young are known. This method is suitable for species with broods that can be readily and periodically surveyed using either fisheries-independent catches or underwater census techniques. Although fisheries-independent collections were used in this study to estimate brood sizes and the fraction of spawning males and females, reliable estimates of these values were also obtained from the underwater survey data alone by caging brooding males (to estimate brood sizes) and tracking changes in the fraction of spawning males and/or females over time (see Fig. 2.3). For species that can be non-lethally sampled in this manner, generalizing the spawning fraction method becomes a particularly useful approach for estimating the spawning frequency and realized annual fecundity of species that are of conservation concern.

*Acknowledgements.* This is a contribution from Project Seahorse. I gratefully acknowledge K. Erzini, and members of his research team, including D. Americano and J. Ribeiro, for providing seahorses captured in their experimental sampling from 2000 – 2002 and for sharing their lab space. Many thanks to R. Adams, H. Balasubramanian, K. Bigney, J. d'Entremont, B. Gunn, S. Lemieux, C.-M. Lesage, E. Murray, J. Nadeau, S. Overington, M. Veillette, K. Wieckowski, and especially S. V. Santos, for excellent field and lab assistance. I thank all members of the Parque Natural da Ria Formosa for their kind logistical support and K. McCann for an insightful discussion of methods for estimating spawning frequency. Many thanks to C. Hall, D. O'Brien and A. Vincent for valuable comments on a previous version of this manuscript. Chocolaterie Guylian generously supported this research. I held a Natural Sciences and Engineering Research Council (NSERC) Postgraduate Scholarship, a Québec (FCAR) Doctoral Research Award and a J.W. McConnell McGill Major Fellowship during the course of this research.

## LITERATURE CITED

Bagenal TB (1978) Aspects of fish fecundity In: Gerking SD (ed) Ecology of freshwater fish production. Blackwell Scientific Publications, Oxford, U.K. pp 75-101

Baylis JR (1981) The evolution of parental care in fishes, with reference to Darwin's rule of male sexual selection. Environmental Biolology of Fishes 6: 223-251

Boisseau J (1967) Régulations hormonales de l'incubation chez un vertébré mâle: recherches sur la reproduction de l'Hippocampe. PhD thesis, University of Bordeaux, Bordeaux, France.

Bone Q, Marshall NB, Blaxter JSH (1995) Biology of fishes (2nd ed). Blackie, London

Brown-Peterson N, Thomas P, Arnold CR (1988) Reproductive biology of the spotted seatrout, *Cynoscion nebulosus*, in south Texas. Fishery Bulletin 86: 373-388

Bye VJ (1984) The role of environmental factors in the timing of reproductive cycles. In: Potts GW, Wooton RJ (eds) Fish reproduction: strategies and tactics. Academic Press, London, U.K. pp. 188-205

Caswell H. (1989) Matrix population models: construction, analysis and interpretation. Sinauer Associates, Sunderland, Massachusetts.

Clarke TA (1987) Fecundity and spawning frequency of the Hawaiian anchovy or Nehu, *Encrasicholina purpurea*. Fishery Bulletin 85:127-138

Cole KS, Sadovy Y (1995) Evaluating the use of spawning success to estimate reproductive success in a Caribbean reef fish. Journal of Fish Biology 47:181-191

Davis TLO, West GJ (1993) Maturation, reproductive seasonality, fecundity, and spawning frequency in *Lutjanus vittus* (Quoy and Gaimard) from the North West shelf of Australia. Fishery Bulletin 91:224-236

deBruyn AMH, Meeuwig JJ (2001) Detecting lunar cycles in marine ecology: periodic regression vs. categorical ANOVA. Marine Ecology Progress Series 214:307-310

DeMartini EE, Fountain RK (1981) Ovarian cycling frequency and batch fecundity in the queenfish, *Seriphus politus* - attributes representative of serial spawning fishes. Fishery Bulletin 79:547-560

Erzini K, Bentes L, Coelho R, Correia C, Lino PG, Monteiro P, Ribeiro J, and Goncalves JMS (2002) Recruitment of sea breams (Sparidae) and other commercially important species in the Algarve (southern Portugal), Final Report. Commission of the European Communities DG XIV/C/1.

Foster S, Vincent, ACJ (2004) The life history and ecology of seahorses, *Hippocampus* spp.: implications for conservation and management. Journal of Fish Biology 65:1-61

Gale WF (1986) Indeterminate fecundity and spawning behaviour of captive red shiners: fractional, crevice spawners. Transactions of the American Fisheries Society 115:429-437.

Gale W, Deutsch W (1985) Fecundity and spawning frequency of captive tessellated darters - fractional spawners. Transactions of the American Fisheries Society 111:35-40

Hunter JR, Macewicz BJ, Sibert JR (1986) The spawning frequency of skipjack tuna, *Katsuwonus pelamis*, from the south Pacific. Fishery Bulletin 84:895-903

Hunter JR, Leong R (1981) The spawning energetics of female northern anchovy, *Engraulis mordax*. Fishery Bulletin 79: 215-230

Hunter JR, Goldberg S (1980) Spawning incidence and batch fecundity in northern Anchovy, *Engraulis mordax*. Fishery Bulletin 79:215-230

Hutchings J (2001) Conservation biology of marine fishes: perceptions and caveats regarding assignment of extinction risk. Canadian Journal of Fisheries and Aquatic Sciences 58:108-121

IUCN (2003) 2003 IUCN Red List of Threatened Species. <www.redlist.org>. Downloaded on 31 August 2004.

Jones AG, Kvarnemo C, Moore GI, Simmons LW, Avise JC (1998) Microsatellite evidence for monogamy and sex-biased recombination in the Western Australian seahorse *Hippocampus angustus*. Molecular Ecology 7:1497-1505

Kolm N (2002) Male size determines reproductive output in a paternal mouthbrooding fish. Animal Behaviour 63:727-733

Kvarnemo C, Svensson O, Forsgren E (1998) Parental behaviour in relation to food availability in the common goby. Animal Behaviour 56:1285-1290

Lasker R (1985) Introduction: An egg production method for anchovy biomass assessment. NOAA, NMFS: 1-3.

Lo Bianco S (1888) Notizie biologiche reguardanti specialmente il periodo di maturita sessuale degli animali del Golfo di Napoli. Mitte. Zool. Stat. Neapel. 8:385-440

Lourie SA, Vincent ACJ, Hall HJ (1999) Seahorses: An identification guide to the world's species and their conservation. Project Seahorse, London, U.K.

Lowerre-Barbieri SK, Chittenden Jr. ME, Barbieri LR (1996) Variable spawning activity and annual fecundity of weakfish in Chesapeake Bay. Transactions of the American Fisheries Society 125:532-545

Machás R, Santos R (1999) Sources of organic matter in Ria Formosa revealed by stable isotope analysis. Acta. Oecol. Int. J. Ecol. 20:463-469

Masonjones HD, Lewis SM (2000) Differences in potential reproductive rates of male and female seahorses related to courtship roles. Animal Behaviour 59:11-20

Masonjones HD (1997) Sexual selection in the dwarf seahorse, *Hippocampus zosterae* (Syngnathidae): An investigation into the mechanisms determining the degree of male vs. female intrasexual competition and intersexual choice. PhD thesis. Tufts University, Medford, Massachusetts

McEvoy LA, McEvoy J (1992) Multiple spawning in several commercial fish species and its consequences for fisheries management, cultivation and experimentation. Journal of Fish Biology 41(Suppl. B):125-136

Milton DA, Blaber JM (1990) Maturation, spawning seasonality, and proximate spawning stimuli of six species of tuna baitfish in the Solomon Islands. Fishery Bulletin 89:221-237

Murua H, Saborido-Rey F (2003) Female reproductive strategies of marine fish species of the north Atlantic. J. Northw. Atl. Fish. Sci.33: 23-31

Myers RA, Bowen KG, Brrowman NJ (1999) Maximum reproductive rate of fish at low population sizes. Canadian Journal of Fisheries and Aquatic Sciences 56: 2404-2419

Nikolsky GV (1963) The ecology of fishes. Academic Press, New York

Okuda N, Tayasu I, Yanagisawa Y (1998) Determinate growth in a paternal mouthbrooding fish whose reproductive success is limited by buccal capacity. Evolutionary Ecology 12:681-699

Parrish RH, Mallicoate DL, Klingbeil RA (1986) Age dependent fecundity, number of spawnings per year, sex ratio, and maturation stages in northern anchovy, *Engraulis mordax*. Fishery Bulletin 84:503-517

Perante NC, Pajaro MG, Meeuwig JJ, Vincent ACJ (2002) Biology of a seahorse species *Hippocampus comes* in the central Philippines. Journal of Fish Biology 60:821-837

Poortenaar CW, Woods CMC, James PJ, Giambartolomei FM, Lokman PM (2004) Reproductive biology of female big-bellied seahorses. J Fish Biology 64:717-725

Reina-Hervás JA (1989) Contribucion al estudio de la F. Syngnathidae (Pisces) en Las Costas del Sureste de España. Arquivos do Museu Bocage 1:325-334

Robert R, Trut G, Laborde JL (1993) Growth, reproduction and gross biochemical composition of the Manila clam *Ruditapes philippinarum* in the Bay of Arcachon, France. Marine Biology 116:291-299

Rothschild BJ (1986) Dynamics of marine fish populations. Harvard University Press, Cambridge, Massachusetts

Sadovy Y (2001) The threat of fishing to highly fecund fishes. Journal of Fish Biology 59(Suppl. A):90-108

Shumway CA (1999) A neglected science: applying behavior to aquatic conservation. Environmental Biology of Fishes 55:183-201

Sprung M (1994a) High larval abundances in the Ria Formosa (Southern Portugal) - methodological or local effect? Journal of Plankton Research 16:151-160

Sprung M (1994b) Macrobenthic secondary production in the intertidal zone of the Ria Formosa - A lagoon in Southern Portugal. Est. Coast. Shelf. Sci. 38:539-558

Strawn K (1958) Life history of the pigmy seahorse, *Hippocampus zosterae* Jordan and Gilbert, at Cedar Key, Florida. Copeia 1:16-22

Tegner M, Basch L, Dayton P (1996) Near extinction of an exploited marine invertebrate. Trends in Ecology and Evolution 11: 278-280

Teixeira RL, Musick JA (2001) Reproduction and food habits of the lined seahorse, *Hippocampus erectus* (Teleostei: Syngnathidae) of Chesapeake Bay, Virginia. Review Brazilian Biology 61:79-90

Vincent ACJ. Giles B (2003) Correlates of reproductive success in a wild population of *Hippocampus whitei*. Journal of Fish Biology 63:344-355

Vincent ACJ (1996) The International Trade in Seahorses. TRAFFIC International, Cambridge, U.K.

Vincent ACJ, Sadler LM (1995) Faithful pair bonds in wild seahorses, *Hippocampus whitei*. Animal Behaviour 50: 1557-1569

Vincent ACJ (1990) Reproductive Ecology of Seahorses. PhD Thesis. Cambridge University, Cambridge, U.K.

Wallace RA, Selman K (1981) Cellular and dynamic aspects of oocyte growth in teleosts. American Zoologist 21:325-343

Weddle GK, Burr BM (1991) Fecundity and the dynamics of multiple spawning in darters: an in-stream study of *Etheostoma rafinesquei*. Copeia 1991:419-433

**Table 2.1** List of symbols and source of data (underwater survey = USD or catch data = CD) used to estimate parameters.

| Symbol | Significance | Source |
|---|---|---|
| $t_s$ | Duration of the reproduction season | CD |
| $t_b$ | Brooding period (males, time to brood a single clutch of eggs) | USD[a] |
| $t_c$ | Time females spent preparing one clutch of eggs | USD |
| $t_{ib}$ | Interbrood interval (males, time between birth and remating) | USD |
| $t_{ic}$ | Interclutch interval (females, time between clutches) | USD |
| $t_{by}$ | Total number of days males spent brooding young $yr^{-1}$ | CD |
| $t_{cy}$ | Total number of days females spent preparing eggs $yr^{-1}$ | USD |
| $s_b^{yr}$ | Number of broods produced $male^{-1}$ $yr^{-1}$ | CD |
| $s_c^{yr}$ | Number of clutches prepared $female^{-1}$ $yr^{-1}$ | CD |
| $s_b^{obs}$ | Number of broods produced $male^{-1}$ per observation period | USD |
| $t_c^{obs}$ | Total time females spent preparing eggs per observation period | USD |
| $f_b$ | Realized batch fecundity (brood size) | CD, USD |
| $f_a = f_b.s_b^{yr}$ | Realized annual fecundity (young produced $male^{-1}$ $yr^{-1}$) | |

[a]estimate from Boisseau (21 days, 1967) used to validate CR and ISR Models

**Table 2.2** Observed number of broods (males) and clutches (females) produced during underwater survey periods in 2001 and 2002. Expected values were calculated by estimating the area beneath fitted curves in Figure 2.3 that corresponded to observation periods in 2001 and 2002. Observed values (mean ± SD) were taken from all males and females that were marked and monitored throughout the entire observation period on the grid.

| | 2001<br>17 Jul - 26 Oct | n | 2002<br>23 May – 12 Sept | n |
|---|---|---|---|---|
| Males | | | | |
| Observed number of broods | 2.07 ± 1.10 | 28 | 2.72 ± 0.86 | 45 |
| (95% CI) | (0 – 4.22) | | (1.03 – 4.40) | |
| CR Model | | | | |
| Predicted number of broods | 5.33[a] | | 5.14[a] | |
| ISR Model | | | | |
| Predicted days with full pouch | 56.65 | | 71.59 | |
| Predicted number of broods | 2.69 | | 3.40 | |
| Females | | | | |
| Observed number of clutches | 3.40 ± 1.07 | 10 | 4.66 ± 1.23 | 18 |
| (95% CI) | (1.30 – 5.49) | | (2.24 – 7.07) | |
| Observed days with prepared eggs | 8.51 ± 3.37 | | 11.58 ± 5.13 | |
| (95% CI) | (1.88 – 15.11) | | (1.52 – 21.65) | |
| ISR Model | | | | |
| Predicted days with prepared eggs | 8.14 | | 10.46 | |

[a] predicted values greater than upper 95% confidence limit for mean observed values

**Table 2.3** Parameter values and model predictions from 3 progressively detailed models for the Ria Formosa population of *H. guttulatus*. Estimates are also given for the Arcachon Basin population using the ISR Model. Refer to the list of symbols in Table 2.1.

| **Model** | Continous Reproduction (CR) | Intermittent Reproduction (IR) | Intermittent and Seasonal Reproduction (ISR) | Intermittent and Seasonal Reproduction (ISR) |
|---|---|---|---|---|
| **Population** | Ria Formosa | Ria Formosa | Ria Formosa | Arcachon Basin |
| **Parameters** | | | | |
| $t_s$ (days) | 245[a] | 245[a] | 250[b] | 102[b] |
| $t_b$ (days) | 21.4 (±5.6) | 21.4 (±5.6) | 21.4 (±5.6) | 21[c] |
| $t_{ib}$ (days) | 0 | 12.9 (±8.2) | | |
| $t_{by}$ (days) | | | 90.4 | 20.7 |
| $f_b$ | 213.9 (±110.9) | 213.9 (±110.9) | 213.9 (±110.9) | 232 (±82.8)[c] |
| **Predictions** | | | | |
| $s_b^{yr}$ (broods $yr^{-1}$) | 11.45 | 7.14 | 4.22 (±1.57)[d] | 0.98 |
| $f_a$ ($f_b \cdot s_b$) | 2449.2 | 1527.2 | 903.6 (±574.7) | 227.4 |

[a] assuming 8 months from March – October (Reina-Hervás 1989)

[b] period when ≥1% of mature males were predicted to have full pouches

[c] values reported in Boisseau (1967)

[d] standard deviation estimated with individual based model

Figure 2.1 The Ria Formosa lagoon in southern Portugal (36°59' N, 7°51' W). The focal study grid (X) was located in the western part of the lagoon (eastern part not shown). Fishery-independent samples were collected at 53 stations within the lagoon in the lower left corner of the map (delimited by rectangle).

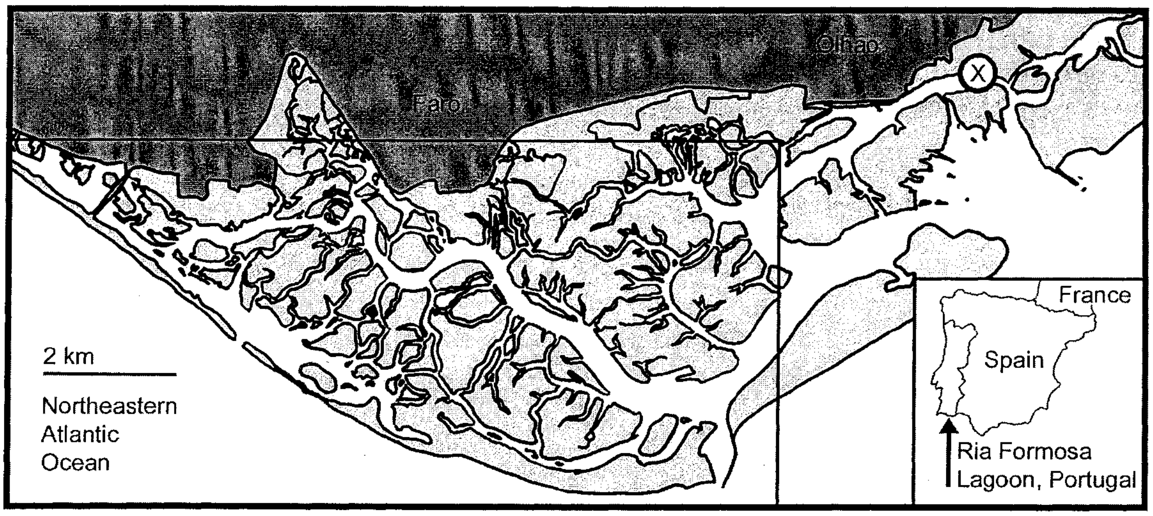


**Figure 2.2** Examples of the changes in reproductive state in one marked male (top) and one marked female (bottom) *H. guttulatus* during the observation period in 2002. Brackets indicate the maximum (A) and minimum (B) duration of three brooding periods. One brief and one lengthy interbrood interval are evident. The interclutch times, inferred from changes in female trunk girth are also denoted with brackets (C).

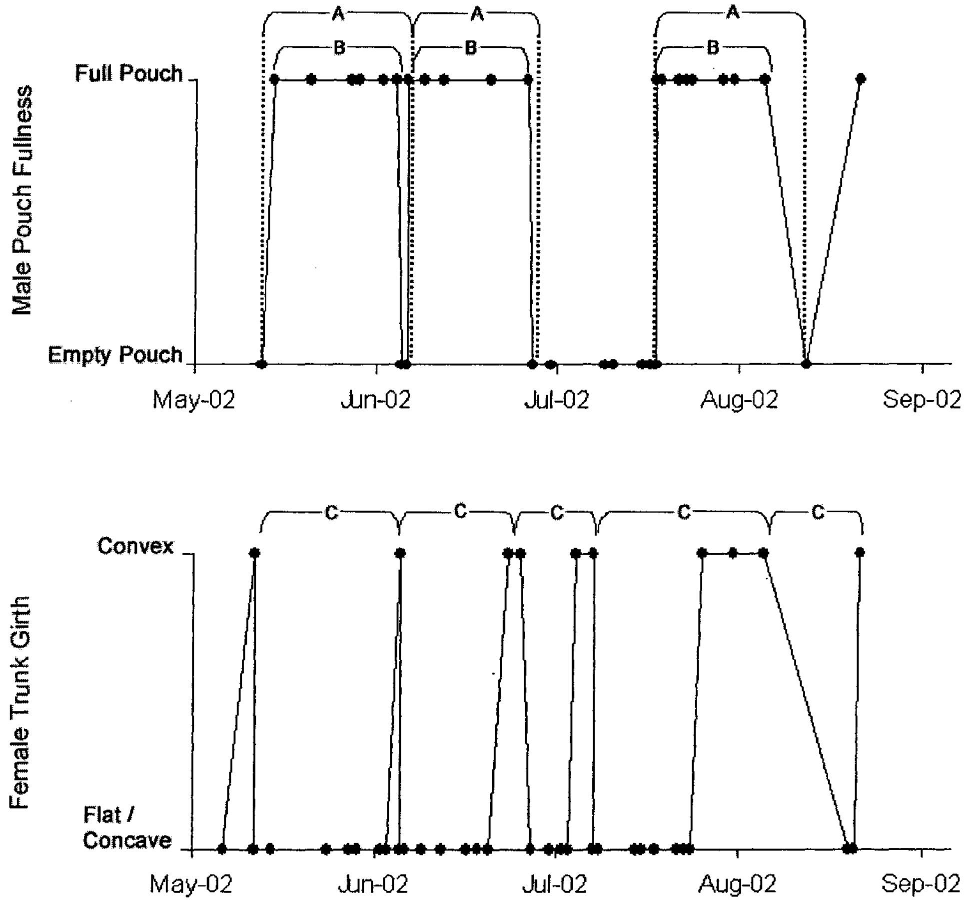


**Figure 2.3** Within-year variation in the reproductive activity of *H. guttulatus* in the Ria Formosa: (a) the proportion of males with full pouches and (b) the proportion of females preparing eggs for mating. The curves (equations given) were fit to catch data from 2000 (solid squares), 2001 (solid circles) and 2002 (solid triangles). The fraction of males with full pouches and females with prepared eggs observed during underwater surveys of the grid (2001-2002) are plotted as a function of underwater survey date for comparison (open triangles).

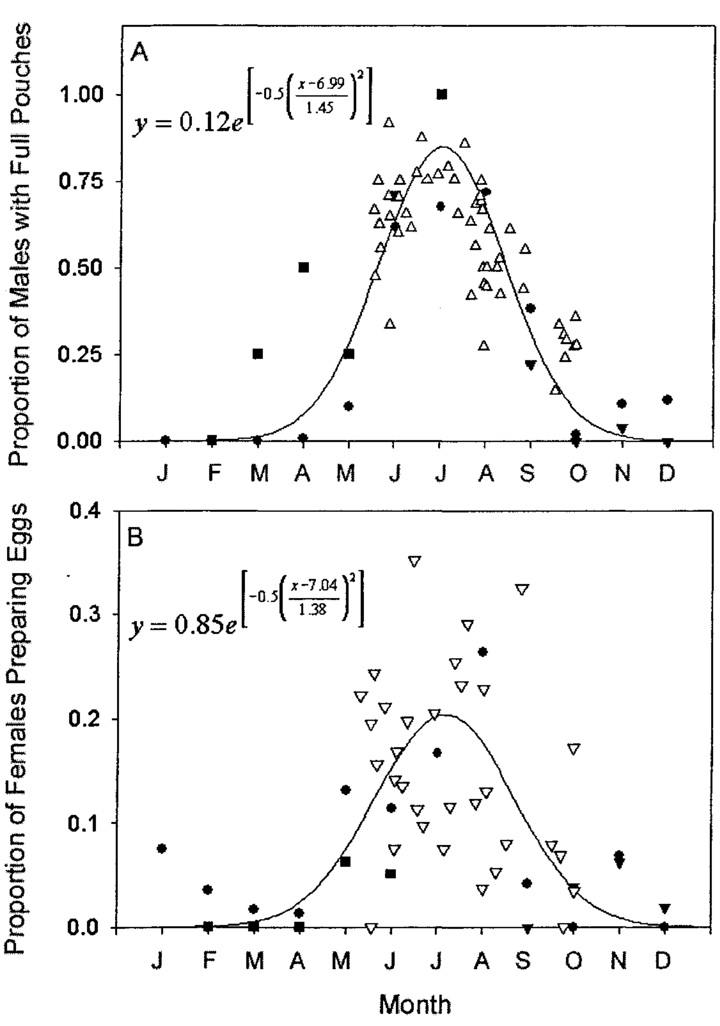


**Figure 2.4** Within-year variation in the reproductive activity of *H. guttulatus* in the Arcachon Basin from June – September 1952. The equation of the solid line fitted to the data (from Boisseau 1967) is given. The dashed line represents the line fitted to catch data from the Ria Formosa lagoon (see Fig 2.3a).

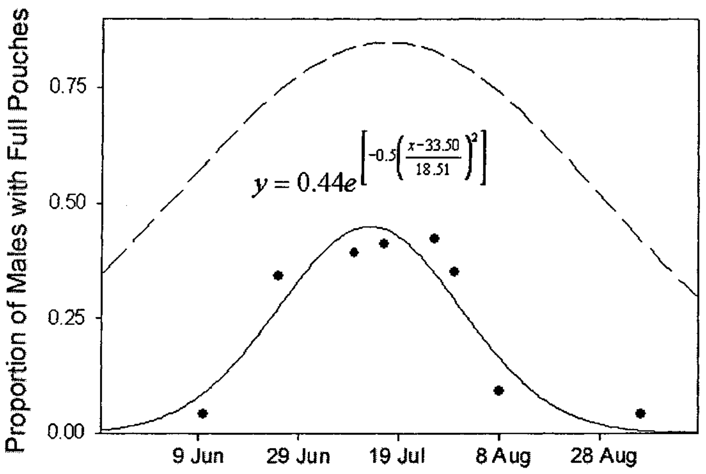


**Figure 2.5** Relationship between *H. guttulatus* male size and brood size (solid circles represent embryos dissected from brood pouches, open circles represent live juveniles born *in situ*): (a) standard length (n=117) and (b) mass (n=40). Equations of the fitted lines are given.

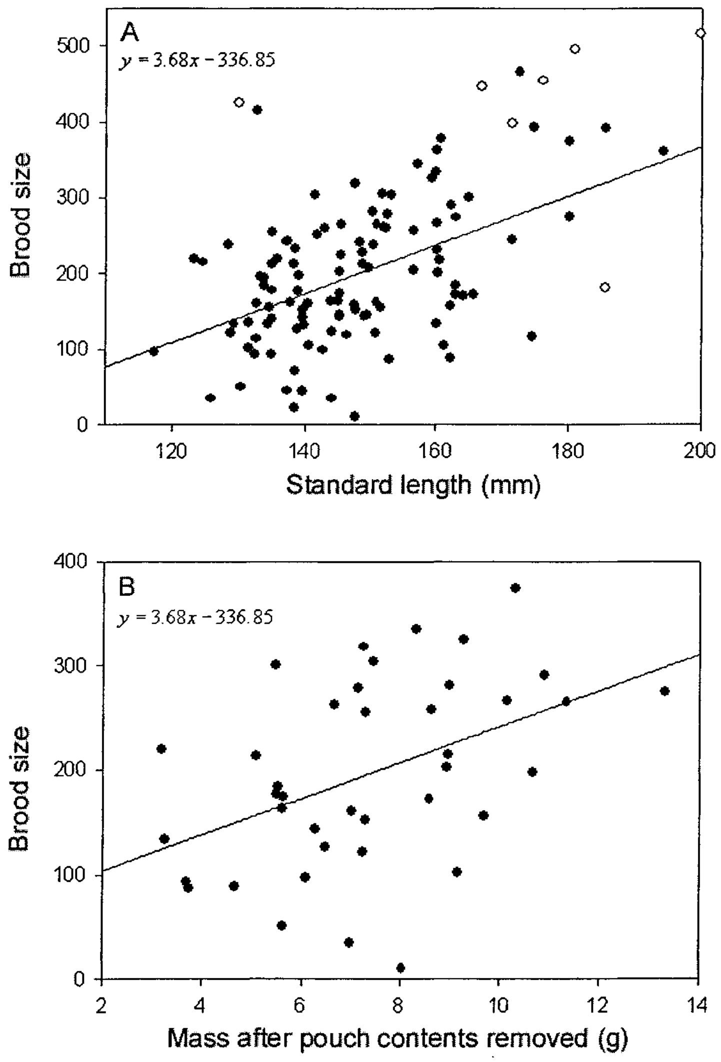
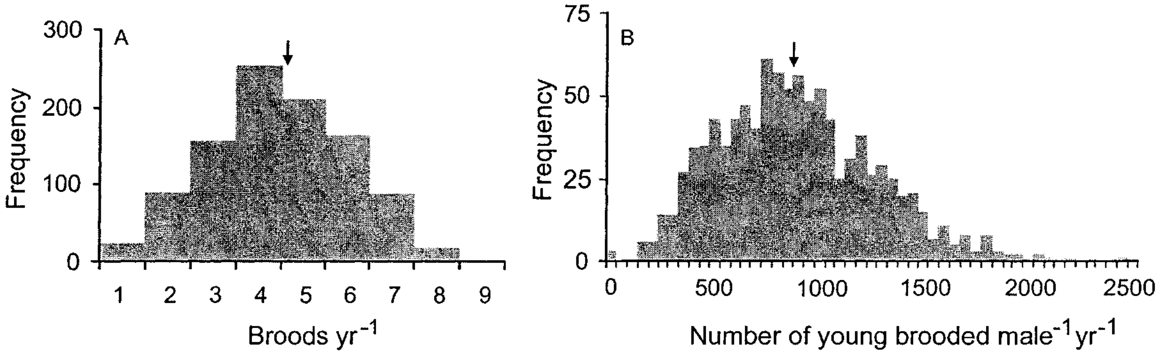


**Figure 2.6** Frequency distributions of (a) number of broods yr$^{-1}$ ($s_b^{yr}$) and (b) number of young brooded male$^{-1}$ yr$^{-1}$ ($f_a$) predicted by an individual based model for *H. guttulatus* (n=1000 simulated males). Mean estimates of $s_b^{yr}$ and $f_a$ from the ISR Model are indicated with arrows.



# LINKING STATEMENT 3

In my first two chapters, I examined the core life history parameters – survival, growth, reproduction and movement – of *H. guttulatus* and discussed the life history strategy of this species in the context of conservation and management. In Chapter 3, I examine patterns in the distribution and abundance of *H. guttulatus* and its sympatric congener, *H. hippocampus* in a subtropical tidal lagoon.

Marine species are often grouped according to their association with conspicuous habitat features including depth, temperature, vegetation type, or bottom structure (e.g. Greene et al. 1999, Foster & Vincent 2004). Such features are often used as habitat suitability indices to predict patterns in the distribution and abundance of species (Prosser & Brooks 1998, Rubec et al. 1998), under the assumption that areas of greater abundance reflect greater habitat suitability. However, inter-specific differences in microhabitat and landscape-level habitat that are too subtle for nominal classification schemes may lead to very different predictions with habitat suitability models. In Chapter 3, I examine how two nominally seagrass-associated species, *H. guttulatus* and *H. hippocampus*, partition their habitat over landscape and microhabitat scales. I also develop a preliminary regression-based model for predicting the relative abundance of *H. guttulatus*.

# CHAPTER 3

# HABITAT USE OF SYMPATRIC SEAHORSE SPECIES ALONG A GRADIENT OF HABITAT COMPLEXITY IN A SUBTROPICAL SEAGRASS-DOMINATED COMMUNITY

## ABSTRACT

We present the first estimates of local population abundance, distribution and habitat preference for two European seahorse species, *Hippocampus guttulatus* and *H. hippocampus*. We predicted that these sympatric species partition their habitat use into two broadly defined habitat types: complex vegetated habitats and sparsely vegetated sand flats. We sampled populations using underwater visual census techniques over landscape (100s – 1000s m) and microhabitat scales (<1 m). Over landscape scales, we estimated abundance and quantified habitat associations using generalized linear models. Over microhabitat scales, we tested for holdfast (attachment site) preferences using habitat selection indices. Both species were patchy in distribution, but *H. guttulatus* mean density (0.073 $m^{-2}$) was one order of magnitude greater than that of *H. hippocampus*. At a landscape scale, *H. guttulatus* abundance was significantly and positively correlated with an index of habitat complexity, the percentage of substrate covered by flora and sessile fauna. Conversely, *H. hippocampus* used more open and less speciose habitats that had greater oceanic influences. At a microhabitat scale, both species significantly preferred grasping holdfasts to using bare microhabitats, but the species differed in holdfast preferences: *H. guttulatus*, a holdfast generalist, grasped all prospective holdfast types with equal probability while *H. hippocampus*, a holdfast specialist, significantly avoided both fauna and flora that formed large colonies or tracts of dense vegetation. Patterns in habitat use were consistent with observed differences in morphology and foraging strategy. Despite similar life histories, these sympatric species may respond differently to disturbances that modify habitat structure and complexity over landscape or microhabitat scales.

# INTRODUCTION

Seagrasses are productive, biogenic habitats in coastal and estuarine ecosystems (Pollard 1984, Sheperd et al. 1989, Short & Wyllie-Escheverria 1996) and usually support a greater abundance and diversity of fishes than surrounding unvegetated habitats (Heck Jr et al. 1989, Edgar & Shaw 1995, Jenkins et al. 1997). This pattern is well documented in a rich body of literature and holds for many species in the family Syngnathidae (seahorses, pipefishes, pipehorses and seadragons) (Teixeira & Musick 1995, Diaz-Ruiz et al. 2000, Kendrick & Hyndes 2003). Greater fish abundance and diversity in seagrass communities (versus surrounding unvegetated habitats) have largely been attributed to lower predation risk (Choat 1982, Orth et al. 1984, Hindell et al. 2000), although greater food availability (Edgar 1990), increased sediment stability, and refuge from hydrodynamic forces have also been hypothesized to explain this pattern (Lewis 1984, Dean & Connell 1987). While hydrodynamic processes can influence recruitment patterns (Jenkins et al. 1997; Stoner 2003), many marine species actively select seagrass beds over unvegetated habitats, as well as microhabitats of different complexities within seagrasses (Bell & Westoby 1986b, Edgar 1990).

As heterogeneous habitats that vary in the degree of structural complexity and exposure to tidal regimes (Bell & Westoby 1986ab, Hovel et al. 2002), seagrass-dominated communities provide opportunities for sympatric species to partition their habitat over multiple spatial scales. Although many studies have examined the effects of habitat structure and complexity on the diversity and abundance of seagrass-associated species (e.g. Lewis 1984, Bell & Westoby 1986ab, Dean & Connell 1987, Heck Jr et al. 1989, Edgar 1990, Hovel et al. 2002, Hyndes et al. 2003), fewer have contrasted the effects of structural complexity on the habitat partitioning of closely related species. Sympatric species of pipefish partition their use of habitat both within and among seagrass beds according to their morphology, mobility, foraging technique and prey use (Howard & Koehn 1985, Kendrick & Hyndes 2003). Within seagrass beds, syngnathids are generally cryptic and sedentary, either occupying the canopy or dwelling at the sediment-water interface (Bell & Westoby 1986a, Teixeira & Musick 1995). Less mobile species (inferred from prehensile tails) consumed mainly planktonic prey and were predicted to rely more on dense macrophyte canopies, while more mobile species (inferred from well developed caudal fins) consumed both planktonic and epibenthic prey and were predicted to use a wider range of microhabitats including bare substrate (Howard & Koehn 1985).

Most temperate seahorse species (genus *Hippocampus*) are nominally associated with seagrass habitats (reviewed in Foster & Vincent 2004), but anecdotal observations suggest that some species exploit bare substrate. Seahorses have highly distinctive morphologies (Lourie et al. 1999) and foraging patterns (James & Heck Jr 1994). Seagrasses provide these sedentary fishes opportunities for crypsis, holdfasts (attachment sites) for prehensile tails, protection from hydrodynamic forces, and abundant faunas of small crustaceans, a primary source of food for syngnathids (Howard & Koehn 1985, Tipton & Bell 1988; Foster & Vincent, in press). However, species including *Hippocampus abdominalis*, *H. capensis, H. guttulatus*, *H. hippocampus* and *H. kuda* have also been encountered on bare substrate distant from potential holdfasts (Bell et al. 2003, Garrick-Maidment & Jones 2004, S. Lourie, personal communication, K. Martin-Smith, personal communication), suggesting that some species may also exploit the open sandy habitats that surround more complex vegetated habitats. This inference is supported by laboratory experiments in which seahorses employed different foraging strategies in vegetated and unvegetated habitats (James & Heck Jr 1994).

Two sympatric seahorse species, *Hippocampus guttulatus* (Cuvier 1829) and *H. hippocampus* L, appear to partition their habitat over both landscape and microhabitat scales (Boisseau 1967, Lythgoe & Lythgoe 1971, Whitehead 1986, Reina-Hervás 1989). Both species occur in the northeastern Atlantic Ocean and Mediterranean Sea (Boisseau 1967, Whitehead 1986, Reina-Hervás 1989, Lourie et al. 1999). While *H. guttulatus* is usually reported from seagrass beds, *H. hippocampus* is usually reported from soft bottoms among rocks and algae. *Hippocampus guttulatus* tends to be dark green or brown in colouration and commonly bears pronounced skin filaments on the head and/or body, suggesting that this species employs primarily vegetated microhabitats. Conversely, *H. hippocampus* is more variable in colouration, typically occurring in shades of brown, orange, or red. Filaments are less common or pronounced than on its larger congener, suggesting that *H. hippocampus* relies less on flora or filamentous structures for crypsis. Recreational diver surveys support these inferences (Garrick-Maidment & Jones 2004). However, no previous studies have examined the extent to which local abundances of these sympatric species can be predicted by habitat structure.

Our study examined the distribution and abundance of *H. guttulatus* and *H. hippocampus* in a coastal lagoon along a habitat gradient from sheltered seagrass and macroalgae to largely unvegetated sand flats exposed to increased tidal flow and water depth. Despite conservation concern (Santos et al. 1995, IUCN 2003, Foster & Vincent, in press), this is one of few ecological studies of either *Hippocampus guttulatus* or *H. hippocampus*. Our objectives were to: (1) estimate local population abundances, (2) quantify differences in habitat preferences over landscape and microhabitat scales and (3) identify components of habitat structure for predicting local abundances. Given the biological characteristics of the study species, we made the following predictions. Over landscape scales, the two main habitat types, vegetated areas and sand flats, would be broadly partitioned by the two species, with *H. guttulatus* exploiting vegetated areas and *H. hippocampus* exploiting more open sandy habitats. Within broadly partitioned habitat types, both species would prefer microhabitats that offered potential holdfasts. We expected that habitat partitioning over both landscape and microhabitat scales would reflect interspecific differences in morphology and foraging strategy. We evaluated these predictions through a multi-scale sampling program covering the lagoon system.

## METHODS

### Species descriptions

We employed the taxonomy of the genus *Hippocampus* as outlined in Lourie et al. (1999), recognizing that *Hippocampus guttulatus* was historically synonymized with *H. ramulosus* (Leach 1814). Further research is required to clarify whether *H. ramulosus* represents a different species (Lourie et al. 1999). In the meantime we retain the name *H. guttulatus*. *Hippocampus guttulatus* and *H. hippocampus* are readily distinguished *in situ* by differences in head, snout and trunk shape (Lourie et al. 1999).

Measuring seahorses precisely is challenging because of the curvature of the trunk and tail and because the head is held at an angle to the trunk. We used the measurement protocol outlined in Lourie et al. (1999) with one important distinction: we measured lengths as straight lines between the appropriate reference points, with the head held at a right angle to the body. All measurements reported in our paper are standard lengths, except where stated otherwise.

Published details about the life histories of *Hippocampus guttulatus* and *H. hippocampus* are sparse (Boisseau 1967, Foster & Vincent, in press). In our study, adult *H. guttulatus* ranged in size from 108 – 210 mm (n = 384). *Hippocampus hippocampus* was on average ~40% smaller, with adults ranging from 87 – 146 mm (n = 41). Juveniles of both species are planktonic (Boisseau 1967, Pérez-Ruzafa et al. 2004) for approximately 8 weeks (Boisseau 1967). The smallest settled *H. guttulatus* and *H. hippocampus* encountered in this study were 65 mm and 62 mm, respectively.

### Site description

We carried out our study in the western and central parts of the Ria Formosa lagoon (36°59' N, 7°51' W), in southern Portugal (Fig. 3.1). The Ria Formosa is a productive coastal lagoon (Sprung 1994a) characterized by high water turnover rates, sand flats, salt marshes and a network of channels and tidal creeks (Sprung 1994a, Machás & Santos 1999). From July 2001 – September 2002, water temperature varied seasonally from 10 – 28 °C. Within our study area, salinity ranged very little (36 – 38 ppt), but has declined occasionally to 33 ppt during previous winters (Monteiro 1989, but see Newton & Mudge 2003). Water is exchanged with the open ocean through 6 tidal inlets, 2 of which have been dredged. Tides are semidiurnal and mean depth varies from 2 – 3 m, but can be as great as 30 m in the main Faro-Olhão channel (Machás & Santos 1999, between sites 11 and 22, Fig. 3.1). Total lagoon area is ~170 km$^2$ (Machás & Santos 1999) with an estimated subtidal area of 26.7 km$^2$ (A. Rodrigues de Matos, Parque Natural da Ria Formosa, personal communication). Subtidal vegetation is dominated by seagrasses (primarily *Cymodocea nodosa*, but also *Zostera noltii* and *Z. marina*) (Alberto et al. 2001) and macroalgae, including *Ulva lactuca* and *Codium* spp., interspersed with sparsely vegetated sand and mud flats.

### Underwater visual census

We surveyed 32 sites (Fig. 3.1) using SCUBA: 16 sites were surveyed from 13 August – 19 September 2001 and 16 were surveyed from 14 June – 22 August 2002. In 2001, coincident with another study (Erzini et al. 2002), sites were selected along a gradient of ocean influences. To verify 2001 results, sites were selected in 2002 from throughout the subtidal area. We did not stratify random sampling by habitat type (e.g. seagrass, macroalgae, sand flat) in either year because the distribution of vegetation had not been mapped at the time of our study, nor could we discern habitat type from the surface because of low water clarity (usually <1 m). Because some parts (21%) of the subtidal area were not accessible due to sand bars, strong currents (e.g. near inlets) or high volumes of boat traffic, sites were surveyed as close to the pre-selected coordinates as possible. Surveys were carried out using standard underwater visual census (UVC) techniques (Samoilys 1997). Three randomly placed 2 m x 30 m transects, placed >5 m apart, were surveyed per site (total surveyed area = 5760 m$^2$). Data were averaged among transects within sites. All sites were >100 m apart.

### Fish counts and observations

The species, sex, trunk length, life history stage (juvenile, adult), holdfast, appearance (colouration, presence of skin filaments) and behaviour were recorded for all seahorses encountered on transects. We used trunk lengths (cleithral ring to the last trunk ring) to measure live seahorses *in situ* because the precision was greater than measuring standard length (tip of the snout to the cleithral ring, and from the cleithral ring to the tip of the straightened tail) underwater. Trunk lengths were converted to standard lengths using regression equations developed for both species (Table 3.1). *Hippocampus guttulatus* and *H. hippocampus* were considered subadults if they were smaller than size at 50% maturity, corresponding to 109 mm and 87 mm SL, respectively (Chapter 1, J. Curtis, unpublished data). In order to characterize interspecific differences in activity patterns associated with habitat use, behaviour was noted upon detection and was classified into 4 categories: (1) stationary, (2) swimming, (3) ambush foraging, when individuals were observed rapidly flicking their heads and sucking from the plankton while in a stationary position, and (4) active foraging, when individuals were observed feeding from the plankton, sediments or vegetation while actively searching for prey.

During underwater visual census surveys, we were confident of detecting at least 90-95% of seahorses even in densely vegetated habitats. Prior to this study, observers had >50 hours of underwater visual census experience with both sympatric seahorse species on a focal study site dominated by a mixture of *Cymodocea nodosa*, macroalgae (*Codium* spp, *Ulva lactuca*) and sessile invertebrates that covered approximately 80% of the substrate (Grid B, Chapter 1). Search efficiency was evaluated in two ways using practice UVCs: a) the first observer censused 10 – 30 $m^2$ of habitat and remove all detected seahorses and the second observer searched the same area to verify that all seahorses were detected; b) both observers recorded the presence and location of seahorses in 10 – 30 $m^2$ of habitat sequentially. Notes were compared to ensure the estimated densities were the same. Out of 17 trials carried out over a three week period, the proportion of seahorses that were not detected by one of the observers ranged from 0 – 6%. Although we carried out these trials in relatively complex habitats, we assumed that detectability was similar or greater in sparsely vegetated habitats.

Few individuals (<1%) swam away from observers during UVCs. Therefore surveys were not time constrained and all transects were thoroughly searched, by lifing and/or displacing macroalgae and sessile invertebrates. Using the same experienced observer (J.M.C.) for all UVCs further reduced observer bias. Population sizes were estimated by extrapolating mean densities to the subtidal area. Confidence intervals (95% CI) were estimated by bootstrapping the density estimates (Efron & Tibshirani 1993).

### Benthic community and habitat characteristics

The abundance of conspicuous (>2.5 cm in height, length or width) seagrasses, macroalgae, invertebrates (sessile and relatively immobile) and artificial structures was estimated as percent cover, defined here as the percentage of substrate (mud, sand or shell fragments) that supported or was overlaid by a given habitat component. Percent cover was quantified using three 30 m line intercepts (English et al. 1994) per site (averaged within sites). These line intercepts corresponded to the same transects used during fish counts. Microhabitat patch sizes (e.g. bryozoan colonies, tufts of macroalgae) were estimated by measuring the length of the line intercept that covered patches. The mean density of seagrass short shoots and mean leaf height were estimated using 15 randomly placed quadrats of 0.01 $m^2$ per site. The presence of mobile benthic invertebrates (e.g. *Aplysia* spp., *Sepia oficinalis*) and fishes (e.g. *Halobatrachus didactylus*) was recorded to estimate total species richness (flora and fauna) at each site. Two composite variables were also considered: (1) a dimensionless index of habitat complexity, $C_t/A_t$ (Bartholomew et al. 2000), which corresponds to the area surveyed within a site ($A_t$) that was covered by vegetation or sessile invertebrates, and (2), $M_t/A_t$, which denotes the proportion of $A_t$ that was covered by all species of macroalgae.

Depth was measured at the beginning and end of each transect and averaged within sites (all UVCs were carried out within two hours of low tide). Distances to the nearest tidal inlet were estimated planimetrically. Substrate particle size structure was characterized by fractionating three randomly placed sediment cores (0.02 m diameter x 0.01 m depth) per site into four size classes (<110 um, 110-1000 um, 1000-1800 um and >1800 um), drying them to constant weight, and estimating percent composition by weight. Potential covariates were recorded and included date, tidal and lunar phases, start time, horizontal visibility, current strength and prevailing weather conditions (wind strength and % cloud cover). Current strength was measured at the beginning of each transect on a scale of 0-5 using the degree of exertion required to maintain position relative to substrate:

0-1 = slack tide, chaotic water movement requiring sculling for stability
2 = minimal kicking to maintain reference point
3 = constant and moderate fin kicking to maintain reference point
4 = strenuous kicking to maintain reference point, but can be maintained
5 = cannot maintain position in current even with strenuous kicking

Recorded values of current strength were averaged within sites. Among site, mean current strength ranged from 0.5 – 4.5 with an overall mean of 2.4 (± 0.98). Although sites were surveyed with 2 hours of slack (low) tide, slack tide lasted no more than 10-20 minutes out of the 1 – 3 hours it took to carry out underwater surveys.

### Landscape level habitat use

The abundance of *Hippocampus guttulatus* approximated a Poisson distribution and we assumed the same for *H. hippocampus*. The landscape level relationships between seahorse counts and habitat variables were therefore characterized using generalized linear models (GLMs), assuming a Poisson distribution (McCullagh & Nelder 1989) with a log link function. Prior to model selection, the data were pooled across sites and summarized in Spearman rank correlation matrices to identify collinear variables as well as suites of variables that were broadly correlated with seahorse abundance. Poisson regressions were carried out for both species in 2001, but only for *H. guttulatus* in 2002 when few *H. hippocampus* were observed. To evaluate the predictive accuracy of our GLMs, we compared the densities of *H. guttulatus* observed in 2002 to expected values based on the 2001 GLM for *H. guttulatus*. We used a jackknife resampling procedure to test whether the correlation coefficient between observed and expected values differed significantly from unity (Manly 1997). In all cases, model selection was carried out using both forward and backward selection, which produced the same results. Variables were retained in the model if p-values associated with type III Wald tests were <0.05. Final regression models are reported. Data were examined for sex- and stage-specific differences in habitat associations.

### Microhabitat/holdfast preferences

Habitat preference is a measure of the strength of selection of one habitat component over others (Johnson 1980). The standardized selection index (Krebs 1999) was used to test whether *Hippocampus guttulatus* and *H. hippocampus* preferred using microhabitats that offered potential holdfasts (covered substrate) to using bare microhabitats. The selection index was estimated as $U_t/(C_t/A_t)$, where $U_t$ was the proportion of individuals grasping a holdfast. A selection index >0.5 indicated relative preference for covered substrate, while a selection index <0.5 indicated a relative preference for bare substrate (Krebs 1999). Rank preference indices (Johnson 1980, Krebs 1999) for using different microhabitats as holdfasts were calculated using PREFER 5.1 (Pankratz 1994). Data were examined for sex and stage-specific differences in microhabitat preferences.

## RESULTS

### Seahorse abundance and distribution

Pooling across years and sites, *Hippocampus guttulatus* was approximately ten times more abundant than *H. hippocampus* and occupied more than twice the number of survey sites (Table 3.2). *Hippocampus guttulatus* was encountered at all but three of the UVC sites. By contrast, *H. hippocampus* occurred primarily in large channels that were strongly influenced by water flowing through tidal inlets (Fig. 3.2a).

### Site characteristics

Survey sites were located from 1 – 6.9 km from the open Atlantic Ocean in 0.25 – 6.75 m of water depth. Horizontal visibility ranged from 0.3 – 6.0 m. Approximately 70% of the total surveyed area (all sites pooled) was bare substrate (fine sand, coarse sand or shell fragments). Of the total area that was covered, 58.6% was seagrass, 20.8% was macroalgae and 17.8% was invertebrates (Table 3.3). Fourteen sites were on sand flats, seven sites were dominated by the seagrass *Cymodocea nodosa*, four sites were dominated by a mixture of *C. nodosa* and macroalgae (mostly *Ulva lactuca* and *Codium* spp.) and seven sites were dominated by a mixture of macroalgae and colonies of benthic invertebrates (mostly the bryozoan *Zoobotryon verticillatum*) interspersed with sand flats (Fig. 3.2b). Where seagrass (*C. nodosa*) was present, mean leaf height ranged from 11.1 – 34.2 cm and mean short shoot density ranged from 233.3 – 848.3 shoots $m^{-2}$.

### Landscape level habitat use

*Hippocampus guttulatus* and *H. hippocampus* were broadly associated with different suites of variables (Table 3.4). After applying a sequential Bonferroni correction to adjust p-values for multiple Spearman rank correlations, the only significant correlation was between *H. guttulatus* density and the % cover of *Ulva lactuca*. However, by examining the direction of the correlations (i.e. positive or negative correlation coefficient), the correlation matrix suggested that *H. guttulatus* density was greater at sites with greater habitat complexity (i.e. sites with greater seagrass shoot density, more vegetation cover and sessile invertebrates and greater species richness), and thus less abundant at sites with lower habitat complexity (i.e. sites with a greater amount of bare sand and shell fragments). Conversely, *H. hippocampus* was generally more abundant in sites that had low habitat complexity (i.e. less speciose sites with a greater amount of bare sand and shell fragments). *Hippocampus hippocampus* density was also greater in sites with stronger oceanic influences, reflected in positive correlations with horizontal visibility, depth and current strength, and a negative correlation with water temperature.

In the GLMs, $C_t/A_t$ was the most significant predictor of *Hippocampus guttulatus* abundance in both years, but $M_t/A_t$ was also retained in the 2001 model and % *Ulva lactuca* was retained in the 2002 model (Fig. 3.3a, Table 3.5). There was a significant correlation between the observed and expected *H. guttulatus* densities for 2002 ($r = 0.471$, $p = 0.033$), but the correlation was significantly less than unity (jackknifed $r = 0.488$, $se = 0.203$, $t = 2.52$, $p < 0.02$) meaning that the predicted values were significantly greater than densities observed in 2002. The density of *H. hippocampus* was significantly explained by (and positively associated with) % bare sand in 2001 (Fig. 3.3b, Table 3.5). Within years, results were robust to the exclusion of the two sites that supported the highest densities of *H. guttulatus* and *H. hippocampus*, respectively, and robust to the exclusion of sites with densities of zero. There were no significant correlations or significant interactions among variables retained in the regression models (even when no sequential Bonferroni corrections were used to adjust critical p-values).

### Microhabitat/holdfast preferences

Despite differences in habitat use over landscape scales, both species significantly preferred using covered substrate to bare substrate at microhabitat scales. Approximately 89% of all seahorses employed holdfasts including seagrass blades, macroalgae, tunicates, bryozoans, polychaete tubes, sea urchins, and artificial structures (e.g. ropes, nets, bricks). The selection index for covered substrate was >0.5 for *Hippocampus guttulatus* at all occupied sites (mean SI = 0.87 ± 0.25 SD, Fig. 3.4a) and >0.5 for *H. hippocampus* at 80% of occupied sites (mean SI = 0.82 ± 0.36 SD, Fig 3.4b). *Hippocampus guttulatus*, did not exhibit a preference for any holdfast type ($F_{19,18} = 1.16$, $W = 3.13$, $K = 100$, all pairwise comparisons <3.13). Conversely, *H. hippocampus*, significantly preferred grasping artificial structures, the small tuft-forming bryozoan *Bugula neritina*, sea urchins, and small or tuft-forming macroalgae (*Colmpomenia sinuosa*, *Dictyota dichotoma*, *Padina pavonia* and *Ulva rigida*) to the abundant bryozoan *Zoobotryon verticillatum* which generally formed large colonies (mean ± SD patch length = 1.25 ± 1.32 m, n=50). *Hippocampus hippocampus* also significantly preferred using small or tuft-forming macroalgae to colonial ascidians, and to seagrasses (mean ± SD patch length = 5.03 ± 4.09 m, n=22) and *Ulva lactuca* (mean ± SD patch length = 1.12 ± 1.28 m, n=47), which generally formed large tracts of densely vegetated substrate.

### Morphology and foraging strategies

The two seahorse species differed significantly in their appearance and foraging strategies. Colouration varied both within and between species (Fisher Exact test, $p < 0.0001$): *H. guttulatus* primarily occurred in shades of brown (69.0%) and green (28.7%) and *H. hippocampus* occurred in shades of mottled brown (61.6%), yellow (26.3%), maroon (7.9%) and rust (4%). A greater percentage (70.3%) of *H. guttulatus* bore skin filaments on their heads and trunks than *H. hippocampus* (36.0%; $\chi^2 = 48.28$, $p < 0.001$). The two species also differed in activity patterns, both in terms of mobility and foraging technique (Fig. 3.5). *Hippocampus hippocampus* swam significantly more frequently than *H. guttulatus*, which tended to sway passively with the currents while grasping holdfasts ($\chi^2 = 33.24$, $p < 0.001$). *Hippocampus hippocampus* was also more active when feeding; a greater proportion was observed actively foraging for planktonic and epibenthic prey than *H. guttulatus* ($\chi^2 = 3.24$, $p = 0.072$). We found no evidence of sex- or stage-specific differences in habitat associations, holdfast preferences, appearance or foraging technique within species.

## DISCUSSION

Temperate seagrass communities support diverse and abundant populations of syngnathids (Pollard 1984, Howard & Koehn 1985, Hindell et al. 2000, Hyndes et al. 2003), which generally prefer complex vegetated habitats to unvegetated habitats (Howard & Koehn 1985, Flynn & Ritz 1999, Kendrick & Hyndes 2003). A rich body of literature examines the structure of fish communities within and among seagrass habitats of varying complexity (e.g. Howard & Koehn 1985, Bell & Westoby 1986ab, Edgar 1990, Hyndes et al. 2003, Kendrick & Hyndes 2003). Our results suggest that closely related fishes also differ in their habitat use between complex vegetated habitats and surrounding sparsely vegetated sand flats. Two nominally seagrass-associated species with similar life histories (reviewed in Foster & Vincent, in press) differed markedly in their habitat use over multiple spatial scales: one species was positively associated with habitat cover at both landscape and microhabitat scales, whereas the other species used more open and less speciose habitats at the landscape scale despite preferring covered microhabitats. As predicted, habitat associations over both spatial scales were linked to differences in morphology (size, colouration, presence of skin filaments), activity and foraging strategy.

### Abundance

We present the first estimates of local population abundance for two European seahorse species, *Hippocampus guttulatus* and *H. hippocampus*. Our estimates of local population size were large given that *Hippocampus* spp. generally occur at low densities (reviewed in Foster & Vincent, in press). The density of *H. guttulatus* was one order of magnitude greater than mean densities observed in similar transect surveys of *H. abdominalis* in Australia (K. Martin-Smith, personal communication), *H. capensis* in South Africa (Bell et al. 2003) and *H. comes* and *H. barbouri* in the Philippines (A. Maypa, personal communication). High seahorse abundance in the Ria Formosa may be attributable to unusually high productivity (Sprung 1994a) and plankton biomass (Sprung 1994b). In some aquatic ecosystems, fish biomass responds positively to increases in primary production (Garg & Bhatnagar 2000). Seahorses, which prey primarily on small vagile crustaceans (Howard & Koehn 1985, James & Heck Jr 1994), may be less prone to food-limitation in growth and reproduction in productive habitats such as the Ria Formosa than in less productive coastal habitats. However, mechanisms underlying differences in abundance between *H. guttulatus* and *H. hippocampus* within the Ria Formosa require further study. Our sampling constraints may have caused us to miss areas of high *H. hippocampus* abundance, which appeared to be linked with greater depths, stronger currents and cooler temperatures (see below).

### Landscape level habitat associations

The sympatric species, *Hippocampus guttulatus* and *H. hippocampus*, differed in their use of two broadly defined habitat types, complex vegetated areas and more exposed, sparsely vegetated sand flats. Over landscape scales, the amount of total habitat covered by vegetation and sessile invertebrates, $C_t/A_t$, appeared to shape the distribution and abundance of *H. guttulatus*. This is consistent with the distributions of many marine organisms, which are positively correlated with the amount of habitat structure (Heck Jr et al. 1989, Kendrick & Hyndes 2003), drift algae (Kulczycki et al. 1981) or seagrass (Kupschus 2003). Although the foraging efficiency of many predators decreases in more complex habitats (Choat 1982), increased habitat complexity either had no impact on (James & Heck Jr 1994) or increased (Flynn & Ritz 1999) the foraging efficiency of syngnathids practicing ambush foraging. By contrast, *H. hippocampus* exploited the more sparsely vegetated sand flats, which were less speciose and subject to stronger oceanic influences. The three sparsely covered sites where *H. hippocampus* outnumbered its congener (5, 17 and 20) were located close to tidal inlets where horizontal visibility, current strength and depth – all positively correlated with *H. hippocampus* abundance – were the greatest. *Hippocampus hippocampus* also outnumbered *H. guttulatus* in more open and exposed coastal habitats near Barcelona (sand flat) and Malaga (seagrass bed), in Spain (J. Curtis personal observation), but were not present in seahorse samples from a protected coastal lagoon in the Mediterranean Sea (Pérez-Ruzafa et al. 2004). Our results are consistent with surveys by recreational divers that encountered *H. guttulatus* more frequently in vegetated habitats and *H. hippocampus* more frequently in relatively bare habitats (Garrick-Maidment & Jones 2004).

Fish distribution and abundance are often predicted by multiple habitat variables including salinity and temperature (e.g. Brown et al. 2000, Kupschus 2003). Despite having quantified 60 habitat variables, the GLMs retained only 1-2 explanatory variables that were related to the extent of local habitat complexity. This may reflect (1) low variance in other important ecological variables within the Ria Formosa lagoon (e.g. salinity, Monteiro 1989), (2) the influence of additional ecological processes (e.g. predation, hydrodynamic processes) on local patterns of abundance (Choat 1982, Orth et al. 1984, Hindell et al. 2000, Hovel et al. 2002, Stoner 2003), or (3) low power to detect weakly correlated variables. Examining patterns of abundance and habitat use over regional spatial scales may reveal other variables for predicting seahorse distribution and abundance, including salinity and water temperature.

### Microhabitat/holdfast preference

Both European seahorse species preferred using covered microhabitats as holdfasts to using bare substrate, although *H. hippocampus* was observed more frequently on bare substrate than *H. guttulatus*. Our results were consistent with an experimental study showing that captive *H. abdominalis* preferred using artificial seagrass microhabitats to bare substrate (Flynn & Ritz 1999). The use of holdfasts with prehensile tails likely helps these relatively poor swimmers to stabilize themselves in strong currents. Seahorses encountered on bare substrate (e.g. *H. capensis, H. guttulatus*, *H. hippocampus*, *H. kuda* and *H. abdominalis*) may be temporarily exploiting open habitats, be in transit between covered microhabitats or have been displaced from their home ranges by strong wave action during storms (as inferred in Moreau & Vincent, 2004).

Although both *Hippocampus guttulatus* and *H. hippocampus* in this study used prehensile tails to grasp structures, they differed significantly in the types of holdfasts they preferred, as reflected by interspecific differences in appearance. Syngnathids mimic vegetation in color, shape and behaviour (Howard & Koehn 1985, Kendrick & Hyndes 2003), which likely reduces their visibility to both predators and prey. The predominantly brown and green colouration of *H. guttulatus*, a holdfast generalist, was consistent with its use of seagrasses, macroalgae and colonial invertebrates for camouflage. With its skin filaments, *H. guttulatus* was well camouflaged when grasping the light brown bryozoan, *Zoobotryon verticillatum*. The colour patterning of *H. hippocampus*, a holdfast specialist, was similar in colour to sand, shell fragments and some sessile invertebrates. This colouration was consistent with *H. hippocampus* being less reliant on vegetation for camouflage than *H. guttulatus*. Being smaller, *H. hippocampus* may also experience less drag in more exposed habitats and be able to maintain crypsis against smaller, preferred structures (e.g. the bryozoan *Bugula neritina*).

Interspecific differences in habitat preferences between *Hippocampus guttulatus* and *H. hippocampus* were also linked to differences in foraging strategy and prey use. *Hippocampus guttulatus* was relatively sedentary and ambushed planktonic prey more frequently than its more active congener. *Hippocampus hippocampus* swam more often and actively foraged for both planktonic and epibenthic prey. Differences in activity and foraging strategy between *H. guttulatus* and *H. hippocampus* were consistent with a study in which *H. erectus* adopted different prey capture techniques in complex and bare habitats: *H. erectus* tended to ambush prey in artificial seagrass habitats and actively forage for prey while on bare substrates (James & Heck Jr 1994). These results were also consistent with observations of four sympatric pipefishes within a seagrass bed: less mobile species consumed mainly planktonic prey and were associated with dense macrophytes, while more mobile species consumed both planktonic and epibenthic prey and probably used a variety of habitats, including bare substrate (Howard & Koehn 1985). Differences in microhabitat use and behaviour coupled with size-selective prey use resulted in little diet overlap among pipefishes during most of the year.

## IMPLICATIONS FOR CONSERVATION

While our results suggest that the two European seahorse species may respond differently to an overall reduction in seagrass cover, both species would likely be affected by a loss of holdfasts, and reductions in exported drift (Melville & Connolly 2003, Vanderklift & Jacoby 2003). Habitat loss has been linked to the extirpation of several marine fish populations (Wolff 2000, Dulvy et al. 2003) and is assumed to threaten many populations of temperate seahorse species including *H. hippocampus* (Santos et al. 1995, Musick et al. 2000, Pogonoski et al. 2002, IUCN 2003). Moreover, syngnathids are incidentally captured in non-selective, bottom-dragged fishing gears, especially trawls (Vincent 1996), which may also reduce habitat structure (Watling & Norse 1998, Turner et al. 1999) and essential holdfast availability.

Although the Ria Formosa's fish assemblages appear to have been stable over the last 20 years (Erzini et al. 2002), local seagrass beds have suffered damage due to dredging (Alberto et al. 2001) and possibly due to fishing gears which are used illegally in the lagoon (Erzini et al. 2002, J. Curtis, personal observation). Seagrasses are threatened marine habitats (Sheperd et al. 1989, Short & Wyllie-Escheverria 1996) and have declined in area along some European coasts (Pasqualini et al. 1999, Wolff 2000). Excessive seagrass damage within the Ria Formosa and in other parts of the North Atlantic Ocean or Mediterranean Sea could result in a reduction of *Hippocampus guttulatus* habitat, and hence local population sizes. Although *H. hippocampus* doesn't rely on seagrasses *per se* for refuge or camouflage, its response is difficult to predict. This is because the implications of seagrass loss reach beyond the physical provisioning of refuge and camouflage for both predators and prey: the production of many marine fishes stems from detritus exported from seagrass beds (Melville & Connolly 2003).

Because fisheries-independent data are often scant and costly to obtain, the development of habitat suitability models for predicting species distributions and responses to environmental change is appealing (Brown et al. 2000, Kupschus 2003). Our preliminary models were strengthened by qualitative concordance between GLMs for *Hippocampus guttulatus*: $C_t/A_t$ was included in both GLMs and densities observed in 2002 were significantly correlated with predicted values. This suggests that our index of habitat complexity, $C_t/A_t$, may be a useful tool for predicting the relative abundance of *H. guttulatus* in similar areas that have not yet been surveyed. Recently, habitat use models extrapolated to broader spatial and temporal scales successfully predicted the relative abundances of several marine fishes (e.g. Brown et al. 2000, Kupschus 2003). Our results also suggest, however, that it is important to examine habitat use and preferences over multiple spatial scales because habitat preferences are not necessarily consistent over a range of spatial scales: while one species preferred complex habitats at microhabitat and landscape scales, the other only preferred structural complexity for use as holdfasts.

*Acknowledgements*. This is a contribution from Project Seahorse. We gratefully acknowledge the following for excellent field and lab assistance: H. Balasubramanian, K. Bigney, B. Gunn, S. Lemieux, C.-M. Lesage, E. Murray, J. Nadeau, Norberto, S. Overington, M. Veillette, K. Wieckowski and especially S. V. Santos. We thank D. Marsden and D. Réale for statistical advice. We also thank members of the Universidade do Algarve and the Parque Natural da Ria Formosa, particularly K. Erzini, N. Grade, E. Marques, A. L. Quaresma, J. Ribeiro, Sr. Silverio, and M. Sprung, for their kind support. A. Gonzalez, S. Lourie, K. Martin-Smith, I. Morgan, S. Morgan, D. O'Brien and four anonymous reviewers provided constructive comments that greatly improved the manuscript. Guylian Chocolates, Belgium, generously funded our study. J.M.C. was supported by NSERC PGS-B (Natural Sciences and Engineering Research Council of Canada), FCAR (Fonds de Recherche et Technologies Québec), and a McConnell McGill Major Fellowship. The John G. Shedd Aquarium supported A.V.

## LITERATURE CITED

Alberto F, Mata L, Santos R (2001) Genetic homogeneity in the seagrass *Cymodocea nodosa* at its northern Atlantic limit revealed through RAPD. Marine Ecology Progress Series 221: 299-301

Bartholomew A, Diaz RJ, Cicchetti G (2000) New dimensionless indices of structural habitat complexity: predicted and actual effects on a predator's foraging success. Marine Ecology Progress Series 206: 45-58

Bell E, Lockyear J, McPherson J, Marsden A, Vincent A (2003) First field studies of an Endangered South African seahorse, *Hippocampus capensis*. Environmental Biology of Fishes 67: 35-46

Bell JD, Westoby M (1986a) Importance of local changes in leaf height and density to fish and decapods associated with seagrasses. J Exp Mar Biol Ecol 104: 249-274

Bell JD, Westoby M (1986b) Abundance of macrofauna in dense seagrass is due to habitat preference, not predation. Oecologia 68: 205-209

Boisseau J (1967) Régulations hormonales de l'incubation chez un vertébré mâle: recherches sur la reproduction de l'Hippocampe. PhD Dissertation, l'Université de Bordeaux, Bordeaux, France

Brown SK, Buja KR, Jury SH, Monaco ME (2000) Habitat suitability index models for eight fish and invertebrate species in Casco and Sheepscot Bays, Maine. North Am J Fish Manage 20: 408-435

Choat JH (1982) Fish feeding and the structure of benthic communities in temperate waters. Annu Rev Ecol Syst 13: 423-449

Dean RL, Connell JH (1987) Marine invertebrates in an algal succession. III. Mechanisms linking habitat complexity with diversity. J Exp Mar Biol Ecol 109: 249-273

Diaz-Ruiz S, Aguirre-Leon A, Perez-Solis O (2000) Distribution and abundance of *Syngnathus louisianae* and *Syngnathus scovelli* (Syngnathidae) in Tamiahua Lagoon, Gulf of Mexico. Ciencias Marinas 26: 125-143

Dulvy NK, Sadovy Y, Reynolds JD (2003) Extinction vulnerability in marine populations. Fish and Fisheries 4: 25-64

Edgar GJ, Shaw C (1995) The production and trophic ecology of shallow-water fish assemblages in southern Australia III. General relationships between sediments, seagrasses, invertebrates and fishes. J Exp Mar Biol Ecol 194: 107-131

Edgar GJ (1990) The influence of plant structure on the species richness, biomass and secondary production of macrofaunal assemblages associated with western Australian seagrass beds. J Exp Mar Biol Ecol 137: 215-240

Efron B, Tibshirani RJ (1993) An introduction to the bootstrap. Chapman and Hall, New York

Erzini K, Bentes L, Coelho R, Correia C, Lino PG, Monteiro P, Ribeiro J, Goncalves JMS (2002) Recruitment of sea breams (Sparidae) and other commercially important species in the Algarve (Southern Portugal). Final Report, DG XIV/C/1, Ref 99/061, University of the Algave, Faro, Portugal 178pp. + annexes

Flynn AJ, Ritz, DA (1999) Effect of the habitat complexity and predatory style on the capture success of fish feeding on aggregated prey. J Mar Biol Ass U K 79:487-494

Foster S, Vincent, ACJ (2004) The life history and ecology of seahorses, *Hippocampus* spp.: implications for conservation and management. Journal of Fish Biology 65:1-61

Garg SK, Bhatnagar A (2000) Effect of fertilization frequency on pond productivity and fish biomass in still water ponds stocked with *Cirrhinus mrigala* (Ham.). Aquaculture Research 31: 409-414

Garrick-Maidment N, Jones L (2004) British Seahorse Survey Report. Topsham, UK: The Seahorse Trust.

Greene HG, Yoklavich MM, Starr RM, O'Connell VM, Wakefield WW, Sullivan ED, McRea Jr JE, Cailliet GM (1999) A classification scheme for deep seafloor habitats. Oceanol Acta 22: 663-678

Heck Jr KL, Able KW, Fahay MP, Roman CT (1989) Fishes and decapod crustaceans of Cape Cod eelgrass meadows - species composition, seasonal abundance patterns and comparison with unvegetated substrates. Estuaries 12: 59-65

Hindell JS, Jenkins GP, Keough MJ (2000) Evaluating the impact of predation by fish on the assemblage structure of fishes associated with seagrass (*Heterozostera tasmanica*) (Martens ex Ascherson) den Hartog, and unvegetated sand habitats. J Exp Mar Biol Ecol 255: 153-174

Hovel KA, Fonseca MS, Myer DL, Kenworthy WJ, Whitfield PE (2002) Effects of seagrass landscape structure, structural complexity and hydrodynamic regime on macrofaunal densities in North Carolina seagrass beds. Marine Ecology Progress Series 243: 11-24

Howard RK, Koehn JD (1985) Population dynamics and feeding ecology of pipefish (Syngnathidae) associated with eelgrass beds of Western Port, Victoria. Australian Journal of Marine Freshwater Research 36: 361-370

Hyndes GA, Kendrick AJ, MacArthur LD, Stewart E (2003) Differences in the species- and size-composition of fish assemblages in three distinct seagrass habitats with differing plant and meadow structure. Marine Biology 142: 1195-1206

IUCN (2003) 2003 IUCN Red List of Threatened Species. <www.redlist.org>. Downloaded on 24 August 2004.

James PL, Heck Jr KL (1994) The effects of habitat complexity and light intensity on ambush predation within a simulated seagrass habitat. J Exp Mar Biol Ecol 176: 187-200

Jenkins GP, Black KP, Wheatley MJ, Hatton DN (1997) Temporal and spatial variability in recruitment of a temperate, seagrass-associated fish is largely determined by physical processes in the pre- and post-settlement phases. Marine Ecology Progress Series 148: 23-35

Johnson DH (1980) The comparison of usage and availability measurements for evaluating resource preference. Ecology 61: 65-71

Kendrick AJ, Hyndes GA (2003) Patterns in the abundance and size-distribution of syngnathid fishes among habitats in a seagrass-dominated marine environment. Est Coast Shelf Sci 57: 631-640

Krebs CJ (1999) Ecological Methodology. Addison-Welsey Educational Publishers, Inc., Menlo Park, California

Kulczycki GR, Virnstein RW, Nelson WG (1981) The relationship between fish abundance and algal biomass in a seagrass drift algae community. Est Coast Shelf Sci 12: 341-348

Kupschus S (2003) Development and evaluation of statistical habitat suitability models: an example based on juvenile spotted seatrout *Cynoscion nebulosus*. Marine Ecology Progress Series 265: 197-212

Lewis FG (1984) Distribution of macrobenthic crustaceans associated with *Thalassia*, *Halodule* and bare sand substrata. Marine Ecology Progress Series 19: 101-113

Lourie SA, Vincent ACJ, Hall HJ (1999) Seahorses: An identification guide to the world's species and their conservation. Project Seahorse, London, UK

Lythgoe J, Lythgoe G (1971) Fishes of the Sea. Blandford Press, London, UK

Machás R, Santos R (1999) Sources of organic matter in Ria Formosa revealed by stable isotope analysis. Acta Oecol 20: 463-469

Manly BFJ (1997) Randomization and Monte Carlo methods in Biology (2nd edition). Chapman and Hall, London, UK

McCullagh P, Nelder JA (1989) Generalized Linear Models. Chapman and Hall, London, UK

Melville AJ, Connolly RM. (2003) Spatial analysis of stable isotope data to determine primary sources of nutrition for fish. Oecologia 136:499-507

Monteiro CC (1989) La faune ichthyologique de la Ria Formosa: Rėpartition et organisation spatio-temporelle des communautés. PhD Dissertation, Université des Sciences et Techniques du Languedoc, Montpellier, France

Moreau, MA, Vincent, ACJ (2004) Social structure and space use in a wild population of the Australian short-headed seahorse, *Hippocampus breviceps* Peters, 1869. Marine and Freshwater Research 55:231-239

Musick JA, Harbin MM, Berkeley SA, Burgess GH, Eklund AM, Findley L, Gilmore RG, Golden JT, Ha DS, Huntsman GR et al. (2000) Marine, estuarine, and diadromous fish stocks at risk of extinction in North America (Exclusive of Pacific Salmonids). Fisheries 25: 6-30

Newton A, Mudge SM (2003) Temperature and salinity regimes in a shallow, mesotidal lagoon, the Ria Formosa, Portugal Estuarine, Coastal and Shelf Science 57:73-85

Orth RJ, Heck Jr KL, Van Montfrans J (1984) Faunal communities in seagrass beds - A review of the influence of plant structure and prey characteristics on predator prey relationships. Estuaries 7:339-350

Pankratz C (1994) PREFER v5.1 Preference Assessment. Jamestown, North Dakota, Northern Prairie Science Center

Pasqualini V, Pergent-Martini C, Pergent G (1999) Environmental impact identification along the Corsican coast (Mediterranean sea) using image processing. Aquatic Botany 65:311-320

Pérez-Ruzafa A, Quispe-Becerra, JI, García-Charton, JA, Marcos, C (2004) Composition, structure and distribution of the ichthyoplankton in a Mediterranean coastal lagoon. Journal of Fish Biology 64:202-218

Pogonoski J, Pollard DA, Paxton JR (2002) Conservation overview and action plan for Australian threatened and potentially threatened marine and estuarine fishes. Environment Australia, Canberra, Australia

Pollard DA (1984) A review of ecological studies on seagrass fish communities, with particular reference to recent studies in Australia. Aquatic Botany 18: 3-42

Prosser DJ, Brooks RP (1998) A verified habitat suitability index for the Louisiana Waterthrush. J Field Ornithol 69: 288-298

Reina-Hervás JA (1989) Contribucion al estudio de la F. Syngnathidae (Pisces) en Las Costas del Sureste de España. Arquivos do Museu Bocage 1:325-334

Rubec PJ, Coyne MS, McMichael RH, Monaco ME (1998) Spatial methods being developed in Florida to determine essential fish habitat. Fisheries 23: 21-25

Samoilys MA (1997) Underwater visual census surveys. In: Samoilys MA (ed) Manual for assessing fish stocks on Pacific coral reefs. Department of Primary Industries, Townsville, Australia

Santos RS, Hawkins S, Monteiro LR, Alves M Isidro EJ (1995) Marine research, resources and conservation in the Azores. Aquatic Conservation - Marine and Freshwater Ecosystems 5:311-354

Sheperd SA, McComb AJ, Bulthuis DA, Neverauskus V, Steffensen DA, West R (1989) Decline of seagrasses. In: Larkum AWD, McComb AJ, Sheperd SA (eds) Biology of Seagrasses. Elsevier, Amsterdam, pp. 346-393

Short FT, Wyllie-Escheverria S (1996) Natural and human-induced disturbances of seagrasses. Environmental Conservation 23:17-27

Sprung M (1994a) Macrobenthic secondary production in the intertidal zone of the Ria Formosa - A lagoon in Southern Portugal. Est Coast Shelf Sci 38: 539-558

Sprung M (1994b) High larval abundances in the Ria Formosa (Southern Portugal) - methodological or local effect? Journal of Plankton Research 16:151-160

Stoner AW (2003) What constitutes essential nursery habitat for a marine species? A case study of habitat form and function for queen conch. Marine Ecology Progress Series 257:275-289

Teixeira RL, Musick JA (1995) Trophic ecology of two congeneric pipefishes (Syngnathidae) of the lower York River, Virginia. Environmental Biology of Fishes 43: 295-309

Tipton K, Bell S (1988) Foraging patterns of two syngnathid fishes: importance of harpacticoid copepods. Marine Ecology Progress Series 47: 31-43

Turner S, Thrush S, Hewitt J, Cummings V and Funnell G (1999) Fishing impacts and the degradation or loss of habitat structure. Fish Manage Ecol 6: 401-420

Vanderklift MA, Jacoby CA (2003) Patterns in fish assemblages 25 years after major seagrass loss. Marine Ecology Progress Series 247: 225-235

Vincent ACJ (1996) The International Trade in Seahorses. TRAFFIC International, Cambridge, UK

Watling L, Norse EA (1998) Disturbance of the seabed by mobile fishing gear: A comparison to forest clearcutting. Conservation Biology 12:1180-1997

Whitehead PJP (1986) Volume II. In: Whitehead, PJP, Bauchot M-L, Hureau J-C, Nielsen J, Tortonese E (eds) Fishes of the north-eastern Atlantic and the Mediterranean. UNESCO, Paris, France

Wolff WJ (2000) Causes of extirpations in the Wadden Sea, an estuarine area in The Netherlands. Conservation Biology 14: 876-885

**Table 3.1** Regression equations for predicting standard length from trunk length for male (m) and female (f) *Hippocampus guttulatus* and *H. hippocampus*. Regressions are from Chapter 1, or unpublished).

| **Species** | **Sex** | **Equation** | **n** | **$r^2$** | **p-value** |
|---|---|---|---|---|---|
| *H. guttulatus* | m | $\log TrL = -0.325 + 0.872 \cdot \log SL$ | 703 | 0.86 | $p < 0.0001$ |
| | f | $\log TrL = -0.474 + 0.950 \cdot \log SL$ | 637 | 0.85 | $p < 0.0001$ |
| *H. hippocampus* | m | $\log TrL = -0.232 + 0.811 \cdot \log SL$ | 56 | 0.90 | $p < 0.0001$ |
| | f | $\log TrL = -0.593 + 1.008 \cdot \log SL$ | 47 | 0.92 | $p < 0.0001$ |

**Table 3.2** Indices of *Hippocampus guttulatus* and *H. hippocampus* abundance. Occupancy (%) refers to the percentage of sites where a species was detected and *n* denotes number of individuals encountered during UVCs (CI = bootstrap-estimated confidence limit).

| **Abundance Index** | ***Hippocampus guttulatus*** | | | ***Hippocampus hippocampus*** | | |
|---|---|---|---|---|---|---|
| | **2001** | **2002** | **Pooled years** | **2001** | **2002** | **Pooled years** |
| *n* – All Individuals | 168 | 216 | 384 | 39 | 2 | 41 |
| *n* – Juveniles | 26 | 22 | 48 | 1 | 0 | 1 |
| % Occupancy | 87.5 | 93.8 | 90.6 | 62.5 | 12.5 | 37.5 |
| Max density ($m^{-2}$) | 0.27 | 0.51 | 0.51 | 0.072 | 0.008 | 0.072 |
| Mean density ($m^{-2}$) | 0.060 | 0.085 | 0.073 | 0.013 | 0.001 | 0.007 |
| *(Lower 95% CI)* | | | *(0.039)* | | | *(0.003)* |
| *(Upper 95% CI)* | | | *(0.110)* | | | *(0.014)* |
| Population Size | | | 1 895 700 | | | 186 900 |
| *(Lower 95% CI)* | | | *(1 041 300)* | | | *(80 100)* |
| *(Upper 95% CI)* | | | *(3 043 800)* | | | *(373 800)* |

**Table 3.3** Primary producers and benthic invertebrates encountered during underwater visual censuses in the Ria Formosa lagoon. Relative abundances (%) are a percentage of the total surveyed area (32 sites pooled) that was covered by benthic flora and fauna. Only taxonomic groups covering >1% of the pooled transects are reported.

| **Habitat Component** | **Dominant Species** | **%** |
|---|---|---|
| Seagrasses | *Cymodocea nodosa* | 54.2 |
| | *Zostera noltii* | 4.4 |
| Macroalgae | *Chaetomorpha* sp. | 1.43 |
| | *Chondrus crispus* | 1.09 |
| | *Codium* spp. | 2.24 |
| | *Dictyopteris* sp. | 1.29 |
| | *Dictyota dichotoma* | 1.19 |
| | *Ulva lactuca* | 9.03 |
| | *Ulva rigida* | 2.43 |
| Bryozoans | *Zoobotryon verticillatum* | 8.4 |
| | *Bugula neritina* | 1.61 |
| Tunicates (pooled) | *Ciona intestinalis*<br>*Clavelina lepadiformis*<br>*Phallusia mammillata*<br>*Styela plicata* | 3.77 |
| Tube-dwelling Polychaetes | *Sabella* spp. | 2.9 |
| Sea urchins | *Paracentrotus lividus* | 1.12 |
| **Total** | | **95.1** |

**Table 3.4** Non-parametric Spearman rank correlations ($r_s$) between habitat variables and seahorse density (data pooled across all sites, n = 32). Included are habitat variables that were most highly correlated ($r_s > 0.3$, $p < 0.1$) with at least one of the species (other variables are not shown).

| **Habitat Variable** | ***Hippocampus guttulatus*** | | ***Hippocampus hippocampus*** | |
|---|---|---|---|---|
| | **$r_s$** | **p** | **$r_s$** | **p** |
| % *Cymodocea nodosa* | 0.406 | 0.021 | 0.196 | 0.282 |
| *C. nodosa* Shoot Density | 0.408 | 0.100 | -0.197 | 0.305 |
| % *Ulva lactuca* | 0.557 | *0.001 | -0.059 | 0.750 |
| % Anemone | -0.334 | 0.064 | -0.193 | 0.289 |
| $M_t/A_t$ | 0.438 | 0.012 | 0.233 | 0.199 |
| $C_t/A_t$ | 0.512 | 0.003 | -0.263 | 0.145 |
| Species Richness | 0.220 | 0.226 | -0.386 | 0.029 |
| % Sand | -0.249 | 0.169 | 0.333 | 0.063 |
| % Shell fragments | -0.433 | 0.013 | 0.142 | 0.437 |
| Horizontal visibility | -0.162 | 0.376 | 0.373 | 0.035 |
| Water temperature | -0.264 | 0.144 | -0.372 | 0.036 |
| Depth | 0.195 | 0.284 | 0.423 | 0.002 |
| Current strength | 0.164 | 0.379 | 0.542 | 0.002 |
| Latitude | -0.110 | 0.556 | -0.304 | 0.096 |
| Lunar Phase | -0.011 | 0.952 | 0.321 | 0.074 |

* Significant after applying a sequential Bonferroni correction to adjust p-values for the multiple tests in this table.

**Table 3.5** Significant explanatory variables retained in generalized linear models for *Hippocampus guttulatus* and *H. hippocampus* density. Regression coefficients (Coeff), corresponding standard errors (se) and p-values (p) are given (n = 16 for all models).

| **Species (year)** | **Variable(s)** | **Coeff** | **se** | **p** |
|---|---|---|---|---|
| *H. guttulatus* (2001) | | | | |
| | % covered substrate | 3.10 | 1.00 | 0.002 |
| | % macroalgae | 3.37 | 1.66 | 0.042 |
| *H. guttulatus* (2002) | | | | |
| | % covered substrate | 2.17 | 0.96 | 0.023 |
| | % *Ulva lactuca* | 7.69 | 2.94 | 0.009 |
| *H. hippocampus* (2001) | | | | |
| | % sand | 25.22 | 0.00 | <0.0001 |

**Figure 3.1** Western and central part of the Ria Formosa lagoon in southern Portugal (inset). Solid (2001) and open (2002) circles correspond to the location of 32 underwater visual census (UVC) sites (site numbers given).

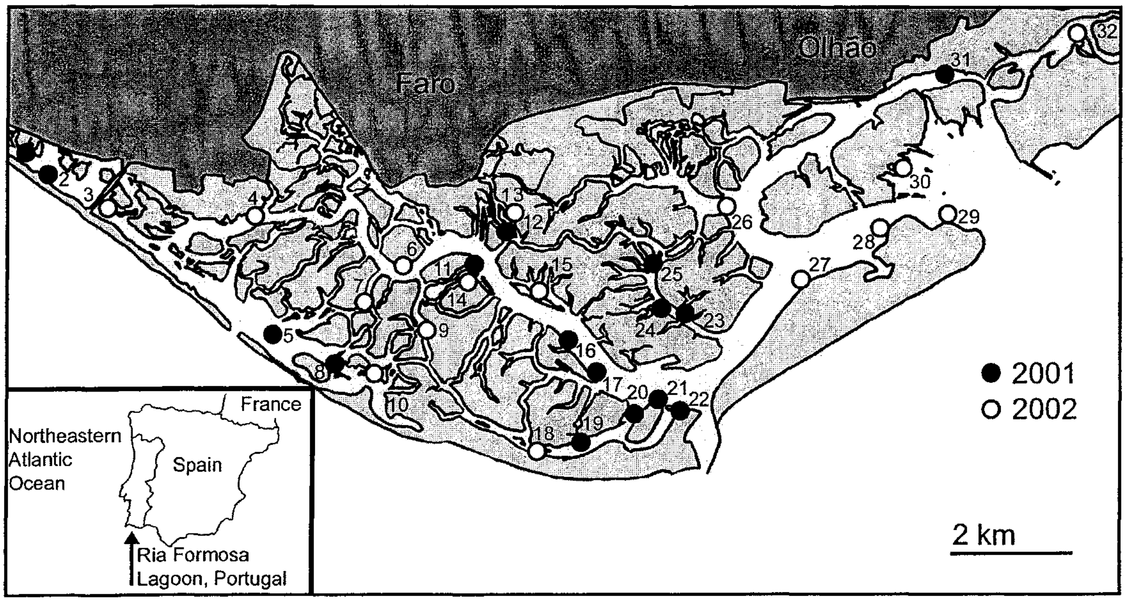


**Figure 3.2** (a) Relative frequencies of *Hippocampus guttulatus* (white) and *H. hippocampus* (black) at each site. The total number of seahorses encountered per site is given. (b) The dominant habitat type at 32 UVC sites: sand flats (open circles), seagrass beds (solid circles), mixed seagrass and macroalgae (horizontal hatching) and mixed macroalgae and sessile invertebrates interspersed with bare sand (vertical hatching).

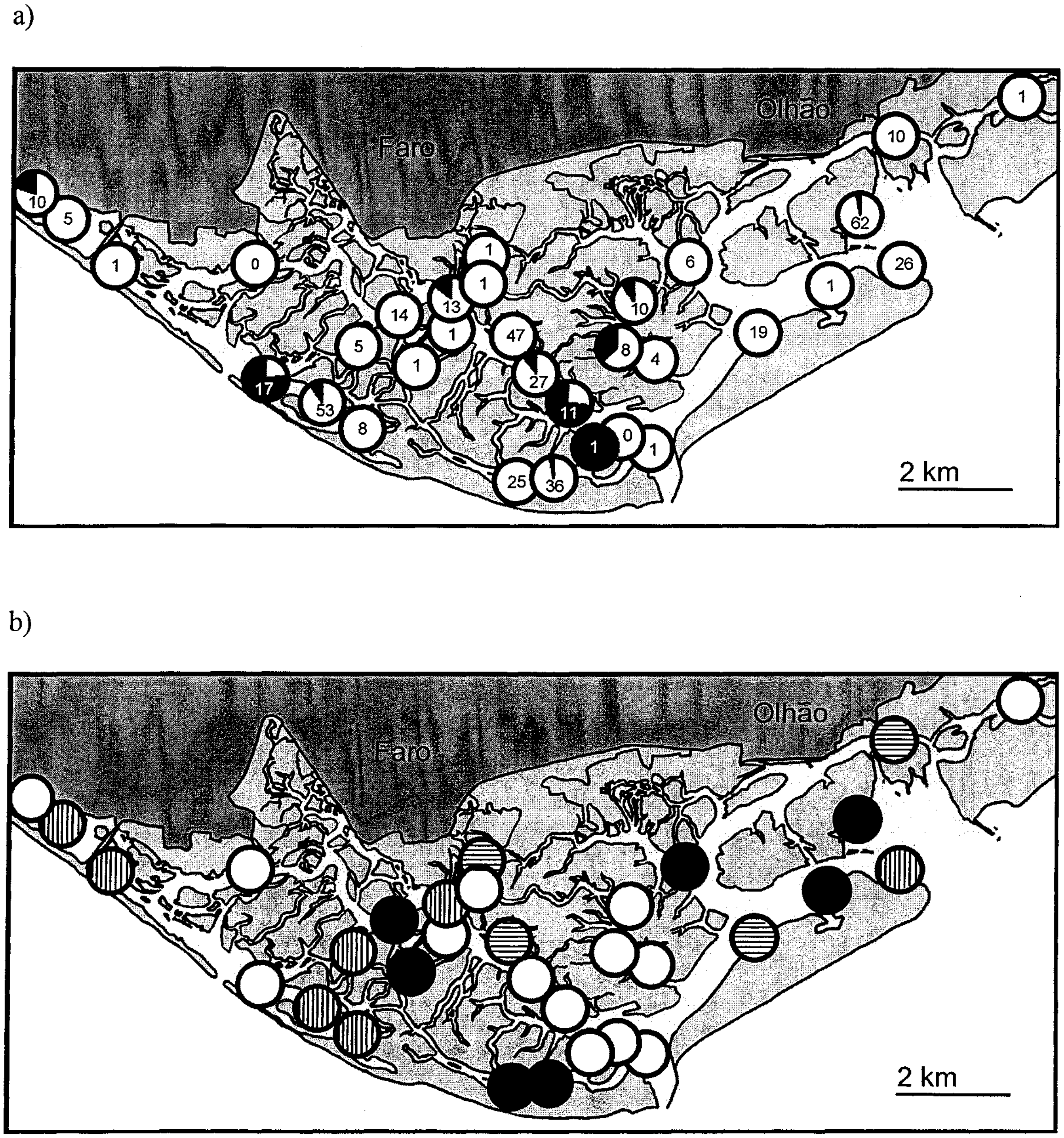


**Figure 3.3** (a) Density of *Hippocampus guttulatus* (solid circles) and *H. hippocampus* (open circles) plotted against $C_t/A_t$ (see text for details). (b) *Hippocampus hippocampus* density plotted against % sand.

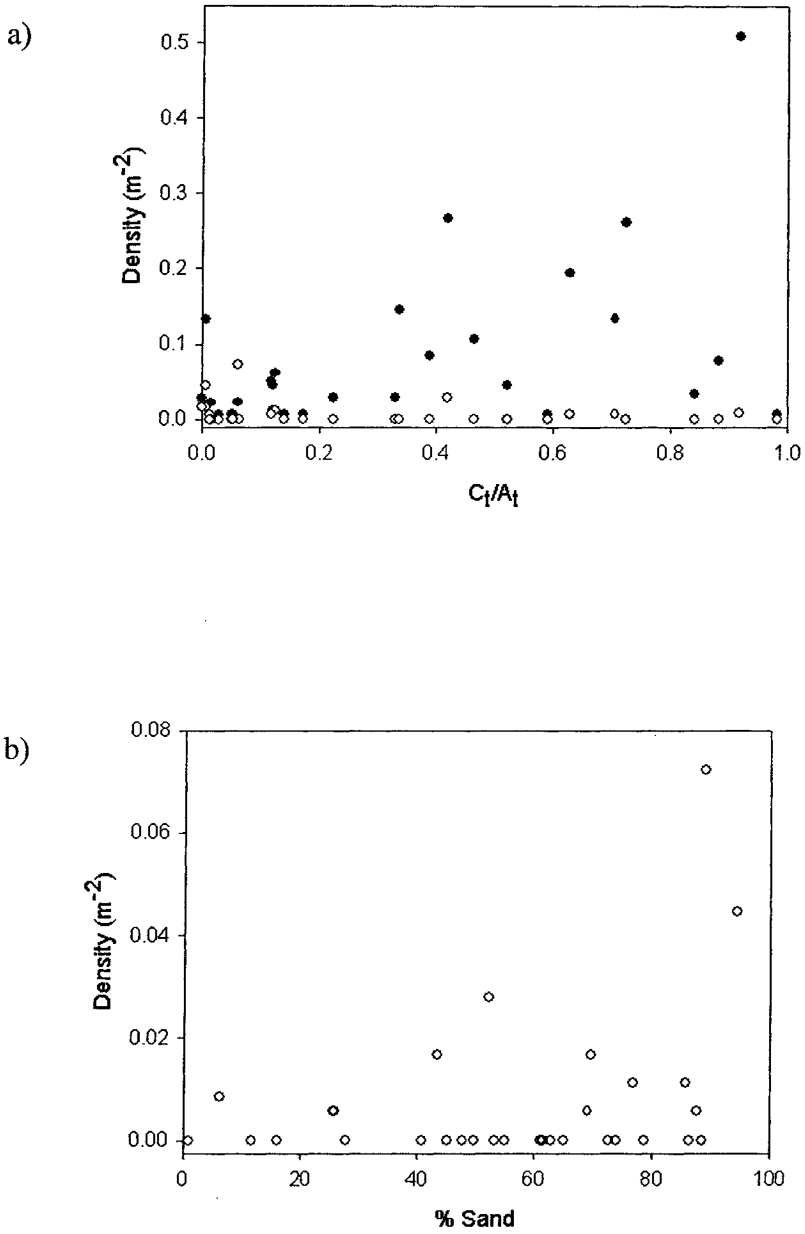
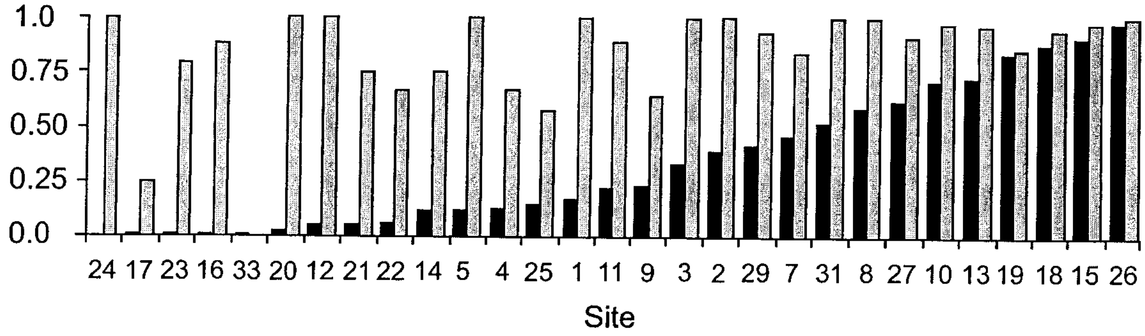


**Figure 3.4** Proportion of available habitat covered by benthic flora and fauna ($C_t/A_t$, black bars) and proportion of individuals using covered habitat ($U_t$, grey bars) for *Hippocampus guttulatus* (a) and *H. hippocampus* (b). Only occupied sites are shown.

a)



b)

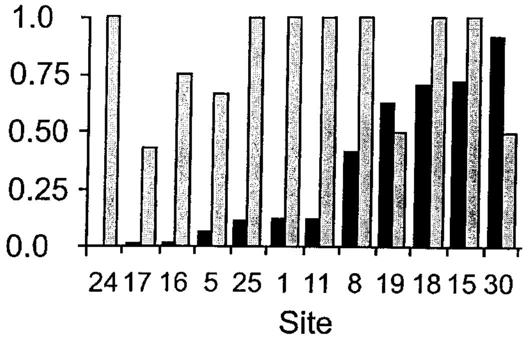
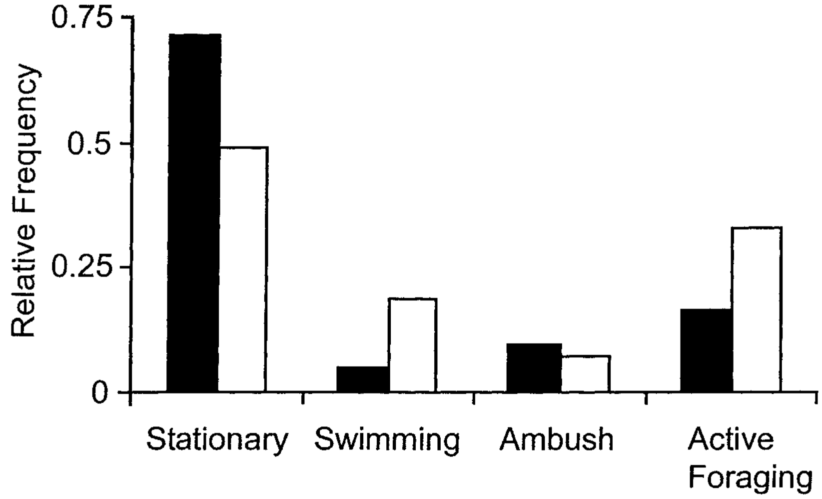


**Figure 3.5** Relative frequency of *Hippocampus guttulatus* (black bars, n = 382) and *H. hippocampus* (white bars, n = 41) individuals observed stationary, swimming, using ambush foraging techniques or actively foraging.



# LINKING STATEMENT 4

In Chapter 3, I reported inter-specific differences in the habitat preferences of two sympatric seahorse species differ in their habitat use along a gradient of habitat complexity and ocean influence. In Chapter 4, I examine the implications of these differences in habitat preference for population-level responses to non-selective bottom fishing, which tends to reduce structural complexity (Turner et al. 1999; Jennings et al. 2001; Cryer et al. 2002; Kaiser 2003).

The use of bottom-dragged fishing gears (e.g. trawls, seines) can affect non-target populations of benthic marine fishes directly through fishing mortality and indirectly by altering the structure of the community that the fishes use as habitat. Although habitat quality is widely accepted as an essential aspect of fish population viability (Kurland 1998; Koslow et al. 2000), few studies of the effects of bottom fishing have related the population-level responses of marine fishes to non-selective exploitation and the associated changes in the habitat structure (but see Rodwell et al. 2003). In Chapter 4, I test whether changes in habitat complexity associated with changes in fishing effort influence the population-level responses of *H. guttulatus* and *H. hippocampus* to reduced fishing effort. I also present the first fisheries-independent evaluation of the impacts of non-selective fishing on seahorse population structure.

# CHAPTER 4

# COMMUNITY IMPACTS AND POPULATION RESPONSES OF SEAHORSES TO EXPERIMENTAL REDUCTIONS IN LOCAL SEINING EFFORT

## ABSTRACT

In this study, we investigated the role of habitat preference in influencing population-level responses of small, benthic marine fishes to changes in community structure induced by non-selective, bottom-dragged fishing. We also quantified the direct effects of bottom fishing on the population structure and relative abundance of two sympatric species of conservation concern. We used a two year experimental seining program coupled with underwater visual surveys to track local changes in the benthic community and populations of two sympatric seahorse species (*Hippocampus guttulatus* and *H. hippocampus*) in response to changes in local fishing effort. Nine sites were experimentally fished each month for one year, but not fished in the second year, while fishing effort was held constant during both years at five control sites. We predicted that the local benthic communities would be more structurally complex after fishing effort was reduced and that the density of both seahorse species would increase in response to reduced fishing mortality. Although both species are nominally associated with seagrasses and macroalgae, *H. guttulatus* prefers complex vegetated habitats and *H. hippocampus* prefers more open, sparsely vegetated habitats. Therefore we also predicted that the magnitude of the increase in density would be greater for *H. guttulatus* than for *H. hippocampus*. As predicted, the structural complexity of the local benthic communities increased significantly in response to a reduction in fishing effort, primarily through increases in the abundance of macroalgae and a colonial bryozoan. Despite similarities in life history characteristics, the two seahorse species responded differently in terms of magnitude and direction to reduced fishing effort: the abundance of *H. guttulatus* increased significantly while its congener decreased in abundance. We conclude that repeated seining, which maintained less complex habitats, favoured greater abundances of *H. hippocampus*. By contrast, a reduction in seining effort resulted in greater habitat cover and favoured higher densities of *H. guttulatus*. Our results suggest that non-selective bottom fishing influences the magnitude and direction of the responses of benthic marine fishes to exploitation through its impacts on preferred habitats. Given differences in habitat preference, simultaneous attempts to protect incidentally exploited populations of both sympatric species would involve different management strategies that sustain both complex and sparsely vegetated habitats.

# INTRODUCTION

A rich body of literature documents the physical and biological effects of bottom-dragged fishing gears on marine benthic communities. Depending on the frequency and intensity of exploitation, bottom fishing can reduce the structural complexity of benthic communities by crushing or removing emergent epifauna and smoothing sedimentary formations (Tuck et al. 1998, Watling & Norse 1998, Turner et al. 1999, Collie et al. 2000ab). Bottom fishing gears also remove or redistribute macrophytes (Meyer et al. 1999, Baum et al. 2003), which generally support a greater abundance and diversity of marine fishes than unvegetated habitats (Heck Jr et al. 1989, Edgar & Shaw 1995, Jenkins et al. 1997). Thus intense and frequent bottom fishing can alter species abundance and composition (Engel & Kvitek 1998, Kaiser et al. 1998, Tuck et al. 1998, Jennings et al. 2001, Cryer et al. 2002, Kaiser 2003, but see Drabsch et al. 2001). When bottom fishing ceases, the physical damage to soft sediment forms is rapidly reversed, while the recovery of species abundance and diversity may occur over longer time scales (e.g. months to years, Kaiser et al. 1998, Tuck et al. 1998).

Fisheries that employ bottom fishing gears capture a rich diversity of fauna and flora (Alverson et al. 1994, Stobutzki et al. 2001b, Baum et al. 2003) that vary widely in life history strategies and habitat requirements (Stobutzki et al. 2001a). Thus, even if a fishery were optimally managed for target species, it might cause population declines in non-target species that are less resistant or resilient to exploitation, or population increases in species that exploit habitat disturbed by trawling (Casey & Myers 1998, Engel & Kvitek 1998, Kaiser et al. 1998, Kaiser 2003). Bycatch in tropical shrimp trawls are often dominated by small (<20 cm in length) benthic marine fishes (Alverson et al. 1994). Because the detailed life histories and habitat preferences of small, benthic marine fishes are usually unknown (Froese & Pauly 2004), it is difficult to evaluate or predict their responses to strategies for managing bottom fisheries (Walters et al. 1999, Stobutzki et al. 2001a).

Large cumulative catches of seahorses (genus *Hippocampus*, Family Syngnathidae) in trawl fisheries (Vincent 1996, Baum et al. 2003) and high volumes in international trade (Vincent 1996) have prompted concern for the group of fishes (IUCN 2003, Foster & Vincent 2004). However, limited biological information is available to assess the impacts of bottom fishing on local seahorse population structure. There are no available estimates of removal rates (catchability) for seahorses and few estimates of population abundance (but see Bell et al. 2003, Chapter 3). In a fishery-dependent study of *H. erectus* bycatch, Baum et al. (2003) inferred that trawling (a) differentially affected cohorts, (b) disrupted social structure by selectively capturing females, (c) reduced seahorse reproduction by disrupting monogamous pair-bonds, (d) damaged habitat by removing seagrasses and (e) injured tails and incidentally killed some seahorses. However, these inferences have not been validated with fishery-independent data.

The two aims of this study were (1) to directly evaluate the population-level impacts of bottom fishing on seahorse populations using fisheries-independent data and (2) to explore whether population responses to non-selective exploitation were mediated by the indirect effects of bottom fishing on habitat structure. We investigated these questions using two sympatric seahorse species (the long-snouted seahorse, *Hippocampus guttulatus* Cuvier 1829, and the short-snouted seahorse, *H. hippocampus* L) that differ in their habitat use along a gradient of structural complexity: *H. guttulatus* prefers structurally complex habitats with high vegetation and invertebrate cover, while *H. hippocampus* preferentially exploits the sparsely vegetated habitats that surround seagrass and macroalgal beds (Garrick-Maidment & Jones 2004, Chapter 3). Our specific objectives were to (a) estimate removal rates (catchability), (b) test whether bottom fishing gears selectively capture seahorses according to size, sex, or reproductive status, (c) quantify changes in the benthic community, (d) quantify population-level responses to reduced fishing effort, and (e) relate inter-specific differences in population response to changes in the structural complexity of benthic communities under reduced fishing pressure.

We investigated the following predictions using an experimental seining program coupled with underwater visual census surveys in a subtropical, seagrass-dominated community.

(a) Reductions in non-selective fishing pressure would result in increased habitat complexity, due in part to an increase in the abundance of benthic organisms as well as the number of species. In particular, we predicted that species with poor attachment capabilities (e.g. drifting macroalgae, sea urchins) would respond most strongly to reduced fishing effort.

(b) The density of both seahorse species would increase in response to reductions in fishing mortality, but the magnitude of numerical increase would be greater for *H. guttulatus* because of its stronger preference for complex, vegetated habitats.

## METHODS

### Species description

Although the life history and ecology of *H. guttulatus* have been investigated recently (Chapter 1, Chapter 2, Chapter 3), biological information about *H. hippocampus* is sparse (Boisseau 1967, reviewed in Foster & Vincent 2004, using taxonomy of Lourie et al. 1999). Both species are associated with seagrass-dominated communities and macroalgae in the northeast Atlantic Ocean and Mediterranean Sea (Boisseau 1967, Lourie et al. 1999, Garrick-Maidment & Jones 2004, Pérez-Ruzafa et al. 2004, Chapter 3). These species differ in both their landscape level habitats, and in their holdfast (attachment site) preferences: *H. guttulatus* grasped all prospective holdfast types with equal probability while *H. hippocampus* significantly avoided fauna and flora that formed large colonies or tracts of dense vegetation (Chapter 3). On average, *H. guttulatus* was ~40% larger (in length and mass) than *H. hippocampus* (Chapter 3), with sizes of benthic juvenile and adult *H. guttulatus* ranging from 65 – 208 mm (Chapter 1). The size of benthic juvenile and adult *H. hippocampus* ranged from 45 – 160 mm (J. Curtis, unpublished data). Adults of both species maintained small home ranges (on average <20 $m^2$) (Chapter 1, J. Curtis, unpublished data). At the height of the reproductive season (June – August), approximately 70% of adult male *H. guttulatus* had full pouches, while approximately 20% of adult females had hydrated eggs (Chapter 2). Sex ratio was approximately 55% female, though not significantly different from 50% (Chapter 1). The newly released juveniles of these multiple spawning fishes (Boisseau 1967, Chapter 2) were planktonic (Boisseau 1967, Pérez-Ruzafa et al. 2004) for at least eight weeks (Boisseau 1967). Juvenile *H. guttulatus* recruited to adult habitat at approximately 3 months of age (~96 mm), but recruited *H. hippocampus* were as small as 45 mm (age unknown, Chapter 1). Both species were locally abundant in our study site (Erzini et al. 2002) with mean densities of 0.07 $m^{-2}$ and 0.007 $m^{-2}$, respectively (Chapter 3).

### Study site

This study was carried out in the Ria Formosa lagoon (36°59' N, 7°51' W), which is part of a natural park in southern Portugal. The Ria Formosa is a highly productive tidal lagoon characterized by seagrass communities interspersed with sparsely vegetated sand flats in a network of channels and tidal creeks (Alberto et al. 2001, Chapter 3). The subtidal vegetation was dominated by seagrasses (primarily *Cymodocea nodosa*, but also *Zostera marina* and *Z. noltii*), but macroalgae (primarily *Ulva lactuca* and *Codium* spp.) were also abundant (Sprung 1994, Alberto et al. 2001, Chapter 3).

### Experimental fishing

Experimental fishing was carried out using a small beach seine (25 m long, 3.5 m maximum height, 9 mm mesh) for two years (October 2000 – 2002) as part of a comprehensive and intensive study of the recruitment of commercially important fishes (primarily sparids) in the lagoon (Erzini et al. 2002). We opportunistically used a subset of sites sampled in Erzini et al. (2002) to address our questions because the experimental fishing effort was known, constant and controlled. Although the recreational and commercial use of seines and trawls are illegal in the Ria Formosa, we are mindful that these illicit gears are occasionally employed in the lagoon (Monteiro 1989, Erzini et al. 2002), and assume that any non-selective fishing effort was constant between years.

In the present study twelve treatment sites were experimentally fished each month (thus seined 12 times $yr^{-1}$) during the first year (October 2000 – October 2001), but not fished during the second (October 2001 – October 2002, Table 4.1). Three additional sites were experimentally fished each month during both years (fished control sites).

During experimental fishing, the outstretched seine was dragged perpendicular to shore for 10 m while one end of the seine was held on shore and the other end was held on a motorized boat. Then the boat end of the seine was driven to shore so that the seine could be hauled out of the water (Erzini et al. 2002). All seahorses captured in the net during the first year were collected and frozen, but a proportion of those caught in the second year was haphazardly returned to the water. The fates of released seahorses (i.e. survival, return to home range) were not known. Thus the fishing mortality associated with experimental seines may have been slightly reduced for some sampling events during the second year. In order to maintain constant fishing effort, the same seine was employed throughout the study and all sites were fished within 2 h of low tide. All sites were at least 100 m apart and located on a gradient of habitat complexity and ocean influence (Erzini et al. 2002).

Our catch data included the species, sex, reproductive state, standard length, and injuries for captured seahorses. *Hippocampus guttulatus* and *H. hippocampus* were considered adults if they were longer than 135 mm and 99 mm, respectively; these lengths corresponded to the maximum observed lengths of juvenile males in the catches (inferred from the presence of an immature brood pouch as in Boisseau 1967, Chapter 1). The sex of adults was determined using the presence (male) or absence (female) of a mature brood pouch. Reproductive state was determined by visual and/or manual inspection of the male's brood pouch (full of eggs or empty) or the female's trunk girth (swollen inferred to mean hydrated eggs, concave inferred to mean immature or spent ovaries) as in Vincent & Sadler (1995). Standard length was measured as a straight line to the nearest mm with a plastic ruler from the cleithral ring to the tip of the straightened tail, and with calipers from the tip of the snout to the cleithral ring.

### Underwater visual census

The seahorse populations and benthic community within sites were surveyed using standard underwater visual census (UVC) techniques (English et al. 1994, Samoilys 1997). All treatment and fished control sites were surveyed using UVCs in both years except for three of the treatment sites, which were only surveyed in 2001 for logistic reasons (Table 4.1). In addition, UVCs were carried out at two unfished control sites that were not experimentally seined in either year. In 2001, UVCs were carried out 11-12 months after the experimental seining began. In 2002, UVCs were carried out 9-10 months after seining ended in the treatment sites (Table 4.1). A global positioning system (GPS) was used to locate the experimental fishing sites to within ± 5 m.

Our UVC data came from three belt transects that were placed parallel to shore at randomly selected distances, up to 20 m from shore, and at least 5 m apart. Because of low precision in our GPS unit (±5 m), as well as variation in the starting point for the seine among sampling dates, we carried out belt transects that were 30 m long and 2 m wide to ensure that UVCs covered the seined area. The total area searched per UVC was 180 $m^2$. The species, sex, reproductive state, trunk length, and injuries of seahorses on transects were recorded underwater. Straight-line trunk lengths were measured (from the cleithral ring to the last trunk ring with a plastic ruler) on live seahorses *in situ* and converted to standard lengths using equations developed for this species (Chapter 1; Chapter 3). During UVCs, transects were also used to calculate % cover of seagrasses, macroalgae and conspicuous (>2.5 cm in height, width or length) sessile epifauna, using the line intercept method (English et al. 1994, Samoily 1997). A dimensionless index of habitat complexity, $C_t/A_t$ (Bartholomew et al. 2000) defined here as the proportion of transect line that supported or was covered by all benthic flora and fauna, was also calculated.

Few (<1%) seahorses swam away from observers during searches (Chapter 3). Therefore UVCs were not time constrained and the benthic habitat was thoroughly searched. The same experienced observer (JC) searched all transects thereby further reducing observer bias.

### Statistical analyses

Whereas most of the treatment sites were located in sparsely or moderately vegetated habitats (<30% vegetation cover), two fished control sites and one unfished control site were located in sites with high (>50%) seagrass cover (Fig. 4.1). Thus, on average, control sites had greater vegetation cover than treatment sites (see results). Because *H. guttulatus* abundance was correlated with the % cover of vegetation and invertebrates (Chapter 3), control sites also had greater densities of *H. guttulatus* (max 1.3 $m^{-2}$) than treatment sites (max 0.15 $m^{-2}$). However, our use of repeated measures statistics (paired t-tests or Wilcoxon signed ranks tests) for evaluating our predictions allowed us to control for these initial differences in habitat structure among sites. We pooled the data from fished and unfished control sites because (a) in both sets of controls the fishing effort was held constant during the entire study and (b) to improve power for paired tests (Zar 1984). These two types of controls did not differ in total amount of vegetation and invertebrate cover (Mann-Whitney tests, all $U \geq 2.0$, $n = 5$, all $p \geq 0.8$) (Fig. 4.1), nor in the densities of *H. guttulatus* (Mann-Whitney tests, all $U \geq 1.0$, $n = 5$, all $p \geq 0.4$) or *H. hippocampus* (Mann-Whitney tests, all $U \geq 2.0$, $n = 5$, all $p \geq 0.8$) in either 2001 or 2002.

*Vulnerability to seining*

Intra-specific differences in vulnerability to the seine were evaluated with paired tests by comparing population structure (sex ratio, size, proportion of males with full pouches, proportion of females with hydrated eggs, and proportion of individuals with injuries) observed at a site during a UVC with the population structure of individuals captured subsequently in seine hauls. For each site in 2001, we pooled catch data from the first two months of seining following the UVC to increase the power of paired tests between UVC and catch data (Note that results were robust to the exclusion of data from the second month after UVCs, but we were unable to carry out all tests for *H. guttulatus* with only the first month of catch data). We only used UVC and catch data from 2001 to test for intra-specific differences in vulnerability because of haphazard release of some seahorses in 2002 seining. In all comparisons of size structure (including those below), only adults were used because the recruitment of juveniles into the Ria Formosa was strongly seasonal and began in August (Chapter 1).

The catchability, q, of *H. guttulatus* and *H. hippocampus* was estimated within each site as:

$$q = \frac{D_{catch}}{1.5 \cdot D_{uvc}}$$

and averaged among sites. $D_{catch}$ was the number of *H. guttulatus* or *H. hippocampus* captured $m^{-2}$ seined during the first month after the UVC, and $D_{uvc}$ was the density of individuals observed during the UVC. $D_{catch}$ was estimated by dividing the number of seahorses captured by the seine area, assumed to be 300 $m^2$ (Erzini et al. 2002). $D_{uvc}$ was multiplied by 1.5 because the probability of detecting tagged *H. guttulatus* during underwater searches was 0.66 (Chapter 1). We assumed the same probability for *H. hippocampus*. We tested for inter-specific differences in CPUE while controlling for local density (both variables log-transformed to normalize data).

### *Community responses to reduced fishing effort*

Differences in the % cover of seagrasses, macroalgae and sessile invertebrates, $C_t/A_t$ and species richness between years were compared with two sets of paired tests, one applied to the nine treatment sites and the other to the five control sites.

### *Population responses to reduced fishing effort*

The population-level responses of *H. guttulatus* and *H. hippocampus* to a reduction in fishing pressure were evaluated by comparing the change in population structure (density, size, sex ratio, proportion of males with full pouches, proportion of females with hydrated eggs and proportion of individuals with injuries) within sites from 2001 to 2002. Thus paired tests were applied to the set of nine treatment sites and the set of five control sites separately.

Some paired comparisons were not carried out for *H. hippocampus* owing to small sample sizes. Thus, in order to increase our power in all the comparisons described above, we also pooled data among treatment sites (and between years), among control sites (and between years) and among seine hauls, respectively, to test for overall differences in population structure using either Kolmogorov-Smirnov tests (for differences in length frequency distributions) or Chi$^2$ tests. All statistical analyses were carried out using SPSS 10.0.7 (SPSS Inc.). Means are reported with standard errors (SE) except where stated otherwise.

## RESULTS

*Hippocampus guttulatus* was more abundant than *H. hippocampus* in UVCs and catch data. In total, 662 *H. guttulatus* and 112 *H. hippocampus* were encountered during UVCs. The total number of *H. guttulatus* and *H. hippocampus* collected in seines from August – November 2001 was 118 and 26, respectively.

### Vulnerability to seining

There were no significant intra-specific differences in vulnerability to the seining for *H. guttulatus* (Table 4.2), but insufficient data were available to test for differences in *H. hippocampus* population structure using paired tests. When 2001 UVC data were pooled among sites and compared to the pooled 2001 catch data, the proportion of brooding male *H. guttulatus* and hydrated female *H. hippocampus* were significantly greater *in situ* than in the catch data (Table 4.3).

On average, the catchability, q, of *H. guttulatus* (0.10 ± 0.12) was greater than that of *H. hippocampus* (0.06 ± 0.04). q was not related to the amount of macrophyte and invertebrate cover ($C_t/A_t$) at sample sites for either species (*H. guttulatus* $r_s = -0.006$, $p = 0.986$; *H. hippocampus*, $r_s = 0.223$, $p = 0.463$). CPUE was linearly related to $D_{uvc}$ of *H. guttulatus* (linear regression, $r^2 = 0.289$, adjusted $r^2 = 0.234$, $F_{1,14} = 5.286$, $p = 0.039$) and to $D_{uvc}$ of *H. hippocampus* (linear regression, $r^2 = 0.34$, adjusted $r^2 = 0.28$, $F_{1,12} = 5.673$, $p = 0.036$). However, CPUE was significantly greater for *H. guttulatus* than for *H. hippocampus* when controlling for local density (ANCOVA, $F_{2,27} = 9.273$, $p = 0.001$) (Fig. 4.2).

### Community responses to reduced fishing effort

Seining had significant impacts on the structure of the benthic community, which supported populations of *H. guttulatus* and *H. hippocampus*. Although the bycatch of macrophytes and sessile invertebrates was not quantified during seining, a rich diversity of benthic organisms (or parts of organisms) was captured with the seine, including ~90 species of fishes (Erzini et al. 2002). As predicted, there was a significant increase in $C_t/A_t$ when fishing effort ceased within treatment sites (Wilcoxon signed ranks test, $n = 9$, $Z = 2.199$, $p = 0.028$), but no change in $C_t/A_t$ between years within control sites (Wilcoxon signed ranks test, $n = 5$, $Z = 0.542$, $p = 0.588$) (Fig. 4.3). On average, $C_t/A_t$ almost doubled within treatment sites after seining ceased. The increase in $C_t/A_t$ within treatment sites was attributable to a significant increase in the % cover of sessile invertebrates ($Z = 2.240$, $n = 9$, $p = 0.025$, Fig. 4.4), primarily the bryozoan *Zoobotryon verticillatum*. The % cover of sessile invertebrates did not increase significantly within control sites ($Z = 1.604$, $n = 5$, $p = 0.109$). On average, the % cover of macroalgae cover also increased within treatment sites (Fig. 4.4). However, changes in the % cover of macroalgae and seagrasses were not significant in either treatment or control sites (all $|Z| < 1.345$, all $p > 0.180$). Species richness ranged from 3 – 28 species during UVCs, but did not differ between years in either treatment sites ($Z = 0.604$, $n = 9$, $p = 0.546$) or control sites ($Z = -1.342$, $n = 5$, $p = 0.180$).

## Population responses to reduced fishing effort

The numerical response of *Hippocampus guttulatus* and *H. hippocampus* differed in both magnitude and direction when seining ceased. The density of *H. guttulatus* increased significantly by 270% within treatment sites in response to a reduction in fishing effort ($Z = -1.96$, $n = 9$, $p = 0.05$: Fig. 4.5a), but did not change within control sites ($Z = 0.674$, $n = 5$, $p = 0.5$). By contrast, the density of *H. hippocampus* decreased significantly (an average of 65%) in treatment sites ($Z = -1.97$, $n = 9$, $p = 0.049$) but did not in the control sites ($Z = 0.00$, $n = 5$, $p = 1.000$) where it only declined on average by 25%.

When data from treatment sites and control sites were pooled respectively within years, differences in the relative frequencies of *H. guttulatus* and *H. hippocampus* held up: the abundance of *H. guttulatus* relative to *H. hippocampus* significantly increased in response to a reduction in fishing effort ($\chi^2 = 28.43$, df = 1, $p < 0.0001$), but did not change in control sites ($\chi^2 = 0.03$, df = 1, p = 0.862). The magnitude of increase in *H. guttulatus* abundance (measured as density in 2002 divided by density in 2001) was significantly and positively correlated with the magnitude of change in $C_t/A_t$ ($r_s = 0.786$, n = 9, p = 0.012). The change in abundance of *H. hippocampus* was not correlated with the magnitude of change in any habitat component (all p > 0.209).

Using paired tests, the size structure, sex ratio, proportion of brooding males, proportion of hydrated females and proportion of injured *H. guttulatus* did not change in response to reduced fishing effort in treatment sites, nor were there differences between years in control sites (Table 4.4). Similarly, *H. hippocampus* population structure did not differ between years in treatment sites (insufficient data for control sites), when paired tests were employed. Increasing power by pooling all individuals within treatment sites, or within control sites, did not reveal significant differences in size structure, sex ratio or the proportion of injured individuals between years, for either species (Table 4.5). However, when all individuals within treatment sites were pooled between years, the proportions of reproductively active male and female *H. guttulatus* increased significantly after fishing ceased. Because the proportions of reductively active males and females also increased between years in the control sites (Table 4.5), these increases probably reflected seasonal variation in reproductive activity (Chapter 2) rather than a real effect of reduced fishing effort on reproduction.

## DISCUSSION

Despite similarities in life history characteristics (Boisseau 1967, Foster & Vincent 2004), two nominally seagrass-associated fishes differed markedly in their numerical responses to reductions in experimental seining effort. Our results suggest that bottom fishing plays a role in determining the magnitude and direction of population-level responses to exploitation through its impacts on habitat. We also present the first fisheries-independent evaluation of the local impacts of bycatch on seahorse population structure.

### Vulnerability to seining

Our underwater visual census surveys suggested that catch data from our seine provided unbiased indices of seahorse population structure and a reliable index of the relative density of settled (benthic) juveniles and adults in these seagrass-dominated communities (though not of planktonic juveniles). Fisheries-dependent data usually do not reflect the true structure of exploited populations because of intra-specific differences in vulnerability to fishing gears and/or the spatial distribution of fishing effort (Hilborn & Walters 1992, King 1995). Nets usually selectively exclude smaller fishes from catches (King 1995). Because the smallest settled *H. guttulatus* and *H. hippocampus* juveniles in the lagoon were at least 65 mm and 45 mm, respectively (Chapter 1), the mesh size of our seine (9 mm) was probably sufficiently small to capture all settled seahorses, regardless of size.

We found no evidence of an effect of sex on vulnerability to seining and an inconclusive effect of reproductive status. Reproductively active male *H. guttulatus* and female *H. hippocampus* were less likely to be caught than observed *in situ*. However, the catch data were collected towards the end of the reproductive season (August – November 2001, Chapter 2, see Table 4.1); these differences probably reflect seasonal variation in reproductive activity, rather than lower vulnerability of reproductively active individuals to seining.

Although the catchability of seahorses was low with the seine (6-10% was captured per seine haul) with the seine, *H. guttulatus* was almost twice as likely to be captured as *H. hippocampus*. Because we found no evidence of size selectivity of the seine within species, it is unlikely that interspecific differences in catchability related to size. However, differences in catchability may be related to differences in microhabitat use or behaviour. *Hippocampus guttulatus* used all prospective holdfasts with equal probability, including the very abundant macroalga *Ulva lactuca* and bryozoan *Zoobotryon verticillatum*, which are easily torn or displaced. By contrast, *H. hippocampus* significantly avoided using *U. lactuca* and *Z. verticillatum* as holdfasts, preferring to use smaller structures that had lower profiles as microhabitats (Chapter 3).

Thus *H. guttulatus* may be more likely to be dragged away by the seine while grasping drifting macroalgae or bryozoan colonies (which have little or no resistance to seining) than *H. hippocampus.*

### Community response to reduced fishing effort

The two groups of invertebrates that increased the most when fishing ceased were species that had poor attachment capabilities (i.e. *Zoobotryon verticillatum*, sea urchins), making them more vulnerable to being dragged away by the seine. On average, most species of macroalgae increased in abundance when seining ceased, although effects were not statistically significant. The impacts of local seining on the total abundance of macroalgae were probably transient because the dominant species of macroalgae, *Ulva lactuca* (and to a lesser extent *Codium* spp.), drifts along the bottom and disperses throughout the Ria Formosa with tidal currents.

Despite a high frequency of seining, we found no detectable changes in seagrass cover in either treatment or control sites. The resistance and resilience of seagrasses to disturbance depend on the frequency and intensity of the disturbance, as well as the biology of the species (Sheperd et al. 1989, Short & Wyllie-Escheverria 1996). The lack of seagrass response within treatment sites suggests one of two hypotheses: (1) seagrass rhizomes provided resistance to removal by seining such that the % cover of seagrass remained unchanged before, during and after the experimental seining was carried out (even if biomass was reduced) or (2), disturbed seagrasses were slow to recolonize disturbed patches.

The lack of consistent seining effects on species richness and abundance of most taxa may reflect the spatial extent of our experimental seining within sites. Detecting impacts of bottom disturbance on species diversity and richness depends on the scale of sampling (Kaiser 2003), as well as the frequency, scale and intensity of the disturbance. Because our seining impact was highly localized, the surrounding (unfished) benthic community probably influenced the structure of the benthic community within our sample sites through local recruitment and/or vegetative growth (i.e. local seining sites had large edge effects).

### Population response to reduced fishing effort

Although experimental seining was only carried out for one year in treatment sites, we anticipated detecting responses to the reduction in seining effort because the frequency of our experimental seining (12 $yr^{-1}$) was considerably greater than that reported in heavily trawled areas where significant biological impacts have been observed (e.g. 0.2 – 6.5 $yr^{-1}$, Jennings et al. 2001).

Our results suggest that sympatric species may respond quite differently to changes in habitat structure driven by bottom fishing. The loss or degradation of habitat is assumed to threaten the persistence of many temperate seahorses including *H. reidi* and *H. zosterae* populations in North America (Musick et al. 2000), *H. breviceps* in Australia (Pogonoski et al. 2002), *H. hippocampus* in the Azores (Santos et al. 1995) and the most endangered species, *Hippocampus capensis*, in South Africa (IUCN 2003). We found, however, that *H. guttulatus* and *H. hippocampus*, which both relied on covered microhabitats for crypsis and stability, differed in the way they used two broadly defined habitat types over landscape scales: complex densely vegetated habitat and more open, sparsely vegetated sand flats (Chapter 3). Thus, a reduction in seining effort resulted in greater % cover and favoured higher densities of *H. guttulatus* and repeated seining, which maintained less complex habitats, favoured greater abundances of *H. hippocampus*. *Hippocampus hippocampus* may prefer habitats that are subject to more frequent natural disturbances; the abundance of this species was correlated with oceanic influences in the Ria Formosa (Chapter 3), and was more common in coastal habitats near Barcelona and Malagá, Spain than *H. guttulatus* (J. Curtis, unpublished data).

It is unclear whether numerical responses to reduced fishing effort arose from changes in local recruitment dynamics (i.e. habitat selection of juveniles recruiting to the lagoon), or of from the redistribution of individuals within and surrounding the treatment sites (Kramer & Chapman 1999, Rodwell et al. 2003). However, tagged *H. guttulatus* adults on a focal study site exhibited high site fidelity, and displaced home ranges between years by only a few metres despite seemingly suitable habitat that surrounded the focal study site (Chapter 1). Thus numerical responses may be primarily related to habitat selection by recruiting juveniles.

Despite significant changes in abundance, frequent seining appeared to be nonselective with respect to size structure and sex ratio, and did not appear to disrupt reproduction or be the primary cause of injuries for either *H. guttulatus* or *H. hippocampus*. However, the effects of repetitive, bottom fishing on seahorse population structure need to be examined over wider spatial and temporal scales that are more commensurate with disturbance regimes typically observed in bottom-fisheries. Seahorses may be more vulnerable to injuries, mortality and disruption of reproduction in large trawls that are deployed for longer and over greater areas (Baum et al. 2003).

## IMPLICATIONS FOR MANAGEMENT AND CONSERVATION

Given the extensive habitat damage and challenges of managing multiple species of fishes, the use of spatial closures (i.e. marine protected areas, MPAs) is a widely supported ecosystem-based strategy for promoting the recovery of benthic communities and their associated fish assemblages (Walters et al. 1999, Martin-Smith et al. 2004). Although our study was carried out over very small spatial scales, we cautiously suggest that sympatric European seahorses may respond quite differently in magnitude and direction to the implementation of spatial closures within exploited populations, depending on the scale of the closure and structure of the community that is protected. Differences in life history and ecology are likely to influence species-specific population-level responses of marine fishes to spatial closures to fishing (Mosquiera et al. 2000).

Given differences in habitat preference, incidentally exploited populations of these sympatric species may benefit from spatial closures that protect both complex vegetated habitats and sparsely vegetated sand flats along a gradient of natural disturbance and ocean influence. In a broader context, we also suggest that in addition to spatial closures to fishing effort (i.e. no-take marine protected areas), there may be a role for the implementation of restricted zones that vary along a gradient of fishing intensity or frequency. The use of MPAs as "exceptional little places where seed stocks and biodiversity can be protected" (discussed in Walters et al. 1999) within a sea that is heavily disturbed by trawling disturbance ignores the potential importance of light or moderate disturbance regimes (whether natural or otherwise) for maintaining diversity in marine ecosystems.

*Acknowledgements*. This is a contribution from Project Seahorse. We gratefully acknowledge excellent field and lab assistance from K. Bigney, H. Balasubramanian, B. Gunn, S. Lemieux, C.-M. Lesage, J. Nadeau, S. Overington, A. M. Santos, S. V. Santos, M. Veillette and K. Wieckowski and the many members of K.E.'s research team. We thank all members of the Parque Natural da Ria Formosa, particularly Fiesta, for providing valuable logistical support from 2000 - 2003. Guylian Chocolates, Belgium generously supported this research. JMC held a Natural Sciences and Engineering Research Council of Canada (NSERC) post-graduate scholarship, a Fonds de Recherches sur la Nature et les Technologies Québec (FCAR) doctoral award, and a McConnell McGill Major Fellowship. The John G Shedd Aquarium in Chicago kindly supported AV's operational costs through its partnership for marine conservation with Project Seahorse.

## LITERATURE CITED

Alberto F, Mata L, Santos R (2001) Genetic homogeneity in the seagrass *Cymodocea nodosa* at its northern Atlantic limit revealed through RAPD. Marine Ecology Progress Series, 221:299-301

Alverson DL, Freeberg MH, Pope JG, Murawski SA (1994) A global assessment of fisheries bycatch and discards. FAO Fisheries Technical Paper No. 339. FAO: Rome

Bartholomew A, Diaz RJ, Cicchetti G (2000) New dimensionless indices of structural habitat complexity: predicted and actual effects on a predator's foraging success. Marine Ecology Progress Series 206:45-58

Baum JK, Meeuwig JJ, Vincent ACJ (2003) Bycatch of lined seahorses (*Hippocampus erectus*) in a Gulf of Mexico shrimp trawl fishery. Fishery Bulletin 101:721-731

Bell E, Lockyear J, McPherson J, Marsden AD, Vincent ACJ (2003) First field studies of an Endangered South African seahorse, *Hippocampus capensis*. Environmental Biology of Fishes 67:35-46

Boisseau J (1967 Régulations hormonales de l'incubation chez un vertébré mâle: recherches sur la reproduction de l'Hippocampe. PhD thesis, l'Université de Bordeaux

Casey JM, Myers RA (1998) Near extinction of a large widely distributed fish. Science 281:690-692

Collie JS, Escanero GA, Valentine PC (2000a) Photographic evaluation of the impacts of bottom fishing on benthic epifauna. ICES journal of Marine Science 57:987-1001

Collie JS, Hall SJ, Kaiser MJ, Poiner IR (2000b) A quantitative analysis of fishing impacts on shelf-sea benthos. Journal of Animal Ecology 69:785-798

Cryer M, Hartill B, O'Shea S (2002) Modification of marine benthos by trawling: toward a generalization for the deep ocean? Ecological Applications 12:1824-1839

Drabsch SL, Tanner JE, Connell SD (2001) Limited infaunal response to experimental trawling in previously untrawled areas. ICES Journal of Marine Sciences 58:1261-1271

Edgar GJ, Shaw CU (1995) The production and trophic ecology of shallow-water fish assemblages in southern Australia I. Species richness, size-structure and production of fishes in Western Port, Victoria. Journal of Experimental Marine Biology and Ecology 194:53-81

Engel J, Kvitek R (1998) Effects of otter trawling on a benthic community in Monterey Bay National Marine Sanctuary. Conservation Biology 12:1204-1214

English S, Wilkinson C, Baker V (1994) Survey manual for tropical marine resources. ASEAN-Australia Science Project. Australian Institute of Marine Science, Townsville.

Erzini K, Bentes L, Coelho R, Correia C, Lino PG, Monteiro P, Ribeiro J, Goncalves J MS (2002) Recruitment of sea breams (Sparidae) and other commercially important species in the Algarve (southern Portugal), Final Report., pp. 178: Commission of the European Communities DG XIV/C/1.

Foster SJ, Vincent ACJ (2004) The life history and ecology of seahorses: implications for conservation and management. Journal of Fish Biology 65:1-61

Froese R, Pauly D (eds) (2004) FishBase. World Wide Web electronic publication. www.fishbase.org, Version 05/2004.

Garrick-Maidment N, Jones L (2004) British Seahorse Survey Report. The Seahorse Trust, Topsham, UK.

Heck Jr. KL, Able KW, Fahay MP, Roman CT (1989) Fishes and decapod crustaceans of Cape Cod eelgrass meadows - species composition, seasonal abundance patterns and comparison with unvegetated substrates. Estuaries 12:59-65

Hilborn R, Walters CJ (1992) Quantitative Fisheries Stock Assessment: Choice, Dynamics and Uncertainty. Kluwer Academic Publishers, Massachussetts, USA.

IUCN (2003) 2003 IUCN Red List of Threatened Species. <www.redlist.org>. Downloaded on 31 August 2004.

Jenkins GP, May HMA, Wheatley MJ, Holloway MG (1997) Comparison of fish assemblages associated with seagrass and adjacent unvegetated habitats of Port Phillip Bay and Corner Inlet, Victoria, Australia, with emphasis on commercial species. Estuarine, Coastal and Shelf Science, 44 569-588

Jennings S, Pinnegar JK, Polunin NVC, Warr KJ (2001) Impacts of trawling disturbance on the trophic structure of benthic invertebrate communities. Marine Ecology Progress Series 213:127-142

Kaiser MJ (2003) Detecting the effects of fishing on seabed community diversity: importance of scale and sample size. Conservation Biology 17:512-520

Kaiser MJ, Edwards DB, Armstrong PJ, Radford K, Lough NEL, Flatt RP, Jones HD (1998) Changes in megafaunal benthic communities in different habitats after trawling disturbance. ICES Journal of Marine Sciences 55:353-361

Kramer DL, Chapman MR (1999) Implications of fish home range size and relocation for marine reserve function. Environmental Biology of Fishes 55:65-79

Lourie SA, Vincent ACJ, Hall HJ (1999) Seahorses: An identification guide to the world's species and their conservation. London: Project Seahorse.

Martin-Smith KM, Samoilys MA, Meeuwig JJ, Vincent ACJ (2004) Collaborative development of management options for an artisanal fishery for seahorses in the central Philippines. Ocean and Coastal Management 47:165-193

Meyer DL (1999) Effects of live-bait shrimp trawling on seagrass beds and fish bycatch in Tampa Bay, Florida. Fishery Bulletin 97:193-199

Monteiro C (1989) La faune ichthyologique de la Ria Formosa; organisation spatio-temporelle. PhD thesis, Université Montpellier.

Mosqueira I, Côté IM, Jennings S, Reynolds JD (2000) Conservation benefits of marine reserves for fish populations. Animal Conservation 3:321-332

Musick JA, Harbin MM, Berkeley SA, Burgess AM, Eklund AM, Findley L, Gilmore RG, Golden JT, Ha DS, Huntsman GR, et al. (2000) Marine, estuarine and diadromous fish stocks at risk of extinction in North America (exclusive of salmonids). Fisheries 25(11):6-30

Pérez-Ruzafa A, Quispe-Becerra JI, García-Charton JA, Marcos C (2004) Composition, structure and distribution of the ichthyoplankton in a Mediterranean coastal lagoon. Journal of Fish Biology, 64:202-218

Pogonoski JJ, Pollard DA, Paxton JR (2002) Conservation overview and action plan for Australian threatened and potentially threatened marine and estuarine fishes. Environment Australia, Canberra, Australia. Available at: http://www.ea.gov.au/coasts/species/marine-fish/index.html

Rodwell LD, Barbier EB, Roberts CM, McClanahan TR (2003) The importance of habitat quality for marine reserve - fishery linkages. Canadian Journal of Fisheries and Aquatic Sciences 60:171-181

Samoilys MA (1997) Underwater visual census surveys. In: Samoilys MA (ed) Manual for assessing fish stocks on Pacific coral reefs, Queensland Department of Primary Industries, Townsville, pp. 16-29

Santos RS, Hawkins S, Monteiro LR, Alves M, Isidro EJ (1995) Marine research, resources and conservation in the Azores. Aquatic Conservation - Marine and Freshwater Ecosystems, 5:311-354

Sheperd SA, McComb AJ, Bulthuis DA, Neverauskus V, Steffensen DA, West R (1989) Decline of seagrasses. In: Larkum AWD, McComb AJ and Shepard SA (eds) Biology of Seagrasses Elsevier, Amsterdam. pp. 346-393

Short FT, Wyllie-Escheverria S (1996) Natural and human-induced disturbances of seagrasses. Environmental Conservation 23:17-27

Sprung M (1994) Macrobenthic secondary production in the intertidal zone of the Ria Formosa - A lagoon in Southern Portugal. Estuarine Coastal and Shelf Science, 38:539-558

Stobutzki I, Miller M, Brewer D (2001a) Sustainability of fishery bycatch: a process for assessing highly diverse and numerous bycatch. Environmental Conservation 28:167-181

Stobutzki IC, Miller M, Jones P, Salini JP (2001b) Bycatch diversity and variation in a tropical Australian penaeid fishery; the implications for monitoring. Fisheries Research 53(3):283-301

Tuck ID, Hall SJ, Robertson MR, Armstrong E, Basford DJ (1998) Effects of physical trawling disturbance in a previously unfished sheltered Scottish sea loch. Marine Ecology Progress Series 162:227-242

Turner S, Thrush S, Hewitt J, Cummings V, Funnell G (1999) Fishing impacts and the degradation or loss of habitat structure. Fisheries Management and Ecology 6:401-420

Vincent ACJ (1996). The International Trade in Seahorses. TRAFFIC International, Cambridge

Vincent ACJ, Sadler LM (1995) Faithful pairbonds in wild seahorses, *Hippocampus whitei*. Animal Behaviour 50:1557-1569

Walters C, Pauly D, Christensen V (1999) Ecospace: prediction of mesoscale spatial patterns in trophic relationships of exploited ecosystems, with emphasis on the impacts of marine protected areas. Ecosystems 2:539-554

Watling L, Norse EA (1998) Disturbance of the seabed by mobile fishing gear: A comparison to forest clearcutting. Conservation Biology 12:1180-1997

Zar JH (1984) Biostatistical analysis (2nd ed). Prentice Hall, Englewood Cliffs, New Jersey

**Table 4.1** Experimental treatments, UVC survey dates and months of catch data used to (A) test for intraspecific differences in vulnerability to the seines and (B) calculate catchability. (Reduced fishing effort = seined monthly during first year, not seined during second year, Fished control = seined monthly in both years, Unfished control = not seined in either year).

| **Experimental Treatment** | **2001 UVC** | **2002 UVC** | **A (2001)** | **B (2001)** |
|---|---|---|---|---|
| Reduced fishing effort[a] | 14 Aug | | Aug – Sept | Aug |
| Reduced fishing effort[a] | 13 Sept | | Sept – Oct | Sept |
| Reduced fishing effort[a] | 13 Sept | | Sept – Oct | Sept |
| Reduced fishing effort | 30 Aug | 30 July | Sept – Oct | Sept |
| Reduced fishing effort | 30 Aug | 29 July | Sept – Oct | Sept |
| Reduced fishing effort | 14 Aug | 24 July | Sept – Oct | Sept |
| Reduced fishing effort | 18 Sept | 28 Jun | Oct – Nov | Oct |
| Reduced fishing effort | 28 Aug | 23 Jul | Sept – Oct | Sept |
| Reduced fishing effort | 28 Aug | 31 Jul | Sept – Oct | Sept |
| Reduced fishing effort | 4 Sept | 29 Jul | Sept – Oct | Sept |
| Reduced fishing effort | 3 Sept | 26 Jul | Sept – Oct | Sept |
| Reduced fishing effort | 3 Sept | 31 Jul | Sept – Oct | Sept |
| Fished control | 13 Aug | 21 Jun | Aug – Sept | Aug |
| Fished control | 4 Sept | 14 Jun | Sept – Oct | Sept |
| Fished control | 19 Sept | 20 Jun | Sept – Oct | Sept |
| Unfished control | 20 Aug | 2 Jul | | |
| Unfished control | 20 Aug | 26 Jun | | |

[a] No UVC in 2002 for logistic reasons

**Table 4.2** Intra-specific differences in vulnerability to seines. Paired comparisons are of *H. guttulatus* population structure in 2001 UVC data and in catch data (paired t-tests for size structure where n is the degrees of freedom; Wilcoxon signed ranks tests for all other comparisons where n is number of sites included in the analyses).

| | **Statistic** | **n** | **P** |
|---|---|---|---|
| Mean size | 1.608 | 9 | 0.142 |
| Max size | 2.061 | 9 | [†]0.069 |
| Sex ratio | 0.514 | 6 | 0.600 |
| Brooding males | -1.521 | 7 | 0.128 |
| Hydrated females | -1.604 | 4 | 0.109 |
| Injuries | -1.095 | 10 | 0.273 |

[†]maximum size was usually greater in UVCs, but there were also larger sample sizes in UVCs

**Table 4.3** Vulnerability of *H. guttulatus* and *H. hippocampus* to seining. Size structure, sex ratio, reproduction and injuries between 2001 UVC data and catch data (all individuals pooled within UVC and catch data, respectively). Chi$^2$ tests were used, except for a Kolmogov-smirnov test for differences in length frequency distributions. Only tests indicated by * were significant after a sequential Bonferroni correction was used to adjust p-values for multiple tests.

| | ***Hippocampus guttulatus*** | | | ***Hippocampus hippocampus*** | | |
|---|---|---|---|---|---|---|
| | **Statistic** | **n** | **p** | **Statistic** | **n** | **p** |
| Size distribution | 0.190 | 270 | 0.090 | 0.390 | 74 | 0.181 |
| Sex ratio | 2.705 | 384 | 0.100 | 0.932 | 76 | 0.334 |
| Brooding males | 19.742 | 198 | [*]*<0.0001* | 2.600 | 43 | 0.529 |
| Hydrated females | 2.636 | 183 | 0.104 | 7.044 | 35 | [*]*0.008* |
| Injuries | 0.311 | 375 | 0.576 | 0.341 | 81 | 0.559 |

[*]Lower proportion of brooding males or hydrated females in catch data

**Table 4.4** Population-level responses to reduced fishing effort. Paired comparisons are of population structure between years in treatment and control sites. All paired tests were Wilcoxon signed ranks tests, except comparisons of mean and maximum sizes which were tested with paired t-tests. The number of sites included in the comparison (or degrees of freedom for t-tests) is given by n. There was insufficient data to test for changes in *H. hippocampus* population structure within control sites using paired tests.

| | ***Hippocampus guttulatus*** | | | ***Hippocampus hippocampus*** | | |
|---|---|---|---|---|---|---|
| | **Statistic** | **N** | **P** | **Statistic** | **n** | **p** |
| **Treatment Sites** | | | | | | |
| Mean size | 1.404 | 6 | 0.210 | 0.244 | 4 | 0.820 |
| Max size | -1.041 | 6 | 0.338 | 1.165 | 4 | 0.309 |
| Sex ratio | 0.105 | 7 | 0.917 | -1.461 | 5 | 0.144 |
| Brooding males | 1.782 | 6 | †0.075 | -0.535 | 4 | 0.593 |
| Hydrated females | -0.944 | 6 | 0.345 | -0.447 | 5 | 0.655 |
| Injuries | -0.730 | 8 | 0.465 | -1.342 | 5 | 0.180 |
| **Control Sites** | | | | | | |
| Mean size | 0.193 | 4 | 0.856 | - | - | - |
| Max size | -1.542 | 4 | 0.193 | - | - | - |
| Sex Ratio | 0.674 | 5 | 0.500 | - | - | - |
| Brooding males | 0.148 | 5 | 0.138 | - | - | - |
| Hydrated females | -0.535 | 4 | 0.593 | - | - | - |
| Injuries | 0.757 | 4 | 0.491 | - | - | - |

† Greater proportion of brooding males in 2002

**Table 4.5** Population-level responses to reduced fishing effort. Size structure, sex ratio, reproductive activity and injuries are compared between years with individuals pooled among treatment and control sites, respectively (Chi$^2$ tests, except for Kolmogov-smirnov tests for length frequency distributions). Only tests indicated by an asterisk were significant after a sequential Bonferroni correction was used to adjust p-values for multiple tests.

| | *Hippocampus guttulatus* | | | *Hippocampus hippocampus* | | |
|---|---|---|---|---|---|---|
| | Statistic | n | P | Statistic | n | P |
| **Treatment Sites** | | | | | | |
| Size distribution | 0.154 | 220 | 0.187 | 0.605 | 51 | 0.858 |
| Sex ratio | 0.035 | 292 | 0.859 | 0.071 | 67 | 0.789 |
| Brooding males | 10.358 | 149 | [*]0.001 | 1.933 | 41 | 0.164 |
| Hydrated females | 24.054 | 143 | [*]*<0.0001* | 0.036 | 29 | 0.847 |
| Injuries | 2.291 | 384 | 0.130 | 0.053 | 68 | 0.817 |
| **Control Sites** | | | | | | |
| Size distribution | 0.144 | 267 | 0.175 | 0.996 | 14 | 0.274 |
| Sex Ratio | 0.160 | 318 | 0.689 | 0.126 | 23 | 0.721 |
| Brooding males | 6.2101 | 162 | [†]0.012 | 0.185 | 19 | 0.666 |
| Hydrated females | 12.90 | 153 | [*]0.0003 | 0.000 | 10 | 1.000 |
| Injuries | 1.804 | 269 | 0.179 | 0.203 | 25 | 0.651 |

[*†] Greater proportion of brooding males or hydrated females in 2002

**Figure 4.1** The proportion of surveyed area covered by vegetation and sessile invertebrates in three fished (solid circles, solid lines) and two unfished (open circles, dashed lines) control sites in 2001 and 2002.

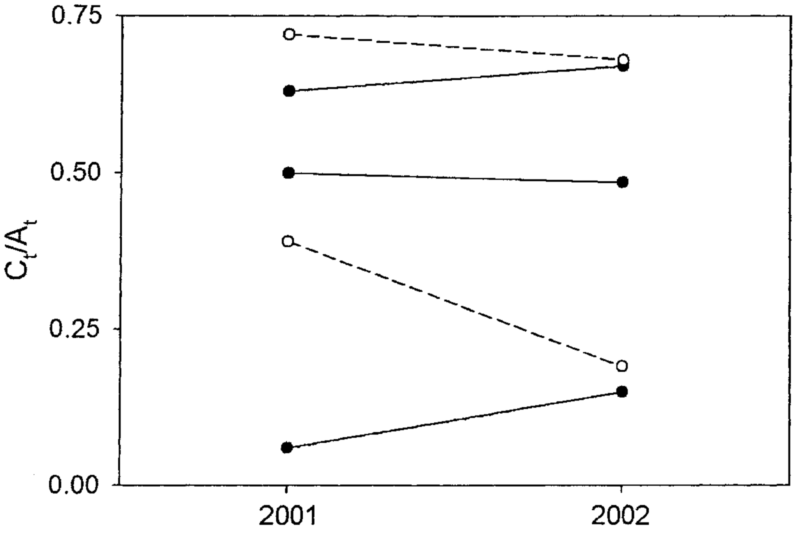


**Figure 4.2** Mean catch per seine (CPUE) as a function of the local UVC density of *H. guttulatus* (closed symbols) and *H. hippocampus* (open symbols). The equations of the fitted curves are y = 0.60x + 0.91 for *H. guttulatus* (top) and y = 0.56x + 0.56 for *H. hippocampus* (bottom). Diamonds represent multiple (2-4) data points.

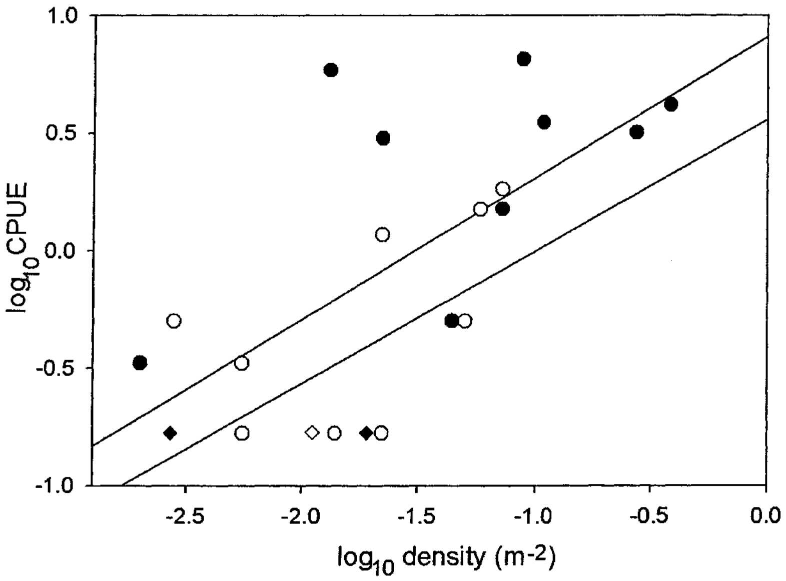


**Figure 4.3** Change in the proportion of substrate covered by all species of vegetation and sessile invertebrates ($C_t/A_t$, mean ± SE) in control sites (n = 5) and in response to a reduction in experimental fishing effort (treatment sites, n = 9) (significant increase in 2002 treatment sites indicated by an asterisk).

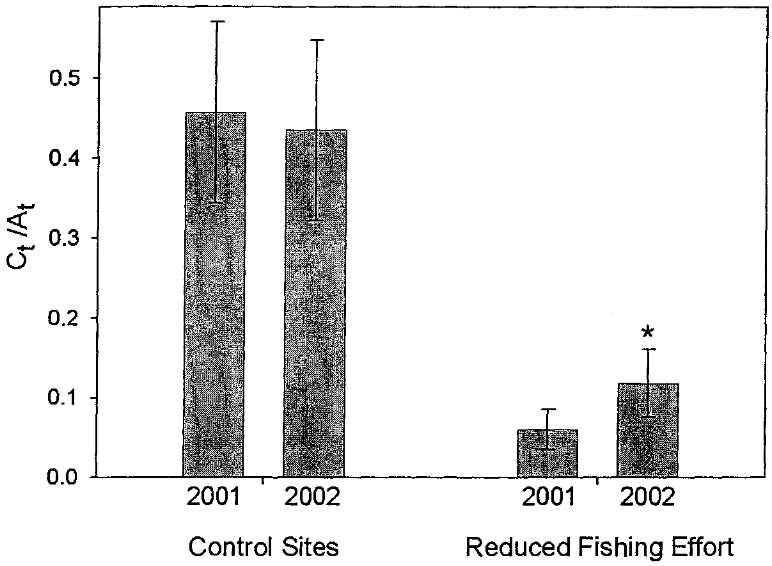


**Figure 4.4** Changes in the % cover (mean ± SE) of seagrasses, macroalgae and sessile invertebrates in response to a reduction in experimental fishing effort (n = 9 treatment sites, top) and in control sites (n = 5, bottom). Significant difference between 2001 (white bars) and 2002 (gray bars) are indicated with an asterisk.

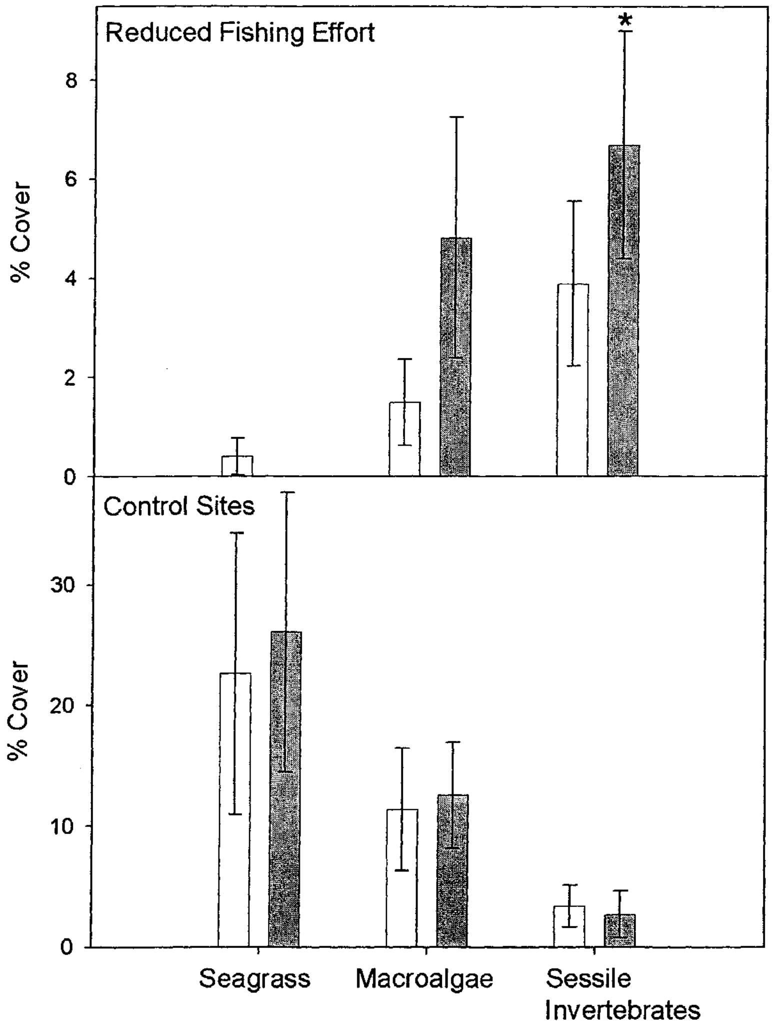
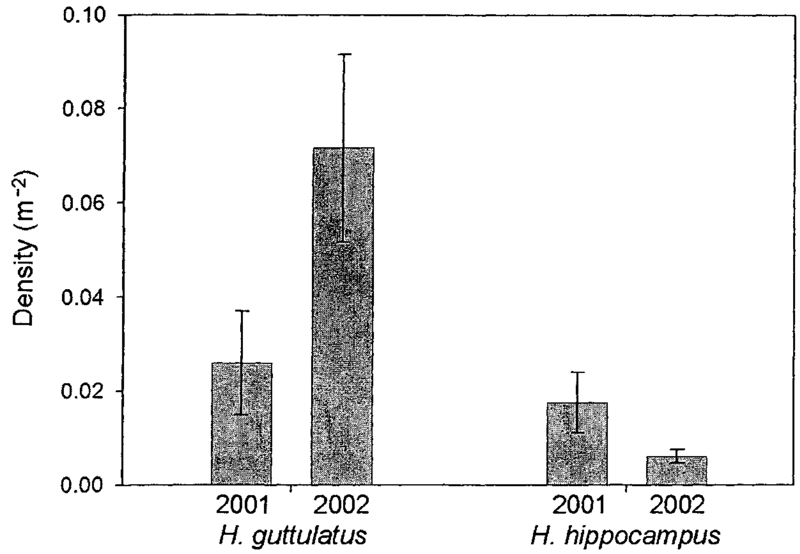


**Figure 4.5** Significant changes in *Hippocampus guttulatus* and *H. hippocampus* densities in response to a reduction in fishing pressure (n = 9 treatment sites).



# LINKING STATEMENT 5

The first four chapters of my thesis provide the basic elements of a quantitative population viability analysis: rates of survival, growth (Chapter 1), and reproduction (Chapter 2), population abundance (Chapter 3) and an understanding of two deterministic factors –habitat alteration and non-selective exploitation – that likely influence the trajectory of wild *Hippocampus guttulatus* populations (Chapter 4). In Chapter 5, I use *H. guttulatus* as a model species to evaluate the biological implications of a generic strategy proposed to manage the exploitation and international trade of all seahorse species, a 10 cm minimum size limit.

A review of the published literature revealed few studies that have explicitly applied population viability analyses (PVA) to marine fishes. Thus Chapter 5 is unusual in applying a PVA model to the prediction of population trajectories under alternative management scenarios for a benthic marine fish.

# CHAPTER 5

# PREDICTING POPULATION-LEVEL RESPONSES TO A GENERIC STRATEGY FOR SEAHORSE CONSERVATION

# ABSTRACT

In this first formal study of seahorse population dynamics, we used a stochastic age-structured matrix model to evaluate the performance of a generic minimum size limit (10 cm) recommended by CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) to help prevent recruitment overfishing in exploited populations of the majority of the ≥34 seahorse species (genus *Hippocampus*, Family Syngnathidae). We used life history and ecological parameters of the European long-snouted seahorse, *Hippocampus guttulatus*, to evaluate the performance of minimum size limits across a spectrum of fishing rates and maximum population growth rates, $r_{max}$, assuming three models of density-dependence: Ricker, Beverton-Holt and ceiling functions. Four size limits were set at: (1) size at maturity, (2) size at first reproduction, (3) size after a full reproductive season and (4) size after two full reproductive seasons. We evaluated these size limits in terms of their ability to prevent declines in population size and minimize extinction risk. Our simulations with *H. guttulatus* suggest that size after one full reproductive season (13 cm for *H. guttulatus*) may be a biologically effective management target for setting a size limit that is robust to a range of plausible fishing rates and assumptions about the nature and strength of density-dependence. A 10 cm size limit, corresponding roughly to size at maturity in *H. guttulatus*, would offer little protection against population declines ≥50% and/or local extirpations that were moderately or intensely exploited, unless there was strong Ricker (over-compensatory) density-dependence in population growth rate. Thus as biological information emerges, the 10 cm minimum size limit will likely need to be adjusted, particularly for highly exploited populations of larger species. Key life-history parameters that need to be quantified in order to refine population assessments and strategies for managing seahorse populations include intrinsic rates of population increase, form of density dependence, and size at first reproduction.

# INTRODUCTION

Most historical extirpations of marine fish populations were causally related to exploitation and its indirect effects on marine habitats (Dulvy et al. 2003). Ensuring sustainable exploitation, however, is considerably hampered by a dearth of basic biological information (Stobutzki et al. 2001ab), particularly for small benthic marine fishes (Froese & Pauly 2004) and/or those of low commercial importance. Managing the exploitation and trade of marine fishes can be particularly challenging when species resemble each other closely and limited biological information is available to identify species-specific management targets (Anonymous 1999, Anonymous 2002).

Although stock assessments of exploited marine fishes are commonly carried out using a variety of robust fisheries models (Pitcher & Hart 1982, Hilborn & Walters 1992, King 1995), relatively few of these models incorporate uncertainty, extinction risk and environmental stochasticity into their design (Akçakaya & Sjorgen-Gulve 2000, Dulvy et al. 2003), which are viewed as important aspects of fisheries assessments (Reynolds & Mace 1999). The application of population viability analysis (PVA) models that explicitly incorporate uncertainty and extinction risk for fish has been limited with most having been applied to freshwater fishes (e.g. Williams et al. 1999, Morita & Yokota 2002, Todd et al. 2004) or anadromous species (e.g. Allendorf et al. 1997, Jager 2001, Jager et al. 2001, McClure et al. 2003). Although demographic and stochastic models have been applied to large marine fishes (e.g. sandbar shark and southern bluefin tuna, Sminkey & Musick 1996, McDonald et al. 2002), the application of PVAs to marine species has largely been to charismatic fauna including coastal cetaceans and otariids (fur seals and sea lions) (e.g. Thompson et al. 2000, Gerber & Hilborn 2001, but see Bearlin et al. 1999 for a study of seagrass dynamics).

Seahorses (genus *Hippocampus,* Family Syngnathidae) are biologically, ecologically, medicinally, culturally and economically important marine fishes (Vincent 1996, reviewed in Foster & Vincent 2004). Broadly speaking, these small-bodied, multiple spawners are characterized by short life spans, rapid growth rates, specialized parental care, and low mobility (Breder & Rosen 1966, Boisseau 1967, Woods 2000, Chapter 1). Newly released juveniles are generally planktonic before settling into seagrass beds, coral reefs, estuaries or mangroves (Foster & Vincent 2004). Adults are usually sedentary and in many species they maintain long-term monogamous pair-bonds (Foster & Vincent 2004).

An apparent increase in the international trade of seahorses since the mid 1980s led to concerns about the sustainability of seahorse exploitation in target fisheries (e.g. Vincent 1996, Perante et al. 1998, Martin-Smith et al. 2004) and in bycatch (Vincent 1996, Baum et al. 2003). In a remarkable example of co-management planning, diverse stakeholders recognized that some exploited populations were likely suffering from growth and recruitment overfishing. They ranked minimum size limits and no take marine protected areas (ntMPAs) as the most biologically effective and socially preferred strategies for sustainable exploitation (Martin-Smith et al. 2004). Concurrently, CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) took a landmark decision that international trade was threatening or had the potential to threaten wild seahorse populations. As a consequence, all *Hippocampus* spp. were added to Appendix II, requiring all of the more than 165 signatory nations (Parties) to ensure that exports after 15 May 2004 not threaten wild populations; such declarations are non-detriment findings.

The need to regulate international seahorse exports for sustainability was felt to pose a considerable challenge to Parties, given that the biology, fisheries, and trades were not well-documented for any of the exploited species. Despite an order of magnitude difference in adult size (2-35 cm, Foster & Vincent 2004), species were also recognized as being difficult to distinguish. In recognition of these challenges, CITES mandated its technical committee on animals to recommend a universal minimum size limit for all seahorses in trade (Decision 12.54: http://www.cites.org/eng/decis/index.shtml). The Animals Committee, in dialogue with Project Seahorse, proposed the implementation of a 10 cm minimum size limit (in height, Ht, Lourie et al. 1999) for all internationally traded seahorses, as an interim means of filing non-detriment findings (Foster & Vincent in review). This management strategy, intended to protect at least part of the spawning biomass of exploited populations (Martin-Smith et al. 2004, Foster & Vincent in review), benefited from the general approval of the stakeholders consulted. It was fully recognised that this measure would serve as an interim means of regulating seahorse exports, and would require further refinement and/or accompanying management measures as new information emerged (Notification 2004/033: http://www.cites.org/eng/notifs/2004.shtml).

Although the implications of a minimum size limit for stakeholders and international trade were comprehensively considered (Martin-Smith et al. 2004, Foster & Vincent in review), the biological implications of a 10 cm minimum size limit for exploited populations were not analytically explored, primarily because of limited biological information for most species (Foster & Vincent, 2004, Foster & Vincent in review). The aim of this paper was to use emerging life history data from the European long-snouted seahorse, *Hippocampus guttulatus* (Cuvier 1829), to evaluate the biological implications of the 10 cm minimum size limit. Although trade in this species has been limited (Vincent & Perry in prep), the estimated size at maturity of *H. guttulatus* falls just below 10 cm Ht (Chapter 1), making this species an appropriate model for evaluating the biological effectiveness of the generic 10 cm minimum size limit.

We used mark-recapture and experimental fisheries data to construct a stochastic age-structured matrix model (Caswell 1989) to evaluate the effectiveness of four alternative minimum size limits that incrementally increased the probability of breeding before capture. We evaluated the performance of alternative minimum size limits in terms of their influence on population stability: (a) ability to prevent declines of 50% or more during a 10 year exploitation period and (b) ability to secure populations against extinction over the same time period. A predicted decline of ≥50% over 3 generations or 10 years (whichever is longer) corresponds to the decline threshold for listing species as Critically Endangered using criterion E (quantitative analyses, IUCN 2001, note that *H. guttulatus* generation time is 1.4 years, Chapter 1).

Population responses to exploitation and management depend on the intensity of disturbance as well as the nature and strength of density-dependence (Boyce 1992, Beissinger 1998). Because the nature and strength of density-dependent changes in population growth rate are not known for seahorses, a minimum size limit will need to perform effectively across a range of fishing rates and be robust to assumptions about intrinsic rates of increase and the form of density-dependence. Therefore we evaluated minimum size limits across a range of plausible fishing rates and maximum rates of population increase ($r_{max}$), assuming different forms of density-dependence (Meffe & Carroll 1997, Groom & Pascual 1998).

## METHODS

To maintain consistency with the use of height, Ht, for specifying the generic size limit, all lengths reported in this paper are expressed in Ht, except where stated otherwise. Lengths obtained from other sources (usually standard length, SL, Lourie et al. 1999), were converted to Ht using equations from Chapter 1. We do, however, use SL when comparing *H. guttulatus* to other fishes, for which this is the standard measurement.

### Species description

*Hippocampus guttulatus* is a seagrass-associated species that is found in the northeastern Atlantic Ocean and Mediterranean Sea (Boisseau 1967, Lourie et al. 1999, Pérez-Ruzafa et al. 2004, Chapter 3). This species is characterized by an early age at maturity (0.5 years), rapid growth rate ($K = 0.57$), small body size (18.0 cm maximum Ht), short life span (4.3 – 5.5 yrs), relatively low fecundity (903 ± 575 fry $yr^{-1}$) and multiple spawnings per year (4.2 ± 1.6 $yr^{-1}$) (Chapter 1, Chapter 2). *Hippocampus guttulatus* also exhibits specialized paternal care within a sealed brood pouch (Boisseau 1967), monogamous mating within reproductive cycles (though not long-term pair bonds, J. Curtis, unpublished data), and fidelity of adults to small (~20 $m^2$), undefended, benthic home ranges (Chapter 1). Males brood young for three weeks and newborn juveniles spend at least eight weeks in the plankton before recruiting to benthic habitats (Boisseau 1967, Pérez-Ruzafa et al. 2004, Chapter 1). Such recruitment coincides with the end of the reproductive season, as well as the onset of the development of the male brood pouch (and thus maturity). Based on the presence of a mature pouch as an index of sexual maturity, *H. guttulatus* usually matures at 9.7 cm Ht, but mean length at first reproduction is 11.3 cm Ht (Chapter 1).

### Model structure and parameters

All model simulations were carried out using RAMAS Metapop Version 4 (Akçakaya 2002), a flexible and commonly employed population viability analysis software package (Brook et al. 2000). We synthesized fisheries-independent data from previous studies of *H. guttulatus* (Chapter 1, Chapter 2, Chapter 3) that were carried out from 2000-2003 in a productive tidal lagoon in southern Portugal (Ria Formosa, 36°59' N, 7°51' W). This model requires an array of life history variables (including age structure, survival rates, fecundities, $r_{max}$ and K), which we derive from these studies in the following sections.

#### *Age structure*

We used a pre-breeding census (birth pulse) Leslie matrix that was modified to model the indeterminate growth pattern of *H. guttulatus* (Caswell 1989). Most reproduction in *H. guttulatus* occurs during a three month period from June – August (Boisseau 1967, Chapter 2), therefore we assumed no mortality between the pre-breeding census and the end of reproduction. Five age classes were modeled: zero year olds, 1 year olds, 2 year olds, 3 year olds and 4+ year olds (Table 5.1).

#### *Survival*

We used monthly adult survival rates reported in Chapter 1 to calculate an annual survival rate, $S_x$, for ages 1 – 4+. There was no evidence of age-specific differences in $S_x$ for adults (Chapter 1) and we assumed that the survival of settled (benthic) juveniles was the same, as assumed in other fishes (King 1995). The average monthly survival rate of adult *H. guttulatus* was 0.90 ± 0.06 (Chapter 1), giving an estimate of annual survival rate of 0.294 ± 0.23.

We used changes in the age distribution of individually tagged *H. guttulatus* on two focal study grids from 2000 – 2003 (see Chapter 1 for census details) to estimate the rate of survival of newborns to age 0 (i.e. from birth to just before they turned 1 year old), $S_0$ as:

$$S_0 = N_0(t+1)/N_{newborn}(t)$$

where $N_0(t+1)$ was the proportion of zero-aged individuals (i.e. just about to turn 1 year old) in the population in year t+1, and $N_{newborn}(t)$ was the proportion of the population in year t that was newborn juveniles produced by individuals from all age classes alive to reproduce in year t. The focal population was censused on two 100 $m^2$ grids that were 60 m apart. The first grid was censused in 2000 and the second grid was censused from 2001 – 2003. All individuals encountered in the focal population during the first month of censuses each year were used to construct yearly tables of the proportion of individuals in each age class, by converting sizes to ages (as described above).

$N_0(t+1)$ was difficult to estimate because maturing juveniles were still in the process of recruiting up to this age (Chapter 1) and may not have been encountered during census dives. Therefore we used the number of individuals in the samples that were just about to turn 2 years old (i.e. age class 1) to back-calculate the expected $N_0(t+1)$ (i.e. zero-age class) in the population, assuming that the survival rate from age class 0 to 1 was 0.294. To estimate $N_{newborn}(t)$, we estimated the total number of newborns produced by all reproductively mature females encountered during census periods each year. This means of estimating $S_0$ assumed that the amount of migration (immigration and emigration) was negligible relative to the level of self-recruitment in this population (see below). Three estimates of $S_0$ ranged from 0.0039 – 0.0058 (Table 5.2).

### *Fecundity*

Age-specific fertilities, $M_x$ (mean number of female or male offspring produced per female of age x, Caswell 1989), were estimated by multiplying the number of male or female young produced per brood by the annual spawning frequency. We used a length at age model developed for *H. guttulatus* (Chapter 1) to group 117 brooding male *H. guttulatus* of known size (Chapter 2) into five age classes (Table 5.1). The number of young brooded by each male was halved (assuming an equal ratio between male and female juveniles), and then averaged within age class. Age-specific fertilities were estimated by multiplying the number of females or males produced per brood by 4.2 ± 1.6, the annual spawning frequency (Chapter 2). We assumed that the mean age-specific reproductive outputs of males and females were the same because (a) there was evidence of size-assortative mating between male and female seahorses (J. Curtis, unpublished data, see also Teixeira & Musick 2001, Vincent & Giles 2003), (b) seahorses mate monogamously within reproductive cycles (Jones et al. 1998), and (c) there was an equal sex ratio in the adult population (Chapter 1).

Age-specific fecundities, $F_x$ (mean number of female or male offspring censused at time t+1, produced per female of age x alive at time t (Caswell 1989, Akçakaya 2002) were estimated by multiplying age-specific fertilities by the survival rate of zero year olds, $S_0$. $F_0$ was adjusted by multiplying the value by 0.06, as the fraction of male and female *H. guttulatus* <11.5 cm Ht (upper size range of zero-aged individuals) that breed in wild populations (Chapter 1) (Akçakaya 2002). All adult females and most adult males reproduced *in situ* during a reproductive season (Chapter 2), therefore $F_x$ was not adjusted for age classes 1 – 4+.

### *Mating pattern*

*Hippocampus guttulatus* populations had no evidence of significantly biased sex ratios, sex-specific differences in reproductive potential or sex-specific differences in the growth and survival of benthic juveniles and adults (Chapter 1, Chapter 2). However, because both sexes of *H. guttulatus* probably mated monogamously within reproductive cycles (as in *H. subelongatus*, Jones et al. 1998), we incorporated a monogamous mating pattern and demographic stochasticity in our model (Morris & Doak 2002).

### *Migration*

Dispersal distances and the degree of philopatry of planktonic juveniles are unknown. Therefore we assumed that any immigration from surrounding populations was negligible relative to local recruitment: the population size of *H. guttulatus* in the Ria Formosa is ~2 000 000 benthic juvenile and adults (Chapter 3) and the closest confirmed population of *H. guttulatus* is approximately one hundred kilometers away (Rio Arade Estuary near Portimão, Karim Erzini, personal communications). Migration of adults (age classes 1 – 4+) among populations is unlikely (Chapter 1).

### *Density-dependence*

We considered three options for modeling density-dependent changes in population growth rate: Ricker function and Beverton-Holt function and a ceiling model, in which population size never exceeds a specified carrying capacity (Morris & Doak 2002). The Ricker model (over-compensatory dynamics) is usually employed to model the dynamics of populations in which all individuals share limiting resources equally (i.e. all individuals do poorly at high densities), whereas the Beverton-Holt model (compensatory dynamics) is typically employed when at least some individuals in a population always get enough resources for survival, growth and reproduction, regardless of population size (i.e. Akçakaya 2002, Morris & Doak 2002). The differences in model assumptions have significant implications for the stability of populations at high maximum population growth rates, ($r_{max}$): populations with Ricker density-dependence exhibit natural fluctuations when $r_{max}$ is high that are independent of environmental fluctuations, whereas populations with Beverton-Holt density-dependence will tend to be stable even when $r_{max}$ is high (Akçakaya 2002, Morris & Doak 2002). To model Ricker and Beverton-Holt functions, the matrix elements were modified at each time step so that the dominant eigenvalue ($\lambda$) was equal to the predicted population growth rate at time t, $r_t$ (Akçakaya 2002):

Ricker: $$r_t = r_{max} e^{\left(\frac{-\ln(r_{max}) N_t}{K}\right)}$$

Beverton-Holt: $$r_t = \frac{r_{max}K}{r_{max}N_t - N_t + K}$$

where $N_t$ was the population size at time t, K was the expected equilibrium population size (carrying capacity) and $r_{max}$ was the maximum population growth rate. Because both Ricker and Beverton-Holt models assume that populations will recover from low densities, we also used a ceiling model which does not make this assumption. When total population size exceeded K in simulations with the ceiling model, population size was reduced to K by proportionally adjusting age-specific abundances (Akçakaya 2002). The expected equilibrium population size, or K was taken to be the actual population size, ~2 000 000 *H. guttulatus* (Chapter 3), because this was the only estimate of population size available for the Ria Formosa lagoon. Fishing mortality in this population is probably low because the use of bottom fishing gears that catch seahorses (e.g. seines, trawls, pushnets) is illegal in the Ria Formosa (Monteiro 1989, Erzini et al. 2002). We therefore had no *a priori* reason for suspecting that *H. guttulatus* was not at or close to its carrying capacity in the Ria Formosa. Thus initial population sizes were also set at 2 000 000 in all simulations. Environmental fluctuations in K were modeled assuming a 10% coefficient of variation (and log-normal distribution). Density-dependence was assumed to affect both fecundities and survival rates and was based on the sum of abundances of age classes 0 – 4+ weighted by age-specific fecundity, as commonly formulated in matrix models of fish populations (Akçakaya 2002).

The intrinsic rate of population increase, r, is difficult to estimate (Myers et al. 1997, Myers et al. 1999, Denney et al. 2002, Froese & Pauly 2004). Therefore we used a regression model (Denney et al. 2002) to predict the number of zero-aged individuals (i.e. individuals at size at first reproduction, just about to turn 1 year of age) produced per adult using the maximum observed body length of *H. guttulatus* (19.7 cm SL, Chapter 1). We predicted $r_{max}$ for *H. guttulatus* using the regression of $\log(r_{max})$ (measured as maximum adult production per adult) against body size for 43 northeastern Atlantic fishes that varied widely in body growth rate, ages at maturity, fecundities, trophic level and habitat use (reported in Denney et al. 2002). Recruitment rates at small population size (measured as the slope at the origin of a stock-recruitment curve, Myers et al. 1997) are probably reliable measures of the intrinsic rate of increase (Denney et al. 2002). Among four life history traits examined for other species, body size was most strongly (and inversely) correlated with maximum production of mature individuals per adult at low population sizes (Denney et al. 2002). The regression analysis predicted an $r_{max}$ of 6.8 for *H. guttulatus*, although the average value of $r_{max}$ reported for these 43 stocks was 3.1. Because of uncertainty about the intrinsic rates of increase for seahorses, as well as $r_{max}$, we carried out simulations using two values of low $r_{max}$ (1.5, 2.5) and two values of high $r_{max}$ (5.0, 7.0). These plausible values approximated the range of estimated values of $r_{max}$ for fishes (Myers et al. 1999).

Allee effects (inverse density-dependence) were not explicitly included in simulations because of insufficient demographic data. However, we recognized that the monogamous mating patterns and small home ranges of adult seahorses could make them vulnerable to Allee effects as exploitation reduced population density. To account for the low mobility of *H. guttulatus*, we set a quasi-extinction threshold at 2 individuals per hectare (below which populations were considered to be extinct). This density was selected because the farthest distance any settled individual was observed dispersing from an initial capture location was 40-45 m (although benthic juveniles probably moved much greater distances, Chapter 1). If 40 m is the radius of the area within which a settled *H. guttulatus* is likely to disperse (roughly 0.5 ha), then the probability of encountering a mate may drop considerably below densities of ~2 $ha^{-1}$. The approximate subtidal area of the Ria Formosa is 26 $km^2$ (A. Rodrigues de Matos, Parque Natural da Ria Formosa, pers. comm.) thus we set the quasi-extinction threshold at 5200 individuals. This threshold was consistent with estimates of minimum viable population sizes for vertebrates (Reed et al. 2003).

## Simulations

### *Size limits*

We examined the effectiveness of four size limits that incrementally increased the probability and frequency of breeding before capture. The first minimum size limit was designed to protect individuals until they reached maturity. Because the range of sizes over which *H. guttulatus* matures coincides with the range of sizes over which this species recruits to benthic habitats (Chapter 1), and become vulnerable to non-selective bottom fishing gears (Erzini et al. 2002, Chapter 4), this size limit was modeled as no restriction on exploitation. The second minimum size limit was designed to protect recruits until they reached size at first reproduction (11.5 cm, ~0.9 yrs, Chapter 1), such that only age classes 1 – 4+ were vulnerable to exploitation. The third minimum size limit was designed to give all individuals at least one full reproductive season to breed before becoming vulnerable to exploitation (≥13 cm, Chapter 1), such that only individuals in age classes 2 – 4+ were vulnerable. The fourth minimum size limit was designed to give all individuals at least two full reproductive seasons to breed before becoming vulnerable to exploitation (≥15 cm, Chapter 1), with age classes 3 and 4+ being vulnerable to exploitation.

### *Fishing rates*

We examined the effectiveness of these four size limits at five fishing rates: 10%, 30%, 50%, 70% and 90%. Here, we define fishing rate as the percentage of the vulnerable population that was removed each year. The catchability of *H. guttulatus* in small beach seines is approximately 0.1 (Chapter 4), thus the lower end of the range in fishing rates represents a population that is exploited once per year by non-selective bottom fishing gears. By contrast, in fisheries where seahorses are directly targeted and hand captured by fishers (e.g. *H. comes* in the Philippines), the fishing mortality can exceed 80% per year (Perante et al. 1998, reviewed in Martin-Smith et al. 2004).

### *Time horizon*

Simulations were initialized for a period of 10 years prior to the onset of exploitation and implementation of minimum size limits. After a 10 year pre-exploitation period, exploitation and management strategies were carried out for a further 10 years. Ten-year periods were selected because the World Conservation Union (IUCN) ranks the risk of population extinctions using quantitative analyses (Criterion E) according to the magnitude of predicted decline during a 10 – 100 year period (IUCN 2001). Carrying out simulations over local temporal scales (10 years) provides a quantitative assessment of population trajectory, quasi-extinction risk and management strategies using contemporary estimates of life history rates.

*Performance criteria*

To compare the effectiveness of minimum size limits under a range of fishing rates from both a biological and social point of view, we summarized the relative final population size (excluding newborns), the relative risk of population quasi-extinction and the relative cumulative catch of *H. guttulatus* over the 10 year exploitation period. Each simulation was replicated 10000 times and we report the mean values of replicates.

## RESULTS

### Dynamics of unexploited populations

Estimates of $F_x$ and $S_x$ (Table 5.3) may have been collected during a period of gradual population decline: the finite rate of increase, $\lambda$ (i.e. dominant eigenvalue of the modified Leslie matrix), was 0.973, suggesting that from 2000 – 2003, the population size was declining on average by 2-3% $yr^{-1}$. However, this should not necessarily be a cause for concern because $\lambda$ does not include any stabilizing effects of density-dependence. Moreover, even when a population is stable, estimates of survival and fecundity rates are unlikely to produce a value of $\lambda$ that falls squarely on 1 (Akçakaya 2002). On average, individuals spent 1.42 years in age class 4+ (calculated from the matrix model in the absence of exploitation).

The Ricker and Beverton-Holt functions affected population size and probability of quasi-extinction at low $r_{max}$ (1.5, 2.5) similarly when there was no exploitation during the 10 years prior to exploitation (Table 5.4). As expected, however, unexploited populations were more stable (i.e. larger mean final population sizes, lower probability of quasi-extinction) at high maximum population growth rates ($r_{max}$ = 5, 7) with a Beverton-Holt density-dependence function than when a Ricker function was assumed (Table 5.4). As $r_{max}$ increased, populations with Ricker functions were increasingly vulnerable to fluctuations in population size and had greater probabilities of quasi-extinction. When the ceiling model was assumed, population size declined the most due to a negative finite rate of increase, $\lambda$, and high variability in demographic rates and environmental carrying capacity, although probability of quasi-extinction was negligible over the 10 year period (Table 5.4).

## Dynamics of exploited populations

*Numerical response to size limits*

Differences in the effectiveness of alternative minimum size limits for preventing population declines were most pronounced at moderate to high fishing rates for all three density-dependence models (Figs. 5.1 & 5.2) and at low $r_{max}$ when Ricker or Beverton-Holt functions were assumed (Fig. 5.1a,b). At the lowest $r_{max}$ (1.5) (Fig. 5.1a), or when the ceiling model was assumed (Fig. 5.2), all population sizes declined when there was exploitation, but the magnitude of the decline was inversely correlated with the minimum size limit. As $r_{max}$ increased for the Ricker & Beverton-Holt models, so did resilience to exploitation.

Setting the size limit at size at maturity almost always resulted in poor performance compared to more stringent size limits, regardless of fishing rate or the nature and strength of density dependence. Notable exceptions were at low to moderate fishing rate and high $r_{max}$ (5, 7) assuming a Ricker function (Fig. 5.1c,e).

Setting the size limit at size at first reproduction reduced the magnitude of population declines in all cases, compared to a size limit set at size at maturity (Figs. 5.1 & 5.2). However, declines of 50% or more were still predicted at low $r_{max}$ (1.5, 2.5) and moderate to high ($\geq$30%) rates of exploitation (Fig. 5.1a,b) for Ricker & Beverton-Holt models. Protecting the small fraction of spawners with this size limit was predicted to prevent declines in population size of >50% across the spectrum of fishing rates, assuming either of these two models and high $r_{max}$ (5, 7) (Fig. 5.3c-f). When the ceiling model was assumed, however, the size limit set at first reproduction only prevented $\geq$50% declines at the lowest fishing rate (10%).

A size limit protecting individuals through to the end of one full reproductive season was robust in terms of preventing declines >50% across the spectrum of fishing rates and $r_{max}$ when Ricker or Beverton-Holt models were assumed. Only at low $r_{max}$ and high fishing rates (50-90%) did populations decline by ≥20% under this minimum size limit (Fig. 5.1a). Thus at most values of $r_{max}$ (2.5-7), setting size limits to protect individuals until they had reproduced during one or two reproductive seasons resulted in stable population sizes across a range of fishing rates, regardless of whether Ricker or Beverton-Holt dynamics were assumed. However, a size limit at first reproduction only precluded ≥50% declines at low fishing rates (10-30%) when a ceiling model was assumed (Fig. 5.2). Under the ceiling model, only a size limit designed to protect individuals until after their second reproductive season prevented declines of 50% or more (Fig. 5.2).

### *Quasi-Extinction risk*

At low $r_{max}$ (1.5, 2.5) and fishing rates ≤70%, the risk of quasi-extinction over the 10 year exploitation period was <2%, whether a Ricker or Beverton-Holt model was assumed (Fig. 5.3a). However, when fishing rate increased from 70%B to 90% at low $r_{max}$, populations in which individuals were only protected until size at maturity or size at first reproduction had probabilities of quasi-extinction equal to unity. As predicted for unexploited populations, the probability of quasi-extinction increased as $r_{max}$ increased assuming a Ricker function (Fig. 5.3a-c), regardless of minimum size limit or fishing rate. By contrast, when a Beverton-Holt model was assumed at high $r_{max}$ (5, 7), the quasi-extinction probability was nil regardless of fishing rate or size limit employed (Fig. 5.3b,c). When a ceiling model was assumed, populations were secured against quasi-extinction across a range of fishing rates if the size limit protected individuals until after their first reproductive season (Fig. 5.3d).

### *Cumulative catches*

In general the relative cumulative catches over 10 years were inversely correlated with the minimum size limit (Fig. 5.4). At low to moderate fishing rates (10-50%), size limits set at size at maturity always resulted in greater cumulative catches, regardless of

$r_{max}$ or density-dependence model. At higher fishing rates (70-90%), however, setting size limits at the size at first reproduction (and in one case, after one reproductive season, Fig. 5.4d) yielded greater cumulative catches (Fig. 5.4b-d) when Ricker or Beverton-Holt models were assumed, except when $r_{max}$ was 1.5.

## DISCUSSION

This is among the few studies to apply a stochastic risk assessment model to predict the population responses of a small benthic marine fish to alternative management strategies. In our study, we synthesized life history and ecological parameters into a structured population dynamics model for seahorses. Our results suggest that a minimum size limit that protects individuals until they have had an opportunity to reproduce during one full reproductive season would help secure populations against declines ≥50% and minimize the probability of local extirpations under a range of fishing mortalities and assumptions about the nature and strength of density-dependence.

### Dynamics of unexploited populations

Our models reveal the aspects of life history that might most influence population persistence and so would bear further research and refinement, for this and other species. The life history characteristics of *H. guttulatus* - small body size, early age at maturity, and rapid growth rates (Chapter 1) - suggest that this species may have a relatively high intrinsic rate of population increase (Myers et al. 1997, Hutchings 2001, Denney et al. 2002, Froese & Pauly 2004). Our empirical estimate of $r_{max}$ (6.8 spawners produced per spawner per year at low population density) was based on a regression that included data from marine fishes that varied in body growth rate, ages at maturity, fecundities, trophic level and habitat use (Denney et al. 2002). This value of $r_{max}$ was at the high end of a spectrum of values observed for exploited fishes (1-7, Myers et al. 1999). Although $r_{max}$ is inversely related to age at maturity (Myers et al. 1997), the implications of social and spatial structures for seahorse population growth rates at low densities have not been investigated. Thus further research is required to determine how monogamy, social interactions, and adult site fidelity influence the way that resources are partitioned as density changes, and to what extent these behaviours stabilize or destabilize the dynamics of *H. guttulatus* populations (Vincent & Sadovy 1998, Shumway 1999).

Our model results would seem to suggest that if the rate of population change follows a Ricker (or logistic) density-dependent function with strong density-dependent responses (i.e. high intrinsic rates of increase), seahorse populations may be prone to local extirpations even in the absence of exploitation and/or regardless of the minimum size limit set. Unfortunately, there are too few long-term studies of seahorse abundance (Foster & Vincent 2004) to characterize natural fluctuations in population density. The longest record of experimental catch data for *H. guttulatus* spans seven years (1980 – 1986, Monteiro 1989) and suggests that population densities in sampling sites may have varied on a yearly basis by as much as seven fold during the early- to mid-1980s, with no seahorses reported in catches in 1984. Such fluctuations in *H. guttulatus* density may occur over small spatial scales: between 2000 and 2001, density decreased from 0.33 $m^{-2}$ to <0.005 $m^{-2}$ on one focal study site, but remained constant from 2001-2003 on a second focal grid merely 60 m away (Chapter 1, J. Curtis, unpublished data). In a large scale sampling program along the Gulf and Atlantic coasts of Florida, seahorse catches (*H. erectus* and/or *H. zosterae*) fluctuated yearly by as much as one order of magnitude from 1992 – 2001 (de Silva et al. 2003).

The apparent fluctuations in population size suggest that seahorse populations may increase under favourable environmental conditions over short temporal scales. The corollary, however, is that local populations of these fishes may be more vulnerable to extended periods of poor recruitment or unfavourable environmental conditions than longer-lived species (Sadovy 2001). Species that exhibit natural fluctuations in population size are probably stabilized to varying degrees by migration among subpopulations (Fagan et al. 2001, Smedbol & Stephenson 2001, Smedbol & Wroblewski 2002). If seahorses falls within the group of so-called refuge-dependent species, then the use of no take marine protected areas (ntMPAs) as spatial refuges and sources of dispersers are likely to play an important and complementary role to minimum size limits for ensuring the long term sustainable exploitation of seahorse populations.

### Minimum size limits for *Hippocampus guttulatus*

Our simulations with *H. guttulatus* suggest that size after one full reproductive season may be a more biologically effective management target than size at maturity when applying minimum size limits for exploitation. In the absence of information about fishing rates and the nature and strength of density-dependence operating in *H. guttulatus* populations, the most biologically and socially appropriate size limit was one that protected individuals until after their first reproductive season. Although a size limit that protected individuals until size at first reproduction allowed at least 6% of zero-aged individuals to reproduce before becoming vulnerable to exploitation, our results suggest that this was insufficient to prevent declines of ≥50% when fishing rates were high, except when Ricker dynamics were assumed with high $r_{max}$. By contrast, protecting individuals until after one full reproductive season helped prevent declines >50% in all scenarios (except when a ceiling model was assumed with high fishing rates): this minimum size limit would correspond to 13 cm for *H. guttulatus*.

In practice the biological implications of minimum size limits for *H. guttulatus* will depend on the direct and indirect effects of exploitation, as well as its population dynamics. We currently have no evidence that *H. guttulatus* is directly targeted in fisheries; most exploitation is probably as bycatch in non-selective fishing gears. Thus fishing rates will likely depend on the catchability of *H. guttulatus* in non-selective gears and the frequency of exploitation. Non-selective gears also cause reductions in habitat complexity, an apparently important aspect of *H. guttulatus* habitat (Chapter 4). Indeed, the decline in *H. guttulatus* density from 2000 to 2001 on the first focal study site described above was associated with the disappearance of most macroalgae (primarily *Ulva lactuca* and *Codium* spp) and invertebrates (primarily *Zoobotryon verticillatum*) from the site (J. Curtis, personal observation). Thus, even though current assumptions suggest that *H. guttulatus* may recover rapidly from the direct effects of exploitation, this species may not be resilient to habitat alteration.

### Biological implications of a 10 cm minimum size limit for the genus *Hippocampus*

The stakeholders who identified minimum size limits as biologically effective and socially acceptable strategies for managing seahorse populations recognized that in order to be effective, the size limits would need to protect individuals at least until after they begin reproducing (Martin-Smith et al. 2004). In setting the recommended minimum size limit at 10 cm, the CITES Animals Committee sought to strike a balance between setting the minimum size limit above size at maturity, but below the maximum body size for most species, thus offering some protection to most exploited populations while allowing trade to continue (Foster & Vincent in review). The CITES Animals Committee also recognised that the limit might need to be raised later, but preferred not to penalise traders too heavily until further information was available. The 10 cm minimum size limit was greater than the inferred size at maturity for all but 4-6 species (depending on whether size at maturity was taken from the literature or predicted using regression analysis, Foster & Vincent in review), and greater than the maximum body length for 10 species.

The generic 10 cm minimum size limit may need to be adjusted, particularly for larger species that are heavily exploited (Martin-Smith et al. 2004, Foster & Vincent in review). Because the average size at first reproduction for most seahorse species was not known (Foster & Vincent 2004, Martin-Smith et al. 2004), size at first maturity was used as an initial proxy for reproduction (Foster & Vincent in review). The presence of a brood pouch was generally used as an index of sexual maturity (e.g. Wilson & Vincent 1998, Baum et al. 2003, Moreau & Vincent, 2004, Foster & Vincent 2004, Foster & Vincent in review, Chapter 1), For *H. guttulatus*, however, there is a 1.6 cm size difference between size at maturity and size at first reproduction (corresponding to an age difference of 0.5 years, Chapter 1). New studies suggest that such delays between mean size at maturity and mean size at first reproduction also exist in other species (*H. hippocampus*: J. Curtis, unpublished data, *H. comes*: A. Vincent et al. unpublished data).

The social implications of increasing the minimum size limits will depend on the fishing rate and strength of density dependence. If fishing rates are low (i.e. 10-30%), increasing a minimum size limit will likely reduce cumulative catches, and thus income, without greatly influencing the probability of extinction. However, when fishing rates are high (e.g. 70-90%), the probability of extinction will be inversely correlated to the minimum size limit. Thus on average, setting smaller size limits when fishing rates are high could result in smaller cumulative catches because of the greater probability of local extinction.

### An important caveat

Seahorses that are caught in non-selective fishing gear, as is the majority of internationally traded seahorses (Vincent 1996, Baum et al. 2003), may not benefit much from minimum size limits, whatever their density-dependent response, fishing mortality or $r_{max}$. Even if fishers discarded seahorses that fall below the 10 cm minimum size limit, small size classes might still suffer fishing mortalities (Baum et al. 2003). Where fishing mortality associated with non-selective fisheries is high, spatial closures would likely help offset the impacts of bycatch on seahorse populations (Martin-Smith et al. 2004, Foster & Vincent in review). Our simulations did not include the indirect effects of non-selective fishing on habitat structure and complexity (Chapter 4), but the possible implications of such indirect effects should be considered when predicting population-level responses to exploitation and management strategies (Chapter 4).

## CONCLUSIONS

No simulation is a substitute for empirical research. In data poor situations, however, models are the first step towards formalizing a management problem, synthesizing available information and predicting responses to precautionary initiatives (Heppell et al. 2002). When data are scarce, precautionary management strategies should be robust to a range of influential parameters including intrinsic rates of increase, nature of density dependence and fishing mortalities. They should also be flexible enough to respond appropriately to emerging data and analyses.

Our paper underscores the importance of estimating the intrinsic rate of population increase and form of density-dependence for understanding the population dynamics of seahorses and predicting population-level responses to alternative management scenarios. We hope that our preliminary findings will stimulate studies designed to quantify the following for species in the genus *Hippocampus*, as well as for other small, benthic marine fishes:

(a) intrinsic rate of population increase
(b) nature and form of density dependence (including Allee effects)
(c) life history rates affected by density dependence
(d) magnitude of fluctuations in relatively unexploited populations
(e) size at first reproduction
(f) biological implications of social and spatial structure for population dynamics

It is noteworthy that the two management strategies that were most highly ranked and socially accepted by a variety of stakeholders – minimum size limits and no take marine protected areas (Martin-Smith et al. 2004) – should, in combination, help ensure the sustainable exploitation of seahorses across a range of fishing rates and be robust to a range of $r_{max}$ and density-dependence models. It is also noteworthy that CITES, as well as the stakeholders who exploit, use or value seahorses, sought to adopt precautionary measures for securing wild populations even for data-poor fisheries (as recommended by Johannes 1998).

*Acknowledgements*. This is a contribution from Project Seahorse. The survival and fecundity estimates used to parameterize the Leslie matrix were based on hard-earned data that were collected with the help of our esteemed research assistants including R. Adams, H. Balasubramanian, K. Bigney, J. d'Entremont, B. Gunn, S. Lemieux, C.-M. Lesage, E. Murray, J. Nadeau, S. Overington, A. M. Santos, S. V. Santos, M. Veillette, and K. Wieckowski. We thank Steven Martell and Resit Akçakaya for useful discussions about density-dependence and estimating $r_{max}$. J.M.C. was generously supported by McGill Major, NSERC, and FCAR awards. The John G. Shedd Aquarium in Chicago kindly provided operating support for AV through its partnership for marine conservation with Project Seahorse.

## LITERATURE CITED

Akçakaya HR (2002) RAMAS Metapop: Viability analysis for stage-structured metapopulations (version 4.0). Applied Biomathematics, Setauket, New York.

Allendorf FW, Bayles D, Bottom DL, Currens KP, Frissell CA, Hankin D, Lichatowich JA, Nehlsen W, Trotter PC, Williams TH (1997) Prioritizing Pacific salmon stocks for conservation. Conservation Biology 11:140-152

Anonymous (2002) Amendments to Chapter 13-95. Hawaii Administrative Rules. Title 13: Department of Land and Natural Resources, Chapter 95: Rules regulating the taking and selling of certain marine resources. HAR Section 19-35. Department of Land and Natural Resources State of Hawaii, Hawaii, USA.

Anonymous (1999) Queensland Coral Reef Fin Fish Fishery: Draft management plan and regulatory impact statement. Queensland Fisheries Management Authority with the Reef Fishery Management Advisory Committee, Queensland, Australia.

Baum JK, Meeuwig JJ, Vincent ACJ (2003) Bycatch of lined seahorses (*Hippocampus erectus*) in a Gulf of Mexico shrimp trawl fishery. Fishery Bulletin 101:721-731

Bearlin AR, Burgman MA, Regan HM (1999) A stochastic model for seagrass (*Zostera muelleri*) in Port Phillip Bay, Victoria, Australia. Ecological Modelling 118:131-148

Beissinger SR (1998) On the use of demographic models of population viability in endangered species management. Journal of Wildlife Management 62:821-841

Boisseau J (1967) Régulations hormonales de l'incubation chez un vertébré mâle: recherches sur la reproduction de l'Hippocampe. PhD thesis, University of Bordeaux, Bordeaux, France.

Boyce MS (1992) Population viability analysis. Annual Review of Ecology and Systematics 23:481-506

Breder CM, Rosen DE (1966) Suborder Syngnathoidei. In: Modes of Reproduction in Fishes. Natural History Press, New York, pp 292-301

Caswell H (1989) Matrix population models: construction, analysis and interpretation. Sinauer Associates, Sunderland, Massachusetts

Denney NH, Jennings S, Reynolds JD (2002) Life-history correlates of maximum population growth rates in marine fishes. Proceedings of the Royal Society of London, Series B 269:2229-2237

de Silva JA, Mahmoudi B, Muller RG, Murphy MD (2003) Florida's Inshore and Nearshore Species: 2002 Status and Trends Report, Appendix A. Florida Fish and Wildlife Conservation Commission - Florida Wildlife Research Institute, St. Petersburg, Florida.

Dulvy NK, Sadovy Y, Reynolds JD (2003) Extinction vulnerability in marine populations. Fish and Fisheries 4:25-64

Erzini K, Bentes L, Coelho R, Correia C, Lino PG, Monteiro P, Ribeiro J, Goncalves J MS (2002) Recruitment of sea breams (Sparidae) and other commercially important species in the Algarve (southern Portugal), Final Report., pp. 178: Commission of the European Communities DG XIV/C/1.

Fagan WF, Meir E, Prendergast J, Folarin A, Karieva P (2001) Characterizing population vulnerability for 758 species. Ecology Letters 4:132-138

Foster SJ, Vincent ACJ. In review. Setting one minimum size limit for the entire genus of seahorses could help ensure sustainable trade.

Foster SJ, Vincent ACJ (2004) The life history and ecology of seahorses: implications for conservation and management. Journal of Fish Biology 65:1-61

Froese R, Pauly D (eds) (2004) FishBase. World Wide Web electronic publication. www.fishbase.org, version (06/2004).

Gerber LR, Hilborn R (2001) Catastrophic events and recovery from low densities in populations of otariids: implications for risk of extinction. Mammal Review 31:131-150

Groom MJ, Pascual MA (1998) The analysis of population persistence: An outlook on the practice of viability analysis. In: Fieldler PL and PM Kareiva. Conservation Biology For the Coming Decade (2$^{nd}$ Ed). Chapman & Hall, New York.

Heppell SS, Crouse DT, Crowder LB (2002) Using matrix models to focus research and management efforts in conservation. In Ferson S, Burgman M (eds) Quantitative methods for conservation biology. Springer-Verlag, New York

Hilborn R, Walters CJ (1992) Quantitative Fisheries Stock Assessment: Choice, Dynamics and Uncertainty. Kluwer Academic Publishers, Massachussetts, USA.

Hutchings J (2001) Conservation biology of marine fishes: perceptions and caveats regarding assignment of extinction risk. Canadian Journal of Fisheries and Aquatic Sciences 58:108-121

IUCN (2001) IUCN red list categories and criteria: Version 3.1. In Commission ISS (ed) IUCN, Gland, Switzerland

Jager HI (2001) Individual variation in life history characteristics can influence extinction risk. Ecological Modelling 144:61-76

Jager HI, Chandler JA, Lepla KB, Van Winkle W (2001) A theoretical study of river fragmentation by dams and its effects on white sturgeon populations. Environmental Biology of Fishes 60:347-361

Johannes RE (1998) The case for data-less marine resource management: examples from tropical nearshore finfisheries. Trends in Ecology and Evolution 13(6):243-246

Jones AG, Kvarnemo C, Moore GI, Simmons LW, Avise JC (1998) Microsatellite evidence for monogamy and sex-biased recombination in the Western Australian seahorse *Hippocampus angustus*. Molecular Ecology 7:1497-1505

King M (1995) Fisheries biology, assessment and management. Fishing News Books, Oxford.

Lourie SA, Vincent ACJ, Hall HJ (1999) Seahorses: An identification guide to the world's species and their conservation. Project Seahorse, London, UK.

Martin-Smith KM, Samoilys MA, Meeuwig JJ, Vincent ACJ (2004) Collaborative development of management options for an artisanal fishery for seahorses in the central Philippines. Ocean and Coastal Management 47:165-193

McClure MM, Holmes EE, Sanderson BL, Jordana CE (2003) A large-scale multispecies status assessment: anadromous salmonids in the Columbia River Basin. Ecological Applications 13:964-989

McDonald AD, Sandal LK, Steinshamn SI (2002) Implications of a nested stochastic/deterministic bio-economic model for a pelagic fishery. Ecological Modelling 149:193-201

Meffe GK, Carroll CR (1997) Principles of Conservation Biology 2nd Ed. Sinauer Associates, Inc. Publishers, Sunderland, Massachussetts.

Monteiro C (1989) La faune ichthyologique de la Ria Formosa; organisation spatio-temporelle. Page 219. Universite Montpellier, France.

Morita K, Yokota A (2002) Population viability of stream-resident salmonids after habitat fragmentation: a case study with white-spotted charr (*Salvelinus leucomaenis*) by an individual based model. Ecological Modelling 155:85-94

Morris WF, Doak DF (2002) Quantitative conservation biology: Theory and practice of population viability analysis. Sinauer Associates, Sunderland, Massachusetts.

Myers RA, Bowen KG, Barrowman NJ (1999) Maximum reproductive rate of fish at low population sizes. Canadian Journal of Fisheries and Aquatic Sciences 56:2404-2419

Myers RA, Mertz G, Fowlow RS (1997) Maximum population growth rates and recovery times for Atlantic cod, *Gadus morhua*. Fishery Bulletin 95:762-772

Perante NC, Vincent ACJ, Pajaro MJ (1998) Demographics of the seahorse *Hippocampus comes* in the central Philippines. Pages 439-448. 3rd International Conference on the Marine Biology of the South China Sea. Hong Kong University Press, Hong Kong.

Pérez-Ruzafa A, Quispe-Becerra JI, Garcia-Charton JA, Marcos C (2004) Composition, structure and distribution of the ichthyoplankton in a Mediterranean coastal lagoon. Journal of Fish Biology 64:202-218

Pitcher TJ, Hart PJB (1982) Fisheries Ecology. Croom Helm, London.

Reed DH, O'Grady JJ, Brook BW, Ballou JD, Frankham R (2003) Estimates of minimum viable population sizes for vertebrates and factors influencing those estimates. Biological Conservation 113:23-34

Reynolds JD, Mace GM (1999) Risk assessments of threatened species. Trends in Ecology & Evolution 14:215-217

Sadovy Y (2001) The threat of fishing to highly fecund fishes. Journal of Fish Biology 59:90-108

Shumway CA (1999) A neglected science: applying behavior to aquatic conservation. Environmental Biology of Fishes 55:183-201

Smedbol R (2002) Metapopulation theory and northern cod population structure: interdependency of subpopulations in recovery of a groundfish population. Fisheries Research 55:161-174

Smedbol R (2001) The importance of managing within-species diversity in cod and herring fisheries of the north-western Atlantic. Journal of Fish Biology 59:109-128

Sminkey TR, Musick JA (1996) Demographic analysis of the sandbar shark, *Carcharhinus plumbeus* in the western North Atlantic. Fishery Bulletin 94:341-347

Stobutzki I C, Miller M J, Brewer D (2001a) Sustainability of fishery bycatch: a process for assessing highly diverse and numerous bycatch. Environmental Conservation 28:167-181

Stobutzki IC, Miller MJ, Jones P, Salini JP (2001b) Bycatch diversity and variation in a tropical Australian penaeid fishery; the implications for monitoring. Fisheries Research 53:283-301

Teixeira RL, Musick JA (2001) Reproduction and food habits of the lined seahorse, *Hippocampus erectus* (Teleostei: Syngnathidae) of Chesapeake Bay, Virginia. Review Brazilian Biology 61:79-90

Thompson PM, Wilson B, Grellier K, Hammond PS (2000) Combining power analysis and population viability analysis to compare traditional and precautionary approaches to conservation of coastal cetaceans. Conservation Biology 14:1253-1263

Todd CR, Nicol SJ, Koehn JD (2004) Density-dependence uncertainty in population models for the conservation management of trout cod, *Maccullochella macquariensis*. Ecological Modelling 171:359-380

Vincent ACJ, Giles BG (2003) Correlates of reproductive success in a wild population of *Hippocampus whitei*. Journal of Fish Biology 63:344-355

Vincent ACJ, Meeuwig JJ, Pajaro MG, Perante NC (2001) Seahorse catches in the central Philippines: characteristics and conservation implications. Briefing document, Project Seahorse Fisheries Workshop, White Point, Nova Scotia. 30 May - 3 June 2001.

Vincent ACJ, Sadovy YJ (1998) Reproductive ecology in the conservation and management of fishes. In: Caro TM (ed) Behavioural Ecology and Conservation Biology. T. M. Caro, ed. Oxford University Press, NY pp 209-245

Vincent ACJ (1996) The International Trade in Seahorses. TRAFFIC International, Cambridge, UK.

Williams LR, Echelle AA, Toepfer CS, Williams MG, Fisher WL (1999) Simulation modeling of population viability for the leopard darter (Percidae : *Percina pantherina*). Southwestern Naturalist 44:470-477

**Table 5.1** Size ranges and fertilities of five age classes of *Hippocampus guttulatus*. Sizes at age were predicted from a von Bertalanffy growth model with parameters $K = 0.571$ and $L_\infty = 197.6$ mm SL (Chapter 1). Height, Ht was converted from standard length SL using the equation $\log_{10}Ht = 0.999\log_{10}SL - 0.052$ (Chapter 1). The number of brooding males used to estimate fertilities, $M_x$, for each age class is given by n (data from Chapter 2). Individuals that were about to turn 5 years old or older (4+ year olds) were grouped because the size differences among these age classes varied by only 2% (Chapter 1).

| Age Class | Stage (at census) | SL (cm) | Ht (cm) | n | $M_x$ ($yr^{-1}$) |
|---|---|---|---|---|---|
| 0 (almost 1 year old) | have reached size at first reproduction | 1.2 – 13.1 | 1.6 – 11.5 | 9 | 260.7 ± 116.7 |
| 1 (almost 2 years old) | Adults: have reproduced once | 13.2 – 16.0 | 11.6 – 14.1 | 79 | 372.4 ± 143.4 |
| 2 (almost 3 years old) | Adults: have reproduced twice | 16.1 – 17.6 | 14.2 – 15.5 | 21 | 501.2 ± 199.6 |
| 3 (almost 4 years old) | Adults: have reproduced three times | 17.7 – 18.5 | 15.6 – 16.3 | 4 | 724.5 ± 286.0 |
| 4+ (almost 5 years old or older) | Adults: have reproduced at least four times | >18.5 | >16.3 | 4 | 756.0 ± 291.2 |

**Table 5.2** The proportion of individuals in each age class and the survival rate of zero aged juveniles, $S_0$. The proportion of newborns was predicted from the number of females in each age class multiplied by their predicted fertilities. See text for more details.

| | Grid 2000 (Aug) | Grid 2001 (July) | Grid 2002 (June) | Grid 2003 (June) |
|---|---|---|---|---|
| N | 62 | 180 | 287 | 192 |
| Newborns | 0.99583 | 0.99393 | 0.99178 | 0.99210 |
| 0 | 0.00238 | 0.00395 | 0.00582 | 0.00556 |
| 1 | 0.00064 | 0.00116 | 0.00172 | 0.00162 |
| 2 | 0.00055 | 0.00062 | 0.00060 | 0.00056 |
| 3 | 0.00009 | 0.00014 | 0.00004 | 0.00007 |
| 4+ | 0.00055 | 0.00018 | 0.00002 | 0.00007 |
| $S_0$ | 0.0039 | 0.0058 | 0.0047 | |

**Table 5.3** Estimated age-specific survival rates, $S_x$, and fecundity rates, $F_x$ (both assumed to be log-normally distributed (Akçakaya 2002).

| Age | $S_x \pm SD$ | $F_x \pm SD$ |
|---|---|---|
| 0 | 0.0048 ± 0.0009 | 0.075 ± 0.036 |
| 1 | 0.294 ± 0.233 | 1.787 ± 0.765 |
| 2 | 0.294 ± 0.233 | 2.405 ± 1.058 |
| 3 | 0.294 ± 0.233 | 3.477 ± 1.519 |
| 4+ | 0.294 ± 0.233 | 3.628 ± 1.554 |

**Table 5.4** Predicted population sizes (N, in millions of individuals aged 0 to 4+ years), probability of quasi-extinction ($P_{ext}$ ± SD) and predicted time at extinction ($t_{ext}$ ± SD) for unexploited populations at the end of a 10 year period.

| Density-Dependence Model | $r_{max}$ | N (millions) ± SD | $P_{ext}$ ± SD | $t_{ext}$ ± SD |
|---|---|---|---|---|
| Ricker | 1.5 | 1.84 ± 1.47 | 0 | 10 ± 0.1 |
| | 2.5 | 2.22 ± 1.66 | 0.0008 | 10 ± 0.4 |
| | 5 | 2.09 ± 2.05 | 0.0256 | 9.4 ± 0.6 |
| | 7 | 1.84 ± 2.47 | 0.1173 | 8.2 ± 1.9 |
| Beverton-Holt | 1.5 | 1.94 ± 1.74 | 0 | >10 |
| | 2.5 | 2.44 ± 1.77 | 0 | >10 |
| | 5 | 2.76 ± 2.35 | 0 | >10 |
| | 7 | 2.84 ± 1.84 | 0 | >10 |
| Ceiling | | 0.39 ± 0.47 | 0 | 10.0 ± 0.2 |

**Figure 5.1** Mean relative population size at the end of a 10 year exploitation period as a function of fishing rate and $r_{max}$ for four different size limits: size at maturity (solid circles), size at first reproduction (open circles), size after first reproductive season (solid triangles) and size after two full reproductive seasons (open triangles). Results using Ricker (solid lines) and Beverton-Holt (dotted lines) functions are plotted together for low $r_{max}$ (1.5, 2.5).

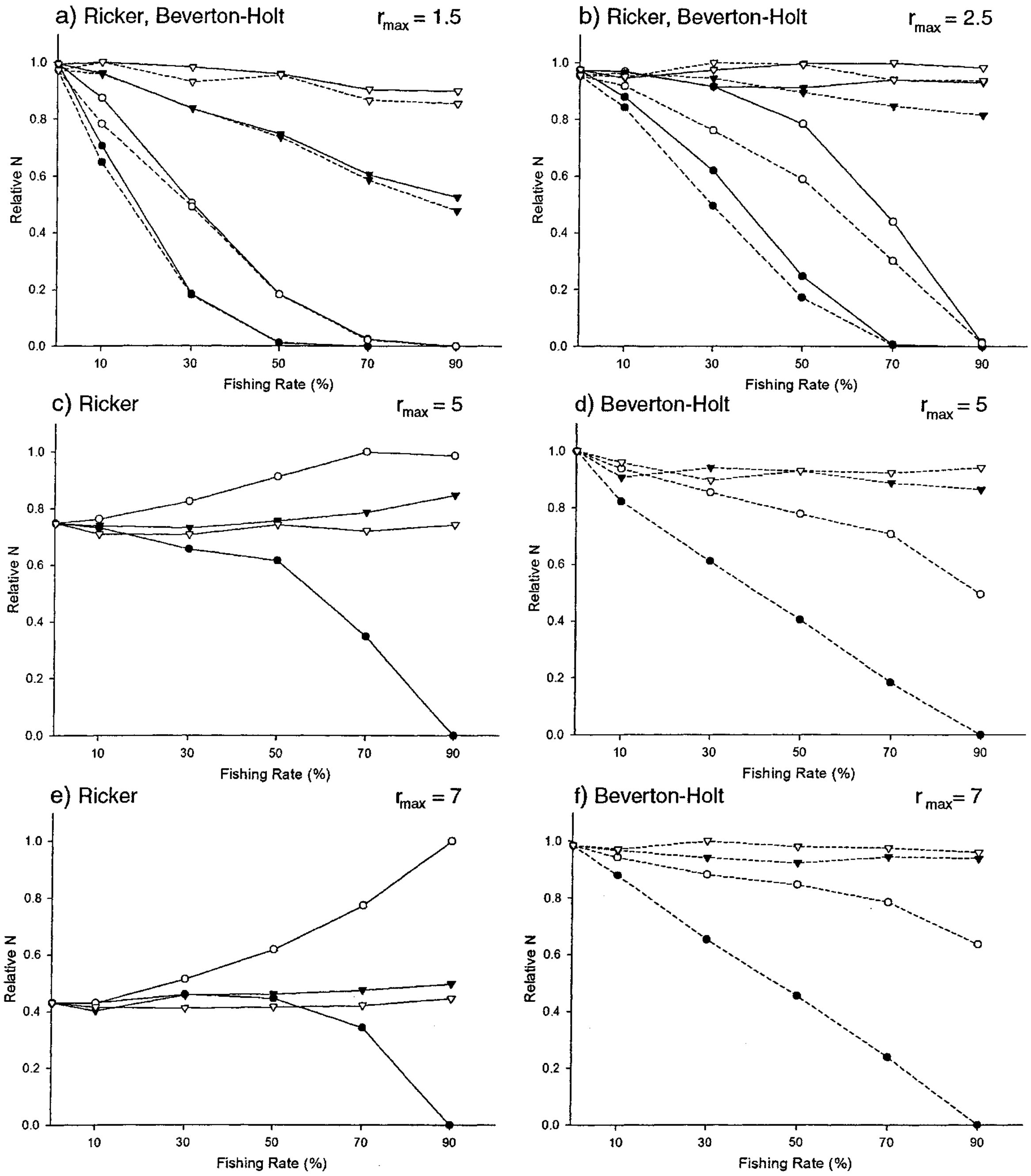


**Figure 5.2** Mean relative population size at the end of a 10 year exploitation period as a function of fishing rate assuming a ceiling model. Size limits set at size at maturity (solid circles), size at first reproduction (open circles), size after first reproductive season (solid triangles) and size after two full reproductive seasons (open triangles).

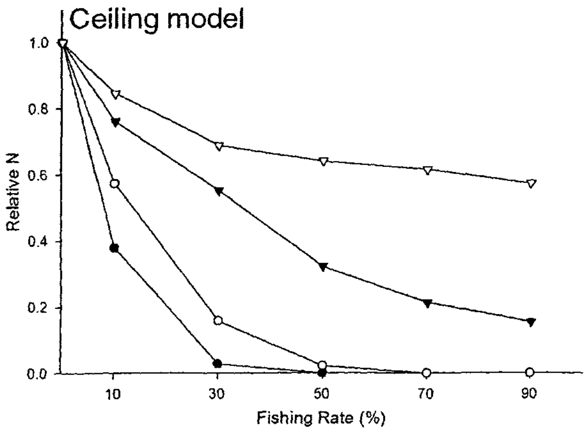


**Figure 5.3** Mean probability of quasi-extinction across a range of fishing rates for size limits set at size at maturity (solid circles), size at first reproduction (open circles), size after first reproductive season (solid triangles) and size after two full reproductive seasons (open triangles). (a) Ricker and Beverton-Holt lines combined, because quasi-extinction probabilities were identical when $r_{max}$ was 1.5 or 2.5. (b and c) Ricker (solid line) and Beverton-Holt (dotted line) results. (d) ceiling model predictions.

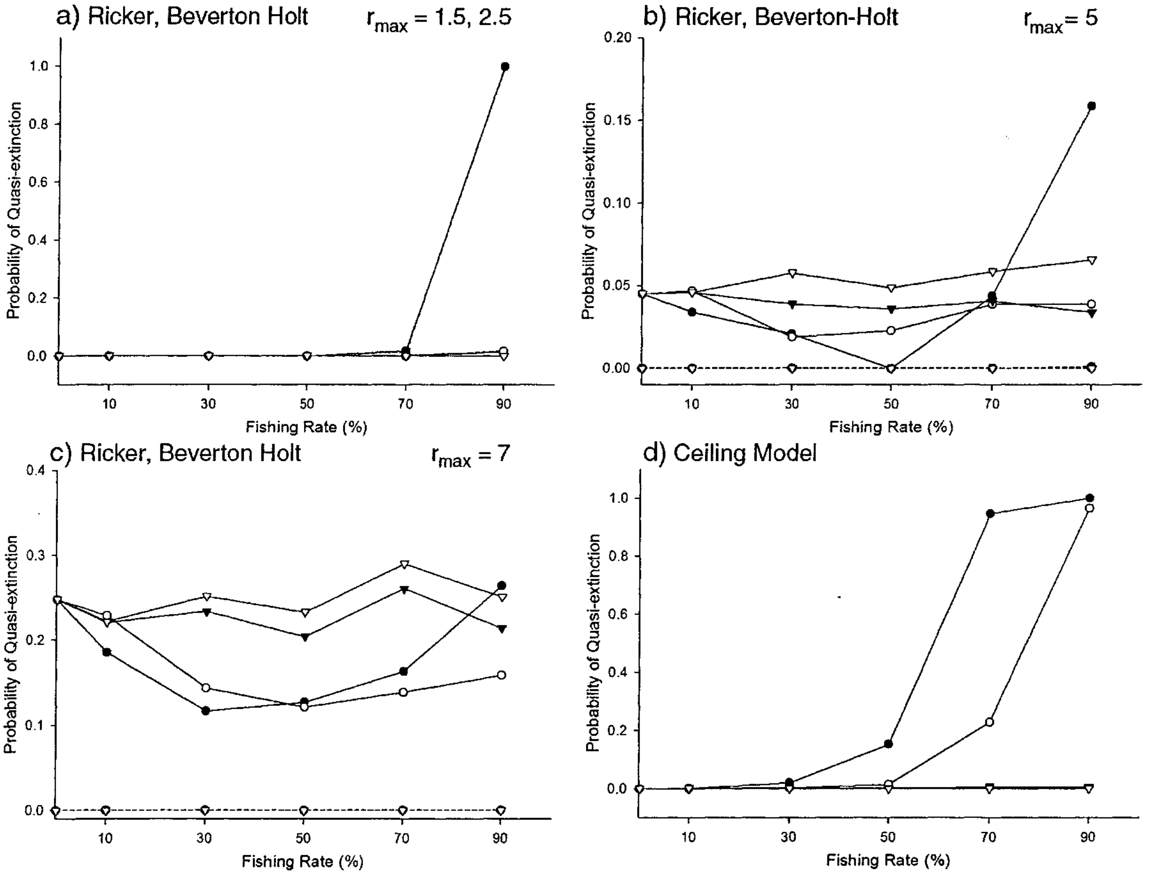
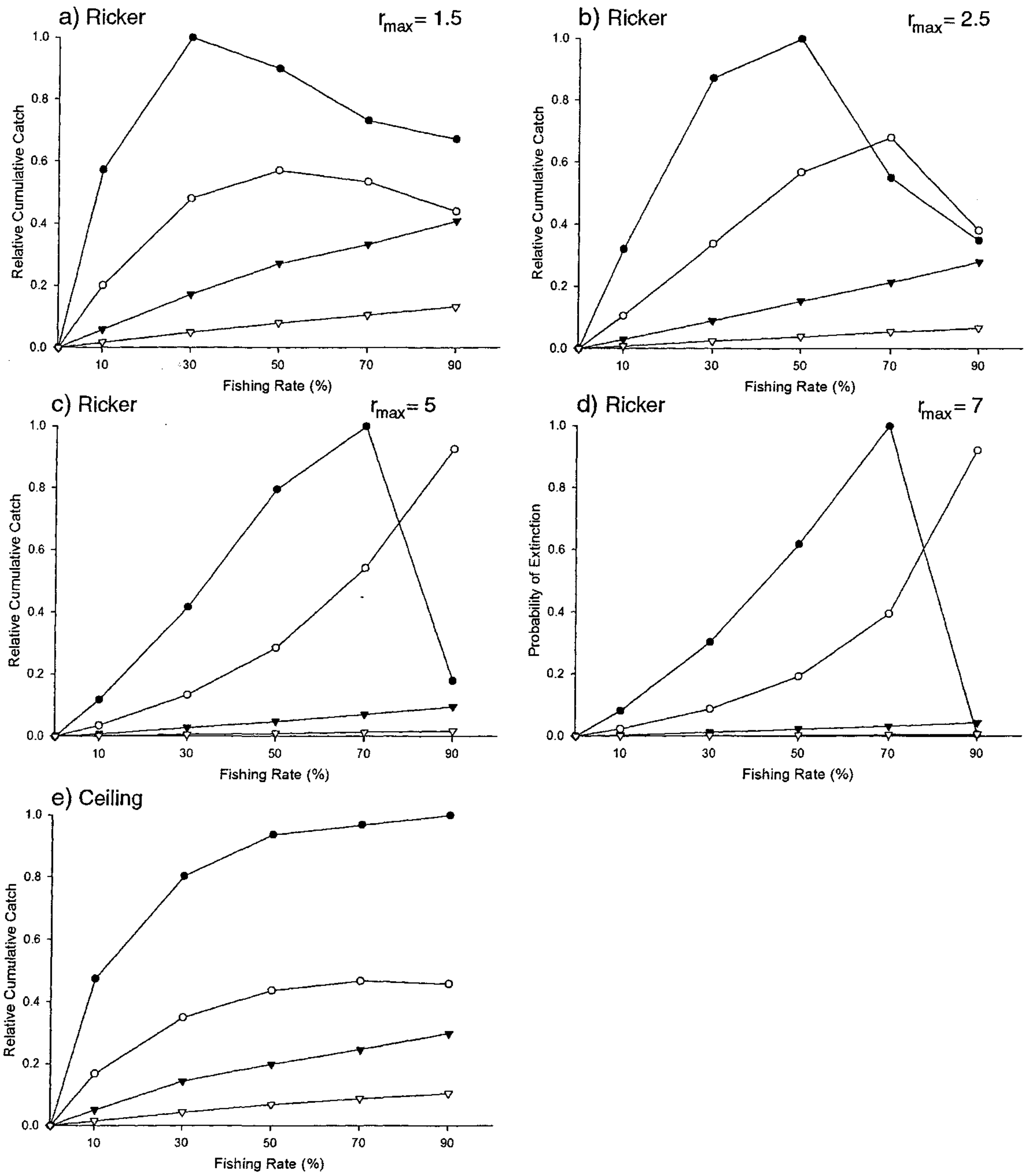


**Figure 5.4** Mean relative cumulative catches over a ten year exploitation period across a range of fishing rates and $r_{max}$ for four size limits set at size at maturity (solid circles), size at first reproduction (open circles), size after first reproductive season (solid triangles) and size after two full reproductive seasons (open triangles). (a to d) simulations with the Ricker function only, as these yielded qualitatively similar results to simulations with the Beverton-Holt function. (e) Relative cumulative catches assuming a ceiling model.



# FINAL CONCLUSIONS

The challenges of studying marine fishes mean that most studies investigating population trajectories have focused on large, commercially valuable species (Roberts & Polunin 1991) using changes in catch data over time to infer changes in distribution, abundance and population structure (Pitcher & Hart 1982, Hilborn & Walters 1992, King 1995, Hall 1999). The tremendous dearth of basic biological information available for most marine fishes (Froese & Pauly 2004) also means that the influences of habitat associations, behaviour (e.g. spawning aggregations, dispersal), and natural environmental variability on population trajectories are often not considered and/or poorly understood (Vincent & Sadovy 1998, Shumway 1999, Akçakaya & Sjorgen-Gulve 2000). In data-poor cases, the World Conservation Union (IUCN 1996), recommends that expert biological opinion should be used as an aid (though not as a substitute) for assessing the implications of complex behaviours for population-level responses to exploitation and/or habitat loss.

My thesis research was prompted by conservation concern for a group of predominantly Data-Deficient marine fishes, seahorses (IUCN 2003, Foster & Vincent 2004), that derived from suspected declines in population size and/or reports from fishers that exploited populations had declined considerably within a short period of time (Vincent 1996). Despite a paucity of basic biological information (life history, abundance, ecology and population dynamics) for most species, seahorses are recognized as vulnerable to exploitation (Foster & Vincent 2004) because of their monogamous mating pattern, specialized parental care, small brood size, sedentary adults, association with threatened habitats, capture as bycatch in non-selective fishing gears, and economic value (Vincent 1996, IUCN 2003, Foster & Vincent 2004). These inferences represented the first step in a multi-tiered risk assessment (Dulvy et al. 2004) for seahorses.

In my thesis, I quantitatively investigated five aspects of risk assessment:

(a) life history (Chapter 1, Chapter 2)

(b) habitat associations (Chapter 3)

(c) ecological interactions (Chapter 3)

(d) behaviour (Chapter 1, Chapter 3, Chapter 4, Chapter 5)

(e) population dynamics in response to habitat loss and exploitation (Chapter 3, Chapter 4, Chapter 5).

Because I considered some aspects of risk assessment separately (e.g. life history strategy in Chapter 1 considered separately from habitat associations in Chapter 3), in my concluding remarks, I synthesize all of my chapters by classifying my estimates of *H. guttulatus* life history and ecological parameters into one of two categories – high or low vulnerability to extinction – using published qualitative and/or quantitative criteria (Roberts & Hawkins 1999; IUCN 2001; Stobutzki et al. 2001) (Table C.1). When all life history and ecological traits are considered simultaneous, suites of characteristics that confer risk or resilience become apparent, as do priorities for research. Here, I draw four general conclusions about the implications of *H. guttulatus* life history and ecology for conservation:

1 Most of the life history parameters relating to the survival, growth, and reproduction of *H. guttulatus* that I quantified (Chapter 1, Chapter 2) are generally considered to confer resilience to disturbance and a greater likelihood of recovery when the disturbance ceases (Hutchings 2001, Hutchings & Reynolds 2004). Three notable life history traits (categorized under high vulnerability in Table C.1) that are exceptions include a short life span, a monogamous mating pattern and parental care. Species with short life spans are more vulnerable to extended periods of poor recruitment than longer-lived species (Sadovy 2001, King & McFarlane 2003). Monogamy means that exploited populations may suffer reductions in population-level reproductive output associated with the loss of either males or females and/or disruptions of pair bonds (for species with long term mate fidelity, Vincent & Sadovy 1998). Extended parental care reduces the probability of successful reproduction prior to capture (Stobutzki et al. 2001). However, the implications of poor recruitment and/or reduced reproductive output for population dynamics are contingent on the capacity for recovery (see below),

population size, intensity and frequency of disturbance, and the nature and strength of density-dependence.

2 An understanding of the capacity for *H. guttulatus* to recover from low population size is a priority for research, as evidenced by a lack of information about dispersal patterns, colonization potential and density-dependence (Table C.1). Previous analyses of seahorse life history and ecology have focussed on traits that confer risk to seahorses, giving little consideration to traits that confer population resilience to disturbance or a capacity for recovery from disturbance (e.g. Foster & Vincent 2004). Predicting the potential for recovery (Stobutzki et al. 2001a) is an important contribution to prioritizing management efforts, and essential for refining risk assessments.

Early age at maturity is inversely correlated with intrinsic rate of population increase (Myers et al. 1997) and may confer resilience to seahorse populations when the direct and indirect effects of disturbance cease. However, resilience to disturbance is also contingent on movement patterns (dispersal distance, migration rates), recruitment patterns, ecological interactions (e.g. competitive ability, habitat associations) and density-dependence in population growth rates. A dearth of information about these parameters limits the predictive accuracy of risk assessment models for seahorses (Chapter 5).

3. The distribution of *H. guttulatus* confers risk to this species (Table C.1). *Hippocampus guttulatus* occupies shallow, inshore seagrass-dominated habitats that are vulnerable to disturbance and pollution (Sheperd et al. 1989, Short & Wyllie-Escheverria 1996, Pasqualini et al. 1999, Wolff 2000). Although *H. guttulatus* was locally abundant in the Ria Formosa (Chapter 3), populations appear to be patchy in distribution (unpublished data). However, *H. guttulatus* is widely distributed in the north-eastern Atlantic Ocean and Mediterranean Sea (Lourie et al. 1999), and therefore likely to be buffered against localized disturbances. Because the Ria Formosa is intermediate (spatially, ecologically,

Monteiro 1989) between the northeastern Atlantic Ocean and the Mediterranean Sea, its abundant populations of *H. guttulatus* and *H. hippocampus* may be important links between populations these two bodies of water.

4. The vulnerability of *H. guttulatus* to exploitation requires further investigation. Although *H. guttulatus* has a low catchability in traditional seines (Chapter 4), catchability will vary according to gear type. The sedentary nature of adults (and to a lesser extent settled juveniles, Chapter 1), as well as low mobility means that local populations of settled juveniles and adults can be rapidly depleted when seahorses are directly targeted or when the frequency of non-selective exploitation is high. More information about the nature, distribution, intensity and frequency of fishing effort relative to the distribution of *H. guttulatus* is required before one can evaluate the extent to which exploitation threatens populations of this species (Table C.1).

The synthesis of *H. guttulatus* life history, discussed in the context of conservation, has prompted the recognition of the importance of reevaluating preconceptions about the population-level responses of seahorses to exploitation (Chapter 1, Chapter 5). Despite considerable parental investment (a trait of equilibrium strategists), *H. guttulatus* shared many characteristics with opportunistic strategists, even when controlling for allometry (Chapter 1, Fig 1.10b). Thus, future research that investigates the life history patterns of small marine fishes may indicate that seahorses are intermediate in their life history strategy, sharing characteristics with both small-bodied opportunists and small-bodied equilibrium strategists. Emerging evidence also suggests that seahorse population sizes may fluctuate (even in the absence of exploitation) over local scales (Monteiro 1989, Grid A, discussed in Chapter 5), landscape scales (Monteiro 1989; Vincent & Martin-smith, unpublished data), and regional scales (de Silva et al. 2003), thus strengthening the need to re-examine assumptions about the dynamics of unexploited populations and their response to disturbance.

By relating interspecific differences in habitat associations to differences in population-level responses to reduced fishing effort (Chapter 3, Chapter 4), my thesis underscored the importance of recognizing that even species with similar life histories and putatively similar habitat associations may repond differently to changes in disturbance regimes. Further research would clarify whether differences in population-level responses to reduced fishing derived from interspecific differences in local recruitment dynamics, or from ecological interactions not investigated in this thesis (e.g. competition, community structure, prey availability).

This study also underscored the importance of considering temporal and spatial scaling in risk assessments; my thesis was carried out over local temporal (4 years) and spatial scales (100 $m^2$ grid, 26 $km^2$ population). Thus assessments carried out over regional scales and longer time periods are needed to validate inferences from this research.

Multivariate approach to risk assessment for *H. guttulatus* provided a useful starting point for identifying general life history strategies and developing successful monitoring and management programs. The predictive accuracy of such multivariate analyses were improved by considering not only the life history traits of fishes, but also the implications of ecology and behaviour (e.g. movement patterns, reproductive behaviour, ecology) when evaluating the dynamics of marine fish populations in response to disturbance (Vincent & Sadovy 1998, Kramer & Chapman 1999, Shumway 1999; Stobutzki et al. 2001).

Transparent risk assessment techniques are powerful when used as a focal point for consensus building, particularly when the aim is to develop adaptive management strategies (Ferson & Burgman 2002). As I demonstrated in Chapter 5, even models that synthesize limited data can be useful for identifying management strategies that are (a) robust to a range of values for unknown parameters, (b) supported by stakeholders, and (c) flexible enough to respond to emerging information (Chapter 5). This formal risk assessment model helped clarify tradeoffs between securing wild populations and maintaining viable fisheries (and thus trade), as well as signally the need to estimate the capacity for population recovery from low densities (i.e. resilience).

While no simulation is a substitute for empirical research, models are the first step towards formalizing a management problem, synthesizing available information and predicting responses to precautionary initiatives (Heppell et al. 2002), even in data poor situations. I hope that my findings will prompt the application of quantitative risk assessment models, including population viability analyses, to marine fishes, even to those considered to be Data-Deficient. The nature and strength of such assessments prompt transparent and objective evaluations of biological assumptions, relative risks and important gaps in understanding (Morris & Doak 2002).

# LITERATURE CITED

Akçakaya HR, Sjorgen-Gulve P (2000) Population viability analyses in conservation planning: an overview. Ecological Bulletins 48:9-21

Dulvy NK, Ellis JR, Goodwin NB, Grant A, Reynolds JD, Jennings S (2004) Methods of assessing extinction risk in marine fishes. Fish and Fisheries 5:255-276

Ferson S, Burgman M (2002) Quantitative methods for conservation biology. Springer-Verlag, New York

Froese R, Pauly D (eds) (2004) FishBase. World Wide Web electronic publication. www.fishbase.org, Version 05/2004.

Hall SJ (1999) Fish Biology and Aquatic Resources Series 1: The effects of fishing on marine ecosystems and communities. Blackwell Science Ltd, Oxford.

Heppell SS, Crouse DT, Crowder LB (2002) Using matrix models to focus research and management efforts in conservation. In Ferson S, Burgman M (eds) Quantitative methods for conservation biology. Springer-Verlag, New York

Hilborn R, Walters CJ (1992) Quantitative Fisheries Stock Assessment: Choice, Dynamics and Uncertainty. Kluwer Academic Publishers, Massachussetts, USA.

Hunter JR, Leong R (1981) The spawning energetics of female northern anchovy, *Engraulis mordax*. Fishery Bulletin 79: 215-230

IUCN (1996) Threatened fish? Initial Guidelines for applying the IUCN Red List Criteria to Marine Fishes. In: Hudson E, Mace GM, Baillie J, Sobel J, Punt A, Safina C, Vincent ACJ, Roberts C, Sadovy Y, Hall H (eds) IUCN/WWF/ZSL Workshop on marine fish and the IUCN red list of threatened animals. IUCN, London, UK

IUCN (2001) IUCN red list categories and criteria: Version 3.1. In: Commission ISS (ed) IUCN, Gland, Switzerland

IUCN (2003) 2003 IUCN Red List of Threatened Species. <www.redlist.org>, Downloaded on 31 August 2004

King JR, McFarlane GA (2003) Marine fish life history strategies: applications to fishery management. Fisheries Management and Ecology 10:249-264

King M (1995) Fisheries biology, assessment and management. Fishing News Books, Oxford.

Kramer DL, Chapman MR (1999) Implications of fish home range size and relocation for marine reserve function. Environmental Biology of Fishes 55:65-79

Lourie SA, Vincent ACJ, Hall HH (1999) Seahorses: An identification guide to the world's species and their conservation. Project Seahorse, London UK.

Monteiro CC (1989) La faune ichthyologique de la Ria Formosa: Répartition et organisation spatio-temporelle des communautés. PhD Dissertation, Université des Sciences et Techniques du Languedoc, Montpellier, France

Pasqualini V, Pergent-Martini C, Pergent G (1999) Environmental impact identification along the Corsican coast (Mediterranean sea) using image processing. Aquatic Botany 65: 311-320

Pitcher TJ, Hart PJB (1982) Fisheries Ecology. Croom Helm, London.

Roberts CM, Polunin NVC (1991) Are marine reserves effective in management of reef fisheries? Reviews in Fish Biology and Fisheries 1: 65-91.

Roberts CM, Hawkins JP (1999) Extinction risk in the sea. Trends in Ecology & Evolution 14(6):241-246

Sadovy Y (2001) The threat of fishing to highly fecund fishes. Journal of Fish Biology 59(Suppl. A):90-108

Sheperd SA, McComb AJ, Bulthuis DA, Neverauskus V, Steffensen DA, West R (1989) Decline of seagrasses. In: Larkum AWD, McComb AJ, Sheperd SA (eds) Biology of Seagrasses. Elsevier, Amsterdam, pp. 346-393

Short FT, Wyllie-Escheverria S (1996) Natural and human-induced disturbances of seagrasses. Environmental Conservation 23:17-27

Shumway, C (1999) A neglected science: applying behavior to aquatic conservation. Environment Biology of Fishes 55: 183-201.

Stobutzki I C, Miller M J, Brewer D (2001a) Sustainability of fishery bycatch: a process for assessing highly diverse and numerous bycatch. Environmental Conservation 28:167-181

Vincent ACJ, Sadovy Y (1998) Reproductive ecology in the conservation and management of fishes. In: Caro, Tim (ed). Behavioural Ecology and Conservation Biology. Oxford University Press, Oxford.

Wolff WJ (2000) Causes of extirpations in the Wadden Sea, an estuarine area in The Netherlands. Conservation Biology 14: 876-885

Table C.1 Summary of *Hippocampus guttulatus* life history and ecological traits, ranked according to their correlations of high or low vulnerability (after Roberts & Hawkins 1999, Stobutzki et al. 2001). Gaps in information are also listed as research priorities.

| **Characteristic** | **High Vulnerability** | **Low Vulnerability** | **Research Priority** |
|---|---|---|---|
| ***Survival and Growth*** | | | |
| Growth rate | | rapid, K = 0.571 | |
| Natural mortality rate | | high, M = 1.22 – 1.15 $yr^{-1}$ | |
| Body size | | small, $L_\infty$ = 197.6 mm | |
| Longevity | short[a] | short[a] (4.3 – 5.5 years) | |
| ***Reproduction*** | | | |
| Reproductive frequency | | multiple spawner (4.2 $yr^{-1}$), March - November | |
| Age or size at maturity | | young (0.5 years) , small (110 mm) | |
| Age or size at first reproduction | | young (~0.9 years), small (~127 mm) | |
| Generation time | | Short (1.4 years) | |
| Sexual dimorphism | | Limited (male trunk shorter) | |
| Sex change | | None, dioecious | |
| Spawning aggregations | | None | |
| Parental care | 3 week brooding | | Implications for population dynamics unknown |
| Mating pattern | Monogamy | | No long term pair bonds in a dense population, implications for low density / population dynamics unknown |
| ***Capacity for population recovery*** | | | |
| Dispersal | | | Eight week planktonic stage, but dispersal distance unknown |
| Competitive ability | | | Ecological interaction unknown |
| Colonizing ability | | | Migration rates unknown |
| Adult mobility | <20 $m^2$ home range | | |
| Recruitment | | annual, strong in Ria Formosa | Recruitment dynamics in other populations unknown |
| $R_{max}$ | | (inferred to be relatively high) | Need to validate indirect estimate based on regression analysis |
| Compensatory dynamics | | | Ricker function suspected, but unknown |
| Allee effects (depensation) | | | Disruption of reproduction due to exploitation is |

|  |  |  |  |
|---|---|---|---|
|  |  |  | expected to reduce fecundity when density is low, but depensatory dynamics are unknown |
| ***Range, distribution and abundance*** |  |  |  |
| Horizontal distribution | Inshore |  |  |
| Vertical depth range | narrow, max 12 m[b] |  |  |
| Geographic range |  | large, north-eastern Atlantic Ocean and Mediterranean Sea |  |
| Habitat specificity | high, vegetated habitats |  |  |
| Habitat vulnerability to anthropogenic destruction | high, seagrasses vulnerable to disturbance and pollution |  |  |
| Demersal | yes, after settlement |  |  |
| Soft or muddy substrate | Yes |  |  |
| Patchiness of populations within range | High, in estuaries and/or lagoons |  |  |
| Commonness/Rarity |  | locally abundant, mean density of 0.07 $m^{-2}$ and max density of 1.5 $m^{-2}$ |  |
| Changes in population size |  |  | Fluctuations in CPUE data, but few estimates of abundance over time and space |
| ***Trophic level*** |  | Low |  |
| ***Vulnerability to Exploitation*** |  |  |  |
| Direct |  |  | requires further study |
| Indirect |  |  | requires further study |
| Catchability |  | low, catchability is 0.1 | requires further study for different gears |
| Survival during discard |  |  | High in *H. erectus* (Baum et al. 2003) |
| Mortality Index[c] |  | low, 0.66 |  |

[a] Short life span probably means relatively high intrinsic rate of increase, but vulnerability to extended periods of poor recruitment.

[b] J. Curtis unpublished data, as reported by Foster & Vincent (2004), <40 m assumed to be vulnerable, Stobutzki et al. (2001).

[C] Mortality index calculated as $= \frac{(L_{\infty} - L_{mean})}{(L_{mean} - L_{\min})}$, where a value >3.44 confers high vulnerability, and a value <1.88 confers low vulnerability (after Stobutzki et al. 2001).

# APPENDIX A: ANIMAL CARE CERTIFICATE